



Effects of herbicides (Atrazine) on the certain blood parameters of *Clarias batrachus*

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Abstract : The toxic effect of Atrazine, one of the commonly used herbicides, has been examined in the Magur (*Clarius Batrachus*) by recording changes in its hematological parameters. The fishes were treated with Atrazine at the dose of 1mg/L for four weeks. At the end of every week their blood sample were collected for haematological study such as total RBC and WBC count, differential count of WBC and Hb%. Due to the exposure, RBCs count were decreased down constantly to 8%, 15%, 29.5% & 43% during 1st, 2nd, 3rd & 4th week respectively when compared to control. Likewise, Hb%

declines significantly on treatment with ATR by 1%, 15%, 19% & 32% respectively. This decrease in Hb% and RBCs count may be due to alteration in pH of water which ultimately affects the oxygen combining capacity of Hb. Similarly, leucocytes also shows quite significant alteration in their values when compared to normal, but white blood cells count increased from normal in first week by 2% showing immunological response and then gradually decreased down by 11%, 20% and 33% in respective weeks due to acute exposure.

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Introduction :

Atrazine (2-Chloro-4-ethylamine-6-isopropyl-amino-1,3,5-triazine) is a chlorinated S-triazine group of herbicides, one of the commonly used herbicide to control broad leaf weeds in the field of crops, corn, sugarcane, including green vegetables (Cui *et al.*, 2002). It enters the aquatic ecosystem through runoff or directly through careless application in such environment. After entering into the water bodies, it affects the aquatic organisms including fresh water fishes such as

Magur, Catla, Rohu etc.

Atrazine effects are evaluated through haematological test since RBCs, WBCs and haemoglobin percentage are significantly affected.

The present study has been designed to study the adverse effect of Atrazine on certain blood parameters of most commonly used freshwater fish *Clarias batrachus*.

Materials and Methods :

Clarias batrachus were selected as a freshwater fish for the present investigation. Healthy, alive and disease free specimens were collected from the fish farm (Mithapur). 30 fishes were taken and divided into two groups. First group having 10 fishes were taken as control group and other group of 20 fishes were taken as treated group, into two separate aquariums. Initially the fishes were acclimatized at normal temperature (i.e. 28°C) for one week in large sized aquarium to rule out the effects of stress on the haematological parameters. During this acclimatization time, the fishes were fed with oil cakes daily twice in the morning and evening without any treatment. The mortality was 0% during the acclimatization.

The fishes which served as control were exposed to tap water in the aquarium with regular and proper maintenance of air and hygiene. The fishes of treated group were exposed to Atrazine. Stock solution of Atrazine was prepared by adopting the dilution technique. Adequate quantity of distilled water was used to get required concentration. Sub lethal level of Atrazine was determined on the fishes by the prohibit analysis method (Finny, 1978). Aquarium was filled with 20 litres of water and Atrazine were added to it with the ratio of 1mg/Litre. After every week 1mg of Atrazine dissolved in 1litre of water was added, at the same time 1 litre of water was removed from aquarium to maintain constant concentration.

After every weeks 2 fishes from controlled and 4 from treated groups were sacrificed. Blood was drawn from posterior caudal vein and was stored in anticoagulant (ETDA) blood collecting vials for haematological examination.

Whole blood were used to calculate red blood cells (RBCs) count, white blood cells (WBCs) count, haemoglobin percentage (Hb%), and differential count of WBCs.

Haematological Analysis :

RBC and WBC count were calculated with the help of Thoma-Zeiss Haemocytometer. Haemoglobin percentage were estimated by Sahli's Acid Haematin Method with the help of haemoglobinometer. Differential count of WBCs were calculated by making a blood film and staining it with Leishman's stain and identifying under the microscope on the basis of characteristics of each cell. This whole experiment was carried out for four weeks.

