Chapter 7

Haematology

INTRODUCTION

Currently it has been realized that a large variety of insecticides used extensively to control insect pests and to augment agricultural yield eventually reach the aquatic ecosystem substantially in run off where they affect the physiology and behaviour of non-target species. Amongst these, fishes are the most vulnerable victims. The possible ill effects of these chemicals on non target organisms have led to an investigation of their influence on animals including fish. Foundation of the work on haematology of fishes was laid down in the early 20th century when Krough studied respiratory function of blood in fishes.

Blood is a fluid connective tissue circulating in the body. It provides one of the methods of communication between the cells of different parts of the body. The study of the fish blood parameters are important for determining factors related to its physiological capacity (Affonso, 2001; Wells, et. al, 2005). The primary functions of blood are: 1) oxygenation of tissues, 2) nutrition of tissues, 3) maintenance of acid-base balance, and 4) removal of metabolic waste products from tissues. Thus, any dysfunctions of blood can have severe effects on the physiological activities of the entire body. Also, certain physiological dysfunctions in the body are reflected as alterations in blood constituents, which can be used as diagnostic indicators.
Erythrocytes, leucocytes and thrombocytes being the essential cellular components of fish blood, their concentration is maintained within well-defined limits in different fish species unless the balance between production and elimination is disturbed by pathological process. Generally, the erythrocytes not only pump out sodium and pump in potassium against electrochemical gradient but also reduce methaemoglobin to haemoglobin (Hb) to transport oxygen to the body tissues. The importance of packed cell volume (PCV) or haematocrit (Hct) as an index of anaemia is well known in clinical medicine (Bell, et. al, 1972). White blood corpuscles (WBC) play a major role in defence mechanism and mainly comprise granulocytes, monocytes and lymphocytes (Jurd 1985); the former two function as phagocytes to salvage debris from injured tissue, and the latter produces antibodies (Wedemeyer and McLeay 1981). Thrombocytes are involved in coagulation of blood (Wepener 1990). Haematological variables of fish under stress are of great significance in assessing the impacts of pollutants in the biota of a particular ecosystem. Therefore, haematology has been widely used as potent bioindicator in aquatic toxicology (Sancho, et. al, 2000).

Haematological studies plays an important role in understanding variation of blood characteristics in relation to factors like phylogenetic position, ecological habitat, pollutants, food selection, etc. The regular monitoring of the fish blood is a diagnostic tool in establishing the health status of the fish in farm. It helps in evaluating the response of different types of blood cells and its components in the conditions of physiological stress due
to toxicity, as it quickly reflect the poor conditions of fish than other commonly measured parameters. The blood composition of a fish reflects to some extent to metabolic and other physiological processes. Accordingly, haematology can be used as clinical tool for the investigations of physiological and metabolic alterations in fish caused by pollution of the aquatic environment (Anandkumar, 1994).

Fish are subjected to many environmental influences which alter the healthy hemogram; i.e., the baseline data for cellular and plasma components. Haematocrit, haemoglobin and the erythrocytic haemoglobin concentration values indicate the oxygen carrying capacity in teleosts. Such parameters are highly variable among the species interfering in the oxygen-carrying capacity (Affonso, 2001; Tavares-Dias and Moraes, 2004; Wells, et. al, 2005). The vital functions that blood cells perform, together with the susceptibility of this highly proliferative tissue to intoxication, makes the hematopoietic system unique as a target organ.

Blood is a vehicle for quickly mobilizing defense against trauma and diseases. Since, fishes differ considerably in their activity patterns and respond to the pollutant. The blood parameters like Red blood corpuscle (RBC), white blood corpuscle (WBC), Haemoglobin (Hb), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) are commonly studied in fishes to assess the impact of pesticides in aquatic biota.
RBC and Hb can be used as an index of anaemia and fluid volume disturbances. Reduction in RBC and haemoglobin is the most prominent haematological effect of toxicants. Anaemia may be due to an increase of plasma volume caused by disturbed water balance, decreased rate of RBC production, loss or destruction of RBC (Sarwath, et al., 2003; Satyanarayana, et al., 2004). Haemoglobin is the main constituent of blood and plays an important role in the transport of oxygen through the blood capillaries. Lowered haemoglobin level might reduce the ability of the fish for seeking food and escaping from predators due to stress syndrome. Many workers have reported alteration in RBC and Hb level (Christensen, et al., 1972; Arunachalam, et al., 1980; Garg, et al., 1989; Magare & Patil, 2000). However, these parameters are more related to the response of the whole organism i.e., to the effect on fish survival, reproduction and growth. In the present investigation an attempt has been made to study the lethal and sublethal effect of cypermethrin on haematological aspect of Labeo rohita.

