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Hematological Responses in a Freshwater Fish *Channa punctatus* Due to Fenvalerate

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Natural pyrethrins, extracted from the flowers of *Chrysanthimum cinerariafolium* have been used as insecticides since the eighteenth century (Green et al 1979). Their importance was realized when synthetic pyrethroids were developed in the twentieth century and now several such compounds are being used all over the world (Elliott 1989).

The role of pyrethroids in the promotion of our economy is very important. These compounds have brought benefits to mankind by destroying the insect pests of various crops, resulting in increased food production and controlling the vectors of human and animal diseases. However, extensive use of pyrethroids for insect pest control has endangered the existence of aquatic life. They enter in the aquatic environment through various routes and cause great pollution in inland water, resulting in the mortality.

The persistence of these toxic chemicals in aquatic environments is dangerous for the survival of fish (Mawdesley 1971; Salyi and Csaba 1994; Banik et al 1996). Harmful effects of three synthetic pyrethroids cypermethrin, permethrin and fenvalerate to a non-target fish, *Channa striatus* has been reported by Singh and Agarwal (1994). Toxicity of these compounds to edible freshwater fishes may be harmful to the human being, because fish form a part of the human diet.

Blood is a pathophysiological reflector of the body because it is highly susceptible to internal and external environmental fluctuations. Physicomorphological changes in blood indicate the changes in the quality of the environment and therefore blood parameters are important in diagnosing the functional status of the animal exposed to toxicants. The present work is related to the effect of fenvalerate on certain blood parameters of an edible freshwater fish *Channa punctatus*. Behavioral changes in *Channa punctatus* exposed to fenvalerate have also been observed.

MATERIALS AND METHODS

Channa punctatus (length 8-10 cm and weight 25-40 g) were obtained from local waters and acclimatized in the laboratory in an aquarium for 10 days and

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then divided into four experimental groups and one control group in different aquaria (75cm X 40cm X 30cm) containing 0.15, 0.25, 0.40 and 0.55mg/L of fenvalerate. In all the experiments dechlorinated tap water was used. Fish were washed with 0.1% $KMnO_4$ solution to avoid dermal infection. If mortality occured during the experimental period, dead fish were removed immediately to avoid depletion of dissolved oxygen (DO) level which adversely affects other fish (Schreck and Brouna 1975).

In the aquaria at least one litre of water was maintained for each gram of fish weight. Artificial aeration was not provided because aeration is reported (APHA et al 1985) to alter the results of toxicity tests. The experiments were repeated twice to confirm the results.

The fishes were maintained upto their death or a maximum period of 30 days whichever was earlier. During this period fishes were sacrificed at an interval of 5 days to examine the effect of this compound on the haematology of fish.

Blood was obtained by cutting the caudal peduncle with capillaries, using heparin as anticoagulant. First few drops were discarded and only the first 5 ml of blood was taken, since the entry of lymph into the blood is reported (Schermer 1954) to affect haematocrit value. The blood was transferred to empty injection glass vials containing anticoagulant (heparin) in requisite quantity.

TEC (Total Erythrocyte Count) and TLC (Total Leucocyte Count) were determined with Neubauer's counting chamber using Hayem's fluid and gentian violet for dilution of blood for TEC and TLC respectively. Haemoglobin concentration was examined by the cyanmethaemoglobin method (Dacie and Lewis, 1969). PCV (Packed Cell Volume) or haematocrit value were measured by using Wintrobe tubes of approximately 3 mm internal diameter and about 110 mm height with graduations at 1mm intervals upto 100 mm. The uncoagulated blood mixed by repeated inversion was filled in Wintrobe tubes upto 100 mm. The tube was then centrifuged at 2800 rpm for 30 minutes. The height of the column of red cells was taken as the PCV and expressed as a percent of the total volume of blood (Dacie and Lewis, 1969).

Bleeding time was determined after giving a slight cut on caudal peduncle of the fish. At regular intervals it was blotted with an absorbent paper. The decrease in size of the blot indicated decrease in haemorrhage. As soon as the bleeding ceased the time was recorded.

Clotting time was determined by filling a thin capillary tube (100 mm in length and 1.5 mm in diameter) with fresh blood and then breaking it at regular intervals until a fibrin thread was observed, indicating coagulation of the blood. The time was recorded using a stop watch.

The data was analyzed for standard deviation from mean, significance (Student's one tailed 't' test) and ANOVA using standard statistical techniques as given by Steel and Torrie 1982.

RESULTS AND DISCUSSION

Observations are summarised in Tables 1- 6. A highly significant decrease can be observed in RBC, haemoglobin concentration and PCV of *Channa punctatus* (Table 1-3). This decrease was greater and quicker with increasing sublethal dose of fenvalerate. Leucocytosis, i.e., increase in TLC, was observed (Table 4) in fishes exposed to fenvalerate. Bleeding time and clotting time were also increased in experimental fishes after their exposure to 0.4 ppm fenvalerate for 30 days (Tables 5 - 6).

Table 1. Effect of fenvalerate on RBC (in millions /mm^3) of *C. punctatus*.

