

Effect of Imidacloprid on Haematological Parameters of a *Channa Punctatus* (Bloch.) And Reversal Responses

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ABSTRACT: The objective of this study was to investigate the reference values for haematological ranges and recovery responses for freshwater teleost *Channa punctatus* (Bloch.). The fish was exposed to sublethal concentration (0.282 ppm) for 24, 48, 72 and 96 hrs to assess the alterations in the level of haematological parameters for acute study. Significant changes in haematological parameters such as RBCs, WBCs and Hb of fish, *Channa punctatus* were observed. These changes were found to be time and dose dependent.

KEYWORDS: Haematological parameters, Imidacloprid, *Channa punctatus*.

I. INTRODUCTION

Environmental pollution problem in the developing world are clearly linked to unbalanced ecosystem. Environment contamination by pesticides has been documented in both biotic and abiotic components. The random use of different pesticides often causes lot of damage to non target organism. The pollution of environment due to use of pesticides has become an increasing problem over the last century with the development of industry, agriculture and increase in population (Syed, *et al.*, 2012). Several workers have studied haematological alterations due to the pesticide toxicity in fish (Kumar and Nelson, 1997; Tilak, *et al.*, 2003; Shukla, *et al.*, 2005 and Remia, *et al.*, 2008). Modern agricultural practices result in indiscriminate use of various pesticides, which usually enter into the aquatic environment. The use of pesticides in the field has the potential to change the aquatic medium, affecting the tolerance limit of aquatic fauna and flora, as well as creating danger to the ecosystem (Adedeji and Okocha, 2012). Water pollution has emerged as one of the most pressing problems of this century. Water quality management, water pollution control and environmental protection are the main issues to save our future. Imidacloprid (Confidor) is one of the most widely used insecticides in India and other parts of the World. It is sold under many trade names which include Confidor, Admire, Gaucho, Merit, Advantage, Provado, and Winner. The International Union of Pure and Applied Chemistry (IUPAC) name is 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine and chemical formula is $C_9H_{10}ClN_5O_2$ (CCME, 2007). Neonicotinoid insecticides are grouped into 1) Nitroguanidine: Clothianidin, Dinotefuran, Imidacloprid, Imidaclothiz, Thiamethoxam. 2) Nitromethylene: Nitenpyram, Nithiazine. 3) Pyridylmethylamine: Acetamiprid, Imidacloprid, Nitenpyram, Paichongding, Thiacloprid. 4) Nereistoxin analogue insecticides: Bensultap, Cartap, Polythialan, Thiocyclam, Thiosultap.

The present study has been undertaken to have a basic understanding of the acute toxicity of Imidacloprid insecticide on the experimental fish *Channa punctatus*. Imidacloprid enters the target pest via ingestion or direct contact. Imidacloprid is used for controlling sucking insects, soil insects, termites, and some chewing insects. India is a major supplier of fish in the world. In 2006 the country exported over 6, 00,000 metric tonnes of fish, to some 90 countries, earning over \$1.8 billion. In 2008 India was the sixth largest producer of marine and freshwater capture fisheries, and the second largest aquaculture farmed fish producer in the world (CIFT, 2008). Man is using a number of chemicals and there are a number of chemicals in the environment now. Some of the chemicals are toxic to living system which get incorporated into food chain, disturb the biochemical processes occurring in the living body causing deformities, diseases and even death are called environmental toxicants (Patnaik, *et al.*, 2010). India is an agricultural

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country with a large rural population (60-80%), where pesticides are freely available and are used extensively. Haematological tests are important diagnostic tools and recent findings have suggested that they may be equally valuable as indicators of disease or stress due to pollutants and environmental fluctuations in fish. Haematological parameters are related to responses of the organisms to the changing environmental conditions and therefore can be used to screen the healthy state of fish experimented to the exposed toxicant.

