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EFFECT OF AMMONIUM SULPHATE ON HAEMATOLOGY PROFILE IN *Channa punctaus* (BLOCH.)

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ABSTRACT

The current study undertaken was the haematological profile changesin the snakehead fish Channa punctatus induced to sublethal concentration (16.5 mg/L) of inorganic fertilizer, ammonium sulphate for chronic (30 days). The treated fishes showed very significant changes at the haematological levels various parameters such as Hb, RBC, WBC decreases while in DLC, Neutrophil, Monocytes and Eosinophil values increase. The present study revealed a severe physiological dysfunction in response to Ammonium sulphate toxicity in the fish Channa punctatus (Bloch.). So, it is suggested that more appropriate condition to culture at water fertilizer, ammonium sulphate concentration of less than 16.5 mg/L in fish culture pond for optimum growth performance.

Key words: *Channa punctatus*, ammonium sulphate, nitrogenous fertilizer, haematology, DLC (Differential leucocytes count), PCV (packed cell volume).

INTRODUCTION :

Nitrogenous fertilizers based water pollution is now considered to be a major problem in many countries of the world (Haygarth and Jarvis, 2002). Fertilizers comprised of nitrogenous compounds exist in two principal forms, organic-N and inorganic-N. Ammonium is the main inorganic form of nitrogen has been produced from fertilizer runoff, degradation of nitrogen-containing organic matter, wastewater treatment plant discharges and industrial sources (Clarkson *et al*., 1986; Palanivelu *et al.*, 2005). Ammonium contamination in fish culture pond may lead to decreased growth (Palanichamy *et al*., 1985), behavioural changes in fish (Rani *et al*., 1997) and increased vulnerability to disease (Thurston *et al.*, 1984). Furthermore, sublethal

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ammonium concentrations in water showed chronic effects on the enzyme activities of fish (Hisar *et al*., 2004) and caused histological degeneration in vital organs (Erdogan *et al.*, 2005, Gupta and Kumari, 2021). It exists in two forms, un-ionized ammonia (NH3) and ammonium ion (NH4+). The equilibrium between the two forms of ammonium is controlled primarily by pH and temperature. The un-ionized ammonia is generally more toxic to aquatic organisms than ammonium ion (Wicks *et al*., 2002).

Ammonium sulfate [(NH4)2SO4] is a synthetic chemical nitrogenous fertilizer widely used for crop production for 150 years. It is soluble in water and release ammonia. When ammonia present in higher concentrations in water bodies is toxic to fish and produces several biochemical and physiological alterations at cellular level (Naidu, *et al.* 2017).

The live fish *Channa punctatus* (Bloch) is an important edible fish. It is airbreathing fish lives in muddy stagnant water and can respire aerially by gulping in air at various intervals when oxygen content of water is reduced below saturation point. This fish is found breed in stagnant water body of this region and most important to paddy cum fish culture practices. The ammonia toxicity from nitrogenous fertilizers occurrence became severe during the indiscriminate use of fertilizers in field (Sangeeta and Anand, 2020).

Hence, in present investigation, efforts have been made to analyze the haematological profile changes in *Channa punctatus* induced to sublethal concentration of ammonium sulphate.

MATERIALS AND METHODS :

Experimental Fish: The air-breathing, snakehead fish, *Channa punctatus* average length (8–10 cm) and weight (30–34 g) procured live from the local fish market, Darbhanga were washed with 0.4% $KMnO_4$ solution to avoid infection if any. 70 healthy fish were acclimated for 15 days to laboratory conditions. Fish fed with commercial feed containing 28% crude protein throughout the experiment period @ 3% of body weight per day. Follow the methods of APHA (1985).

