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Effects of Deltamethrin on Behaviour Response and Haematology Profile of Air Breathing Fish *Clarias batrachus* (Linn.)

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A B S T R A C T

The current investigation was undertaken to study the haematological profile alterations induced by chronic (30 days) exposure of the fish *Clarias batrachus* (Linn.) to a sublethal concentrations (0.015 ppm) of synthetic pyrethroid, deltamethrin. At haematological levels various parameters, such as Hb, RBC, WBC decreases while in DLC, Neutrophil, Monocytes and Eosinophil values increase. So, it is suggested that more suitable to culture at water pesticides deltamethrin concentration of < 0.015 mg/l for optimum growth performance and survival rate than other water conditions.

Introduction

The environment is contaminated with different kinds of pollutants. Pesticides are one of such anthropogenic pollutants which play an important role in controlling different types of pests that cause damage to the crops and to improve agricultural production. Insecticides, fungicides and herbicides constitute the major source of potential environmental hazards not only to birds, fish, and other animals but also to humans when they become a part of food chains (Khan *et al.,* 2012). Indiscriminate use of pesticides on crops causes serious environmental hazards affecting aquatic fauna. Unfortunately, most of the pesticides are not biodegradable and tend to persist for years together in the environment. Haematological studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture and greater awareness of the pollution of natural freshwater resources in the tropics. Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Summarwar and Verma, 2012). The fish, *Clarias batrachus*, locally known as "Mangur", having the presence of suprabranchial accessory respiratory organs, an air-breathing teleost and deltamethrin were selected for present study. Deltamethrin belongs to a group of pesticides called synthetic pyrethroids. Deltamethrin is known to be toxic to fish and various other aquatic organisms (Mittal, 1994).

Materials and Methods

The air-breathing teleost *Clarias batrachus* procured live from the local fish market were washed with 0.1% $KMnO_4$ solution to remove dermal infection if any. Healthy fish of average length (10–12cm) and weight (21–25 g) were acclimated for 15 days to laboratory conditions. The fish were fed with chopped goat liver every day adlibitum. Running tap water was used in all the experiments and the fish were adjusted to natural photoperiod and ambient temperature. No aeration was done.

Static acute bioassays were performed to determine LC_{50} values of deltamethrin for 24, 48, 72 and 96 hours following the methods of APHA, AWWA & WPCF (1985). The LC_{50} values for these periods were 1.5 ppm, 0.85 ppm, 0.45 ppm and 0.15 ppm respectively. The sub-lethal concentration was determined following the formula of Hart *et al.,* (1945). Twenty acclimated fish were exposed to a sub-lethal concentration (0.015 ppm) of deltamethrin for 30 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 30^{th} 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).

Results and Discussion

Fish Behavioral Response

The control fish shows a tendency to remain at the bottom of the aquarium with little disturbance. However, mortalities were removed immediately, and behavioural abnormalities were assessed at these regular intervals using a modified behavioural protocol checklist (Klesius *et al.,* 2000). Scores were assigned daily to individual fish in the experiment and were based on the following scoring system: 0, no observed changes in behaviour; 1, swimming abnormally, lethargic or unresponsive, changes in skin coloration; 2, hyperactive or excitable, rapid operculation; 3, death. Mean behaviour scores were calculated per replicate treatment.

Just after introduction to test solution fishes showed increased swimming, surfacing and hyperactivity. Restlessness, rapid surfacing, peeling of skin and colour fading were prominent after 24 hr exposure. After 48 hr exposure the fishes showed slightly reduced activity and gradual increase in colour fading. Gill adhesion and a thin film of mucous were noticed on gills, operculum and general body surface at this stage. After 72 hr exposure increased surfacing and gulping of air was observed. At this stage fishes showed loss of balance and jerky movements during swimming. The school formation, a characteristic of this fish, was found weakened in test animals as compared to controls at this stage. After 96 hr ulceration on trunk, base of caudal and pectoral fins were prominent in 95% of the animals. A thick film of mucous on whole body and gills was observed in almost all test fishes. Test fishes lost their natural colouration and. Loss of equilibrium before death is a symptom shown all the test fish. Similar steps of behavioral response also found in sublethal dose of deltamethrin i.e. 0.015 ppm for 30 days of duration of experiment.

Haematological studies

From Table: –1.) it is quite clear that Hb in control value is 12.56 ±0.06 gm/dl which is decreases under Deltamethrin as 7.79 ±0.11

gm/dl and showed highly significant (P<0.001). Similarly the value of RBC decreases under Deltamethrin at 4.68 ± 0.08 in contrast to control value 6.79± 0.01. the decreased value under treatment showed highly significant (P < 0.001) (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, Leuco-cytopaemia, Neutropaemia, Lymphopaemia, Eosinophilia and Erythropaemia.

