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Effect of a pesticide lambda cyhalothrin on haematological changes in the fresh water fish, *Labeo rohita* under short and long term exposure periods

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Abstract
The aim of the present study was to analyse the haematological changes in the fish *Labeo rohita* under short term and long term exposure of the pesticide lambda cyhalothrin. Wide spread use of lambda cyhalothrin and other pesticides damages terrestrial and aquatic organisms. Haematological parameters such as Red blood corpuscles (RBC), White blood corpuscles (WBC), Haemoglobin (Hb) content, in the blood of fish, *Labeo rohita* were studied after exposure to pesticide Lambda cyhalothrin at short term (24, 48, 72 and 96 hours) and long term (10, 20 and 30 days) exposure periods.

Keywords: pesticide, lambda cyhalothrin, haematological study

1. Introduction
Pesticides are used in India depends on the higher population and increased food demand. The lamda-cyhalothrin belongs to the pyrethroid class of pesticides. In the agricultural field farmers choose the synthetic pyrethroids because of their effectiveness when compared to other insecticides (Pauluhn, 1999) [13]. Wide spread use of lambda cyhalothrin and other pesticides damages terrestrial and aquatic organisms. Recent evidences demonstrates the ability of pesticides to act as endocrine disruptors, contributing to various adverse effects associated with reproductive and developmental toxicity (Eskenazi *et al.*, 1999; Colborn, 2006) [7, 4]. The aim of the present study was to analyse the haematological changes in the fish *Labeo rohita* under short term and long term exposure of the pesticide lambda cyhalothrin.

2. Materials and Methods
2.1 Blood collection
Blood was drawn by gill puncture and the syringe rinsed with EDTA (ethylenediaminetetraacetic acid) was used to draw blood dripping out from the cut and then drained into a sterile eppendorf tube. The pooled up blood of ten fish was taken as a single sample for a period of fish exposed under lambda cyhalothrin. Similarly, the blood sample was collected from control and analysed.
Blood was taken immediately after the exposure to minimize the stress of sampling time. For the haematological tests, 0.5ml of blood was taken from the gills of 10 fishes after the fishes were anaesthetized at the end of the exposure. Each blood sample was placed in a tube on ice. Approximately 2ml of blood was collected from another 10 fish with an unheparinized syringe for blood serum chemistry. Each of these sample was transferred into a glass test tube and allowed to clot at room temperature for 1hr. Serum was obtained by centrifugation at 720g for 15minutes and stored at -80^{o}C for 3 days until analyzed.

2.2 Estimation of haemoglobin content
The estimation of haemoglobin content was done using the standard procedure by Sahli, 1962. The pipette was filled with blood sample slightly above the 0.02 ml mark and the tip was wiped out with a filter paper and the excess of blood was removed and volume was adjusted to exactly 0.02 ml. The pipette was rinsed several times in the acid solution and the sample was allowed to remain for 15 minutes. The principle behind this method is the conversion of haemoglobin to acid haematin.

The acid haematin was diluted with distilled water till the colour matched with the colour of the standard in the haemoglobinometer. The height of column at which the match was obtained gives the values of haemoglobin in g%. The same procedure was followed for the control and treated animals. Haemoglobin is a compound of globin with haematin. A molecule of haematin contains 1 atom of iron. This atom of iron enables haemoglobin to carry oxygen in large amounts Haeme is the iron containing split portion of haemoglobin.

2.3 Counting of red blood corpuscles (RBC)

Enumeration of formed elements (blood cells) is a quantitative measure of the population of blood cells in circulation. The counting of cells can be done manually with the help of a microscope after diluting blood and making a special type of wet mount as per method given in Mukherjee (1988). The technique is popularly known as haemocyto metry and added by Neubaur grid, on the haemocyto meter which show cell counting areas for the estimation.

Total red blood cells (RBC) were counted using the improved Neubaur haemocyto meter (Shah *et al.,* 2010) [14]. Blood was diluted (1:200) with Hayem's fluid. Erythrocytes were counted in the loaded haemocyto meter chamber and total numbers were reported as 10^6 mm^3 (Wintrobe, 1967) [15].