Experiment designed: Static acute bioassays were performed to determine LC_{50} values of ammonium sulphate, the mortality was recorded after 24, 48, 72 and 96 h, and were calculated

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by the Finney method (1978). The LC_{50} values for these periods were 318 mg/l, 242 mg/l, 198 mg/l and 165 mg/l respectively. 1/10th value of the LC_{50} value for 96 hr was taken as the sublethal concentration (Sprague, 1971). Twenty acclimated fish were exposed to a sub-lethal concentration (16.5 mg) of ammonium sulphate for 20 days. Side by side same number of fish as that of experimental one was maintained as the control group. At the end of exposure period the fish were anaesthetized with 1:4000 MS 222 (tricane, methane, sulfonate, sandoz) for two minutes. On 30th day blood samples were extracted from the caudal vien of the test fish. Estimation of haemoglobin, RBC, WBC, Lymphocytes, neutrophil, monocytes, basophil, eosinophil and determination of PCV (packed cell volume), MCV, MCH and MCHC levels as method (Akela *et al.* 1996; Shrivastav, 1979). MCV, MCH and MCHC were calculated according to standard formulae (Dacie and Lewis, 1991):

MCV (fI) = [PCV (%) x 10] / [RBC count in millions/mm^3]

MCH (pg) = [HB (g/dl) x 10] / [RBC count in millions/mm^3]

MCHC (g/dl) = [HB (g/dl) x 100] / [PCV (%)]

RESULT AND DISCUSSION

The present study undertaken was the fish, *Channa punctatus* induced to ammonium sulphate (16.5 mg/l) sublethal concentration for 30 days showed alteration in haematological profile i.e. a highly significant ($P<0.001$) decreases (about 36.44%) was observed in haemoglobin (Hb) (5.65±0.10 gm/dl) ammonium sulphate induced fish than control (8.89 ±0.05 gm/dl). The study revealed that RBC count in control fish was 5.35±0.05 x 10^6µl of blood. The result showed decreasing (about 21.49%) a significant value ($P<0.001$) was observed in treated fish 4.20±0.05 x 10^6µl of blood (Table-1, Figure-1).

The values of Neutrophil, Monocytes and Eosinophil were increasing under treatment groups such as 13.10±0.05, 7.10±0.05 and 3.30±0.05 in compare to control value such as 5.45±2.05, 4.0±0.05, 2.2±0.05. Neutrophil was highly significant ($P< 0.001$), while Eosinophils showed significant ($P < 0.01$). The decrease value was exhibited by Basophil 1.44 ±0.02 in treated fish and 1.8 ±0.02 control has non significant ($P < 0.05$) (Table-1, Figure-3).

In DLC (Differential leucocytes count) the values of Lymphocytes was decreases (about 37.65%) under treatment groups 32.50±0.02 from control 52.13±2.40, the Lymphocytes showed

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significant ($P < 0.01$). Similarly PCV (Packed Cell Volume) also decreases (about 61.18%) in treated fish group as 13.55±0.01 compared to control fish group as 34.91±0.06. Its value showed significant ($P < 0.01$) (Table-1, Figure-2)

In the present study ammonium sulphate treated *Channa punctatus* exhibited distinguishable haematological variable. The decreases was observed in haemoglobin (Hb) (5.65±0.10 gm/dl) of ammonium sulphate induced fish in present study was conformity with observation of Raizada and Gupta, (1982) have observed a decrease in haemoglobin when *Trichogaster fasciatus* treated with fungicide RH-216. Similar changes of haematological parameter in *Channa striata* reported by Sasikala *et al*. (2011). Arjun *et al.* (2009) also reported highly significant ($P<0.001$) reduction in the level of haemoglobin in catfish, *Clarias batrachus* induced to chromium. Similar haematological alterations results were observed by earlier workers with various toxicants treated fish; Hb decline was reported by Revathi *et al.* (2003), Shipra *et al.* (2005), Bruska *et al.* (2005), Anwar and Choudhary (2009) and Roy and Nath, (2011).

TABLE-1

Haematological profile changes in ammonium sulphate sublethal conc. 16.5 mg/l for 30 days induced *Channa punctatus*

Variable		Ammonium sulphate
Parameter	**Control**	**16.5 mg/l (30 days)**
Blood Hb (gm/dl)	8.89 ±0.05	5.65±0.10 ***
TEC(RBC) (10^6 cell/mm^3)	5.35±0.05	4.20±0.05 ***
WBC (10^4 cell/mm^3)	4.92±0.05	2.30±0.05 ***
Neutrophil (x 10^3µl)	5.45±2.05	13.10±0.05 ***
Lymphocytes(x 10^3µl)	52.13±2.40	32.50±0.02 **
Monocytes (x 10^3µl)	4.0±0.05	7.10±0.05 *

