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EFFECTS OF CYPERMETHRIN ON SOME HAEMATOLOGICAL PARAMETERS IN *HETEROPNEUSTES FOSSILIS* (BLOCH)

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ABSTRACT
The present experimental project was designed to assess the impacts of Cypermethrin, a 4th generation insecticide on haematological parameters in *Heteropneustes fossilis* viz. total erythrocyte count (TEC) and hemoglobin content (Hb). Fishes were subjected to 0.1 μg/L (1/6th of 96h. LC_{50}) of Cypermethrin 10% emulsified concentration for 24h, 48h, 72h and 96h to study the toxic effects of the chemical pesticide. The 96h LC_{50} value of cypermethrin for the test fish was found to be 0.67μg/L. There was a significant decrease ($p < 0.05$) in total erythrocyte count (TEC) and haemoglobin content (Hb) of treated fishes compared with the control group.

INTRODUCTION

The synthetic pyrethroids are among the most potent and effective insecticides available accounting for more than 30% of the world market for insecticides (Moore and Waring, 2001). The main advantage of pyrethroids is their photostability, high effectiveness already in low concentration, easy disintegration and low toxicity to birds and mammals. (Bradbury and Coats, 1989; Maud *et al*., 1998) Cypermethrin is a type of cyanophenoxy-benzyl pyrethroid and is categorized as restricted use pesticide (RUP) by United States Environmental Protection Agency (USEPA) because of its high toxicity to fish (Extension Toxicology Network, 1996). This 4th generation synthetic pyrethroid is used to control pests of cotton, fruits and vegetable crops (Pedigo, 1996). Use of cypermethrin is rapidly increasing throughout the world because of its low toxicity to birds and mammals (USEPA, 1989). Although toxicity of synthetic pyrethroids in birds and domestic animals is low, fishes are extremely sensitive to the neurotoxic effects of these pesticides. The excess use of this pesticide may enter into natural waters through agricultural run-off and ultimately cause damage to non-target organisms such as fish. (Stephenson, 1982; Prashanth and Neelagund, 2008; Singh *et al*., 2010). The freshwater catfish *Heteropneustes fossilis* is widely cultivated in rice fields, swamps and derelict water bodies (Chondar, 1999) and is thus frequently exposed to agricultural runoff. Fish mortality may occur because of the use of cypermethrin in normal agricultural practice (Shires, 1983).

Blood represents an index of physiological disorder as it immediately responds to any change in surrounding environment or physiological stress. Various workers (Chauhan *et al*., 1994; Agarwal and Chaturvedi, 1995; Nath and Banerjee, 1996) have also reported a decrease in RBC count, haemoglobin and PCV of some fish sps after their exposure to insecticides. Hence, in the present study an attempt has been made to study the effects of cypermethrin on some haematological parameters, selecting *H. fossilis* as an experimental model.

MATERIALS AND METHODS

Healthy and sexually matured living fishes of length 12 $\pm$ 0.5cm and weight 25 $\pm$ 0.5g were procured from a local fish farm in Guwahati, Assam and disinfected in 0.1% solution of potassium permanganate for 5 minutes to avoid dermal infection. The fishes were allowed to acclimate in a glass aquarium in the laboratory for one month. The water of the aquarium was changed daily. Fishes were fed daily with commercial fish food C. P. Classic to avoid effects of starvation. The feeding was discontinued 24h prior to exposure. Commercial grade cypermethrin (10%EC) of liquid formations manufactured by United Phosphorus Ltd. was purchased from local agro-chemical stores. The LC_{50} value of cypermethrin 10% emulsified concentration with 95% confidence limit was estimated for 24, 48, 72 and 96h by probit analysis (Finney, 1971). The 96h. LC_{50} cypermethrin for *H. fossilis* was found to be 0.67μg/L. The selected concentration for the present experiment was 0.1 μg/L, which is 1/6th of 96h. LC_{50} value. The experiments were conducted for 24h, 48h, 72h and 96h

to study the short-term exposure effects and each treatment experiment was repeated for 7 times. At the end of each exposure, fishes were thoroughly rubbed with a clean piece of cloth and blood was collected by serving off the caudal region of the fish. Clotting of collected blood was prevented by using anticoagulant EDTA (of India Drugs and Pharmaceuticals Ltd. Hyderabad).Total count of RBC was done by Neubaur haemocytometer and haemoglobin content (Hb) of blood was determined with the help of Sahli's Haemoglobinometer following the routine procedure.

Student's t-test was used to analyze the statistical significance between the control and cypermethrin treated fishes.