Conc mg/L	0 DAY	05 DAYS	10 DAYS	15 DAYS	20 DAYS	25 DAYS	30 DAYS
Ctrl	2.4 ± 0.9[a]	2.3 ± 0.9[a] 0.4[b]	2.3 ± 0.9 2.5	2.3 ± 0.9 0.8	2.3 ± 0.9 1.3	2.3 ± 0.9 3.0	2.3 ± 0.9 1.3
0.15	-	2.3 ± 0.9 1.3	2.3 ± 0.9 2.5	2.3 ± 0.9 3.4	2.2 ± 0.9 5.2	2.2 ± 0.8 4.8	2.1 ± 0.8 8.6
0.25	-	2.2 ± 0.9 6.0	2.2 ± 0.8 6.1	2.1 ± 0.8 10.3	2.0 ± 0.8 12.9	1.9 ± 0.8 14.8	1.9 ± 0.8 19.3
0.40	-	2.1 ± 0.8 9.8	2.1 ± 0.8 10.9	1.9 ± 0.8 17.1	1.9 ± 0.8 19.7	1.8 ± 0.8 21.4	1.7 ± 0.8 25.7

a = mean of 6 individuals ± S.E.

b =percent decrease in RBC above control

Fish are known to be affected upon exposure to low levels of pyrethroid pesticide (Ohtsuka 1993, Madsen et al 1996). Pyrethroids have a high toxicity to fish in water without particulate matter and cause adverse changes in the populations or productivity of the aquatic ecosystem (Hill 1989). The haematological parameters such as RBC, TLC, PCV, bleeding time and clotting time have been effectively used as sensitive diagnostic indicators of pyrethroid poisoning. The present investigations show that prolonged exposure of *Channa punctatus* to fenvalerate in water induces a variety of anomalies in the haematology of this fish.

In the present observations reduction in RBC count and Hb concentration indicate the occurance of acute anaemia, which is one of the most sensitive pathological situations developed as a result of pyrethroid poisoning in fishes. Pyrethroids are known to induce anaemia in fishes (Reddy and Bhashamohideen 1989). Anaemia associated with erythropenia has also been reported by Srivastava and Mishra

1979) in *Colisa fasciatus* after acute exposure to lead. PCV appears to be positively correlated with RBC counts. Similar results have been reported for several freshwater fishes (Khalaf Allah 1999; Balathakur and Bais 2000; Rehulka 2000).

Table 2. Effect of fenvalerate on blood Hb (in g/100ml) of *C. punctatus*.

Conc mg/L	0 DAY	05 DAYS	10 DAYS	15 DAYS	20 DAYS	25 DAYS	30 DAYS
Ctrl	13.8 ± 2.1[a]	13.7 ± 2.1[a] 0.1[b]	13.3 ± 2.1 1.9	13.4 ± 2.1 2.4	13.7 ± 2.1 0.1	13.6 ± 2.1 0.9	13.6 ± 2.1 1.2
0.15	-	13.4 ± 2.1 2.4	13.3 ± 2.1 1.0	13.3 ± 2.1 1.0	13.2 ± 2.1 4.0	13.1 ± 2.1 3.8	13.0 ± 2.1 2.6
0.25	-	13.3 ± 2.1 2.9	13.3 ± 2.1 1.0	13.3 ± 2.1 1.0	13.2 ± 2.1 3.5	13.2 ± 2.1 2.9	13.2 ± 2.1 2.6
0.40	-	13.2 ± 2.1 3.9	13.1 ± 2.1 3.0	12.8 ± 2.1 4.4	12.7 ± 2.1 7.6	12.1 ± 2.0 11.3	11.3 ± 1.9 16.5

a = mean of 6 individuals ± S.E.

b = percent decrease in blood Hb above control

Table 3. Effect of fenvalerate on packed cell volume (in %) of *C. punctatus*.

Conc mg/L	0 DAY	05 DAYS	10 DAYS	15 DAYS	20 DAYS	25 DAYS	30 DAYS
Ctrl	38.7 ± 3.6[a]	38.5 ± 3.6[a] 0.5[b]	38.0 ± 3.6 1.6	37.9 ± 3.6 1.9	38.0 ± 3.6 1.6	37.8 ± 3.5 2.2	36.5 ± 3.5 5.5
0.15	-	37.4 ± 5.6 2.8	36.8 ± 3.5 3.3	37.7 ± 3.4 5.8	35.0 ± 3.4 7.9	34.4 ± 3.4 9.0	33.2 ± 3.3 9.0
0.25	-	35.6 ± 3.4 7.3	34.6 ± 3.4 9.0	33.8 ± 3.4 10.8	32.9 ± 3.3 13.4	31.4 ± 3.2 17.0	30.2 ± 3.2 17.4
0.40	-	34.2 ± 3.4 11.0	32.9 ± 3.3 13.4	31.0 ± 3.2 18.2	30.0 ± 3.2 21.2	28.7 ± 3.1 24.1	27.2 ± 3.0 25.4

a = mean of 6 individuals ± S.E.

b = percent decrease in PCV above control

Leucocytosis was evidenced by the increase in WBC counts during fenvalerate

intoxication and this has been reported by Arora (1995) and Singh (1992) in *Channa punctatus* and *Heteropneustes fossilis* after exposure to derma- orange and γ-BHC, respectively.