RESULTS

The results of the haematological parameters are found to be time dependent. The summary of the results after exposing fish Labeo rohita to lethal (24, 48, 72, and 96 h) and sublethal (1, 5, 10, and 15 days) concentrations of cypermethrin is as follows.

**Red blood corpuscle (RBC) count:** Results of total erythrocyte count (Table 29 and Fig. 26) indicated alteration at both lethal and sublethal concentrations. At lethal concentration maximum reduction of -63.97% was observed at 96 h.
At sublethal concentration increase in the reduction was observed up to day 10 (-24.86%), reduction was continued on day 15 (-22.04%) also. Under lethal concentration the RBC count was ranged -10 % to -63.97% and under sublethal concentration the count ranged from -10.67% to -22.04%

**White blood corpuscle (WBC):** The WBC count altered specifically (Table 29 and fig. 28). Maximum count 35.72% was observed at 96 h under lethal concentration regressed at 24 h (10.08%), 48h (21.05%), and 72h (11.56%). In sublethal concentration of cypermethrin, WBC count increased continuously from day 1 (8.11%) to day 10 (24.33%) and later intensity of decrease was reduced on day 15 (21.58%)

**Haemoglobin (Hb):** Results of haemoglobin levels suggests the progressive reduction at lethal concentration. The decrease was 55.57% up to 96 h. At sublethal concentration day 1 (-2.66%), 5 (13.96%) and 10 (12.67%) registered reduction in decrease on day 15 (8.08%) over control (Table 30 and fig.26).

**Packed cell volume (PCV):** The trend of PCV value was like that of RBC and Hb at lethal and sublethal concentration (Table, 30 and fig. 26). In the lethal concentration decrease was up to 96 h (55.52%). During sublethal concentration PCV was decreased from day 1 (-0.59%) to day 10 (-19.41%). While approaching towards 15th day, decrease was reduced to 12.46% over control and was increased compared to day 10.

**Mean corpuscular volume (MCV):** MCV value calculated on the basis of PCV and RBC values and presented in the table, 31 and fig. 27. The values exhibited enhancement at lethal concentration. At lethal concentration the
percent elevation ranged between 2.08% to 30.81%. In contrary at sublethal concentration the elevation was seen up to day 5 (11.72%), which later decreased on day 10 (3.74%) and 15 (1.06%) over earlier periods of exposure. The maximum elevation was recorded on day 5.

**Mean corpuscular haemoglobin (MCH):** MCH represents the average weight of haemoglobin in RBC. MCH exhibited increase in all exposure periods of lethal concentration (Table 31 and fig. 27). Maximum increase was observed at 96 h (23.34%). At sublethal, the increase was up to day 5 (19.27%).

**Mean corpuscular haemoglobin concentration (MCHC):** MCHC is the indication of average concentration of haemoglobin in RBC cells, calculated based on Hb and PCV. Decrease in Hb and PCV coupled with increase in MCV might be the reason for the decreased MCHC. At lethal concentration MCV was decreased with maximum of -17.37% at 48 h. At sublethal concentration, the value was increased in all exposure periods (Table 32 and fig. 28). The maximum increase registered at 5th day (27.49%) of the exposure.

**DISCUSSION**

Blood is a pathophysiological reflector of the whole body and, therefore, haematological components may be considered as promising bioindicators in insecticidal toxicosis as in diagnosing the structural and functional status of fish exposed to toxicants. Haematological study play vital role in monitoring fish health, pollution load, stress and disease. Therefore, it is pertinent to study the impacts of pollutants on fishes. In the present investigation, fish *Labeo rohita* on exposure to lethal and sublethal
concentrations of cypermethrin showed considerable alteration in the level of different blood parameters.

According to the haematological findings, the general health status of cypermethrin exposed fish was severely affected. Decrease in total erythrocyte count, haemoglobin percentage, PCV values indicates the occurrence of anemia associated with erythropenia. The anemia may be due to the inhibition of erythropoiesis and hemosynthesis and to an increase in the rate of erythrocyte destruction in hemopoietic organs. However, the underlying mechanism which led to the anaemic condition might differ according to the compound. Anaemia in fish is a consequence of inductive haemolytic effects on exposure to cypermethrin, since it has a higher affinity for membrane phospholipids, which accounts for its lytic activity. In addition cypermethrin, with a slightly higher lipophilicity (OECD, 2006), and is known to partition extensively into the phospholipid component of biological membranes. Hence it is suggested, that anaemia found in cypermethrin exposed fish in the present study is a consequence of an interaction between cypermethrin and the erythrocyte membrane. Due to the influence of cypemethrin on the composition of erythrocyte phospholipids may lead to an increased osmotic fragility of these cells, disrupting iron-synthesizing mechanisms which also may lead to decrease in oxygen carrying capacity of the blood and contribute to the haematological effects observed.