RESULTS

The total count of erythrocytes after 24h of exposure to 96h of exposure in treated fishes showed gradual decrease as compared to that of control. Alterations in the total count of RBC in *Heteropneustes fossilis* is given in Table 1. Total erythrocyte count (TEC) in the control set stood at the range of $4.10 \pm 0.009 \times 10^6/mm^3$ to $4.20 \pm 0.009 \times 10^6/mm^3$ of blood with an average count of $4.15 \pm 0.009 \times 10^6/mm^3$ of blood . In treated fishes, the total count of erythrocytes after 24h of exposure to 96h of exposure showed gradual decrease from $3.75 \pm 0.011 \times 10^6/mm^3$ to $3.60 \pm 0.014 \times 10^6/mm^3$ of blood as compared to control. Haemoglobin content of *H. fossilis* in the control and treated fishes is presented in the Table 2. The haemoglobin content of blood of control fishes ranged from 18.9 ± 0.075 g/100mL to 18.3 ± 0.075 g/100mL with an average value of 18.6 ± 0.075 g/100mL.In treated fishes, haemoglobin content showed gradual decline from 17.7 ± 0.092 g/100mL to 15.5 ± 0.092g/100mL of blood after 24h. of exposure to 96h. of exposure. A significant decrease ($p < 0.05$) of RBC and Hb content was observed with exposure to cypermethrin.

Table 1: Total Erythrocyte count (TEC) of control and cypermethrin (10% EC) treated *Heteropneustes fossilis* expressed in million cells/ mm³ of blood

Period of exposure	Control (Mean ± S.Em.)	Treated (Mean ± S.Em.)	t-value
24h	4.10 ± 0.009	3.75 ± 0.011	t_1 = 22.786*
48h	4.20 ± 0.007	3.71 ± 0.014	t_2 = 28.770*
72h	4.10 ± 0.007	3.68 ± 0.014	t_3 = 25.798*
96h	4.20 ± 0.009	3.60 ± 0.014	t_4 = 33.609*
Control average value = $4.15 \pm 0.009 \times 10^6/mm^3$			

Values are mean ± S.E. of 7 observations; *indicates that significant difference at 5% level; t_1 = t value of 24h control and treated fishes; t_2 = t value of 48h control and treated fishes; t_3 = t value of 72h control and treated fishes; t_4 = t value of 96h control and treated fishes.

Table 2: Haemoglobin content (Hb) of control and cypermethrin (10% EC) treated *Heteropneustes fossilis* expressed in g/100mL of blood

Period of exposure	Control (Mean ± S.Em.)	Treated (Mean ± S.Em.)	t-value
24h	18.9 ± 0.075	17.7 ± 0.092	t_1 = 10.212*
48h	18.6 ± 0.075	16.9 ± 0.100	t_2 = 13.561*
72h	18.4 ± 0.075	16.6 ± 0.100	t_3 = 13.558*
96h	18.3 ± 0.075	15.5 ± 0.092	t_4 = 23.134*
Control average value = 18.6 ± 0.075 g/100mL			

Values are means ± S.E. of 7 observations;*indicates that significant difference at 5% level, t_1 = t value of 24h control and treated fishes, t_2 = t value of 48h control and treated fishes, t_3 = t value of 72h control and treated fishes, t_4 = t value of 96h control and treated fishes.

DISCUSSION

The gills are the routes of entry for any toxicant in fishes from which it is transported to other parts of the body through circulating blood. Therefore alterations in the haematological parameters are considered to be a sensitive indicator for toxic insult and environmental pollution. The findings of the present experimental project clearly revealed the haematoxic effects of cypermethrin. It is commonly believed that the properties of blood are very sensitive to physiological and pathological changes in fish. The most common observation in the haematological picture of the fishes exposed to cypermethrin was marked loss of erythrocytes accompanied by loss of haemoglobin content in the peripheral blood with the progress of the exposure paradiagm. A decrease in total erythrocyte count (TEC) and Hb content were reported in *H. fossilis* (Nath and Banerjee, 1996) and in common carp (Svobodova *et al*., 2003) after their treatment with cypermethrin. Various workers (Saxena and Seth, 2002; Adhikari *et al*., 2004; Parma *et al*., 2007) also reported significant decrease in RBC and Hb content in different freshwater fishes after their exposure to cypermethrin. Some other workers (Pandey *et al*., 1976; Ranganathan and Rammurthi, 1979; Goel *et al*., 1982) reported the decrease in RBC count and Hb content in fishes after their exposure to various pesticides. Another study reported that due to high lipophilicity cypermethrin becomes adsorbed on the particulate matter in natural environment which reduces the bioavailability of this compound (Hill, 1989). Mortality of fishes by cypermethrin in natural environment was also reported by some workers (Shires, 1983; Edwards *et al*., 1987). In the present study cypermethrin was found to exhibit high toxicity to common fresh water fish *H.*fossilis under laboratory condition by causing a significant change in the haematology. Although under field conditions, synthetic pyrethroids are considered to pose less risk due to high adsorption to soil, these data should be considered when assessing potential ecosystem risks.

From the present study it can be concluded that when fishes are exposed to the fourth generation pesticides like cypermethrin, they have various haematoxic effects which make the fishes less fit for survival. This in turn will affect the fecundity of the fish population and also other organisms including human beings through food chain.

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