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Research Article

EFFECTS OF DELTAMETHRIN ON HAEMATOLOGICAL INDICES OF INDIAN MAJOR CARP, *CIRRHINUS MRIGALA* (HAMILTON)

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ABSTRACT

Deltamethrin is a pyrethroid ester insecticide widely used in agricultural and non agricultural purposes in India, creates a serious threat to the environment as well as target and non target organisms. The present investigation was undertaken to study the effect of lethal (8mg/L) and sublethal (0.8mg/L) deltamethrin level on selected haematological indices of *Cirrhinus mrigala*. The target fish were exposed to lethal (1, 2, 3 and 4 days) and sublethal (1st, 5th, 10th and 15th day) periods. The results showed that the erythrocytes, Hb, and hematocrit values decreased continuously for both lethal and sublethal concentration of deltamethrin. WBC, MCV and MCH were increased considerably, while the MCHC remained unchanged during the study. In conclusion, the study confirmed the lethal and sublethal effect of deltamethrin on *C. mrigala*. The decrease in levels of RBCs, Hb, PCV and increase in WBCs revealed the haematological toxicity of this pesticide pollutant and they may be helpful as a tool to monitor the health status of the fish species.

Keywords: Deltamethrin, *Cirrhinus mrigala*, Haematological indices, Pesticides, Lethal Concentration, Sublethal concentration.

INTRODUCTION

The environment is plagued with different kinds of pollutants. Pesticides are one of such pollutants which play an important role in controlling different types of pests that cause damage to the crop plants 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 (Abd-Alla *et al*., 2002). Long term exposure to these pollutants causes countless abnormalities and reduces the life span of organisms (Hussain *et al*., 2011; Naz *et al*., 2011; Khan *et al*., 2012). Indiscriminate use of pesticides on crops causes serious environmental hazards affecting aquatic and land dwelling animals. Unfortunately, most of the pesticides are not biodegradable and tend to persist for years together in the environment.

There are many reports available related to the toxicity of pesticides on different fish species (Farombi *et al*., 2008). Fishes can serve as bioindicators of environmental pollution and therefore can be used for the assessment of the quality of aquatic environment. Fishes are directly exposed to chemicals, resulting from agricultural production via surface runoff of water (Ateeq *et al*., 2002). One of the difficulties in assessing the state of health of natural fish population has been the paucity of reliable references of the normal condition. In pursuant to this, many fish physiologists have focussed on to study the haematology, because it has proved a valuable diagnostic tool in evaluating the fish health. 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).

Deltamethrin is one of the most important and widely used pyrethroid insecticide, since the application of pyrethroid as insecticide and antiparasitary preparations has been accepted on

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a large scale for agricultural purposes and very markedly increased during last 10–15 years. If these pesticides are used in excessive dosage, then these become a part of food chain, triggering a series of haematological changes (Atamanalp and Yanik, 2003; Ahmad *et al*., 2009; Hussain *et al*., 2011). In third world nations, annually more than 25-77 million poisoning cases (Zhang *et al*., 2011) and 0.22 million casualties (Yashmashito *et al*., 1997) have been reported due to contamination (Ahmad *et al*., 2009).

Contamination of aquatic ecosystem by pesticides has gained increasing attention in recent decades. The acute and chronic exposure of these chemicals can result in tissue burdens that produce adverse effects not only in the exposed organisms, but also organisms including human beings; therefore, it seems essential to study detrimental effects of pollutants to formulate the strategies for safe guarding aquatic organisms (Ali and Rani, 2009).

Blood is a fluid connective tissue circulating in the body; it provides one of the methods of communication between the cells of different parts of the body. Haematological studies play an important role in understanding variation of blood characteristics in relation to factors like phylogenetic position, ecological habitat, pollutants, food selection, etc. The regular monitoring of the fish blood is a diagnostic tool in establishing the health status of the fish farm. It helps evaluating the response of different types of blood cells and its components in the condition of physiological stress due to toxicity, as it is quickly reflect the poor condition of fish than other commonly measured parameters. Accordingly, haematology can be used as clinical tool for the investigations of physiological and metabolic alteration in fish caused by pollution of the aquatic environment (Kumar, 1994). The present study was designed to assess the effect of deltamethrin on haematological parameters of fish *C. mrigala,* on exposure to lethal and sublethal concentrations.

MATERIALS AND METHODS

Experimental design

Healthy and active fresh water fish *C. mrigala* (20-30 g; 15-20 cm) were procured from Karnataka State Fisheries Department, Dharwad, Karnataka, India. The fishes were treated with potassium permanganate solution (0.5% w/v) for 2 min to remove dermal adherent. All fishes were acclimatized to laboratory condition for fifteen days. Effects of lethal and sub-lethal concentrations of deltamethrin were studied at different periods of exposure in order to understand influence of time over toxicity. In the lethal concentration (8 mg/l) 1, 2, 3 and 4 days and in the sub-lethal (0.8 mg/l) concentration 1st, 5th, 10th and 15th day were chosen to observe the short term and long term effects of deltamethrin on the fish, *C. mrigala*.

