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### **Haematological Changes In *Clarias Batrachus* Due To Cypermethrin Insecticide.**

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#### **ABSTRACT**

The objective of this study was to observe the effect of Synthetic Pyrethroid (Cypermethrin) on the haematology of *Clarias batrachus* fish. The haematological parameters studied such as Red Blood Cell (RBC), White Blood Cell (WBC), differential leucocytes counts (neutrophils, lymphocytes, eosinophils and monocytes), Hb %, platelets, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Packed Cell Volume (PCV). The results revealed a significant decrease in Red Blood Cell (RBC), Hb %, platelets, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Packed Cell Volume (PCV) levels but White blood cells (WBCs) count and neutrophils, eosinophils, basophils, monocytes were increased and then decreased significantly in experimental group compare with control group. Thus on the basis of obtain result in the present investigation it can be concluded that 96 hrs. exposure of 80 ppm of Cypermethrin aqueous solution has toxic effect and suggests that exposure of cypermethrin could cause some level of stress as indicated by changes in the haematological indices of the fish under consideration.

**KEY WORDS:** Haematology, Cypermethrin, *Clarias batrachus*, Synthetic Pyrethroid, Toxic, Blood

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## INTRODUCTION

Cypermethrin is a synthetic pyrethroid broad-spectrum insecticide, used extensively in households, industrial and agriculture fields<sup>1</sup> for control of several insect pests<sup>2</sup>. Due to indiscriminate use, cypermethrin makes their entrance into natural water bodies through agriculture run-off and ultimately affects the several target aquatic organisms and thus adversely affects fish metabolism<sup>3</sup>, haematology<sup>4</sup>, meat quality and fish population<sup>5</sup>.

To determine the effect of disease and unsuitable environmental conditions on fish, the normal haematological parameters have been investigated. It is known that diseases, pollution and the presence of agricultural chemical in water cause changes in blood cells of fish resulting in losses to aquaculture<sup>6</sup>. A major part of the world's food is being supplied from fish source, so it is essential to secure the health of fishes<sup>7</sup>.

Fish in close association with their aquatic environment and any changes in this environment would be reflected in alterations in their haematological studies<sup>8,9</sup>. Haematological parameters are important for reflecting the pathophysiological status of a fish. These parameters have been widely used as indicators of disease or stress due to pollutants. Blood is an important component for studying the effects of toxicants as it is highly susceptible to environmental fluctuations<sup>10</sup>. Blood is a pathophysiological indicator of the body as it is highly susceptible to internal and external environmental fluctuations in stress conditions. It is affected by the toxic pollutants that have gained momentum in recent years and are in fact important diagnosing tools to investigate disease or stress in fish<sup>11</sup>.

In assessing the toxic effects of chemicals in aquatic organisms the use of haematological techniques has become more useful in recent times, as a result of the intimate relationship between fish and its aqueous environment<sup>12, 13, 14</sup>. Sampathet *al.* (1993)<sup>15</sup> observed that haematological studies in fish, lies in the possibility that the blood will reveal abnormally within the body of the fish long before there is any outward manifestation of symptoms of disease or effects of unfavourable environmental factors.

Around the world, it is being used in aquaculture as a chemotherapeutic agent against lice infiltration and preventing invasion by copepod parasites<sup>16, 17</sup>. Thus, use of CYP on commercial level could cause ecological toxicity in water reservoirs, which in turn could have adverse implications on the aquatic biota generally and fish particularly<sup>18</sup>. The current investigation was undertaken to investigate **Haematological changes in *clariasbatrachus* due to cypermethrin insecticide.**

## MATERIAL AND METHODOLOGY

**Experimental animal:** Healthy *Clariasbatrachus* were used as a experimental animal and it is was collected from local fish market & acclimatized to the laboratory for one week during which they were regularly feed with prawn powder & soya meal.

**Test chemical:** Cypermethrin was used as a test chemical. Test fishes were exposed to sub-lethal doses (80ul/l) for maximum 96 hrs.

### **Experimental design:**

In the present investigation experimental fishes were divided into two groups.

