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Analysis of haematological manifestation of air breathing fish *Clarias batrachus* (Linn.) induced to deltamethrin

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Abstract
Indiscriminate use of pesticides in agriculture fields caused water pollution in many regions of the world. The aquatic ecosystem has been getting contaminated with pesticides faced with threat of fish biodiversity loss. The present study includes the haematological parameters changes of the fish *Clarias batrachus* (Linn.) exposed to a sublethal concentrations (0.02 ppm) of synthetic pyrethroid, deltamethrin for 20 days. It was found that in treated fish various parameters of haematology, such as Hb, RBC, WBC values decreases while in DLC, Neutrophil, Monocytes and Eosinophil values increase. So, the present study could be helpful to monitering the aquatic pollution and in fish production. It is suggested that more suitable to culture at water pesticides deltamethrin concentration of < 0.02 mg/l for optimum growth performance and survival rate than other water conditions.

Keywords: *Clarias batrachus*, deltamethrin, hematology, pesticides, water pollution

Introduction
Indiscriminate and injudicious use of pesticides in agriculture fields and public health programme to control vectors caused water pollution in many regions of the world. Pesticides reach and persist in aquatic ecosystems through run-off and trophic transfers. On reaching aquatic ecosystems, such chemicals can cause serious harm to aquatic organisms as well as organisms depended on water bodies. The aquatic ecosystem has been getting contaminated now a days with pesticides faced with threat of fish biodiversity loss. The pesticides are designed to kill a very narrow range of undesirable organisms but these have capacity to harm non-target organisms inhabiting treated ecosystems. Deltamethrin is one of the synthetic pyrethroid –II, insecticides widely used in many countries worldwide including India for agricultural, public health and livestock applications such as in the control of mosquitoes, fleas, cockroaches and tsetse flies. It kills insects on contact and through digestion. Due to its powerful property it is preferred, like very broad spectrum control and relatively stable and non-persistent in the environment (Nunoo and Dankwa, 2006) [11].
The study of Haematological profile is today has important role in diagnosis of diseases as well as toxic effects on fishes. There are increasing emphasis of haematology in field of pisciculture and greater awareness of the pollution of natural freshwater resources. According to Summarwar and Verma, (2012) [21] haematological tests are effective and sensitive index to monitor physiological and pathological changes in fishes. Hematological indices like Hb, RBC, PCV and MCHC have regularly been used to monitoring metal pollution in aquatic environment (Kumar and Benerjee, 2016) [8].
Therefore, the present paper contains study of the haematological profile alteration of fish, *Clarias batrachus*, exposed by synthetic pyrethroid, deltamethrin.

Materials and Methods
The walking catfish, *Clarias batrachus*, locally called "Mangur" haing suprabrancheal accessory breathing organ, procured live from the local fish market and brought to lab were washed with 1% $KMnO_4$ solution to avoid external infection if any. Healthy fish of average length (11.0±2 cm) and weight (30.2±4 g) were acclimated for 15 days to laboratory conditions. The fish were fed with feed (crude protein 32%) at the rate 3% body wt. of fish once a day. Running tap water and without airation was used in all the experiments and the fish were adjusted to natural photoperiod and ambient temperature.

To determine LC_{50} values of deltamethrin were performed stataic acute bioassay, following the methods of APHA, AWWA & WPCF (2005) [2]. The LC_{50} values for 24, 48, 72 and 96 hours were resulted as 1.5 ppm, 0.85 ppm, 0.45 ppm and 0.2 ppm of deltamethrin conc. respectively. For the chronic values the sub-lethal concentration of deltamethrin was determined by method of Hart *et al.* (1945) [6] and Finney (1978) [5] for statics analysis. Ten acclimated fish were exposed to a sub-lethal concentration (0.02 ppm) of deltamethrin and side by side ten fish maintained as control for 20 days. At the end of defined exposure period the fish were anaesthetized with 1:4000 MS 222 (Tricane, methane, sulfonate, sandoz) for two minutes. On 20th day blood samples was extracted from the caudal dorsal of the test fish and were then processed for estimation of haemoglobin, RBC, WBC, Lymphocytes, neutrophil, monocytes, basophil, eosinophil and determination of PCV (Packed cell volume) as method (Akela *et al.* 1996; Shrivastav, 1979) [22, 20].