Reduction in the Hb content and Ht values evidenced erythropenia in the present study. Erythropenia had also been reported in Gobius

The subsequent rise in RBC count and haemoglobin concentration, which occurred, on exposure to sublethal concentration of cypermethrin beyond 10 days was probably due to enhanced erythropoiesis. Few earlier studies reported with increase in RBC and Hb in fishes exposed to pollutants in several other teleost species, for instance *C. carpio* and *Puntius ticto* chronically exposed to sublethal doses of aldrin, dieldrin, DDT, BHC and
chlordane for 10, 20 and 30 days, respectively, showed decrease in RBC count with increase in exposure period, but Hct values increased with respect to aldrin and dieldrin and decreased with DDT, BHC and chlordane (Satyanarayana, et. al, 2004). Matthiessen (2006) noted that during six sequential application of endosulfan for controlling tsetse fly in Botswana, there was a rise in erythrocyte count and Hb content in several teleosts during spray only; these began returning to normal before the spraying ceased and reached prespraying levels within 6 months. *Puntius conchohius* exposed to mercury (Gill and Pant, 1988), *S. mossambica* exposed to endosulfan (Sarvanan and Harikrishna, 1999). They suggested that, the increment was might be due to the enhancement of erythropoiesis, which triggered as a typical stress response.

In the present investigation WBC count under lethal concentration increased on first day of exposure period, which later decreased consecutively. But under sublethal the level increased from day 1 to day 10, which decreased on day 15 showing normalcy. The initial increase in the WBC count may be the result of direct stimulation of immunological defense due to the presence of toxic substance or may be associated with induced tissue damage. Decrease in WBC count in later days might be due to series of changes in the immunological set up of the fish under pesticide stress (Anandakumar, 1994). Kalavathy, et. al, (2001) suggested that decrease in WBC count could be because of autolysis, caused due to haemolytic enzymes leaked out by cells on exposure to cypermethrin.
Increase in the number of WBC (leucocytes) of treated fish reflects a general state of toxaemia exhibiting impairment of the defence mechanism, and is manifested into leucocytosis to cope with such a situation. Similar results were reported in teleosts by Dutta, *et. al*, (1992); Ramesh and Saravanann (2008) Tyagi, *et. al*, (1989) and Kalair, *et. al*, (1993) on exposure to different pesticides. Lymphocytes are numerically predominant white blood cells in fish (Swarnlata 1995; Kumar, *et. al*, 1999). Lymphocytes of fish have been regarded as immunocompetent. Thus, they are responsible for the production of antibodies (Ellis, *et. al*, 1978).