In the present study, certain deformities and unusual swimming patterns were found in fish exposed to 0.015 mg/L and above concentrations of deltamethrin. The responses recorded for the fish in this study are similar to those reported by other authors under various stress conditions (Paul and Banerjee, 1996; Rani *et al.,* 1997; Palanivelu *et al.,* 2005; Lata *et al.,* 2008). Behavioural responses of fish to most toxicants are the most sensitive indicators of potential toxic effects (EIFAC, 1983). Acute toxic effect mercuric chloride was observed on zebrafish by Vutukuru, Basani K. (2013); Pathak P. & Anand, (2020). The toxic effects of ammonium chloride fertilizer on fish *C. batrachus*, Sangeeta *et al.,* (2020). The toxic effects of surfactant, dodecyl dimethyl benzyl ammonium chloride (1227) on larval locomotors of zebra fish was observed by Yanan, *et al.,* (2015). It is, therefore, conclude that the toxicity of the pesticide deltamethrin depend upon a number of physical, chemical and biological factors. Each of which may be used as a tool for pesticides toxicity to fish.

Haematological study

Haemoglobin

The present findings were in conformity of Raizada and Gupta, (1982) has explained a decrease in RBC number and haemoglobin was observed in the fish *Trichogaster fasciatus* after exposure to the fungicide RH-216. In *C. batrachus*, haemoglobin content decreased from 15.34 to 10.30 g% in 96 hr with 10 ppm of Rogor. Muthalagi (2006) has explained different circulation of sewage of the Hb to the fish *C. mirgala.*

Again Arjun *et al.,* (2009) 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 Revathi *et al.,* (2003), Shipra *et al.,* (2005), Anwar and Choudhary (2009) has been reported in rat also. Again Pratibha and Kumar (2013) 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). In fishes, Muthalagi (2006) has found similar nature of decrease under sewage treatment to the fish *C. mrigala.* Again Arjun *et al.,* (2009) has found similar nature of decline in RBC under the treatment of chromium to the fish *C. batrachus.* Very recently Pratibha and Kumar (2013) has found similar decline nature of RBC under the treatment of mercury chloride to the fish *H. fossilis* (Bloch). The present findings, i.e. decrease in RBC level was close conformity with fish and mammals studies.

On the basis of above facts that the chemicals disrupts in form of synthesis machinery because of Glycolysis inhibition. Further it has been explained that toxicants could destiny the blood cells leading to significant decrease. Shipra *et al.*, (2005) clearly explained the decline of RBC, which reduces Hb. Very recently Muthulagi (2006) showed similar significant reduction under domestic sewage while Pratibha and Kumar (2013) has showed similar decline in RBC under the treatment of mercury chloride to the fish *H. fossilis* (Bloch).

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) 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.

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

Variable		
Parameter	**Control**	**Deltamethrin (96 hrs) exposure 0.015 mg/l**
Blood Hb (gm/l)	12.56 ±0.06	7.49±0.12 ***
TEC(RBC) (x 10^6µl)	6.79±0.01	4.68±0.08 ***
DLC (WBC) (% values)		
Neutrophil	7.74±2.04	16.2±0.04 ***
Lymphocytes	66.43±2.42	48.0±0.02 **
Monocytes	5.0±0.03	8.0±0.03 *
Eosinophil	2.0±0.03	3.0±0.02 **
Basophil	1.1 ±0.02	1.2 ±0.02 *
PC (%values)	35.95±0.06	12.02±0.03 **

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.

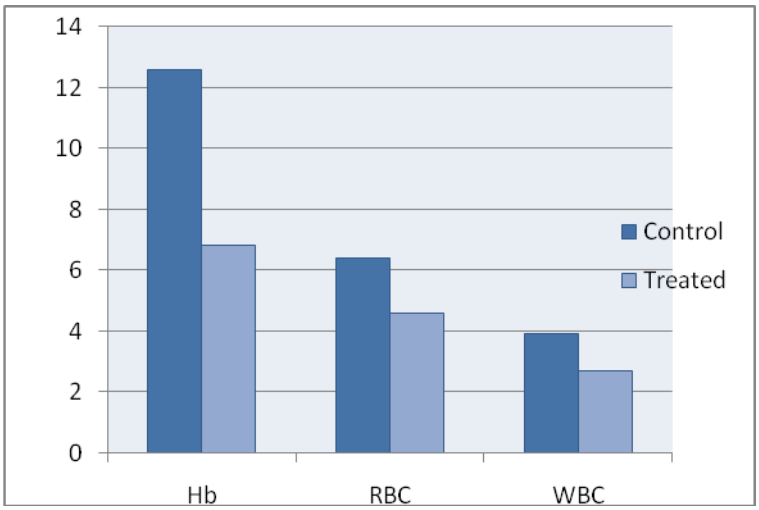


Fig.2 Showing the effect of Deltamethrin on Neutrophil, Monocytes, Basophil in *Clarias batrachus* (96 hrs) *P<0.05, *** P<0.001.

