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BLOOD COMPONANT CHANGES IN *CLARIAS BATRACHUS* DUE TO ALPHAMETHRIN INSECTICIDE

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Abstract:

This study investigates the toxic effects of Alphamethrin (75 ppm) on the haematology of *Clarias batrachus* after 96 hours of exposure. The results showed a significant decrease in Red Blood Cell (RBC) count, Haemoglobin (Hb), platelets, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), and Packed Cell Volume (PCV), while White Blood Cell (WBC) count and immune cell populations (neutrophils, eosinophils, monocytes) increased, indicating a stress response. These haematological changes suggest that Alphamethrin induces toxic effects, impairing normal blood function and triggering an immune response, ultimately leading to physiological stress in the fish.

Key Words: Haematology, Alphamethrin, *Clarias batrachus,* Synthetic Pyrethroid, Toxic, Blood

1. Introduction:

The synthetic pyrethroid insecticide alphamethrin is not only used in agricultural practices during crop production, but also in public health programs and as an ectoparasiticide in animals. This account for 30% of global pesticide consumption. (Prama *et al.*,2007).

Alphamethrin have been reported to damage vital organs of various target and non-target species (Joshi *et al*.,2007), also reduces reproductive ability (Singh *et al*., 2010) in fishes

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The effect of diseases and unsuitable environmental conditions on fish health has been extensively studied through the investigation of normal haematological parameters. It is well-documented that factors such as diseases, pollution, and the presence of agricultural chemicals in water can lead to significant changes in the blood cells of fish, which in turn can cause substantial losses in aquaculture (Mustafa Dorucu and Asiye Girgin, 2001). Since a significant portion of the world's food supply comes from fish, ensuring the health of fish populations is crucial for food security and sustainability (Tripathi *et al*., 2002). These findings emphasize the importance of monitoring environmental conditions and disease impacts on fish, as they directly affect both the health of the fish and the viability of aquaculture industries.

Fish are closely linked to their aquatic environment, and any changes in this environment are often reflected in alterations in their haematological parameters (Hughes *et al*., 1979; Suzana Golemi *et al*., 2012). Haematological parameters are crucial for assessing the pathophysiological status of fish, as they can indicate the presence of disease or stress induced by pollutants. Blood is particularly sensitive to environmental fluctuations, making it an important medium for studying the effects of toxicants. As such, changes in blood composition can serve as reliable indicators of the impact of pollutants and other stressors on fish health (Pandey and Pandey, 2001). This underscores the value of haematological studies in evaluating the overall health and environmental stress experienced by fish in aquaculture and natural habitats.

Blood serves as a key pathophysiological indicator, being highly sensitive to both internal and external environmental fluctuations, particularly under stress conditions. Toxic pollutants, which have become more prevalent in recent years, significantly affect the blood composition of fish. As a result, haematological parameters are crucial diagnostic tools for investigating diseases or stress in fish, helping to assess the impact of environmental contaminants on fish health (Seth and Saxena, 2003). This highlights the importance of blood analysis in monitoring the well-being of fish in polluted or stressful environments

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In recent years, haematological techniques have become increasingly valuable for assessing the toxic effects of chemicals on aquatic organisms, due to the close relationship between fish and their aqueous environment (Musa and Omoregie, 1999; Gabriel *et al*., 2006; Akinrotimi *et al*., 2010). Haematological studies are particularly useful because changes in blood parameters can reveal abnormalities within the fish's body long before any visible symptoms of disease or environmental stress are apparent (Sampath *et al*., 1993). This makes blood analysis an early and effective diagnostic tool for detecting the impact of pollutants and other stressors on fish health

The current investigation was undertaken to investigate haematological changes in *Clarias batrachus* due to alphamethrin insecticide.

2. Material and Methodology:

Experimental animal: Healthy *Clarias batrachus* 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: Alphamethrin was used as a test chemical. Test fishes were exposed to sub-lethal doses (75ul/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 75 µl concentration of alphamethrin 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 *Clarias batrachus* then processed for various haematological tests.