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Eosinophil (x 10^3µl)	2.2±0.05	3.30±0.05 **
Basophil (x 10^3µl)	1.8 ±0.02	1.45 ±0.02 *
PVC (%values)	34.91±0.06	13.55±0.01 **
MCV(fl/cell)	150.25±0.86	158.50±1.30
MCH(pg)	42.53±1.02	48.10±1.40
MCHC(g/dl)	24.05±1.05	29.70±1.05

Values are mean ± SE of 5 individual observations:-

* *P<0.5 Non Significant,*

** *P<0.01 Significant,*

*** *P<0.001 Highly Significant*

Figure-1

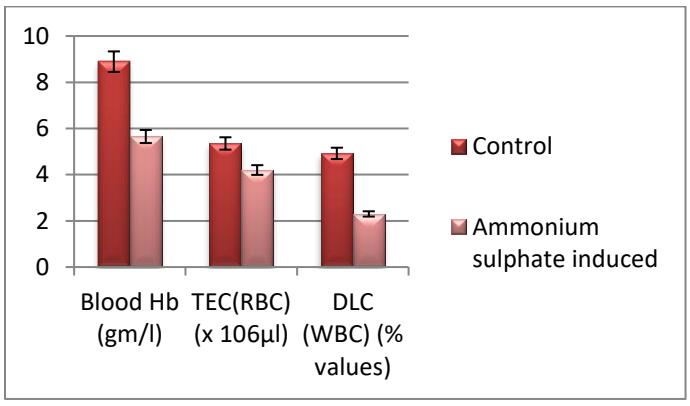

Figure-2

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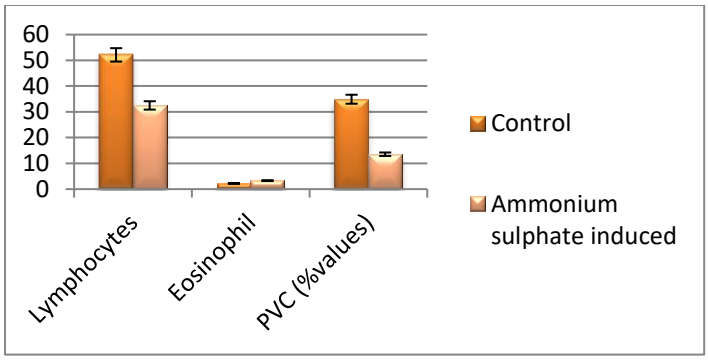
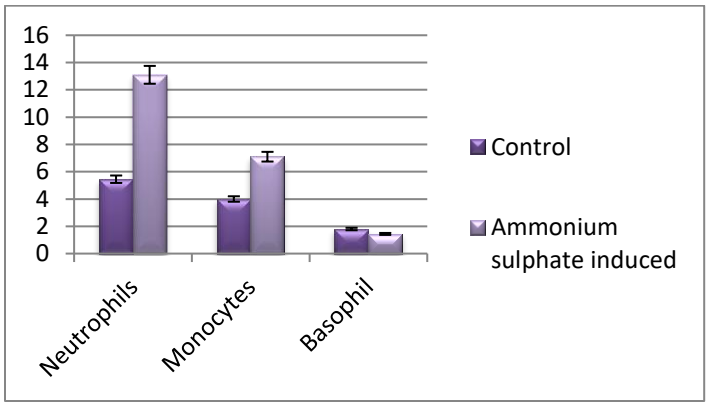


Figure-3



Haematological profiles have been recognized as indicator of stress induced by fertilizers, pesticides, heavy metals and infection of parasites, and variation in RBCs count and haemoglobin concentration was due to deleterious effect of toxicants on the erythropoietic tissue (Verma *et al.* (1982); Pratibha, (2013) Sangeeta *et al.* 2020). *Heteropneustes fossilis* exposed to the pesticide, malathion at the rate conc. 7.6 ppm for 96 hrs resulted in a decrease in RBC count from 6,400,000 to 3,460,000/cm in reported by Mishra and Srivastava, (1983). Similarly Muthalagi (2006) has found a decrease in RBC count under sewage treatment given to the fish *C. mrigala.* The present study showed also conformity with Arjun *et al.* (2009) observed a decline in RBC count under the treatment of chromium to the fish *C. batrachus.* Recently Pratibha, (2013) have observed the *H. fossilis* (Bloch) induced to mercury chloride showed similar decline nature of RBC.