1. Control group: - In this group 10 fishes were kept and exposed to normal water.
2. Experimental group: - In this group 40 fishes were exposed to 80 µl concentration of cypermethrin solution.

**Experimental duration:** In both control and experimental group fishes were exposed to maximum 96 hrs.

**Autopsy:** Fishes of control and experimental groups were sacrificed at 0 hrs, 24 hrs, 48hrs, 72 hrs and 96 hrs. Blood collected by cardiac puncture of *Clariasbatrachus* then processed for various haematological tests.

### **Haematological analysis**

#### **a. RBC & WBC Counting**

RBC & WBC counting were done by MANUAL METHOD<sup>19</sup>.

#### **b. Differential Leukocytes counting**

DLC Counting was done by LEISHMANN METHOD<sup>19</sup>.

#### **c. Haemoglobin % Analysis**

Hb% analysis was done by SAHIL'S METHOD<sup>19</sup>.

#### **d. Platelets, MCV, MCH & PCV counting**

Platelets, MCV, MCH & PCV counting were done by MANUAL METHOD<sup>19</sup>.

## RESULTS

In the present investigation haematological estimation of control and experimental fish were done. The haematological parameter were RBC ,WBC , DLC ( Neutrophiles, Eosinophiles, Lymphocytes, Basophiles and Monocytes ), platelets, PCV, MCV and MCH.

In control haematological values (Tables and Figures ) were RBC (8.23 million/ml), WBC ( $5.21 \times 10^9$  cells), Hb (10.76 g/dl), Neutrophiles (20.54%), Eosiniphiles (9.23 %), lymphocytes (71.33%), Basophiles (4.33 %), Monocytes (3.60%), Platletes (125%) , PCV (32.53%) MCV (80.68fl ) and MCH (35.00 pg).

In the present investigation at the 24 hrs. haematological values were RBC (7.28 million/ml), WBC (6.50 x10<sup>9</sup>cells), Hb (8.79 g/dl), Neutrophiles (21.76 %), Eosiniphiles (10.42%), lymphocytes (73.25%), Basophiles (5.20%), Monocytes (4.23 %), Platletes (121.6%) , PCV (28.00%), MCV (75.86 fl ) and MCH (33.24 pg).

In the present investigation at the 48 hrs haematological values were RBC (5.60 million/ml), WBC (7.23 x10<sup>9</sup>cells), Hb (7.43 g/dl), Neutrophiles (22.63 %), Eosiniphiles (11.03%), lymphocytes (76.21%), Basophiles (5.80 %), Monocytes (4.90 %), Platletes (119.0%) , PCV (26.11%) , MCV (71.43 fl ) and MCH (31.42 pg).