Haematology

Collection of blood: Blood sample was collected by the method of Steuke and Schoettgr (1970), by severing the caudal peduncle. Before considering the blood for haematological observation, a drop was placed on a cleaned slide and examined for the presence of parasites if any. Blood free from any infection was used to study the haematological parameters to avoid variation. The samples were taken at a particular time during early hours of the day. Ethylene diamine tetra acetic acid (EDTA) was used as an anticoagulant.

Determination of red blood corpuscles (RBC) count: RBC count was determined with a Neubauer crystalline counting chamber as described by Davidson and Henry (1969). The blood was sucked up to 0.5 marks on the RBC pipette and immediately RBC diluting fluid (Hayem's fluid) was drawn up to 101 mark and the pipette was rotated between the thumb and the forefinger to facilitate adequate mixing of the solution. A cover glass was placed in position over the ruled area. A drop of blood was placed holding the pipette at an angle 45° on the central platform near the edge of the cover slip. It was allowed to settle for 2 to 3 min. The ruled area was focussed under the microscope and the number of RBC's was counted in 80 small squares. The cells touching the upper and left hand lines were counted. The cells touching the lower and right hand lines were omitted.

The number of RBC's per sq mm was calculated as follows:

Area of a small square	: 1/400 sq mm
Depth of the counting chamber	: 1/10 mm
The volume of Small Square is	: 1/4000 cu mm
The dilution of the blood is	: 1/200

$$\text{Total RBC} = \frac{N \times 4000 \times 200}{80}$$

N = No of cells in 80 small squares.

Determination of white blood corpuscles (WBC) count: WBC count was done as per the procedure described by Donald Hunter and Bonford (1963). The blood was drawn up to 0.5 mark of WBC pipette and the diluting fluid was drawn up to the 101 mark above the bulb. The solution was allowed to mix and stand for 2 to 3 minutes. A drop of blood was placed from the tip of the pipette under the cover slip by holding the pipette at an angle of 40° and allowed to stand for 2 to 3 min. WBC was counted in the four corner square millimetres and the number of WBC per cubic millimetre was calculated.

Estimation of haemoglobin (Hb): Hb content in the blood was estimated by cyanmethaemoglobin method as described by Dacie and Lewis (1975). Haemoglobin is converted in to cyanmethaemoglobin by the addition of potassium ferricyanide (KCN). The colour of cyanmethaemoglobin is read in a photoelectric colourimeter at 540 nm against a standard solution.

Determination of packed cell volume (PCV) or haematocrit value: PCV was determined by micro haematocrit method of Schalm *et al.*, (1975). The heparinised blood was filled up to the mark 100 of the haematocrit tube with the help of pasteur pipette and centrifuged at 3000 rpm for 30 minutes. The relative volume of the height of the RBC's packed at the bottom of the haematocrit tube was recorded as (PCV) in terms of percentage of total blood column taken in the haematocrit tube.

Determination of mean corpuscular volume (MCV): MCV indicates the average size of the RBC in a given sample of blood. MCV was calculated by the following formula and represented in cubic microns.

$$\text{MCV} = \frac{\text{PCV} \times 10}{\text{RBC count}}$$

Determination of mean corpuscular haemoglobin (MCH): MCH represents the average weight of the haemoglobin contained in each RBC in a given volume of the blood. MCH is influenced by the size of the cell and concentration of the haemoglobin. MCH was calculated by the following formula and expressed in picograms (pg).

$$\text{MCH} = \frac{\text{Haemoglobin (g/dL)} \times 10}{\text{RBC count}}$$

Mean corpuscular haemoglobin concentration (MCHC): MCHC reflects the average concentration of the haemoglobin in the RBC in a given volume of the blood. MCHC was obtained by the following formula and expressed in terms of percentage.