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 (%)]

Results

The present study undertaken was the fish, *Clarias batrachus* induced to deltamethrin (0.02 ppm) sublethal concentration for 20 days showed alteration in haematological profile i.e. a highly significant (p<0.001) decreases was observed in haemoglobin (Hb) (7.79±0.11 gm/dl) ammonium sulphate induced fish than control (12.56 ±0.06 gm/dl),(Table-1). The study revealed that RBC count in control fish was 6.79±0.01 x 10^6µl of blood. The result showed decreasing a significant value (p<0.001) was observed in treated fish 4.68±0.08 x 10^6µl of blood. (Table: –1, Figure:-1), values of Neutrophil, Monocytes, Eosinophil is increasing under treatment groups such as 16.1±0.04, 8±0.03 and 1.4±0.02 in compare to control value such as 7.74±2.01, 5±0.05, 1.1±0.03. Neutrophils are highly significant (p<0.001), while Eosinophils showed significant (p<0.01) and Basophil showed non-significant (p<0.05). In DLC (Differential leucocytes count) the values of Lymphocytes and Basophil are decreases under treatment groups. In control to values are 66.43±242 and 1.1±0.02 while under treatment the values decreases as 48±0.02 and 1.3±0.02. The Lymphocytes showed significant (p<0.01) while Basophil showed non-significant (p<0.05). Similarly PCV (Packed Cell Volume) also decreases under treatment group as 12.01±0.03 compared to control group as 35.95±0.06. It showed significant (p<0.01) (Table:-1, Figure:-2).

At haematological levels various parameters, such as Hb, RBC, WBC decreases while in DLC, Neutrophil, Monocytes and Eosinophil values increases while Lymphocytes and PC value decreases. The increase or decrease value showed either significant, highly significant or non-significant. It causes various diseases Erythropoesis, anaemia, Leucocytopaemia, Neutropaemia, Lymphopaemia, Eosinophilia and Erythropaemia.

Table 1: Showing the effects of Deltamethrin on Hb, RBC, WBC, DLC, PCV of *Clarias batrachus.*

Variable		Deltamethrin (96 hrs) exposure 0.02 mg/l
Parameter	**Control**	
Blood Hb (gm/l)	12.56 ±0.06	7.49±0.12 ***
TEC(RBC) (x 10^6 cell/mm^3)	6.79±0.01	4.68±0.08 ***
DLC (WBC) (% values)	4.92±0.05	2.30±0.05 ***
Neutrophil (x 10^3µl)	7.74±2.04	16.2±0.04 ***
Lymphocytes (x 10^3µl)	66.43±2.42	48.0±0.02 **
Monocytes (x 10^3µl)	5.0±0.03	8.0±0.03 *
Eosinophil (x 10^3µl)	2.0±0.03	3.0±0.02 **
Basophil (x 10^3µl)	1.1 ±0.02	1.2 ±0.02 *
PC (%values)	35.95±0.06	12.02±0.03 **
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