II. MATERIAL AND METHODS

Healthy fresh water fish, *Channa punctatus* (av. length 08.40±1.14 to 10.50 ±1.56 cm and av. weight 12.14±0.45 to 15.54±0.48 gm) of both sexes were purchased from local market of Amalner, district, Jalgaon and used as the test animal, carefully brought to the laboratory avoiding any injury to the fish during transport. Identification of fish was done with the help of standard keys and available literature. The fish were disinfected with 0.01% solution of KMnO_4 for two minutes and then transferred to a 45 litre (60×30×45 cm) capacity glass aquarium to acclimatized for 15 days in laboratory conditions before use. The tank water was aerated continuously and food was provided daily in the morning in the form of chopped goat liver, only white part of boiled egg, meat, chicken as well as pieces of earthworm. After acclimatization, fish were kept in different concentrations of Imidacloprid in to 5 litre capacity plastic tubs. Finney (1971) Probit Analysis LC_{50} determination method was used to find out the LC_{50} concentration. The formula used to assess the mortality of the fish recommended by the WHO (2004) and FAO (2002). The commercial grade Imidacloprid (1-[6-chloro-3-pyridylmethyl]-N-nitroimidazolidin-2-ylideneamine) was chosen for the experiment and was obtained from the local market, Amalner. The toxicity of the Imidacloprid on the fresh water fish, *Channa punctatus* was assessed and determined LC_{10} , LC_{50} and LC_{90} for 24, 48, 72 and 96 hrs by probit analysis as per Organisation for Economic Co-operation and Development (OECD) guidelines for testing of chemicals (OECD 203 "Fish, acute toxicity test", 1992 and 2010). After completion of experimental and control groups, five fish were taken without anesthesia after an interval of 24, 48, 72 and 96 hrs for haematological study and five fish were remained for recovery study. Blood was collected from five fish from each group by heart puncturing as well as posterior caudal vein using Ethylene-diaminetetraacetate (EDTA) as anticoagulant. Red blood corpuscles (RBCs), White blood corpuscles (WBCs) counts were done with an improved Neubauer haemocytometer (Shah and Altindag, 2004) under the Microscope (Olympus Microscope 20i BIMEF, Olympus (India) Pvt, Ltd, Noida) and Hemoglobin (Hb) concentration was measured by Sahli's method (Sahli, 1962). The results obtained were subjected to analysis for mean and standard deviation. The mean values of treatment were subjected to statistical analysis using two-way analysis of variance (ANOVA) to test the (0.05) level of significance between the various sublethal concentrations of Imidacloprid.

III. RESULT AND DISCUSSION

In present study White Blood corpuscles increased, red blood corpuscles counts exhibited a decrease in their numbers and hemoglobin values also decreased significantly were reported after exposure to the sublethal dose of Imidacloprid. Malathi, *et al.* (2012) studied comparatively ranges values of haematological parameters in *C. punctatus* and *C. striatus*. They reported as RBC 3.29×10^3 to $3.42 \times 10^6/\text{cumm}$; WBC 4.3 to $4.6 \times 10^3/\text{cumm}$ and haemoglobin 9.37 to 10.84 g/dl. In present study normal values of haematological parameters were reported as RBC $3.28 \times 10^6/\mu\text{l}$; WBC $14.44 \times 10^3/\mu\text{l}$ and haemoglobin 13.1 g/dl. RBCs decreased to $2.67 \times 10^6/\mu\text{l}$ (18.59%), $2.33 \times 10^6/\mu\text{l}$ (28.96%), $2.19 \times 10^6/\mu\text{l}$ (33.23%) as well as $2.47 \times 10^6/\mu\text{l}$ (24.69%) respectively after 24 hrs, 48 hrs, 72 hrs and 96 hrs respectively and $3.066 \times 10^6/\mu\text{l}$ (6.52%) recovered after 10 days (Fig. 1.1). Whereas in experimental group WBCs mean level increased to 42.32 ± 2.174 (193.07%), 31.38 ± 3.356 (117.31%), 62.16 ± 4.926 (330.47%) as well as 55.14 ± 1.443 (281.85%) for 24 hrs, 48 hrs, 72 hrs, and 96 hrs for sub lethal exposure of Imidacloprid respectively and after 10 days recovery 16.2 ± 3.043 (12.18%) (Fig. 1.3) and The fish were exposed to sublethal concentration of Imidacloprid showed the haemoglobin mean values for 24 hrs, 48 hrs, 72 hrs as well as 96 hrs 11.42 ± 0.356 g/dl (12.82%), 10.96 ± 0.55 g/dl (16.33%), 10.4 ± 0.223 g/dl (20.61%) and 11.3 ± 0.839 g/dl (13.74%) respectively and after 10 days recovery mean value 12.9 ± 0.353 g/dl (1.526%) (Fig. 1.5). The percentage increase/decrease of RBCs, WBCs and Hb in various time exposures for 24, 48, 72 and 96 hrs and recovery values were showed in figure 1.2, 1.4 and 1.6 respectively. Variations and percent increase/decrease in Red blood corpuscles (RBCs), WBCs and Haemoglobin count

