

Consequences of Fame (Flubendiamide 480 sc) Exposure on Haematological Parameters of the Fish *Oreochromis Mossambicus*

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Abstract- The haematological parameters such as Total Erythrocyte Count (TEC), Total Leucocyte Count and Differential leucocyte count (TLC and DLC), Haemoglobin Content (Hb), Packed Cell Volume (PCV), the Erythrocyte constants like Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) and Enzymes (AST/SGOT and ALT/SGPT) were estimated in the fish *Oreochromis mossambicus* exposed to sub-lethal concentration (10ppm) of FAME (Flubendiamide 480 SC) pesticide for four weeks. Studies in fish exposed to this chemical pesticide (amide derivative) indicated significant reduction in the blood parameters except WBC throughout the experiment. Total leucocytes showed significant increase throughout the experiment. The enzyme profile showed significant increase after 4weeks of exposure confirming the significant impact of the pesticide on the organism.

Index Terms- flubendiamide, haematological, *Oreochromis mossambicus*, sub-lethal concentration

I. INTRODUCTION

Seasonal utilization of paddy fields for fish culture is quite common in Kerala and West Bengal. In recent years, with the advent of high yielding varieties of paddy, the use of pesticide has become very popular. Agricultural inputs in terms of pesticides/insecticides are increasing day by day and its residues are being drained off in to water bodies. Use of pesticides in agriculture sector and its ultimate discharge in to aquatic ecosystem have a potential toxicological concern to biota of the ecosystem including fishes (Tilak *et al.*, 1980). Environmental pollution is one of the serious plights of the modern world. During the last decade, due to the significant increase in the environmental pollutants and lack of precautionary measure or observance of the environmental regulations, it has become the global problem (Bondarenko *et al.*, 2004, Devi *et al.*, 2008, Sachar and Raina, 2014). Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, because they are sprayed or spread across entire agricultural fields (Gaafar *et al.*, 2010, Saeed *et al.* 2012; Shankar *et al.*, 2013). Pesticides in the aquatic environments can negatively affect the ecosystem.

Haematological parameters are closely related to the response of the animal to the environment, an indication that the environment where fishes live could exert some influence on the haematological characteristics (Gabriel *et al.*, 2004, Kavitha *et al.*, 2010). Blood offers important profile to study the toxicological impact on animal tissues. Different blood parameters are often subjected to change depending upon stress condition and various other environmental factors (Goel *et al.*, 1985, Goel and Sharma 1987, Suvetha *et al.*, 2010; Chaudhary *et al.*, 2015; Prasad *et al.*, 2015). Fishes are very sensitive to a wide variety of toxicants in water; various species of fish show uptake and accumulation of many contaminants or toxicants such as pesticides (Herger *et al.*, 1995). Accumulation of pesticides in tissues produces many physiological and biochemical changes in the fishes and freshwater fauna by influencing the activities of several enzymes and metabolites (Nagarathnamma and Ramamurthi, 1982). Bouck and Ball (1966), Johnson (1968) and Srivastava *et al.* (2010) stated that fish serves as a bio indicator species as it responds with great sensitivity to changes in the aquatic environment and has an important role in the monitoring of water pollution. Blood analysis is crucial in many fields of Ichthyologic research, fish farming and in toxicology and environmental monitoring as an indicator of physiological and pathological changes in fishery management and disease investigation (Adedeji *et al.*, 2000).

Several researchers studied the effect of various toxicants on the haematology of fishes such as hemato-biological characteristics of *Heteropneustes fossilis* under the stress of Zinc (Goel *et al.*, 1985), effect of zinc sulphate on haematology of dog fish (Flos *et al.*, 1987). Ramanujan and Mohanty (1977) studied the effect of sub-lethal concentration of Endosulphan on the haematological parameters of *H.fossilis*. Srivastava and Mishra (1979) have reported anaemia associated with erythropenia in *Iolisa faciatas* after exposure to lead. The significant increase in RBC number and percentage haemoglobin could be ascribed to enhanced erythropoiesis which is triggered as a typical stress response (Gill and Pant, 1985). Goel *et al.* (1992) reported that the results of reduced activities of AST, ALT, ACP, ALP, LDH in various organs of fish (*Pontius conchoniis*) exposed to mercury in implies destruction in the tissues of the animals. Serum AST and ALT are important diagnostic tools in medicine and clinics, and are used to detect the toxic effects of various pollutants (Nelson and Cox, 2000). Roy and Bhattacharya (2005) noted significant changes in SGOT (AST) and SGPT (ALT) in

Channa punctatus exposed to As_2O_3 and indicated that the changes may be due to histopathological lesions in liver. Kallagadda *et al.*, (2016) observed marked changes in behavioural morphology and haematological parameters in *L.rohita* exposed to flubendiamide.