Haematological analysis

a. **RBC & WBC Counting**

RBC &WBC counting were done by MANUAL METHOD **(Sharma and Singh, 2000).**

b. **Differential Leukocytes counting**

DLC Counting was done by LEISHMANN METHOD **(Sharma and Singh, 2000).**

c. **Haemoglobin % Analysis**

Hb% analysis was done by SAHIL'S METHOD **(Sharma and Singh, 2000).**

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

Platelets, MCV, MCH & PCV counting were done by MANUAL METHOD **(Sharma and Singh, 2000)**

3. Results:

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

In control haematological values (Table 01 and Figure 01) were RBC (8.42 million/ml), WBC ($5.31x10^9$cells), Hb (10.87 g/dl), Neutrophiles (20.71%), Eosinophiles (9.22 %), lymphocytes (71.56%), Basophiles (4.38 %), Monocytes (3.68%), Platelets (131%), PCV (32.83%) MCV (80.80fl) and MCH (36.00 pg).

In the present investigation at the 24 hrs. haematological values were RBC (7.47million/ml), WBC ($6.60 x10^9$cells), Hb (8.90 g/dl), Neutrophiles (21.93%), Eosinophiles (10.41%),

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lymphocytes (73.48%), Basophiles (5.25%), Monocytes (4.31 %), Platelets (117.6%) , PCV (28.30%), MCV (75.98 fl) and MCH (34.24 pg).

In the present investigation at the 48 hrs haematological values were RBC (5.79 million/ml), WBC (7.33 x10^{9}cells), Hb (7.54 g/dl), Neutrophiles (22.80 %), Eosinophiles (11.02%), lymphocytes (76.41%), Basophiles (5.85%), Monocytes (4.98%), Platelets (125.00%) , PCV (26.41%) , MCV (71.55 fl) and MCH (32.42 pg).

In the present investigation at the 72 hrs haematological values were RBC (4.51 million/ml), WBC (10.77 x10^{9}cells), Hb (6.21 g/dl), Neutrophiles (19.48 %), Eosinophiles (10.00%), lymphocytes (70.61%), Basophiles (3.95 %), Monocytes 3.231 %), Platelets (116.2%), PCV (23.41%) MCV (69.13 fl) and MCH (30.10 pg).

In the present investigation at the 96 hrs haematological values were RBC (3.67 million/ml), WBC (14.43 x10^{9}cells), Hb (3.79 g/dl), Neutrophiles (18.77 %), Eosinophiles (8.67 %), lymphocytes (69.52%), Basophiles (3.06 %), Monocytes (3.18 %), Platelets (105.23%), PCV (14.63 %) MCV (68.58 fl) and MCH (28.69 pg).

RBC, Hb, Platelets, PCV, MCV and MCH values were decreased as compared to control value at 24, 48, 72 and 96 hrs. WBC value were increased as compared to control at 24, 48, 72 and 96 hrs. Neutrophil, Basophiles, Eosinophiles, Lymphocytes and Monocytes values increased at 24 and 48 hrs and then decreased at 72 and 96 hrs as compared to control value.

Table 01:- Haematological changes in *Clarias batrachus* due to alphamethrin insecticide