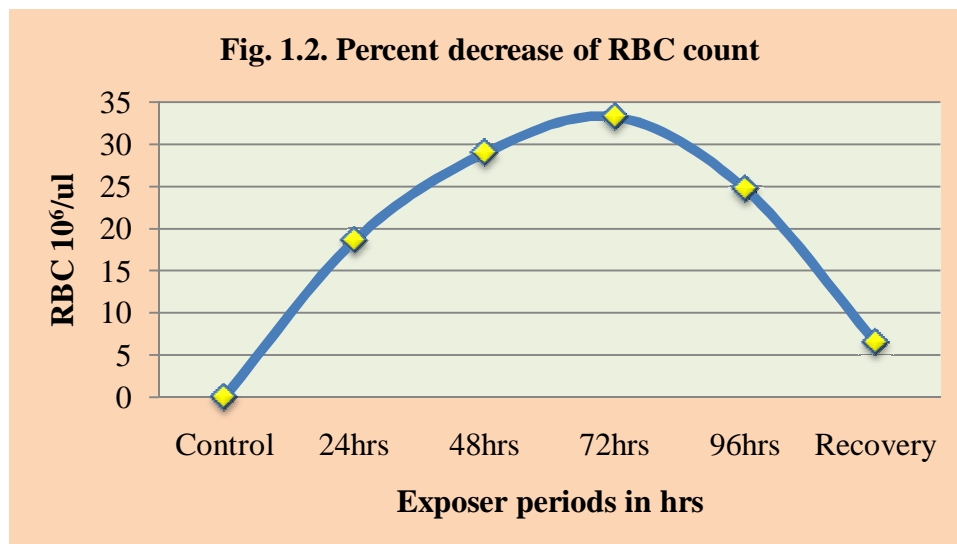
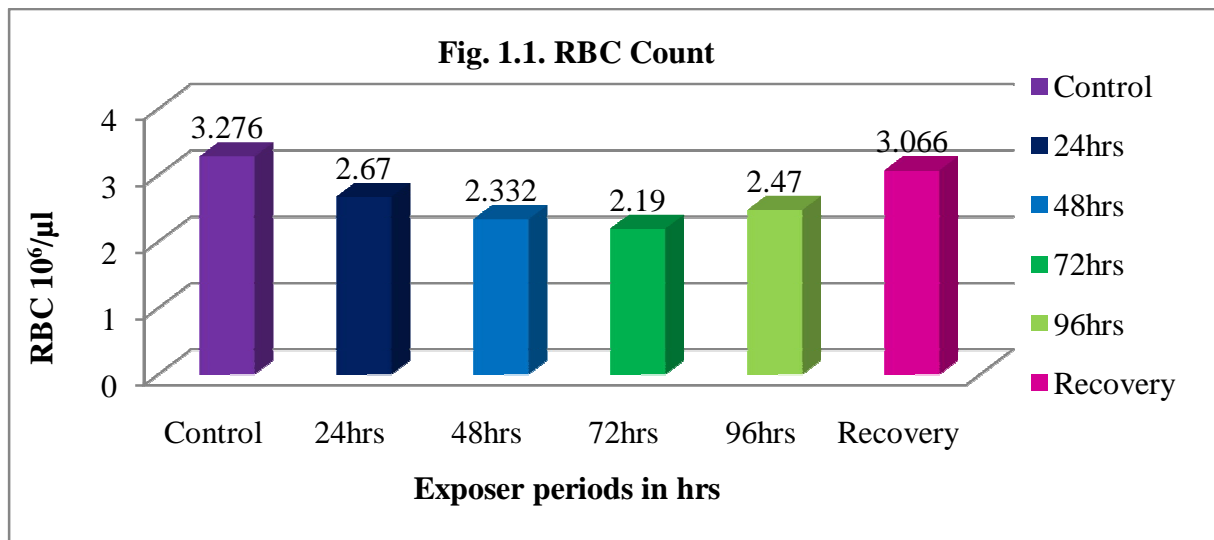
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of control and treated fish with Imidacloprid are depicted in table.1.1.1, 1.2.1 and 1.3.1 respectively. Comparison of data of control with that of the treated groups very clearly indicates. Reduction of RBC in treated groups at sublethal concentrations of Imidacloprid (0.282ppm). RBC count value decreased significantly ($p < 0.05$) as compared to control group. After 10 days recovery period RBCs count was observed normally as compared to control fish.