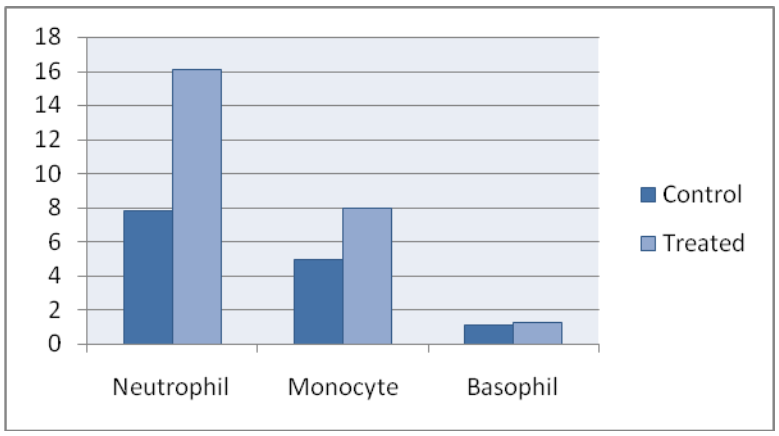
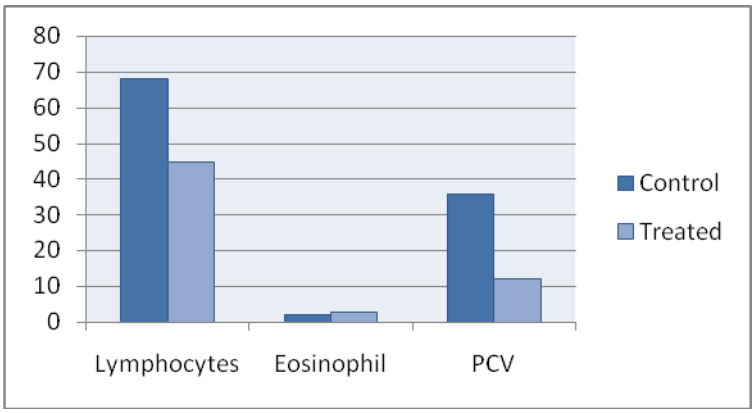


Fig.3 Showing the effect of Deltamethrin on Lymphocytes, Eosinophil, PCV, in *Clarias batrachus* (96 hrs) ** P<0.01.



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 Revathi *et al.,* (2003), Shipra *et al.,* (2005), Anwar and Choudhary (2009). Pratibha and Kumar (2013) 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), Arjun (2010) and Pratibha and Kumar (2011) under various exposure of sewage, chromium and mercury chloride to the fishes.

While Revathi *et al.,* (2003), Shipra *et al.*,(2005), Anwar & Choudhary (2009) has found same levels of decrease or increase in Rat.CCl_4 (injected at 0.03 and 0.06 ml/100 g body weight at intervals of 3 days) caused considerable lymphocytosis; i.e., within 6 days, lymphocytes increased from 33 to 72% (Sharma and Gupta, 1982).

Neutropaemia might be under exposure of Di-ammonium phosphate under 20 days Lymphopaemia in 20 days exposure to Di-ammonium phosphate increased secretion of Adraline (Table:-2, Fig.-2 & 3).

PVC

The present study (Table-1, Fig.-3) is conformity with Muthalagi (2006), Arjun (2010) and Pratibha (2013) in fish water fishes under exposure of sewage, chromium as well as cadmium chloride. Similarly Revathi *et al.,* (2003) has explained similar decrease in PC, MCV, MCH and MCHC with increase in tannery effluent concentrations. Similar results was obtained by Arjun under exposure of chromium to *Clarias batrachus*. Pratibha and Kumar (2013) has explained to same decrease of PCV, MCV, MCH and MCHC under the exposure of Cadmium chloride to the fish *H. fossilis* (Bloch).

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 < 0.015 mg/l for optimum growth performance and survival rate than other water conditions.

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