Table 4. Effect of fenvalerate on TLC (in millions/mm^3) of *C. punctatus*.

Conc mg/L	0 DAY	05 DAYS	10 DAYS	15 DAYS	20 DAYS	25 DAYS	30 DAYS
Ctrl	32.1 ± 3.3[a]	32.0 ± 3.2[a] 0.2[b]	31.8 ± 3.3 0.8	32.1 ± 3.3 0.1	32.0 ± 3.2 0.2	31.9 ± 3.2 0.6	31.9 ± 3.3 0.6
0.15	-	32.3 ± 3.3 0.9	32.4 ± 3.3 2.0	32.6 ± 3.3 1.5	32.8 ± 3.3 2.6	33.0 ± 3.3 3.4	33.1 ± 3.3 3.8
0.25	-	32.7 ± 3.3 2.2	33.0 ± 3.3 3.9	33.3 ± 3.3 3.7	33.7 ± 3.5 5.4	34.3 ± 3.4 7.5	34.7 ± 3.4 8.9
0.40	-	33.3 ± 3.3 4.1	33.7 ± 3.4 6.1	34.2 ± 3.4 6.5	34.7 ± 3.4 8.4	35.6 ± 3.4 11.5	40.2 ± 3.7 26.0

a = mean of 6 individuals ± S.E.

b = percent decrease in TLC above control

Table 5. Effect of fenvalerate on bleeding time (in seconds) of *C. punctatus*.

Conc mg/L	0 DAY	05 DAYS	10 DAYS	15 DAYS	20 DAYS	25 DAYS	30 DAYS
Ctrl	24.8 ± 2.9[a]	32.6 ± 3.3[a] 31.6[b]	32.5 ± 3.3 30.9	34.0 ± 3.4 37.0	35.1 ± 3.4 41.6	35.8 ± 3.4 44.3	36.8 ± 3.5 48.5
0.15	-	34.3 ± 3.4 5.1	35.3 ± 3.4 8.7	37.0 ± 3.5 8.8	38.5 ± 3.6 9.5	39.8 ± 3.6 11.2	41.6 ± 3.7 13.1
0.25	-	36.5 ± 3.5 11.7	38.8 ± 3.6 19.5	40.5 ± 3.7 19.1	42.5 ± 3.8 20.8	44.5 ± 3.8 24.2	46.6 ± 3.9 26.6
0.40	-	38.3 ± 3.6 17.3	40.6 ± 3.7 25.1	43.5 ± 3.8 28.0	46.5 ± 4.0 32.2	48.0 ± 4.0 34.0	50.6 ± 4.1 37.6

a = mean of 6 individuals ± S.E.

b = percent increase in bleeding time above control

Table 6. Effect of fenvalerate on clotting time (in seconds) of *C. punctatus*.

Conc mg/L	0 DAY	05 DAYS	10 DAYS	15 DAYS	20 DAYS	25 DAYS	30 DAYS
Ctrl	38.7 ± 3.6[a]	38.5 ± 3.6[a] 0.5[b]	38.0 ± 3.6 1.6	37.9 ± 3.6 1.9	38.0 ± 3.6 1.6	37.8 ± 3.5 2.2	36.5 ± 3.5 5.5
0.15	-	37.4 ± 5.6 2.8	36.8 ± 3.5 3.3	37.7 ± 3.4 5.8	35.0 ± 3.4 7.9	34.4 ± 3.4 9.0	33.2 ± 3.3 9.0
0.25	-	35.6 ± 3.4 7.3	34.6 ± 3.4 8.9	33.9 ± 3.3 10.8	32.9 ± 3.3 13.4	31.4 ± 3.2 17.0	30.2 ± 3.1 17.4
0.40	-	34.2 ± 3.4 11.0	32.9 ± 3.3 13.4	31.0 ± 3.2 18.2	30.0 ± 3.2 21.2	28.7 ± 3.1 24.1	27.2 ± 3.0 25.4

a = mean of 6 individuals ± S.E.

b = percent increase in clotting time above control

The increase in WBC count can be correlated with an increase in antibody production which helps in survival and recovery of the fish exposed to sublethal concentrations of pesticide (Joshi et al 2002). The present findings also show hypersensitivity of leucocytes for fenvalerate and these changes may be due to immunological reactions to produce antibodies to cope up with stress induced by fenvalerate.

The increased blood clotting time in *Channa punctatus* after exposure to fenvalerate might be due to a thrombocytopenic effect. Hougie (1971) and Lone and Javaid (1976) demonstrated that fish with disease or organophosphate toxicosis had increased blood clotting time.

The results of the present investigations show that the entire physiology of fish was disturbed and they were under stress during fenvalerate exposure. This may be due to residue accumulating in their blood and other tissues. Fenvalerate creates haematological disturbances and causes metabolic disorders in *Channa punctatus* which ultimately lead to the deterioration of general health of this fish.

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