Haematological parameters can be detected rapidly and hence can be used for prediction and diagnosis of pesticide toxicity. Haematologic indices are important parameters for the evaluation of fish physiological status, the changes in which depend on fish species, age, sexual maturity of spawners and disease (Zhitenera *et al.*, 1989, Luskova, 1997). These indices have been employed in effectively monitoring the response of the fishes to the stressors and thus their health status under such adverse conditions. The haematological indices were widely used by fish biologists and researchers (Adhikari *et al.*, 2004; Cazenave *et al.*, 2005; Kori-Siakpere *et al.*, 2006; Ali and Rani, 2009; Sharaf *et al.*, 2010; Kori-Siakpere and Ikomi, 2011 and Nte, 2011). Sequel of pesticides on haematological factors of a number of fish species have been investigated by several researchers: in *Cyprinus carpio* (Satyanarayan *et al.*, 2004; Salvo *et al.*, 2008); in *Clarias batrachus* (Patnaik and Patra, 2006; Kharat and Kothavade, 2012; Summarwar and Verma, 2012); in *Oreochromis mossambicus* (Ali and Rani, 2009; Desai and Parikh, 2012); in *Heteropneustes fossilis* (Deka and Dutta, 2012; Chaudhary *et al.*, 2015; Prasad *et al.*, 2015), and in *Puntius mesopotamicus* (Saxena and Seth, 2002; Carraschi, 2012). Present study was carried out to analyse the effect of sub-lethal concentration of amide derivative pesticide, flubendiamide on the haematological parameters of the fish *Oreochromis mossambicus* and to estimate the impact of long term use of pesticide on the vital organ such as liver of fish by analyzing the liver marker enzymes.

II. MATERIALS AND METHODS

Oreochromis mossambicus weighing 20-30 g from the local fish farm were used for the experiment. The fish were acclimatised to the laboratory conditions in a glass aquarium filled with aerated de-chlorinated water under natural photo period for one week. The fish were fed every day. Water in the tank was renewed every day. Only healthy and active fishes of similar size were randomly selected from the aquarium tanks and used for haematological studies.

96 hour LC_{50} value for the pesticide was determined using bioassay methods proposed by Doudoroff *et al.* (1951). From the LC_{50} value a sub-lethal concentration ($1/10^{th}$ of LC_{50}) was calculated for the pesticide and was fixed as the experimental concentration. Fish were exposed to sub-lethal concentration (10ppm) of fame (Flubendiamide) for a period of 28 days (4week), and blood samples were taken by severing the caudal vein of the fish. Haematological parameters like Total Erythrocyte count (TEC), Haemoglobin Count (Hb gm %), Packed cell volume (%), Total and differential Leucocyte Count (TLC and DLC) and erythrocyte constants (MCV, MCH, MCHC) were determined by standard methods. Blood samples were also used for the analysis of enzymes such as Alkaline transaminase (ALT/SGPT) and Aspartate aminotransferase (AST/SGOT).

III. RESULT

Data on haematological parameters such as Total Erythrocyte count (TEC), Total Leucocyte Count (TLC), Haemoglobin Count (Hb), Packed cell volume or Haematocrit (PCV) and erythrocyte constants such as Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) of *O.mossambicus* exposed to sub-lethal concentration (10 ppm) of pesticide FAME (Flubendiamide 480 SC) for a duration of 24 hrs to 4 weeks are represented in Tables I and II. Table III delineates the enzyme profile after exposure of 4 weeks. Data is represented as mean values \pm Standard Error.

IV. DISCUSSION

Water pollution is recognized globally as a potential threat to both human and other animal populations that interact with aquatic environments. Any alteration in the environment upsets homeostasis. Basically all toxicants produce adverse effect on some physiological functions in aquatic organisms. According to studies conducted by Shayeghi *et al.* (2001) and Honarpajouh (2003), traces of insecticides were detected several months after spraying. Peripheral haematological parameters are a reliable indicator of physiological status of fish and an effective parameter to assess the background effects of environment perturbation (Natarajan *et al.*, 1998). At sub-lethal concentrations, the fish survive even after prolonged periods of exposure (Natarajan, 1984). Prolonged exposure in sub-lethal concentrations of the toxicant may cause a strict decline in many of haematological parameters.