Reduction in the number of circulating leucocytes can be viewed as stress response of fish on exposure to cypermethrin. It could be attributed to possible increase in the level of circulating ACTH and corticosteroid stress hormones as suggested by Srivastava and Agrawal (1977). Hickey (1976) has suggested that leucopenia is a specific response of fish to stress. They attributed leucopenia to increased activity of pituitary interrenal stress axis. Reddy and Bashamohideen (1989) suggested that leucopenia might be due to depression of leucopoiesis, alteration in the cell membrane or disintegration of WBC. Kaattari and Piagnelli (1996) and Adedeji, *et. al*, (2009) were of the view that a prolonged stimulation of the immunological defence system after insecticide exposure resulted in the development of leucopenia, accompanied by increase in the number of neutrophils and eosinophils which drastically inhibits the capacity of the fish to withstand non-specific stress.
Packed cell volume or Haematocrit values showed declining trend at both lethal and sublethal concentration of cypermethrin in the exposed fish. This may be due to impaired oxygen supply to various tissues, resulting in slow metabolic rate and low energy production. The decline in PCV might shrink cell size due to intoxication. Similar results were noted in rainbow trout following mancozeb treatment (Atamanalp and Yanik 2003). Recently, Ramesh and Saravanan (2008) also opined that the decrease in the PCV of C. carpio treated with sublethal concentration of chlorpyrifos was the result of either rapid oxidation of haemoglobin to methaemoglobin or release of oxygen radical brought about by toxic stress of the insecticide. It is well recognized that xenobiotics capable of undergoing redox cycling can exert toxic effect via generation of free radicals oxygen (Markovics and Witas 1981). Tovassoli (1975) suggested that haemopoiesis (production of constituent elements of blood) may be affected by insecticides as the nervous system and neural elements are associated with blood vessels, stroma and insecticides alter the cell membrane by hydrolysis of acetylcholine in the body fluids by cholinesterase of erythrocytes and consequently, responsible for development of the erythropenia with consequent fall in Hb content and PCV (Plack, et. al, 1979; Srivastava and Mishra 1987; Reddy and Bashamohideen 1989; Singh, et. al, 1992; Sancho, et. al, 2000). Adedeji, et. al, (2009) opined that a decrease in PCV in the test fish could be ascribed to reduction in erythrocyte lifespan and/or a suppressive effect of the active substance of the insecticide on the erythropoietic tissues, resulting in the failure of erythrocyte production.
MCV values exhibited gradual reduced at lethal concentration. But in sublethal concentration the increase was registered up to day 5, which later decreased. Anandkumar, (1994) suggested increase in MCV may be caused due to endosmosis. Endosmosis leads to the passage of solvent from less concentrated solution to more concentrated one. This results in haemodilution, further increasing the MCV value. Previously Garg, et. al, (1989), Anandkumar, et. al, (1994), Dhanapakiam and Ramasamy, (2001) reported increase in MCV of fishes exposed to toxicants. In contrast to this (Srivastav and Mishra, 1979; Nath and Banerjee, 1996) reported decrease in MCV value in fish. Janardan Reddy, (1991) reasoned decrease in MCV for the variation in red cell volume that attributes the occurrence of exosmosis indicated by increased electrolyte concentration inside the red cell after insecticide treatment. David, (1995) observed a decrease and then a subsequent increase of MCV in *L. rohita* exposed to low and high sublethal concentration of fenvalerate. Koundinya and Ramamurthy, (1980) observed increase in MCV of *Tilapia mossambica* exposed to sublethal concentration of sumithion. As MCH and MCHC are derived from Hb and RBC, any sort of alteration in the levels of Hb and RBC would result in the alteration of MCH and MCHC. In addition, similar observations have been reported in teleost species treated either to lethal or sublethal concentrations of various insecticides. Atamanalp, et. al, (2002) reported a marked rise in the values of MCHC, MCH and MCV in *O. mykiss* on exposure cypermethrin. Parma, et. al, (2007), however, noticed minor alterations in the values of MCV, MCH, and
MCHC concentrations in *Prochilodus lineatus* treated with 0.6 ppm of cypermethrin.

Thus, cypermethrin in the aquatic medium is a major factor responsible for drastic changes in the fish blood. Perhaps it would be more rational to state that a rise or fall in RBC and WBC count, Hb content, PCV or haematocrit and MCV value, MCH and MCHC concentration in insecticide-exposed fish provide important information on the general physiology and health status of fish under investigation. Thus, fish blood following exposure to contaminants is at the best suitable bioindicators in the field of environmental toxicology. It may be added here that changes observed in the whole animal oxygen consumption and oxidative stress–inducing potential of cypermethrin on important vital organs including liver of fish corroborates early warning signals of oxidative damage. As the oxidative damages are expressed as ultrastructural reactions, resulting change in subcellular architecture which are evidenced in the present study.
Table 29: RBC count (x 10^6 / mm^3) and WBC count (x 10^3 / mm^3) in the blood of the fish, *Labeo rohita* on exposure to lethal and sub lethal concentration of cypermethrin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Exposure Periods</th>
<th>Lethal (h)</th>
<th>Sublethal (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>RBC</td>
<td>3.73^A</td>
<td>3.35^B</td>
<td>2.23^G</td>
<td>1.93^H</td>
</tr>
<tr>
<td>SD ±</td>
<td>0.13</td>
<td>0.23</td>
<td>0.17</td>
<td>0.13</td>
</tr>
<tr>
<td>% Change</td>
<td>----</td>
<td>-10.00</td>
<td>-40.03</td>
<td>-48.25</td>
</tr>
<tr>
<td>SD ±</td>
<td>0.17</td>
<td>0.19</td>
<td>0.14</td>
<td>0.11</td>
</tr>
<tr>
<td>% Change</td>
<td>----</td>
<td>10.08</td>
<td>-21.05</td>
<td>-11.56</td>
</tr>
</tbody>
</table>