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During present study the WBC decreases are close conformity with various workers, under the treatment of fertilizers, pesticides, alkaloids to fishes or mammals. In fishes Muthalagi (2006) has been reported similar decrease of WBC under domestic sewage to the fish *C. mrigala.* Recently Arjun (2010) has explained similar decrease of WBC under chromium exposure to *Clarias batrachus.* The present findings are conformity with various mammals, such as rat, rabbit etc. under the exposure of metals, pesticides, alkaloids etc.

WBC plays a very important role in the defense mechanism of body. The present findings are conformity with Revathi *et al.* (2003), Shipra *et al.* (2005), Anwar and Choudhary (2009). Pratibha, (2013) have explained exposure of mercury chloride to the fish *H. fossilis.* A decrease in WBC count in exposed fish is termed as leucopaemia. Another observation support the present work, Vasait and Patil (2005) found decreasing lymphocyte count in *Nemacheilus botia* fish induced to organophosphorous insecticide.

Goger and Sawant (1989) reported that differential count (DC) of leukocytes is a reliable haematological index to investigate the environmental contamination by various pollutants. During present study under DLC- Neutrophil, Monocytes and Esnophil increase while Lymphocytes, Basophil decreases in ammonium sulphate treated fish. The increase and decrease values in such parameters are in close conformity with earlier works, Muthalagi (2006), Arjun (2010) under various exposure of sewage, chromium and mercury chloride to the fishes. Gomulka *et al.* (2014) reported a significant reduction in the counts of lymphocytes, neutrophils and monocytes in haematolog profile of European whitefish exposed to propofol. Neutropaemia might be under exposure of ammonium sulphate under 20 days Lymphopaemia in 20 days exposure to urea increased secretion of Adraline (Table:-1).

The present study (Table-1) is conformity with Muthalagi (2006), Arjun (2010) and Pratibha (2013) in experiment with fish under exposure of various toxicants like sewage, chromium cadmium chloride etc. Increase in MCH and MCHC levels of the ammonium sulphate induced fish may be attributed to increased haemolysis of RBCs and a decrease in cellular blood iron which depicts the reduction in the Hb concentration. Revathi, *et al.* (2003) has explained similar decrease in PC, MCV, MCH and MCHC with increase in tannery effluent concentrations. Similar decrease was reported by Arjun under exposure of chromium to *Clarias batrachus*.

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Pratibha, (2013) have explained to same decrease of PCV, MCV, MCH and MCHC under the exposure of Cadmium chloride to the fish *H. fossilis* (Bloch). Olojo and Ladeji, (2012) found an increase in MCV, MCH and MCHC levels of *C. gariepinus* in response to manganese exposure. Sharma and Langer, (2014) found that *Garra gotyla gotyla* exposed to various concentrations of manganese showed an increase in lymphocyte, eosinophils and monocytes with a decrease in neutrophils and basophils.

The haematological profile was assessed on various parameters, such as Hb, RBC, WBC decreases while in DLC, Neutrophil, Monocytes and Eosinophil values increases while Lymphocytes and PCV values decrease. The increase or decrease value analyzed and appeared in three ways as significant, highly significant or non-significant. Such physiological changes causes various diseases in ammonium sulphate induced fishes like Erythropoesis, anaemia, Leucocytopaemia, Neutropaemia, Lymphopaemia, Eosinophilia and Erythropaemia.

CONCLUSION:

It could be concluded that *Channa punctatus* with average weight 30.0± 4.0 g, exposed to fertilizer, ammonium sulphate concentration of < 16.5 mg/l for 30 days showed haematological alterations. It is, therefore, also conclude that the water contaminated with fertilizer ammonium sulphate should be less than 16.5 mg/l for the fish culture. Haematological parameters analysis may be used as a tool for fertilizer toxicity to fish culture monitoring.

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