The red blood cells have the important function of carrying oxygen to all tissues in the body via haemoglobin in erythrocytes. The decreased red blood cell number following exposure to pesticide could be as a result of haemolysis or destruction of red blood cells. In the present study, the total RBC count was decreased significantly on the last day of the experiment. The decrease found in total RBC might be due to the disruptive action of pesticide on the erythropoietic tissue (Larsson *et al.*, 1985). Similar result has been reported by previous studies in *Cyprinion watsoni* due to malathion exposure (Khattak and Hafeez, 1996), in *Piaractus mesopotamicus* due to trichlorophon exposure (Tavares *et al.*, 1999) and in carp due to chlorpyrifos (Ramesh and Saravanan, 2008, Vani *et al.* 2011 and Yonar *et al.*, 2012). It has been widely reported that many pollutants denature or oxidise the haemoglobin by inhibiting glycolysis or hexose monophosphate shunt resulting in anaemia (Buckley *et al.*, 1976). In our study too Hb content is reduced. The significant decrease of TEC and Hb during four weeks of exposure is the result of decreased erythropoietic activity.

Haematocrit is used to determine the ratio of plasma to corpuscles in the blood as well as the oxygen carrying capacity of the blood. The findings of present study agree with the previous results (Larsson *et al.*, 1985) in which recorded a decrease in haematocrit level in brown trout after exposure to diclofenac for 7 days. The haematocrit values decrease when a fish loses its appetite or diseased (Blaxhall, 1973) or poisoned by pesticides (Gill and Pant, 1985). The reduction in packed cell volume (PCV) of *C. c. var. communis* may also be due to decreased rate of erythropoiesis as well as haemolysis as

observed in the *Flounder pleuronectes flesus* when subjected to cadmium toxicant (Larsson, 1975). In the present study a gradual decline in blood parameters like haemoglobin content and PCV were noticed with increased exposure period. Same observations were made by Ramanujan and Mohanty (1977) while studying the effects of pesticide endosulphan on *Heteropneustes fossilis*. Thus it appears that the fish experienced a mild anaemia due to haemolysis and haemorrhage of blood cells. Similar observations were reported earlier by Tilak *et al.* (2001), Saravanan *et al.* 2010 and Saeed *et al.* (2012). Decreased haemoglobin content leads to decreased O₂ content, which affects the physiological state of the fish in an unhealthy way. Simultaneously increase in MCV reported in this study can be correlated with erythrocyte swelling, probably resulting from plasma acidosis and a reduction in plasma ions as given in earlier studies of Wood *et al.* (1989) and Milligan and Wood (1982).

Increase in TLC in the test fish could be due to stimulated lymphopoiesis and enhanced release of lymphocyte. Such lymphocyte response might be due to the presence of toxic substances or may be associated with the pollutant induced tissue damage as opined by Haniffa (1991). Increased WBC count established leucocytosis, which is considered to be of an adaptive value for the tissue under chemical stress. This also helps in the removal of cellular debris of necrosed tissue at a faster rate (Rivas, 1986). According to Sudha (2012) the increase in WBC count can be correlated with a natural response of the fish against toxic invasion. The findings of present study are also in tune with the results of previous studies.

But the increased MCV values from 24 Hrs to 4 week of exposure indicate cellular swelling and consequent macrocytosis and decrease of value due to the iron deficiency *etc* and thus lower haemoglobin concentration. The decreased PCV value (24 Hrs to 4 week) and MCHC (24 Hrs to 4weeks) indicates the occurrence of microcytic anaemia and due to the deficiency of iron and reduction in haemoglobin synthesis and the value increased due to haemolysis. The values of blood cell indices were enhanced after the exposure and this tune with the findings of earlier studies (Ko:pru:s *et al.*,2006, Rao, 2010, Kallagadda *et al.*,2016).