Peramaters	**Control value**	**Experimental value**

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		24 hrs	48 hrs	72 hrs	96 hrs
RBC (million/ml)	8.42	7.47±0.22	5.79±0.17	4.51±0.26	3 .67±0.29
WBC (cells/cumm)	5.31	6.60±0.19	7.33±0.22	10.77±0.18	14.43±0.18
Hb% (g/dl)	10.87	8.90±0.31	7.54±0.33	6.21±0.116	3.79±0.12
Neutrophiles (%)	20.71	21.93±0.12	22.80±0.19	19.41±0.28	18.77±0.23
Eosinophiles (%)	9.22	10.41±0.14	11.02±0.11	10.00±0.20	8.67±0.18
Lymphocytes (%)	71.56	73.48±0.34	76.41±0.23	70.61±0.32	69.52±0.34
Basophiles (%)	4.38	5.25±0.12	5.85±0.29	3.95±0.12	3.06±0.16
Monocytes (%)	3.68	4.31±0.26	4.98±0.20	3.31±0.24	3.18±0.12
Platelets (%)	131	127.60±0.18	125.00±0.30	116.20±0.38	105.23±0.24
PCV (%)	32.83	28.30±0.27	26.41±0.18	23.41±0.14	14.63±0.16
MCV (fl)	80.80	75.98±0.23	71.55±0.32	69.13±0.14	68.58±0.35
MCH (pg)	36.00	34.24±0.20	32.42±0.32	30.10±0.22	28.69±0.27

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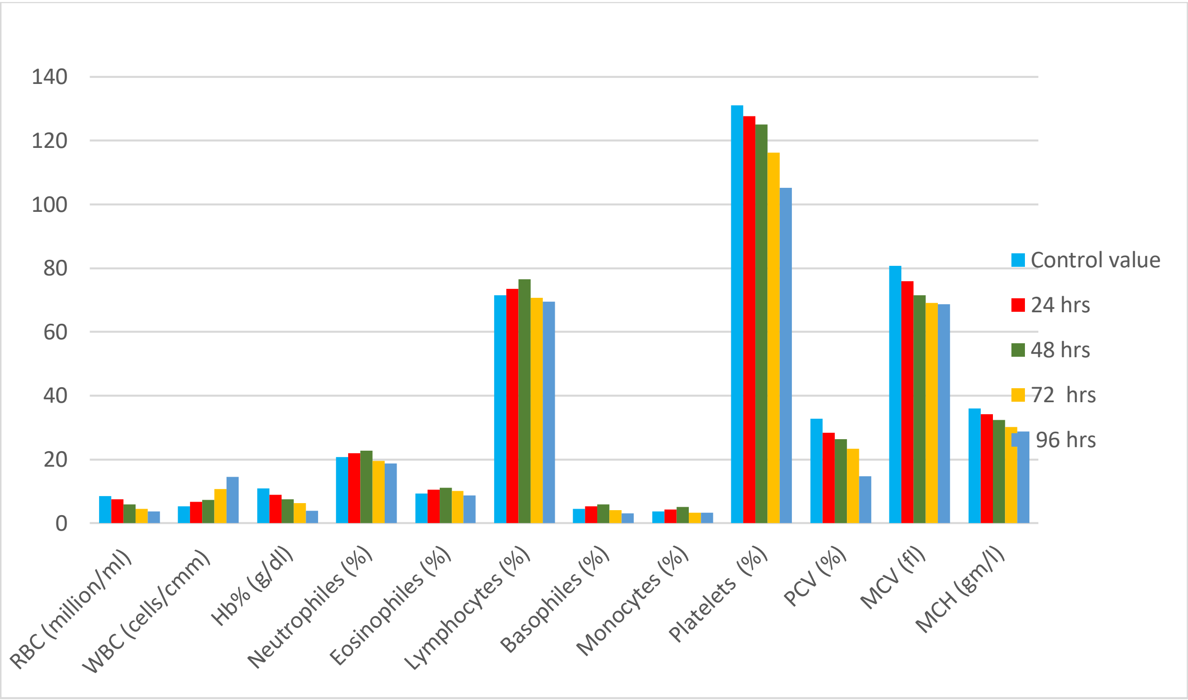

Figure 01: Hematological changes in *clarias batrachus* due to alphamethrin insectiside

4. Disscussion:

Blood is the key factor which affect all pathophysiological factors of the body, making blood parameters essential for diagnosing the structural and functional status of fish exposed to toxicants. In this study, significant alterations were observed in the blood components of fish treated with alphamethrin. Notably, there was a reduction in red blood cell count, hemoglobin (Hb) levels, platelets, packed cell volume (PCV), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). Conversely, white blood cell (WBC) counts increased, and the differential leucocyte count, including neutrophils, eosinophils, lymphocytes, basophils, and monocytes, showed fluctuations. These changes suggest that alphamethrin exposure has a profound impact on the haematological profile of fish, indicating stress and potential toxicity.