The blood was drawn into the RBC pipette up to 0.5 mark and immediately the diluting fluid containing, formal citrate solution was drawn up to 101 mark. This gives a dilution of 1:200 (Blood: Formal citrate solution). The solution was added by shaking gently and allowed to be settled for 2 to 3 minutes. The counting chamber and cover glass were properly cleaned and the cover glass was placed over the ruled area. Again the solution was mixed gently and the stemfull of solution was expelled and a drop of fluid was allowed to flow under the cover slip by holding the pipette at an angle of 45°. It was allowed to settle for 2 to 3 minutes, the

RBCs after settling without air bubble under the coverslip were counted. Then the ruled counting area was focused under the microscope and the number of RBC's were counted in fine small squares of the counting area under high power lence and the number of RBC per sq.mm were calculated by using the following formula:

$$\text{Total RBC} = \frac{\text{No. of Cells} \times \text{dilution factor} \times \text{depth factor} = \text{number cubic mm}}{\text{Total No. of small squares}}$$

2.4 Counting of white blood corpuscles (WBC)

Total white blood cells (WBC) were counted using an improved Neubaur haemocytometer (Mgbenka and Oluah, 2003) [11]. Blood was diluted (1:20) with Turk's diluting fluid and placed in haemocytometer. Four large (1Sq.mm) corner squares of the haemocytometer were counted under the microscope (Olympus) at 640x. The total number of WBC was calculated in mm^3x10^3 (Wintrobe, 1967) [15]. The following formula was taken for the enumeration of WBC.

$$\text{Total WBC} = \frac{\text{No. of cells counted} \times \text{Volume of the Square} \times \text{Dilution factor} = \text{cubic mm}}{\text{No. of Squares (4)}}$$

3. Results

3.1 Short term exposure

Haematological parameters such as Red blood corpuscles (RBC), White blood corpuscles (WBC), Haemoglobin (Hb) content, in the blood of fish, *Labeo rohita* were studied after exposure to pesticide lambda cyhalothrin at short term (24, 48, 72 and 96 hours) exposure periods. The results obtained were compared with the fish under control. The values obtained are presented in table 1 and graph 1.

The RBC count in the blood of fishes exposed to pesticide lambda cyhalothrin showed significant decrease (P< 0.05) over control. The values of RBC count in fish exposed to short term exposure to pesticide lambda cyhalothrin water were 1.08, 1.01, 0.92, and 0.61 for 24, 48,72 and 96 hours respectively. The control value for RBC is 2.01. The values are expressed as million/cu.mm. The results are presented in the Table 1 and graph 1

The WBC count of fish exposed to pesticide lambda cyhalothrin at short term exposure periods showing significant increase (P> 0.05) over control. The values of WBC count of fish treated with short term exposure of the pesticide lambda cyhalothrin water over control at 24, 48, 72 and 96 hours were 20.09, 24.12, 29.12, and 30.20 respectively. The control value noted is 12.21. The values are expressed as 1000/cu.mm. The results are presented in the Table 1 and graph.1.

The haemoglobin content observed in the present study in the pesticide lambda cyhalothrin treated fish at short term exposure periods showed significant decrease (P< 0.05) over control. Haemoglobin content of fish treated with short term exposure of the pesticide lambda cyhalothrin over control at 24, 48, 72 and 96 hours were 4.30, 4.04, 3.66, and 2.17 respectively. The control value noted is 5.01. The values are expressed as g/dl. The results are presented in the Table 1 and graph. 1.

Table 1: Haematological changes in blood due to the pesticide lambda cyhalothrin on the fresh water fish, *Labeo rohita* on short term exposure periods

Blood parameters	Exposure periods				
	Control	24hrs	48hrs	72hrs	96hrs
RBC(10^6/mm^3) 't'	2.01±0.01	1.08±0.01 4.46**	1.01±0.01 6.87**	0.92±0.007 11.29**	0.61±0.007 15.11**
WBC(cells/mm^3) 't'	12.21±0.008	20.09±0.01 26.41**	24.12±0.01 59.47**	29.12±0.01 58.86**	30.20±0.01 56.21**
HB(mg/dl) 't'	5.01±0.005	4.30±0.007 5.91**	4.04±0.04 37.57**	3.66±0.03 9.155**	2.17±0.007 23.92**