The liver function tests, analysis of serum AST/SGOT and ALT/SGPT, are widely used to demonstrate liver function or toxicant-induced hepato-toxicity (Yang and Chen, 2003; Dutta *et al.* 1993, 1996 and 2006). Roy and Bhattacharya (2005) noted significant changes in serum, SGOT and SGPT in *Channa punctatus* exposed to As₂O₃, and indicated that the changes may be due to histopathological lesions in liver. After sub-chronic dietary, Cu exposure for 40 days increased serum AST and ALT concentrations with increasing time and dose were observed in the rockfish, *Sebastes schlegeli* (Kim and Kang, 2004). In the present study, the significant increase in AST and ALT levels in Fame treated fish indicates hepatic damage due to accumulation of the toxicant, which in turn releases these enzymes into the bloodstream.

The study suggested that, the perturbations in these haematological indices attributes to a defence reaction against toxicity of FAME through the stimulation of erythropoiesis or may be due to the disturbances that occurred in both metabolic and haemopoietic activities of fish exposed to sub-lethal concentration. The toxicant induced haematological disturbance

could lead to the impairment of the fish's ability to combat diseases, reduce its chances for survival and potential for growth and reproduction.

V. CONCLUSION

Among pesticides, amide derivative pesticide like FAME (flubendiamide 480 SC) plays an important role in polluting the aquatic ecosystem. Fish were exposed to sub-lethal concentration (10 ppm) of flubendiamide 480 SC for 4 weeks. Haematological parameters like TEC, TLC & DLC, Hb and PCV were recorded after 24 Hrs to 4 weeks of exposure. Haematological constants like MCV, MCH and MCHC were obtained for control and experimental fishes. The TEC was recorded a significant decrease in the value at 24 Hrs to 4 week. The PCV and Hb values also showed a decreasing trend. The MCV values fluctuated during the study period. The MCH values showed decrease at 24 Hrs to 4 week. The MCHC showed a fluctuating trend from 24 Hrs to 4 week of exposures. MCH is mostly associated with haemoglobin concentration. The decreased values indicated iron deficiency and reticulocytosis and increased value may be due to haemolysis. The observations on haematological indices have provided valuable information on the fish health and its stress response.

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Table I: Haematological parameters of *Oreochromis mossambicus* exposed to sub- lethal concentration (10ppm) of fame (Mean ± Standard Error)

Haematological parameters Duration of exposure	RBC (X 10 ⁶ /mm ³)	WBC (cells/mm ³)	Hb (g %)	PCV (%)
CONTROL	5.47 ± 0.082	7950 ± 46.78	9.12 ± 0.143	41.40±0.425
24 HOURS	5.09 ± 0.021	8950 ± 20.00	8.44 ± 0.372	39.40±0.201
1 WEEK	4.72 ± 0.020	10100 ± 2 6.07	7.84 ± 0.312	35.00±0.282
2 WEEKS	4.05 ± 0.208	11320 ± 30.06	6.96 ± 0.057	31.00±0.282
3 WEEKS	3.93 ± 0.022	11970 ± 17.57	6.40 ± 0.282	28.60±0.203
4 WEEKS	3.21 ± 0.024	13350 ± 59.66	4.94 ± 0.051	22.40±0.483
't' value	3.88, p<0.01	4.12, p<0.01	3.61, p<0.01	3.29, p<0.01

Table II: Haematological indices of *Oreochromis mossambicus* exposed to sub-lethal concentration (10ppm) of fame (Mean ± Standard Error)

Haematological indices Duration of exposure	MCV fL	MCH pg	MCHC g/dL
CONTROL	75.68±0.881	16.67±0.193	22.08±0.235
24 HOURS	77.37±0.625	16.58±0.023	21.44±0.198
1 WEEK	74.86±0.397	16.61±0.020	22.43±0.154
2 WEEKS	76.52±0.959	17.18±0.021	22.50±0.284
3 WEEKS	72.70±0.446	16.28±0.017	22.41±0.257
4 WEEKS	69.63±0.280	15.38±0.085	23.00±0.583
<i>'t' value</i>	0.56, p<0.10	3.86, p<0.01	0.425, p<0.10

Table III: Enzyme Profile of control and fish *Oreochromis mossambicus* exposed to 10ppm of pesticide for 4 weeks

	CONTROL		FISH EXPOSED TO PESTICIDE (10ppm) FOR 4 WEEKS	
	SGPT (U/L)	SGOT(U/L)	SGPT (U/L)	SGOT(U/L)
Mean	58.4 ±1.00	70.8±0.51	116.4±1.90	149.4±0.85
<i>'t' value</i>	-	-	17.55, p<0.01	31.57, p<0.01