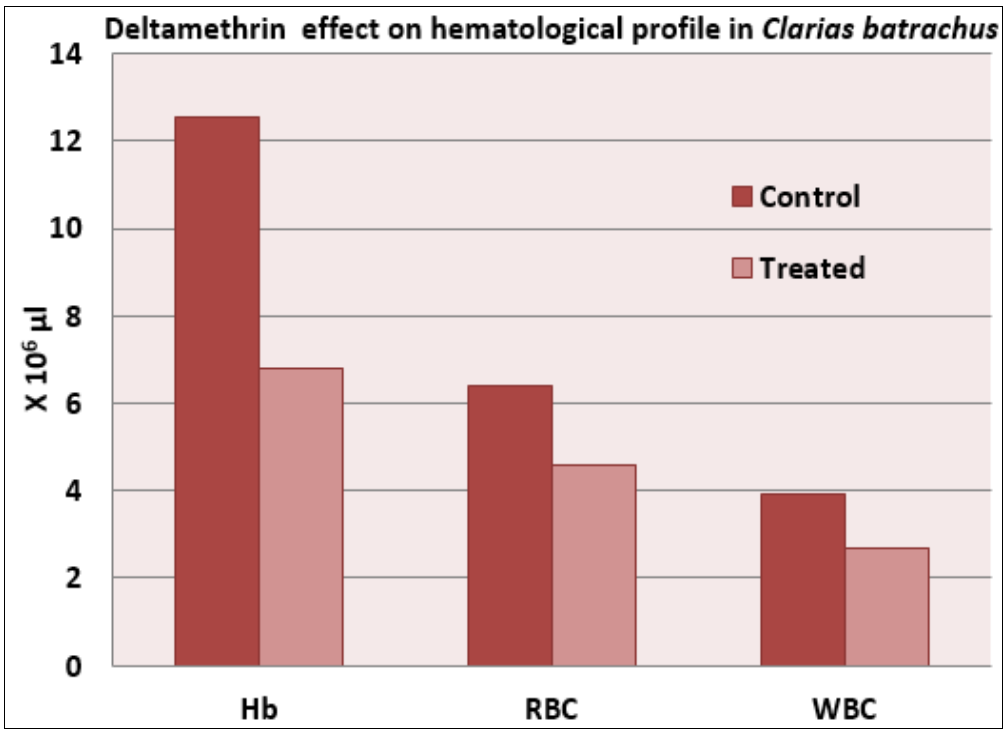

Fig 1: Showing the effect of Deltamethrin on Hb, RBC, WBC in *Clarias batrachus* (96 hrs) ***p<0.001.

Parameters

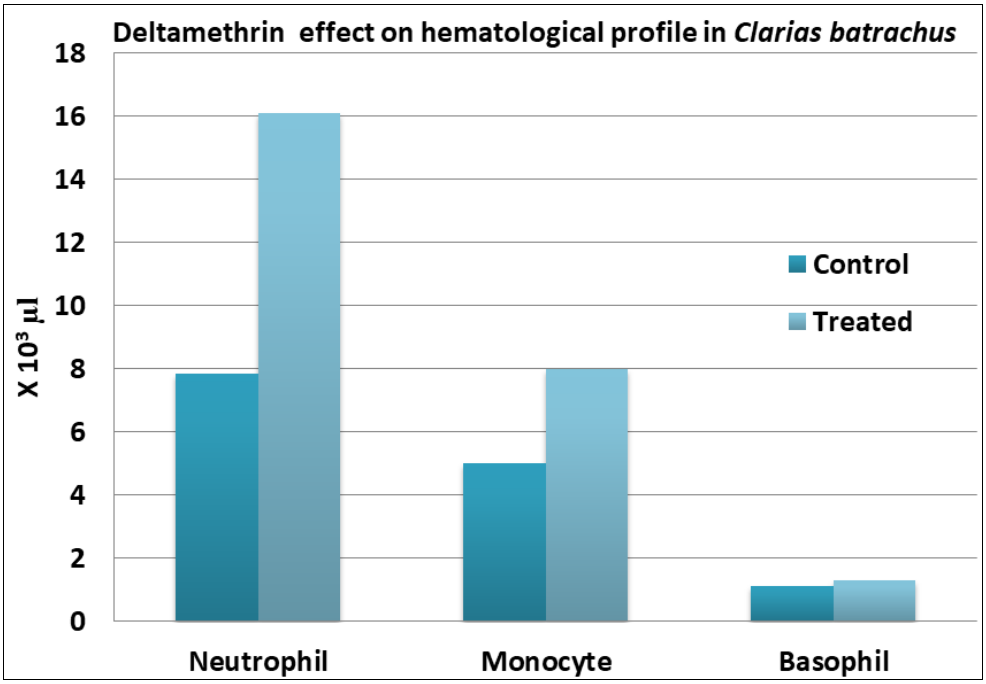

Fig 2: Showing the effect of Deltamethrin on Neutrophil, Monocytes, Basophil in *Clarias batrachus* (96 hrs) *p<0.05, *** p<0.001.

Discussion

In the present study deltamethrin treated *C. batrachus* exhibited distinguishable haematological variable which are discussed here.