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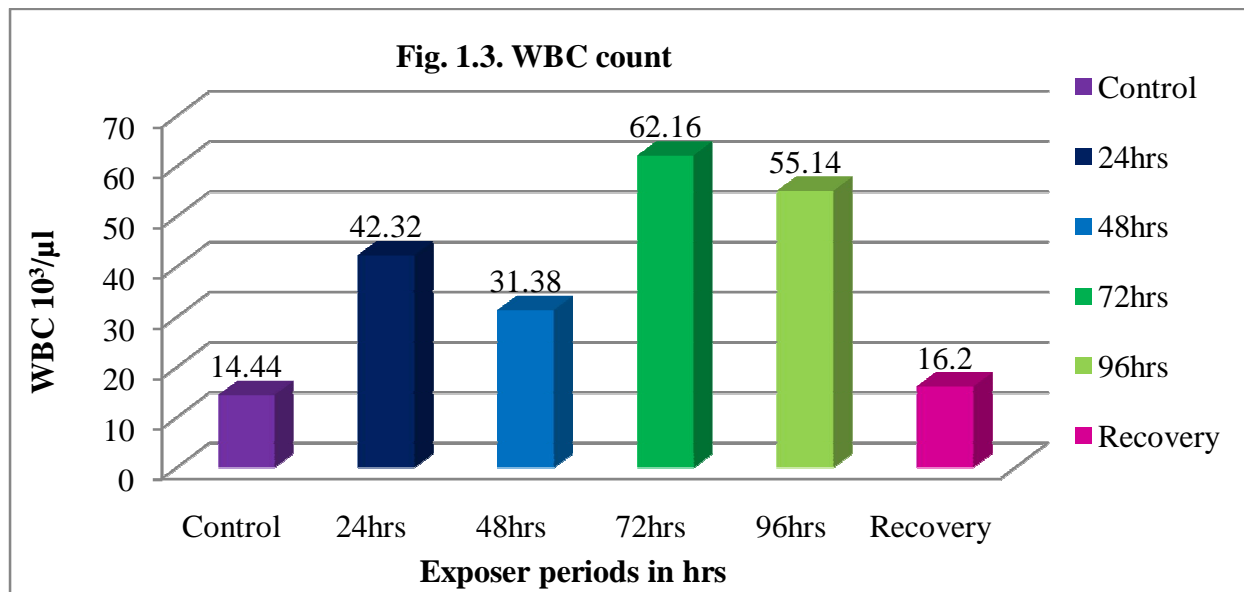
Table 1.1.1. The variation in RBC, WBC and Hb count of *Channa punctatus* exposed to sublethal concentration of Imidacloprid.

Exposure period (hrs)	RBC ($10^6/\mu\text{l}$)	Percent decrease
Control	3.28±0.461	-
24	2.67±0.157*	18.59*
48	2.33±0.129*	28.96*
72	2.19±0.106*	33.23*
96	2.47±0.152*	24.69*
Recovery	3.066±0.358	6.52

Data are represented as mean±SD.

Data were analyzed through two-way ANOVA.

*Significant ($P < 0.05$), when treated groups were compared with control.



