RESEARCH ARTICLE

Modulation of blood profile of juvenile *Cyprinus carpio* exposed to imidacloprid

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ABSTRACT

Cyprinus carpio is a common fresh water fish abundantly distributed in ponds, beels and canals of India. The fish is regularly consumed because of its high nutritional value. Pesticides are common pollutants of the aquatic environment because of their persistent and tendency to concentrate in aquatic organisms. This freshwater fish is continuously exposed to imidacloprid toxicity as this pesticide enters the body through gills and contaminated food. Fresh water *C. carpio* were exposed to different concentrations of imidacloprid for varied span of time in controlled laboratory condition to measure haematological responses. Data is indicative of cellular stress in *C. carpio* that may lead to decline population size in its natural habitat.

Keywords: *Cyprinus carpio*, Imidacloprid, Blood.

INTRODUCTION

The aquatic environment is continuously being contaminated with chemicals from agriculture and urban activities. Fish constitute a dietary item for human consumption and aquatic pollution affects health and survival status of the organisms. The extensive use of pesticides are widely practiced to enhance the crop production and other benefits has raised concerns about potential adverse effects on the environment, human health and non-target animals such as freshwater fishes and other aquatic organisms even at very low concentration (El-Sayeed et al., 2007). Fish can serve as vital indicators of imidacloprid toxicity as they are continuously exposed to the pesticide through gill ingestion and ingestion of pesticide contaminated food. In the present study, the toxic effects of pesticide were screened in freshwater edible fish, *Cyprinus carpio* in relation to blood parameters and homeostasis.

MATERIALS AND METHODS

Collection & acclimatization of fish

The small size freshwater fish, *Cyprinus carpio*, weighing 10±0.4 gram and

measuring 7±0.5 cm were collected with the help of local fisher man from water bodies located in Halisahar, North 24 Parganas district of West Bengal. The fish was properly washed in tap water and treated with 0.02% $KMnO_4$ and 0.004% formalin solution to remove external infection of algae, fungi etc. Fishes were separately maintained at temperature ranging between 14^0C-30^0C in aquarium of 50 litre capacity with continuously aerated and dechlorinated tap water (pH 7.2-7.4; hardness 185- 200 mg/l as $CaCO_3$; alkalinity 170-175 mg/l as $CaCO_3$) for 15 days before taken for experimentation. The animals were fed with boiled eggs and earthworms. Water was renewed periodically so as to maintain the dissolved oxygen. The specimens were devoid of feeding prior to the test period to reduce the quantum of excretory products in the aquarium to avoid vomiting of the fish.

Determination of LC_{50}

Prior to treatment, LC_{50} value of imidacloprid for *Cyprinus carpio* was calculated following Trimmed Spearman Karber Method (Hamilton et al., 1977). During determination of the median lethal concentration (LC_{50}) of imidacloprid to *Cyprinus carpio*, the fishes were divided into three equal groups consisting of 10 each and each group was transferred separately to glass aquaria of 50 litre volume. The group I fish were maintained as control without any treatment, the group II and III fishes were exposed to various concentrations of imidacloprid for four days to determine the median lethal concentration (LC_{50}) for selection of sublethal dose.

Experimental design

The experiment was conducted in a static system in glass aquarium of 50 litre capacity. The acclimatized fishes were grouped into three experimental groups each consisting of five fishes. The experimental groups were categorized based on the LC_{50} value and from the reports of highest level of imidacloprid contamination of natural freshwater bodies.

Group 1: Fish subjected to zero pesticide level (control).
Group 2: Fish subjected to 20 ppm of imidacloprid.
Group 3: Fish subjected to 40 ppm of imidacloprid.

The fish were exposed to sublethal concentrations of imidacloprid for 4, 7, 10, 20 and 30 days. The blood was collected from control and treated fish for haematological investigation.

Blood collection

Blood was collected from the caudal vein by using plastic disposable syringe fitted with 26-gauge needle. The collected blood was then taken immediately into a plastic eppendroff containing small amount of anticoagulant EDTA and placed on ice.

***Enumeration of leucocyte* (*cells/cu.mm*)**

Blood was collected from the caudal vein of the fish by using plastic disposable syringe fitted with 26-gauge needle. Then it was drawn to 0.5 mark of WBC pipette, extra blood is wiped out from the tip, and WBC diluting fluid was drawn to 11 marks, mixing the solution well by the bead present in the pipette; i.e., dilution became 1:20. The counting was done with the help of Neubauer Hemocytometer (Wintrobe, 1967).

Haemoglobin percentage (%) estimation

Haemoglobin was tested using Haemometer (Humtsoe et al., 2007). At first, N/10 HCl was taken upto mark 2 of the graduated tube of Haemometer.Blood collected from treated and control fishes from caudal vein, taken in blood pipette up to 20 microliter marks. Then the blood was blown out into previously taken N/10 HCl of graduated tube. It was mixed well by a glass stirrer and waited for 3-5 minutes, then the distilled water was added till the colour of the sample gets same intensity with the standard tubes. Percentage marks were noted from the graduated tube. Haemoglobin concentrations were expressed in g/100ml.