**-Significant at one per cent level;*-significant at five per cent level; NS-Non significant.

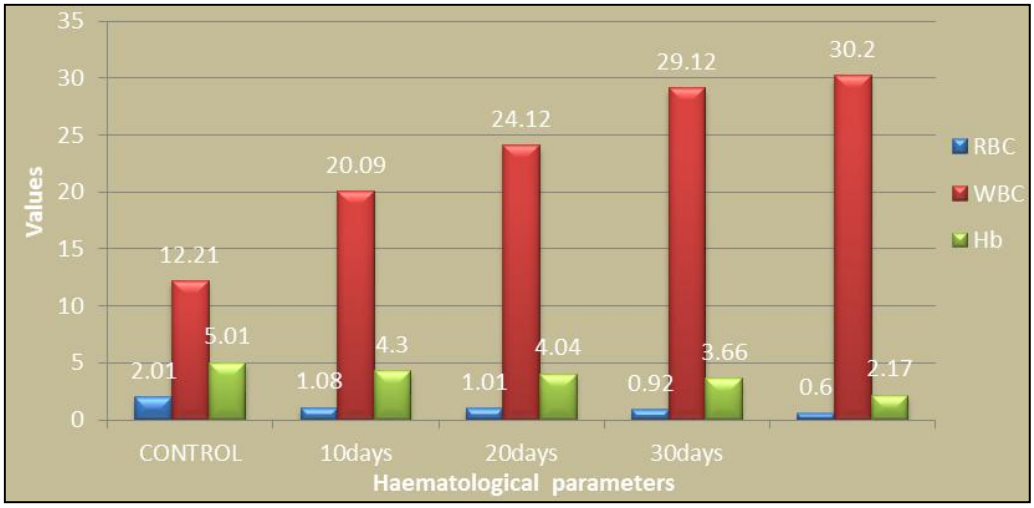

Fig 1: Haematological changes in blood due to the pesticide lambda cyhalothrin on the fresh water fish, *Labeo rohita* on short term exposure periods

3.2 Long term exposure

Haematological parameters such as Red blood corpuscles (RBC), White blood corpuscles (WBC), Haemoglobin (Hb) content, in the blood of fish, *Labeo rohita* were studied after exposure to pesticide Lambda cyhalothrin at long term (10, 20 and 30 days) exposure periods. The results obtained were compared with the fish under control. The values obtained are presented in Table 2 and graph. 2.

The RBC values of fish exposed to long term exposure of the pesticide lambda cyhalothrin are 1.42, 1.30 and 1.19 for 10, 20 and 30 days respectively. The control value noted was 2.12. The values are expressed as million/cu.mm. The result of RBC content in both treated and control fish are presented in Table. 2 and Figure 2.

The values of WBC count over control at 10, 20 and 30 days were 36.3, 37.9, and 40.02 respectively. The control value noted was 13.99. The values are expressed as 1000/cu.mm. The results of WBC content in both treated and control fish are presented in Table 2. And Figure 2.

Haemoglobin content in fish treated with long term exposure of the pesticide lambda cyhalothrin water over control at 10, 20 and 30 days were 1.97, 1.63 and 1.42 respectively. The control value noted is 4.93. The values are expressed as g/dl. The results of haemoglobin content in both treated and control fish are presented in Table 2 and Figure 2.

Table 2: Haematological changes in blood due to the pesticide lambda cyhalothrin on the fresh water fish, *Labeo rohita* on long term exposure periods

Blood parameters	Exposure periods			
	Control	10days	20days	30days
RBC(10^6/mm^3) 't'	2.12±0.02	1.42±0.01 16.06**	1.30±0.004 13.28**	1.19±0.01 19.43**
WBC(cells/mm^3) 't'	13.99±0.007	36.3±0.12 86.23**	37.9±0.10 71.68**	40.02±0.12 80.49**
HB(mg/dl) 't'	4.93±0.01	1.97±0.12 93.1**	1.63±0.007 154.0**	1.42±0.008 27.75**