The reduction in red blood cell (RBC) count, haemoglobin (Hb) levels, and packed cell volume (PCV), which are essential for oxygen transport, can result from the inhibition of erythropoiesis and haemoglobin synthesis, as well as increased erythrocyte destruction in hematopoietic organs. Studies in fish species like *Catla catla* (Vani *et al*., 2011) and *C.gariepinus* (Adamu, 2009) have shown that exposure to environmental stressors such as plant extracts (e.g., *Moringa oleifera* seed extract) (Kavitha *et al*., 2012) or toxins like tobacco leaf extracts and cassava effluents leads to significant reductions in RBCs and Hb. This may be due to mechanisms such as erythroblastosis, which causes anaemia by producing immature RBCs that are ineffective at oxygen transport. Such findings underscore the detrimental effects of environmental pollutants and toxins on blood health in aquatic organisms, leading to impaired oxygen-carrying capacity and overall haematological dysfunction.

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 Akinrotimi *et al*. (2009) 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, including RBC count, haemoglobin concentration, and PCV, are recognized as indicators of secondary responses to pollutants in organisms (Rogers *et al*., 2003; Akinrotimi *et al*., 2012b; Akinrotimi *et al*., 2012c). Exposure to alphamethrin caused a significant reduction in platelet counts in fish. A similar decrease in platelet counts was reported in *O. mykiss* exposed to cypermethrin (Velisek *et al*., 2006). This decline may be associated with platelet sequestration in the spleen, reduced production, or increased destruction of platelets.

The Differential Leucocyte Count (DLC) exhibited fluctuations in this study. An increase in neutrophils, monocytes, and eosinophils was observed, while lymphocytes and basophils showed a decrease in monogenean-infected fishes. Comparable findings were reported in helminth-infected *Schizothorax* spp. and *Cyprinus* spp. (Shah *et al*., 2009).

A significant increase in WBC count was observed as a haematological response to alphamethrin exposure. Similar findings have been reported in common carp (*Cyprinus carpio*) following acute exposure to phenitrothion, imidan, and dichlorvos (Svobodova, 1991, 1996, 1998). This increase is likely due to the release of white blood cells from the spleen into the bloodstream to counteract the toxicant. In the present study, the substantial rise in leukocyte count may be attributed to a general immune activation and a defensive response to cypermethrin. White blood cells play a crucial role in protecting the organism during injury, haemorrhage, or the invasion of foreign antigens (Velmurugan *et al*., 2016). During stress, leukocyte levels increase significantly to help the organism cope with the adverse conditions and strengthen its defense mechanisms (Deshmukh, 2016).

Exposure of *Clarias batrachus* to alphamethrin (75 µl/l) for 96 hours in the present study proved toxic, leading to significant haematological changes. Red blood cell count, haemoglobin level, platelet count, PCV, mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) values decreased, while the Differential Leucocyte Count (DLC) fluctuated, and white blood cell (WBC) count increased. These findings align with those reported in previous studies.

5. Conclusion:

Based on the results obtained in this study, it can be concluded that a 96-hour exposure to 75 ppm of alphamethrin (aqueous solution) has toxic effects, altering the haematology of the fish. Our findings clearly indicate that alphamethrin disrupts the fish's physiology, causing haematological disturbances that could impair its ability to combat diseases and reduce its

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chances of survival and growth. Therefore, it is recommended that users of this pyrethroid pesticide (alphamethrin) exercise caution regarding the dosage applied.

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