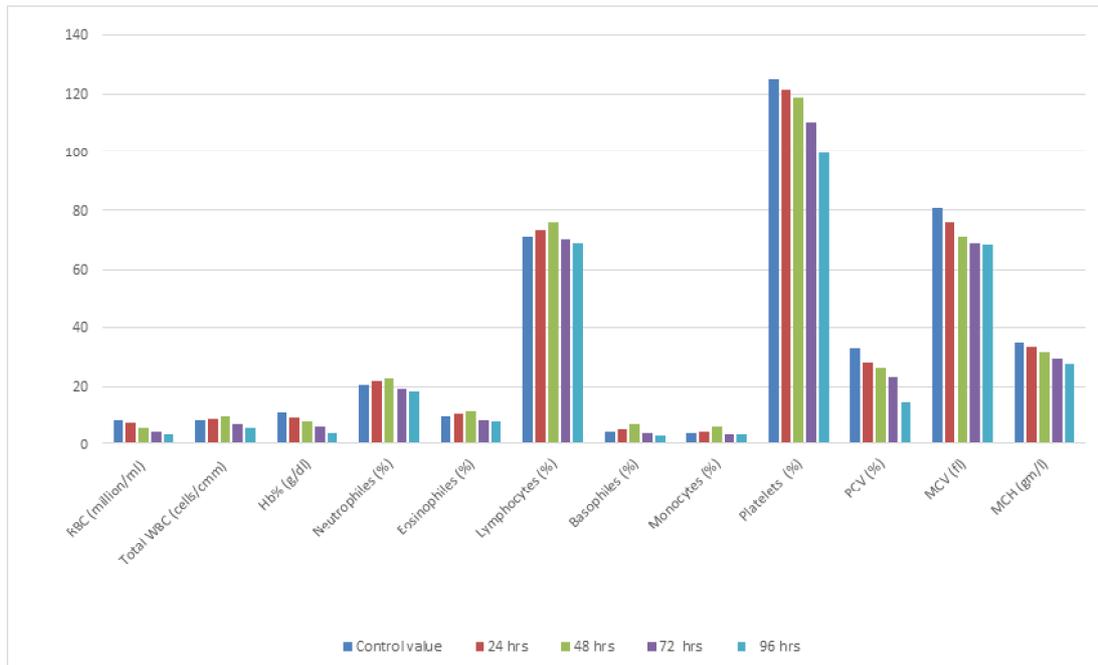
In the present investigation at the 72 hrs haematological values were RBC (4.32 million/ml), WBC (10.67 x10<sup>9</sup>cells), Hb (6.10 g/dl), Neutrophiles (19.31 %), Eosiniphiles (10.01%), lymphocytes (70.41%), Basophiles (3.90 %), Monocytes (3.23 %), Platletes (110.2%) , PCV (23.11%) MCV (69.01 fl ) and MCH (29.10 pg).

In the present investigation at the 96 hrs haematological values were RBC (3.48 million/ml), WBC (14.33 x10<sup>9</sup>cells), Hb (3.68 g/dl), Neutrophiles (18.60 %), Eosiniphiles (8.68 %), lymphocytes (69.32%), Basophiles (3.01 %), Monocytes (3.10 %), Platletes (99.23%) , PCV (14.33 %) MCV (68.46 fl ) and MCH (27.69 pg).

RBC, Hb, Platelets, PCV, MCV and MCH values were decreased as compared to control value at 24, 48, 72 and 96 hrs. WBCs value were increased as compared to control at 24, 48 hrs and then decreased at 72 and 96 hrs as compared to control value .

**Table 1:-Haematological changes in *clariabatrachus* due to cypermethrininsectiside**

Peramaters	Control value	Experimental value			
		24 hrs	48 hrs	72 hrs	96 hrs
<b>RBC (million/ml)</b>	8.23	7.28±0.20	5.60±0.16	4.32±0.28	3.48±0.27
<b>Total WBC (cells/cmm)</b>	8.01	8.31±0.18	8.85±0.21	6.67±0.17	5.33±0.16
<b>Hb% (g/dl)</b>	10.76	8.79±0.30	7.43±0.32	6.10±0.14	3.68±0.11
<b>Neutrophiles (%)</b>	20.54	21.76±0.12	22.63±0.18	19.31±0.26	18.60±0.22
<b>Eosiniphiles (%)</b>	9.23	10.42±0.14	11.03±0.12	8.01±0.19	7.68±0.19
<b>Lymphocytes (%)</b>	71.33	73.25±0.33	76.21±0.22	70.41±0.30	69.32±0.32
<b>Basophiles (%)</b>	4.33	5.20±0.11	6.80±0.28	3.90±0.11	3.01±0.14
<b>Monocytes (%)</b>	3.60	4.23±0.25	5.90±0.19	3.23±0.24	3.10±0.11
<b>Platelets (%)</b>	125	121.6±0.16	119.00±0.9	110.2±0.36	99.23±0.22
<b>PCV (%)</b>	32.53	28.00±0.26	26.11±0.17	23.11±0.13	14.33±0.15
<b>MCV (fl)</b>	80.68	75.86±0.22	71.43±0.30	69.01±0.29	68.46±0.34
<b>MCH (gm/l)</b>	35.00	33.24±0.19	31.42±0.31	29.10±0.25	27.69±0.26

Figure 1 : Haematological changes in *Clariasbatrachus* due to cypermethrin insecticide

## DISCUSSION

Blood is a pathophysiological reflector of the whole body and, therefore, blood parameters are important in diagnosing the structural and functional status of fish exposed to toxicants. In this study, significant changes were noticed in blood components of treated fish. These changes included reduction in the red blood cells count, Hb level, Platelets, PCV, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values. On the other hand white blood cells increased and the Differential Leucocytes Count (neutrophils, eosinophiles, lymphocytes, basophiles and monocytes) value showed fluctuations as a result of cypermethrin exposure.