$$\text{MCHC} = \frac{\text{Haemoglobin (g/dL)} \times 100}{\text{PCV}}$$

Statistical Analysis

Data obtained from replicates were used to calculate mean values. The difference in mortality values was analyzed by chi-square test. The values of haematological parameters were presented as mean ± standard deviation. Data for haematological parameters were tested for normality and then analyzed by one-way analysis of variance (ANOVA) to test the significant differences among different haematological parameters. All statistical analyses were carried out using SPSS 10.1 and $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The results showed in table 1 revealed that deltamethrin is highly toxic to the Indian major carp *C. mrigala*. Toxicity of deltamethrin on carp increased with increasing their concentration. Pesticides in the aquatic environment can negatively affect the ecosystem. Although the aquatic environment is not the target of such pesticides, but the widespread use of them has led to some serious problem in aquatic biota. Moreover, the effects of pollutants on cellular distributions in fish hematopoietic tissues results in decreased lymphocyte, haemoblast counts and increased granulocyte counts (Peters and Schwarzer, 1985). Haematology is an indicator of immunological status and can provide definitive diagnoses (Duncan *et al.*, 1994; Campbell and Ellis, 2007). Haematological indices are of different sensitivity to various environmental factors and chemicals (Sanchez-Fortun and Barahona, 2005). Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of haematological parameters of aquatic organisms. Thus, water quality is one of the major factors, responsible for individual variations in fish haematology, since they are sensitive to trivial fluctuation that may occur within their internal milieu (Fernades and Mazon, 2003). High concentration of pesticides or long term exposure to sub lethal concentration usually decreases erythrocyte indices (Masoud Saeedi *et al*., 2012). A haematological index plays a vital role in the study of fish health, pollution load, stress and disease.

Haematological indices are of different sensitivity to various environmental factors and chemicals. Haematology and clinical chemistry analysis, although not often used in fish medicine, can provide substantial diagnostic information. On the basis of haematological studies, it would be possible to predict the physiological state of fish in natural water bodies. Haematology studies in teleosts have indicated that haematocrit values might be useful as a general indicator of fish health, since fish given iron deficient diets, or those exhibiting anaemia; all possess reduced haematocrit (PCV) values. Erythrocytes reflect the state of the organism over a prolonged period of time. High concentration of pesticides or long term exposure of fish to sublethal concentration usually decreases erythrocyte indices (Adedeji *et al*. 2009). The study of blood characteristics may corroborate important subsidies of diagnoses and prognoses of morbid conditions in fish populations (Tavares *et al.,* 1999) and therefore, contribute to better comprehending comparative physiology, feeding conditions and other ecological parameters.

Results of the present study shows the haematological profile such as RBC, Hb, WBC count of exposed fish decreased significantly after acute exposure to deltamethrin ($p < 0.05$). On the other hand, the MCV and MCH values of exposed fish showed a significant increase ($p < 0.05$), while the MCHC values of both groups were not significantly different from each other during the study (Table 1). In the present study, fish samples treated with lethal and sub-lethal concentration of deltamethrin, showed considerable alteration in the level of different blood parameters. Decrease in total erythrocyte count, haemoglobin percentage, PCV values indicates the occurrence of anaemia associated with erythropenia. This is in coordination with various workers (Sen *et al*, 1993; Olufayo, 2009; Jawale and Dama, 2010). Decrease in RBC count, Hb percentage and haematocrit value reflects to the anaemic state of fish. Studies shows similar kind of results when fishes treated with heavy metals (Christensen el al, 1972; Garg *et al*, 1989).

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. Decrease in WBC count was seen in the later days of exposure might be due to the series of changes in immunological set up of the fish under pesticide stress (Kumar, 1994). Kalavathy *et al*. (2001) suggested that decrease in WBC count could be the result of autolysis caused due to haemolytic enzymes leaked out by the cells under toxicant stress. It is well known that the changes in leucocytes counts after exposure to pollutants may be associated to a decrease in nonspecific immunity of the fish. Leucocytes are involved in the regulation of immunological function in many organisms and the increase in WBC in stressed animals indicates a protective response to stress (Witeska 2004). In the present investigation the significant increase in WBC during sublethal treatment might have resulted from stimulation of immune system by deltamethrin and to protect the fish against toxicity. PCV values of the fish in the present study showed decreased at both lethal and sublethal concentration. Drop in PCV could be attributed to low RBC count or haemodilution. MCV values exhibited gradual decrease at sub-lethal concentration. But in lethal concentration the increase was registered up to day 5, which later decreased. According to Kumar (1994), endosmosis is the reason for increase in MCV. This results in haemodilution, further increasing the MCV value. This was in co-relation with many workers (Garg *et al*, 1989; Kumar *et al.*, 1994; Dhanapakiam and Ramaswamy, 2001). David (1995) observed a decrease and then a subsequent increase of MCV in *Labeo rohita* exposed to low and high sub lethal concentration of fenvalerate. As MCH and MCHC are derived from Hb and RBC some of alteration in the levels of Hb and RBC would result in the alteration of MCH and MCHC. These findings are consistent with the findings of some other authors studying the responses of other fishes exposed to organophosphorus pesticides. This regards changes in RBC indices after acute and sub acute exposure to malathion in *Cyprinion watsoni* (Khattak and Hafeez, 1996), formothion in *Heteropneustes fossilis* (Singh and Srivastav, 1994), trichlorfon in *Piaractus mesopotamicus* (Tavares *et al*., 1999), chlorpyrifos in *Cyprinus carpio* (Ramesh and Saravanan, 2008), and phosalone in *Oreochromis mossambicus* (Alt and Rant, 2009). Similarly, decreased RBC, Hb, and PCV values after acute and sub acute exposure to deltamethrin have been reported in fingerling European catfish (*Silurus glanis*) , male brood stock (*Rutilus frisii* kutum), common carp (*Cyprinus carpio*), and African catfish (*Clarias gariepinus*). The reduction in RBC count and Hb are often accompanied by a decrease in PVC and demonstrates the physiological dysfunction of the hemopoietic system. In order to overcome hypoxic conditions in stressful media, fish usually respond by increasing the MCV and MCH of erythrocytes. In the present study, a significant increase in these indices was observed in the test fish after acute and sub acute exposure to deltamethrin. The increase in MCV and MCH values after exposure to deltamethrin indicates that a reduced RBC count may be due to the destruction of erythrocytes or their decreased synthesis in bone marrow (Rauf and Arian, 2013).