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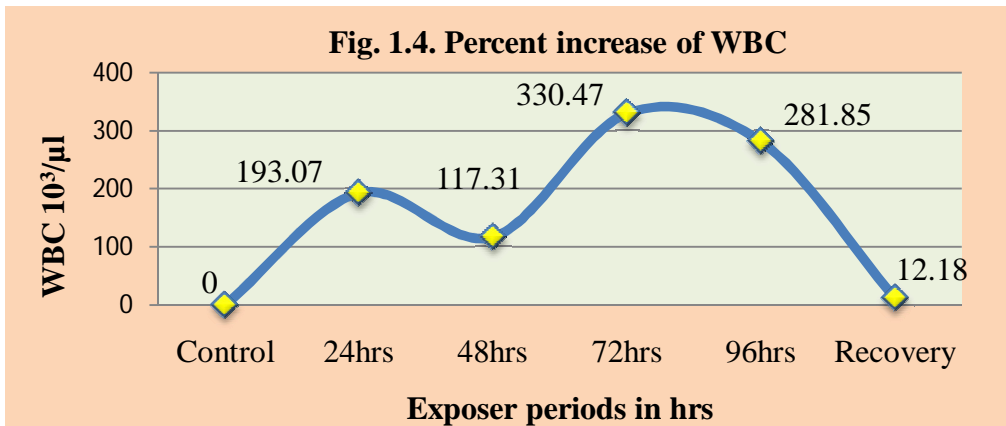


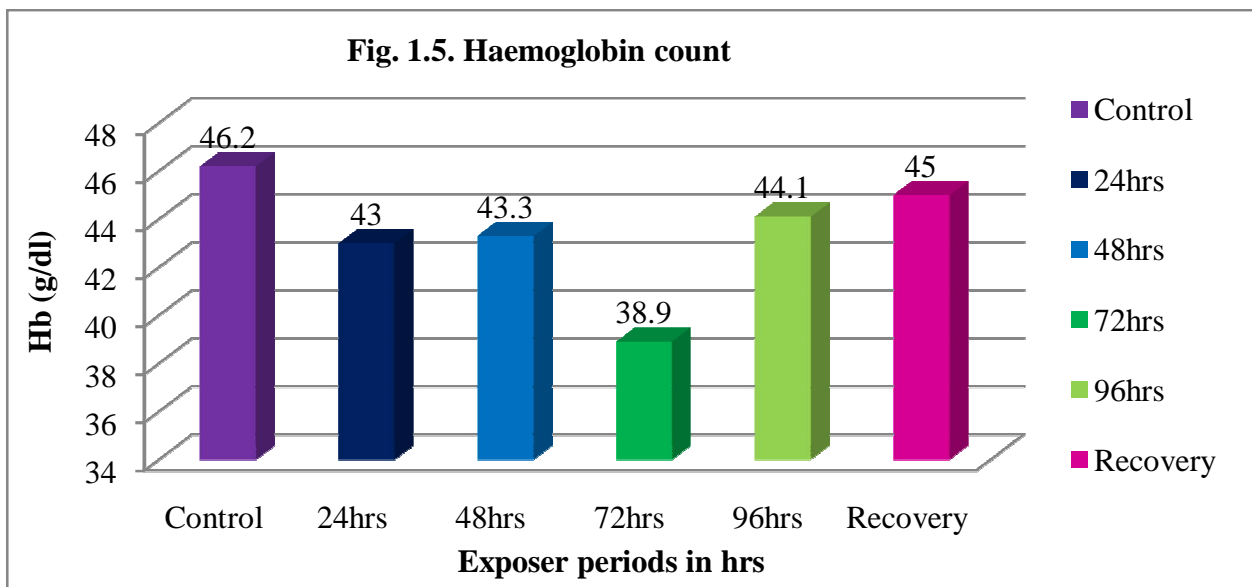
Table 1.2.1: The variation in WBC of *Channa punctatus* exposed to sublethal concentration of Imidacloprid.

Exposure period (hrs)	WBC (10 ³ /µl)	Percent increase
Control	14.44±0.661	-
24	42.32±2.174*	193.07*
48	31.38±3.356*	117.31*
72	62.16±4.926*	330.47*
96	55.14±1.443*	281.85*
Recovery	16.2±3.043	12.18

Data are represented as mean±SD.

Data were analyzed through two-way ANOVA.

*Significant (P < 0.05), when treated groups were compared with control.