**-Significant at one per cent level;*-significant at five per cent level; NS-Non significant

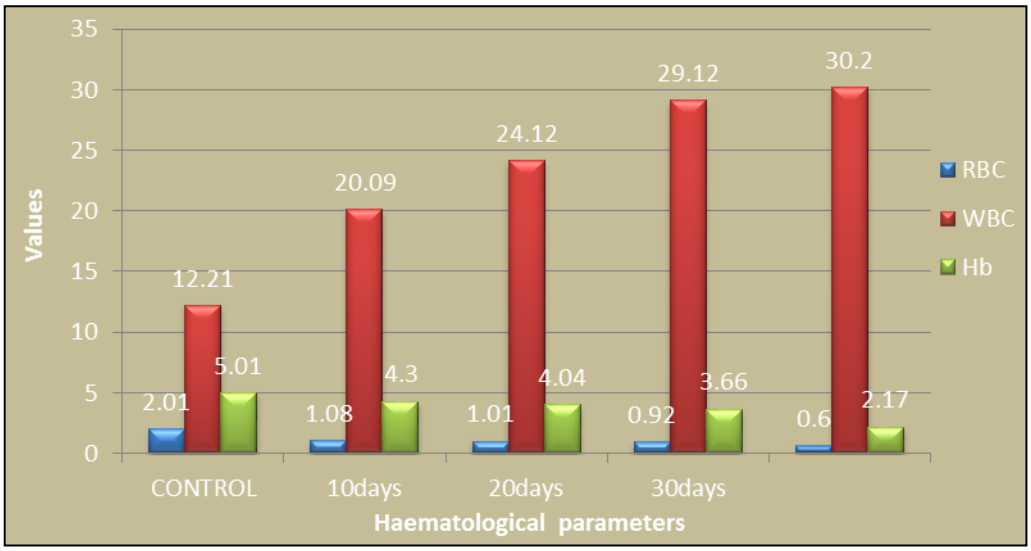

Fig 2: Haematological changes in blood due to the pesticide lambda cyhalothrin on the fresh water fish, *Labeo rohita* on long term exposure periods

4. Discussion

Haematological parameters helps to understand and study the blood characteristics and physiological situation of an organism. Red blood cells counts are of immense physiopatho logical importance. A lower count of RBC found in the fish *Labeo rohita* may be due to the direct effect of lambda cyhalothrin. The anaemic condition resulted in the present study may be due to the destruction of mature RBC or inhibition of erythrocyte production. Such decreases in RBC and anaemic condition had been observed by Koundinya and Ramamurthy (1979) [10] in *Sarotherodon mossambicus* after exposure to different concentrations of submithion. Studies on the effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* by Adhikari *et al.* 2004 [1] showed decrease in RBC, Hb, leading to anaemia. Anaemia could be due to the effect of insecticide on haemopoisis or attraction of cell membrane. When fishes were in stressful condition the erythrocyte count was found to be decreased. The lower count may be due to the destruction of red blood cells or either by the inhibition of erythropoiesis.

In the present study the significant increase in WBC during treatment might have resulted from stimulation of immune system by lambda cyhalothrin and to protect the fish against toxicity. Effect of lindane and malathion exposure to certain blood parameters in a fresh water teleost fish *Clarias batrachus* was studied by Joshi *et al*. (2002) [9] and concluded that the WBC count increased and it might be due to an increase in antibody production which helps in survival and recovery of the fish exposed to sub lethal concentrations of pesticide. El-Sayed *et al.* (2007) [6] found similar findings in *Oreochromis niloticus* exposed to acute concentration of deltamethrin.

The reduction in haemoglobin content in the present study may be due to the stress response due to toxicant. The freshwater teleost fish *Clarias batrachus* when exposed to lindane and malathion and showed reduction in haemoglobin level. Lower haemoglobin level might decrease the ability of fish to enhance its activity in order to meet occasional demands in the study of Joshi *et al*. (2002b) [9] in *Clarias batrachus*. The reduction of oxygen-carrying capacity in fish may be associated due to introduction of toxicant so there is a decrease in haemoglobin concentration. *Oncorhynchus mykiss*, exposed to Mancozeb also showed similar results of decreased haemoglobin in the study done by Atamanalp and Yanik (2003) [3]. All these studies supports the present condition and finally conclude that the test organism *Labeo rohita* is highly affected by the pyrethroid insecticide lambda cyhalothrin.

5. Conclusion

Haematological parameters such as Red blood corpuscles (RBC), White blood corpuscles (WBC), Haemoglobin (Hb) content, in the blood of fish, *Labeo rohita* were studied after exposure to pesticide lambda cyhalothrin at short term (24, 48, 72 and 96 hours) and long term (10, 20 and 30 days) exposure periods. The RBC count and haemoglobin content in the blood of fishes exposed to pesticide lambda cyhalothrin showed significant decrease ($P< 0.05$) over control. The WBC count of fish exposed to pesticide lambda cyhalothrin at short term exposure periods showing significant increase ($P> 0.05$) over control. The results and discussions conclude that the insecticide lambda cyhaothrin negatively affects the haematology and physiological activities of *Labeo rohita.*

6. References

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