Results :

The results showed that Atrazine has a decreasing effect on various hematological parameters i.e RBCs, WBCs and Hb of *Clarias batrachus*. The parameters showed a significant change between treated group and control group, revealing the adverse effect of herbicide. During the acute treatment, RBC count decreased to 8%, 15%, 29.5%, and 43% respectively during 1st, 2nd, 3rd and 4th week when compared to their control group (Graph-I). Whereas elevation in WBC count were observed in 1st week by 2% and then gradually decreased to 11%, 20%, and 33% in 2nd, 3rd, and 4th week respectively (Graph -II).

Haemoglobin concentration also showed a significant decrease of 1%, 15%, 19% and 32% for 1st, 2nd, 3rd and 4th week respectively after exposure to Atrazine (Graph-III).

The results also showed that WBCs count in the treated groups were significantly different when compared to control group. Initially the elevated level of lymphocytes and monocytes were observed which gradually decreased. The neutrophils and eosinophils also decreased with the exposure to Atrazine. Basophils being least abundant granulocyte in the blood of *C.batrachus*, no effects were observed as such.

Blood Parameters	Control	Test A	Test B	Test C	Test D
RBC($\times 10^6/\mu\text{L}$)	2.34	2.15	2	1.65	1.34
WBC($\times 10^3/\mu\text{L}$)	224.1	230.2	200.4	179.3	150.5
Hb%(g/dL)	12.3	12.2	10.4	10	8.4

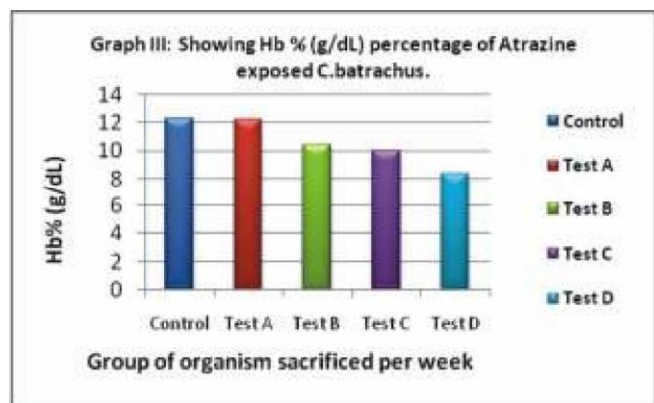
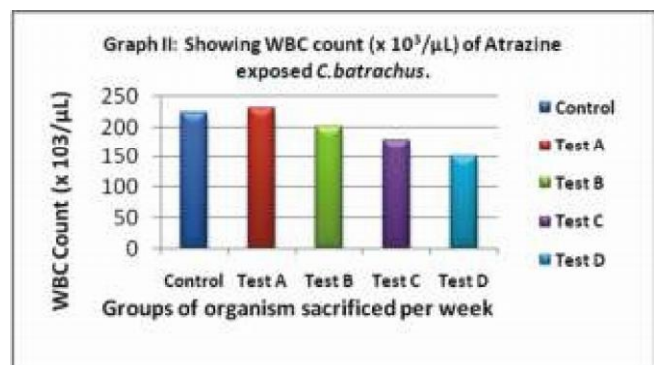
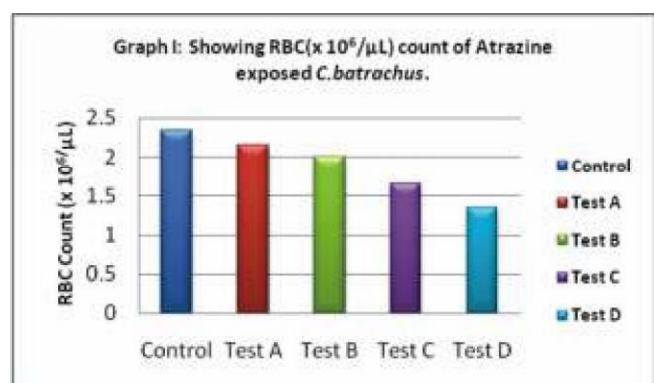
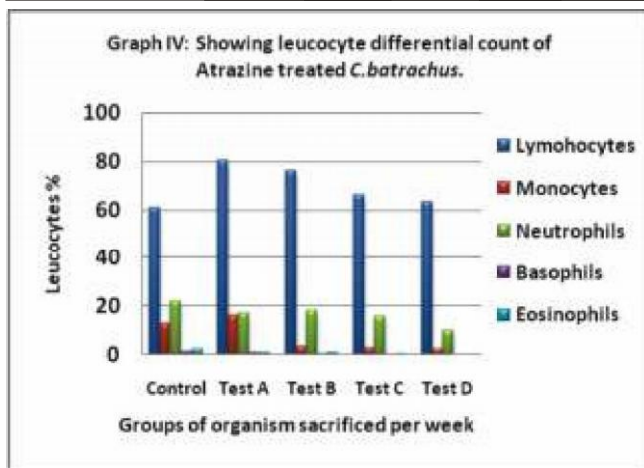