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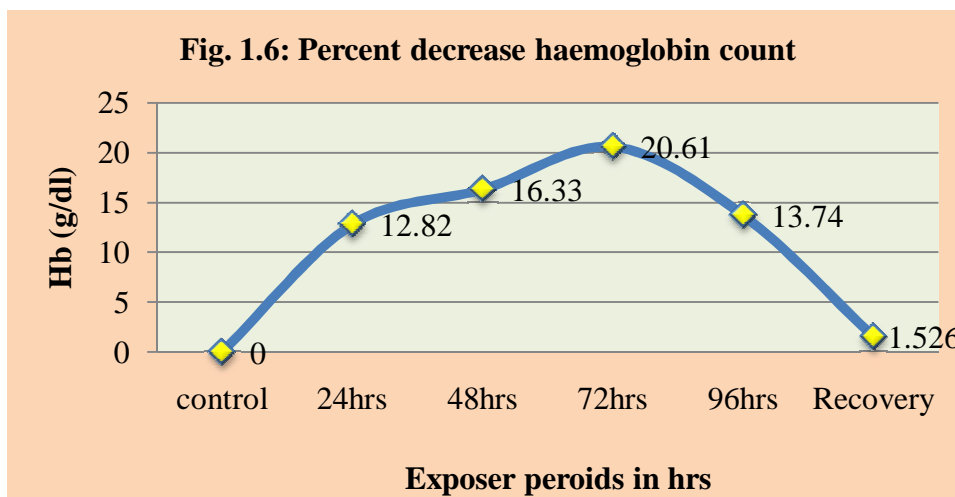


Table 1.3.1: The variation in Haemoglobin of *Channa punctatus* exposed to sublethal concentration of Imidacloprid.

Exposure period (hrs)	Hb (g/dl)	Percent decrease
Control	13.1±0.387	-
24	11.42±0.356*	12.82*
48	10.96±0.55*	16.33*
72	10.4±0.223*	20.61*
96	11.3±0.839*	13.74*
Recovery	12.9±0.353	1.526

Data are represented as mean±SD.

Data were analyzed through two-way ANOVA.

*Significant (P< 0.05), when treated groups were compared with control.

An increase in the WBCs count was mostly observed during the exposure period of stress reaction when fish tried to restore the disturbed homeostasis. High white blood cell counts indicate damage due to infection of body tissues, severe physical stress, and as well leukemia. The value mentioned above showed a significant decrease RBCs, Hb and increase WBCs when compared with control ($p < 0.05$). Similar level as a control fish were observed in the *C. punctatus* after recovery period. Padma, *et al.* (2012) reported different concentrations (0.002 ppm, 0.004 ppm, 0.006 ppm, 0.008 ppm and 0.010 ppm) of Imidacloprid insecticide on serum biochemical parameters of fresh water teleost *Channa punctatus*. Saxena and Seth (2002) noticed the toxic effects of Cypermethrin on certain hematological aspects of fresh water fish *Channa punctatus* and observed a significant change in the hematology of the fish. Anees (1978) observed a decrease in erythrocyte count and haemoglobin content in freshwater fish *Channa punctatus* after acute exposure to Diazinon. Singh, *et al.* (2008) showed effect of Copper on haematological profile of freshwater fish, *Channa punctatus* a significant decrease in the haemoglobin content from 10.73 to 6.60%, red blood cells from 2.86 to $1.84 \times 10^6/\text{mm}^3$ and packed cell volume from 31.00 to 23.33%. Whereas the white blood cells increased from 60.00 to $92.48 \times 10^3/\text{mm}^3$ at the end of 45th day as compared to control. Reddy, *et al.* (2015) obtained the static LC_{50} values were lethal (14,260 mg/l) and 13,710 mg/l and 11,900 mg/l for 24 and 96 hrs respectively in fresh water fish *Labeo rohita* (Hamilton) exposed to Confidor (Imidacloprid ingredient). Therefore, present results are in agreement with the observations of the previous workers.

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IV. CONCLUSION

Decreased Hb%, RBC count and increased WBC counts indicate damage due to infection of body tissues, severe physical stress, and as well leukemia. In most cases, abnormal red cell morphology is noted. Haematological parameters related to Oxygen transport (RBC and Hb), defense mechanisms (WBC) all exhibited marked alterations between control and experimental groups in response to insecticide Imidacloprid in presently studied fish, *C. punctatus*. Present studies thus confirm that haematological parameters are very sensitive indicators in fish under toxicity of chemicals and ultimately become the causative for affecting the general health status of the fish. Water quality can thus be regarded as one of the major factors responsible for individual variations in fish haematology.

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