The reduction in RBC, Hb and PCV which are related to oxygen carrying capacity of the blood, may be due to the inhibition of erythropoiesis hemossynthesis, and increase in the rate of erythrocyte destruction in haemopoietic organs. The reduction in Hb and several other blood components might be due to the inhibition of RBCs' and haem synthesis, osmoregulatory dysfunction and destruction of RBCs in hematopoietic organs as reported earlier in *Catlacatla*<sup>20</sup>. Kavitha et al. (2012)<sup>21</sup> observed a significant reduction in Hb, and RBC levels in fish exposed to *Moringaoleiferaseed* extract. Generally, reduction in hemoglobin level and number of red blood cells might be owing to erythroblastosis causing anemia<sup>22</sup>. Similar observations were also previously reported in *C. gariepinus*, which was exposed to tobacco leaf extracts, and cassava effluents<sup>23</sup>.

In the light of the present study, the mean value of PCV decreased progressively in the experimented group compared to the control. The result agreed with the work of Akinrotimiet al. (2009)<sup>24</sup> in haematological indices of *Tilapia guineensis* subjected to handling stress. The decrease in

the PCV indicates the worsening of the condition of the organism and developing of anaemia. Platelets are nucleated cells which are responsible for blood clotting in fish; slight decrease in values observed in this study may signify the effect on platelet (thrombocyte) production.

Haematological indices like RBC count, concentration of haemoglobin and PCV have been reported to indicate secondary responses of an organism to pollutants<sup>25, 26, 27</sup>. There was significant reduction in platelets counts with the exposure of the fish to cypermethrin. Similar reduction in platelets counts had been also reported in *O. mykiss* exposed to cypermethrin<sup>28</sup>. The decrease of this parameter can be related with trapping of platelets in the spleen, decreased platelets production or increased destruction of platelets.

The Differential Leucocytes Count (DLC) value showed fluctuations. In this study neutrophils, monocytes and eosinophils increased whereas lymphocytes and basophils decreased in monogenean infected fishes. Similar results were found in helminth infected *Schizothorax*spp. and *Cyprinus*spp.<sup>29</sup>.

Another type of haematological response to the effect of cypermethrin was a significant increment in WBC. This response was equally observed in common carp *Cyprinus carpio* after acute effect of phenitrothion, imidan and dichlorvos<sup>30</sup>. This may be due to release of white blood cells from spleen into the blood stream to combat the toxicant. The substantial increment in leukocyte count in the current investigation may be attributed to general immune response and a defensive response of cypermethrin. White blood cells are the main components of the blood that shield the organism in the time of injury, haemorrhage and entry of foreign antigen particle in the body<sup>31</sup>. During stress, the number of leukocytes increases significantly to cope with conditions of stress and defend organism<sup>32</sup>.

In the present investigation cypermethrin (80ul/l) exposure for 96 hrs to *Clarias batrachus* was found toxic as it altered rather decreased the red blood cells count, Hb level, Platelets, PCV, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values, fluctuated Differential leucocytes count and increased the WBC. Thus the results of present study corroborate with observation of previous authors.

**CONCLUSION: - Thus** on the basis of obtain result in the present investigation it can be concluded that 96 hrs. exposure of 80 ppm of Cypermethrin (aqueous solution) has toxic effect and alter the haematology of fish. In conclusion, our study clearly indicated that the physiology of the fish was disturbed by the cypermethrin. The cypermethrin caused hematological disturbances which could lead to impairment ability to combat diseases, reduce its chances for survival for growth of fish. Therefore it is recommended to the user of this pyrethroid pesticide (Cypermethrin) that they should be careful about the dose they are using.

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