Determination of Clotting Time (seconds)

Clotting time was determined from the blood collected from caudal vein of treated and control fish. First drop of blood was wiped off, then the one end of 12 cm long glass capillary tube was placed at bleeding site and filled by blood. With 5 seconds interval, tube was broken off about 5 mm each time, until the thin fibrin thread appeared. Stopping the watch, time was noted.

RESULTS AND DISCUSSION

LC_{50} of imidacloprid in C. carpio

Median and lethal concentrations of imidacloprid in *C. carpio* of 7±0.5 cm length were estimated against different span of exposure i.e., 1, 2, 3, & 4 days in static water environment. The highest LC_{50} value against imidacloprid was determined as 200 ppm for 96 hours exposure. Based on the LC_{50} value, sublethal

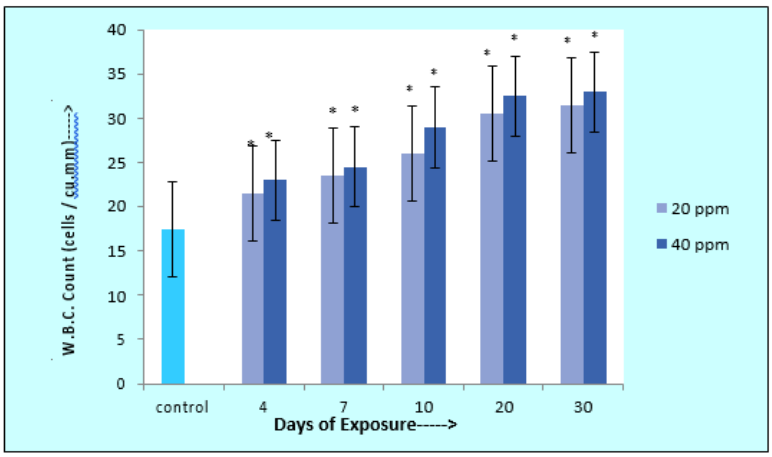

Fig. 1: Leucocyte count of control and experimental *Cyprinus carpio* exposed to sublethal concentration of imidacloprid. Data is represented as Mean ± S.D. Stastical significance is shown at $p < 0.05$* (n=3).

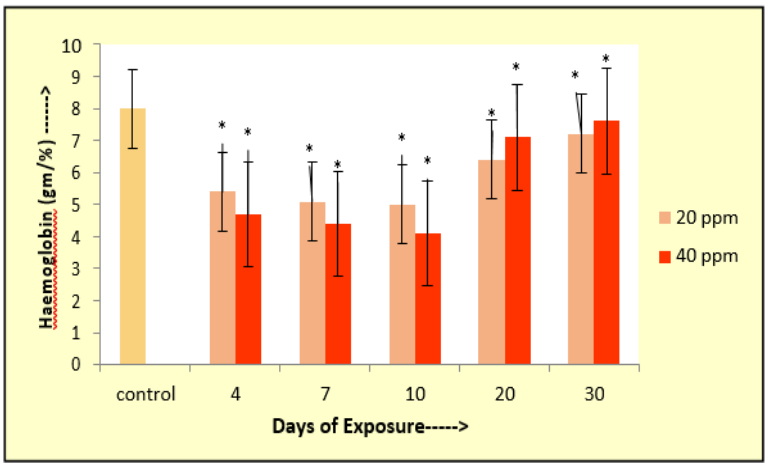

Fig. 2: Effect of imidacloprid on haemoglobin concentration of *Cyprinus carpio*. Data is represented as mean ± S.D. Statistical significance is shown at $p < 0.05$ * (n=3).

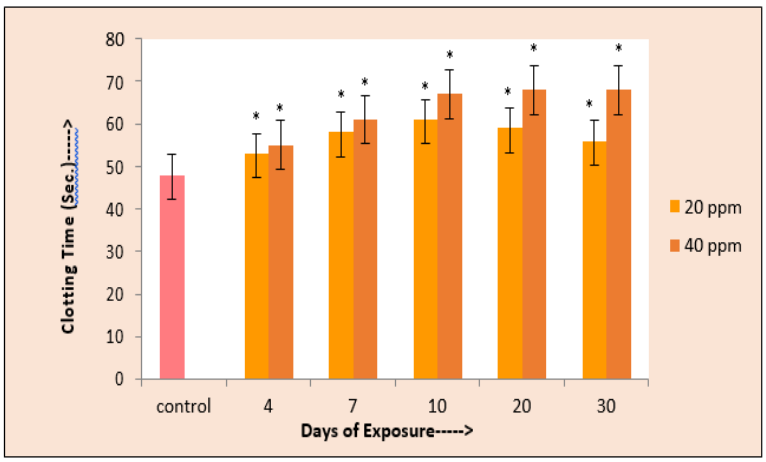

Figure 3: Effect of imidacloprid on clotting time of *Cyprinus carpio*. Data is represented as Mean ± S.D. Statistical significance is shown at $p < 0.05$ * (n=3).

concentration of 20 ppm and 40 ppm of imidacloprid were selected as experimental concentration for animal.