Haemoglobin: The present findings were in conformity of Raizada and Gupta, (1982) [15] has explained a decrease in RBC number and haemoglobin was observed in the fish *Trichogaster fasciata* after exposure to the fungicide RH-216 and Kumar and Baberjee (2016) [8] used arsenic on *C. batrachus*. Muthalagi (2006) [10] has explained different circulation of sewage of the Hb to the fish *C. mrigala.* Again Arjun *et al.* (2009) [3] has explained the Hb treatment under chromium exposure to the fish *C. batrachus* and found similar decrease in the level of Hb and Hb treatment under chromium showed highly significant (p<0.001). Similar results of Hb decline has been also reported by Rewathi *et al.* (2003) [16], Shipra *et al.* (2005) [19], Anwar and Choudhary (2009) [1] has been reported in rat also. Again Pathak *et al.* (2013; 2020) [13, 14] has explained Hb treatment under mercury chloride and showed similar decrease level results in Hb and also showed Hb is highly significant (p<0.001).

RBC: The present study showed also conformity with *Heteropneustes fossilis*, the pesticide malathion resulted in a decrease in RBC count from 6,400,000 to 3,460,000/cm* in 96 hr at 7.6 ppm (Mishra and Srivastava, 1983) [9]. In fishes, Muthalagi (2006) [10] has found similar nature of decrease under sewage treatment to the fish *C. mrigala.* Again Arjun *et al.* (2009) [3] has found similar nature of decline in RBC under the treatment of chromium to the fish *C. batrachus.* Pathak *et al.* (2013) [14] has found similar decline nature of RBC under the treatment of mercury chloride to the fish *H. fossilis* (Bloch) and recently, Kumar and Benerjee (2016) [8] explained to same decrease under the exposure to arsenic to *C. batrachus*. The present findings, i.e. decrease in RBC level was close conformity with fish and mammals studies.

WBC: 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) [10] has been reported similar decrease of WBC under domestic sewage to the fish *C. mrigala.* Recently Arjun (2010) [4] 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.

Initial increase in the WBC count was might be the result of direct stimulation of immunological defence due to the presence of toxic substance or may be associated with induced tissue damage. The present findings are conformity with Rewathi *et al.* (2003) [16], Shipra *et al.* (2005) [19], Anwar and Choudhary (2009) [1]. Pathak *et al.* (2013) [14] has explained exposure of mercury chloride to the fish *H. fossilis.* These workers has been found same decrease in WBC in Rat under various exposure of sewage, alkaloids etc. On the basis of above facts it is quite clear that WBC plays a very important role in the defense mechanism of body. A decrease in WBC count is termed as leucopaemia. The common cause of this includes blood poisoning in which the body literally runs out of WBC.

DLC: During present study under DLC Neutrophil, Monocytes and Esnophil increase while Lymphocytes, Basophil decreases. The increase and decrease values are in close conformity with Muthalagi (2006) [10], Arjun (2010) [4] and under various exposure of sewage, chromium and mercury chloride to the fishes. While Rewathi *et al.* (2003) [16], Shah *et al.* (2004) [23]; Shipra *et al.* (2005) [19], Anwar & Choudhary (2009) [1] has found same levels of decrease or increase in Rat. (Table:-1, Fig.-2).

The present study (Table-1) is conformity with Muthalagi (2006) [10], Arjun (2010) [4] in fish water fishes under exposure of sewage, chromium as well as cadmium chloride. 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. Rewathi *et al.* (2003) [16] has explained similar decrease in PCV, MCV, MCH and MCHC with increase in tannery effluent concentrations. Similar result was obtained by Arjun under exposure of chromium to *Clarias batrachus*. Pathak and Kumar (2013; 2020) [13, 14] 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) [12] found an increase in MCV, MCH and MCHC levels of *C. gariepinus* in exposure to manganese and Khan *et al.* (2012) [7] also found similar result in pyrethroid pesticide exposed to fish. Sharma and Langer (2014) [18] reported an increase in lymphocyte, eosinophils and monocytes with a decrease in neutrophils and basophils concentration in Garra gotyla gotyla exposed to various concentrations of manganese. Recently, Kumar and Benerjee (2016) [8] explained to same pattern found under the exposure to arsenic to *C. batrachus*. In the present study also same observation found.

Conclusion

The reduction in RBC count and Hb are often accompanied by a decrease in PVC and demonstrates the physiological dysfunction of the haemopoietic system. It could be concluded that *Clarias batrachus* with average weight 30.0± 4.0 g, were more suitable to culture at water pesticide, Deltamethrin concentration of < 0.02 mg/l for optimum growth performance and survival rate than other water conditions.

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