Table 1. Different blood parameters in *Cirrhinus mrigala* on exposure to lethal and sublethal concentration of deltamethrin.

Parameter	Control	Exposure period in days							
		Lethal				Sublethal			
		1	2	3	4	1	5	10	15
RBC (x10^6/mm^3)	1.7733A	1.5007 C	1.2438F	0.8693 H	0.6052I	1.6185 B	1.2119 G	1.2646E	1.3817 D
±SD	0.0107	0.01	0.012	0.01	0.019	0.0113	0.012	0.015	0.0117
% change		-15.37	-29.85	-50.97	-65.87	-8.729	-31.65	-28.68	-22.08
WBC (x10^6/mm^3)	10.475F	10.664 E	9.671G	8.776H	6.3784I	10.728 D	12.148 B	12.338A	12.065 C
±SD	0.0137	0.0131	0.0123	0.0124	0.013	0.0142	0.014	0.0138	0.0131
% change		4.29	-7.67	-16.21	-39.1	15.49	15.97	17.78	15.18
Haemoglobin (g/dL)	7.3868A	6.5356 C	5.645G	3.9136 H	3.1091I	6.8118 B	6.0208F	6.1109E	6.4325 D
±SD	0.0121	0.0147	0.0121	0.016	0.0121	0.0151	0.0184	0.0163	0.0121
% change		-9.21	-18.49	-35.88	-57.86	-5.82	-23.65	-26.02	-24.56
PCV (%)	25.652A	23.28C	20.907 D	16.447 H	10.809I	24.157 B	19.585E	18.975G	19.351F
±SD	0.0125	0.0128	0.0127	0.0121	0.0121	0.0123	0.012	0.0119	0.0121
% change		-9.21	-18.49	-35.88	-57.86	-5.82	-23.65	-26.02	-24.56
MCV(mm^3)	71.6384 G	76.283F	83.246 C	93.708 A	88.4612 B	79.721E	80.036 D	76.7023 I	73.359 H
±SD	0.1065	0.1092	0.1327	0.2057	0.2083	0.2071	0.2043	0.1596	0.1525
% change		6.48	16.2	30.8	23.48	11.28	11.72	6.89	3.18
MCH (pg)	22.9618I	23.83H	25.018 G	27.188 C	28.322A	25.021F	27.387 B	27.691D	25.663 C
±SD	0.1231	0.1132	0.1327	0.1057	0.2011	0.2071	0.2032	0.1314	0.1566
% change		3.78	8.957	18.408	23.34	8.97	19.27	16.02	11.76
MCHC (%)	22.53A	20.873 E	21.058 D	19.055 G	18.975I	20.108F	18.957 H	21.16C	21.625 B
±SD	0.0456	0.0236	0.0541	0.0587	0.0581	0.0683	0.0671	0.0633	0.0642
% change		-7.354	-6.533	-15.423	-15.778	-10.75	-15.858	-6.08	-4.016

Means are ±SD (n=6) for a parameter in a row followed by the same letter are not significantly different (P≤ 0.05) from each other according to Dunchan's multiple range test.

CONCLUSION

In the present study, fish samples treated with lethal and sub-lethal concentration of deltamethrin, showed considerable alteration in the level of different blood parameters. Our results confirm the lethal and sublethal effect of deltamethrin on *C. mrigala*. The decrease in levels of RBCs, Hb, PCV and increase in WBC, MCHC revealed the haematotoxic effects of this chemical pollutant and they may be helpful as a tool to monitor the health status of this and other related fish species. The evaluation of haematological parameters will help in early detection of clinical pathology as well as the presence of disturbance in the environment.

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