Table II: The mean total and differential white blood cell count in *C.batrachus* exposed to Atrazine.

Leucocyte types (%)	Control	1 st Week	2 nd Week	3 rd Week	4 th week
Agranulocyte(%)					
Lymphocytes	61	80.5	76.5	66.5	63.5
Monocytes	13	16.00	3.5	3.0	2.5
Granulocytes(%)					
Neutrophill	22	17.0	18.0	15.5	10.0
Eosinophill	2.5	1.0	0.5	0.5	—
Basophill	1.5	1.0	0.5	—	—



Discussion :

The result shows that Atrazine has adverse effect on different blood parameters of *Clarias batrachus*. Atrazine is toxic; often bio-accumulative and persistent (Fernado *et al.*1992). Hussein *et al.*,(1996) reported decreased RBCs number and Hb concentration of *Oreochromis nitoticus* and *Chrysichthyes auatus* when exposed to 3 and 6 mg/LATR.

Horn and Hanke (1980) found the decline in number of erythrocytes after exposure of *C.carpio* to 0.1mg/L ATR. Inhibition of erythropoiesis and increase in rate of RBCs destruction in haematopoietic organs is the cause of decrease in RBCs count (Joshi *et al*, 2002). Rehwoldt (1978) found significant decrease of RBCs and Hb in ATR exposed fish species and indicated the toxic effect of Atrazine on spleen, liver and anterior kidney.

M.Ramesh *et al* (2009) found that significant decrease in RBCs and Hb content might have resulted from the lowering of oxygen content of the

water due to presence of Atrazine in treated media. Reduced TEC (total erythrocytes count) or haemolysis is due to haemodilution resulting from impaired osmoregulation across the gill epithelium as reported by Wedemeyer *et al.* (1984) and Svoboda *et al.* (2001). However, the decrease in erythropoietic activity of kidney (Santhakumar *et al.* 1999) may also be the cause of decrease in RBCs count of *C.batrachus* exposed to ATR concentration. In the present investigation it has been observed that in the treated fishes the number of red blood cells and haemoglobin percentage decreased significantly from the normal values.

The decreased haemoglobin was apparently due to decreased RBCs count. The reduction of RBCs is mainly due to development of hypoxic condition during the treatment which in turn leads to increase in destruction of RBC or decrease in rate of formation of RBC due to non availability of Hb content in the cellular medium (Chen *et al.*, 2004). Anaemia characterized by reduced RBC and Hb content have also been reported by several workers after insecticides feeding (Mandal *et al.* 1986; Ali, 1989).

White blood cells (leucocytes) are involved in the regulation of immunological function. In the current study WBC count increased and gradually decreased after chronic exposure to Atrazine as compared to the control. The increase in WBC is perhaps due to typical defensive response of fish against the toxic invasion.

Moreover the oxygen carrying capacity is also reduced due to atrazine exposure. It also affects oxygen consumption. Significant changes were seen in the constituents of the blood and O₂ consumption of fish suggesting the existence of respiratory distress in the fish as a consequence of atrazine toxicity (Prasad *et al.*, 1991).

Conclusion :

From the present study, it is concluded that Atrazine has a profound influence on the blood profiles of treated fishes. A very small amount of Atrazine could result in significant haematological alteration, such as Hb% and RBC were decreased significantly showing RBC destruction. Whereas WBC count increased initially to show immunological response but later it decreases due to acute exposure.

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