Means are SD ± (n= 6) for a parameter in a row followed by the same letter are not significantly different (p ≤ 0.05) from each other according to Duncan’s Multiple Range (DMR) Test.
Table 30: Haemoglobin level (g/100 ml) and PCV level (%) in the blood of the fish, *Labeo rohita* on exposure to lethal and sub lethal concentration of cypermethrin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Exposure Periods</th>
<th>Sublethal (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lethal (h)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>8.16^A</td>
<td>7.39^D</td>
<td>5.33^G</td>
</tr>
<tr>
<td>SD ±</td>
<td>0.28</td>
<td>0.53</td>
<td>0.41</td>
</tr>
<tr>
<td>% Change</td>
<td></td>
<td>-9.45</td>
<td>-34.65</td>
</tr>
<tr>
<td>SD ±</td>
<td>0.099</td>
<td>0.09</td>
<td>0.05</td>
</tr>
<tr>
<td>% Change</td>
<td>--------</td>
<td>-8.13</td>
<td>-30.31</td>
</tr>
</tbody>
</table>

Means are SD ± (n= 6) for a parameter in a row followed by the same letter are not significantly different (p ≤ 0.05) from each other according to Duncan’s Multiple Range (DMR) Test.
Table 31: MCV level (cu μm) and MCH level (pg) in the blood of the fish, *Labeo rohita* on exposure to lethal and sublethal concentration of cypermethrin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Expos</th>
<th>Lethal (h)</th>
<th>Sublethal (days)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td><strong>MCV</strong></td>
<td>75.22&lt;sub&gt;G&lt;/sub&gt;</td>
<td>76.79&lt;sub&gt;F&lt;/sub&gt;</td>
<td>87.41&lt;sub&gt;C&lt;/sub&gt;</td>
<td>98.39&lt;sub&gt;A&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>SD ±</strong></td>
<td>0.07</td>
<td>0.02</td>
<td>0.01</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>% Change</strong></td>
<td>---</td>
<td>2.08</td>
<td>16.20</td>
<td>30.81</td>
</tr>
<tr>
<td><strong>MCH</strong></td>
<td>21.86&lt;sub&gt;G&lt;/sub&gt;</td>
<td>22.00&lt;sub&gt;F&lt;/sub&gt;</td>
<td>23.82&lt;sub&gt;D&lt;/sub&gt;</td>
<td>23.63&lt;sub&gt;E&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>SD ±</strong></td>
<td>0.08</td>
<td>0.07</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>% Change</strong></td>
<td>---</td>
<td>0.61</td>
<td>8.95</td>
<td>8.08</td>
</tr>
</tbody>
</table>

Means are SD ± (n= 6) for a parameter in a row followed by the same letter are not significantly different (p ≤ 0.05) from each other according to Duncan’s Multiple Range (DMR) Test.
Table 32: MCHC level (%) in the blood of the fish, *Labeo rohita* on exposure to lethal and sub lethal concentration of cypermethrin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Exposure Periods</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Lethal (h)</td>
<td>Sublethal (days)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>48</td>
<td>72</td>
<td>96</td>
<td>1</td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>MCHC</td>
<td>29.07\textsuperscript{D}</td>
<td>28.65\textsuperscript{E}</td>
<td>27.25\textsuperscript{F}</td>
<td>24.02\textsuperscript{G}</td>
<td>29.03\textsuperscript{D}</td>
<td>31.63\textsuperscript{C}</td>
<td>31.03\textsuperscript{C}</td>
<td>32.51\textsuperscript{B}</td>
<td>33.91\textsuperscript{A}</td>
</tr>
<tr>
<td>SD ±</td>
<td>0.08</td>
<td>0.02</td>
<td>0.09</td>
<td>0.08</td>
<td>0.02</td>
<td>0.03</td>
<td>0.07</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>% Change</td>
<td>--</td>
<td>-1.43</td>
<td>-6.23</td>
<td>-17.37</td>
<td>-0.11</td>
<td>8.80</td>
<td>6.75</td>
<td>11.83</td>
<td>16.66</td>
</tr>
</tbody>
</table>

Means are SD ± (n= 6) for a parameter in a row followed by the same letter are not significantly different (p ≤ 0.05) from each other according to Duncan’s Multiple Range (DMR) Test.
Fig 26: Percent change over control in RBC count (x 10^6 / mm^3), Haemoglobin level (g / 100 ml) and PCV level (%) in the blood of the fish, *Labeo rohita* on exposure to lethal and sub lethal concentration of cypermethrin.
Fig 27: Percent change over control in MCV level (cu μm) and MCH level (pg) in the blood of the fish, *Labeo rohita* on exposure to lethal and sub lethal concentration of cypermethrin.
Fig 28: Percent change over control in MCHC level (%) and WBC count (x $10^3 / \text{mm}^3$) in the blood of the fish, *Labeo rohita* on exposure to lethal and sub lethal concentration of cypermethrin.