Total Leucocyte Count (cells/cu.mm)

High white blood cell count indicates damage due to infection of body tissues, severe physiological stress as well as leukemia. White blood cell counts were found to increase significantly following imidacloprid exposure as shown in Figure 1.

Haemoglobin (gm/%)

The exposure of *Cyprinus carpio* to sublethal concentration of imidacloprid for varied span of exposure exhibit decrease in haemoglobin concentration (Figure 2) that may lead to anaemia. Anaemia, under imidacloprid induced stress may also due to disruption in haemoglobin synthesis.

Clotting Time (seconds)

In the present study, exposure of fish to sublethal concentration of imidacloprid for varied span of exposure caused significant increment in clotting time in result to control (Figure 3).

Measurements of haematological parameters are important in diagnosing the structural and functional status of animals exposed to toxicant because blood parameters are highly sensitive to environmental and physiological changes and health conditions (Sarvanam et al., 2011). White blood cells are involved in the regulation of immunological functions and their number increase as a protective response in fish to stress (Mishra and Niyogi, 2011). Increased total leucocyte count in *C. carpio* exposed to sublethal concentration of imidacloprid may be due to stimulated lymphopoiesis.

In the present study, the significant increase in the number of WBC (Figure 1) indicates the stress condition of fish caused by pesticide toxicity which might

have produced hypoxia and gill damage. The exposure of *C. carpio* to sublethal concentration of imidacloprid significantly decreased Hb% (Figure 2). Sarvanam et al. (2011) reported that reduced haemoglobin content in toxicant exposed fish may be due to disruption of haemopoetic process and accelerated disintegration of erythrocyte cell membrane. A clot is formed as the end product of the blood coagulation (Balw and Sinha, 1999). *C. carpio* under sublethal exposure of pesticide exhibits impairment in clotting time (Figure 3) that may lead to onset of physiological stress and disruption of blood homeostasis.

CONCLUSION

The focus of environmental monitoring has evolved from measuring the discrete sources of pollution towards defining the effects of multiple sometimes unknown, stressors (Cairns et al., 1993). Haematological parameters of fish can be helpful to identify the target organs of effects of toxic effect and also the general health condition of harmful changes in stressed organism. The findings of the present study reflect that imidacloprid exposure of *C. carpio* affects its haematological profile. This parameter would be effectively used as potential biomarker of imidacloprid to the fresh water fish in the field of environmental biomonitoring.

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

Balw S and Sinha MP (1999) Haematological effects of toxicity of sublethal concentration of some heavy metal salts on *Channa punctatus* leucocytes. *J. Environ. Poll,*2 (4): 219-222.

Cairns J Jr, McCormick P V and Niederlehner, B R (1993) A proposed framework for developing indicators of ecosystem health. *Hydrobiologia,* 263(1):1-44.

El-Sayed YS, Saad TT and El-Bahr SM (2007) Acute intoxication of deltamethrin in monosex Nile tilapia, *Oreochromis niloticus* with special reference to the clinical, biochemical and haematological effects. *Environ*. *Toxicol. Pharmacol*, 24: 212–217.

Hamilton MA, Russo RC and Thurston RV (1977) Trimmed Spearman- Karber Method for Estimating Median Lethal Concentration in Toxicity Bioassays 11: 714- 719; Correction (1978), 12: 417.

Humtsoe N, Davoodi R, Kuikarni B G and Chavan B (2007) Effect of arsenic on the enzymes of the Rohu carp. *Labeo rohita* (Hami Hon, 1882). *Ruff. Bull*, 14:17-19.

Mishra A and Niyogi PA (2011) Haematological changes in the Indian Murrel (*Channa punctatus*, Bloch) in response to phenolic industrial wastes of the Bhilai steel plant Chhaittisgarh, India). *Int. J. Res. Chem. Environ*,1(2):83-91.

Saravanam M, Ramesh M, Malarvizhi A and Petkam R (2011) Toxicity of neem leaf extracts(*Azadirachta indica*, A. Juss) on some haematological, ionoregulatory, biochemical and enzymological parameters of Indian major carp, *Cirrhinus mrigala*. *J. Trop.Environ*, 1(1):14-26.

Wintrobe MM (1967) Clinical hematology, 6th Eds, Lea and Febiger, Philadelphia, Library of Congress, Print USA.

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