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ANALYSIS OF HAEMATOLOGICAL AND HISTOPATHOLOGICAL MANIFESTATIONS IN GILLS AND MUSCLE OF FISH *Channa punctatus* (Bloch 1793) EXPOSED TO SYNTHETIC PYRETHROID DELTAMETHRIN

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aquatic ecosystem was especially faced with the threat of fish biodiversity loss due to indiscriminate use of pesticides in agriculture fields. The pesticides were entered into the aquatic regimes and affected their non-target animals like fishes. The present study aims the analysis of deleterious effects like haematological and histopathological alterations in type II synthetic pyrethroid deltamethrin exposed fish *Channa punctatus*. In experiment, the acclimatized fishes were exposed to sub-lethal concentration, 0.026 ppm (10% of 96 h-LC_{50} of deltamethrin) along with a control. The blood, gills and muscle tissues of fish were sampled out after 7, 14 and 28 days of exposure intervals. The significant ($p < 0.05$) decrement in red blood corpuscles count, hemoglobin and hematocrit, while, augmentation in white blood corpuscles count were found in deltamethrin exposed blood of fish. The noticeable histopathological abnormalities were observed in the gills and muscle of fish *Channa punctatus*. The control fish showed no histological alterations. In exposed fishes, the breakdown of the epithelial cell framework with numerous changes such as epithelial lifting (EL), congestion (C) and bulging (B) observed

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in fish gills. Also, the anomalies like fragmentation (FS) and bursting (Br) were found in the fish muscle. The findings illustrated the prominent haematological and histopathological perturbations in deltamethrin exposed blood, gills and muscle tissues of fish. These changes can be used as indicators for monitoring the aquatic pollution and helpful in the conservation of aquatic biodiversity including fishes.

Keywords: *Channa punctatus*; deltamethrin; epithelial lifting; hematological; histopathological abnormalities.

1. INTRODUCTION

Pesticides principally utilized in three major customs such as insecticides, herbicides and fungicides to control the different pests in agriculture fields. From the agricultural fields, they perceived in aquatic ecosystem due to their seepage. Among pesticides, pyrethroids (mostly synthetic pyrethroids), such as deltamethrin, cypermethrin and others, posed their adverse effects on the aquatic animals like fishes. Synthetic pyrethroid deltamethrin was made analogy with pyrethrins present in the blossoms of *Chrysanthemum cinerariafolium* [1]. Deltamethrin was first portrayed in 1974 and entered the commercial centre in 1978 [2]. Structurally, deltamethrin was a pyrethroid made up of a stereoisomer specifically prepared by the esterification of (1R, 3R)- or cis-2, 2-dimethyl-3-(2, 2-dibromovinyl) cyclopropane carboxylic corrosive with (alpha-S)- or (+)- alpha-cyano-3-phenoxybenzyl liquor or by particular recrystallization of the racemic esters got by esterification of the (1R, 3R)- or cis-corrosive with the racemic or (alpha-R, alpha-S, or alpha-R/S)- or + or – liquor [2]. The prolonged exposure of deltamethrin created the deleterious effects on aquatic fauna. Among aquatic animals, fishes stand at top of its food chain. Therefore, this pesticide highly and differentially concentrated and posed the deleterious effects on tissues as well as on hematological components of fish. The hematological parameters referred as good indicators of the overall physiological disturbances in fish. The physiological status of metal exposed fish described in terms of the determination of hematological indices such as red blood cells (RBCs), haematocrit (Hct), haemoglobin (Hb) and white blood cells (WBCs) [3]. Besides, the histopathological changes have been used as biomonitoring tools for knowing the structural and functional changes in the xenobiotics exposed fishes [4, 5]. In various fish species, the histopathological and haematological alterations used as well established biomarkers [6]. The objective of present study is to determine the hematological and histopathological changes in blood, gills and muscle of freshwater fish, *Channa punctatus* exposed to deltamethrin.

2. MATERIALS AND METHODS

Chemicals: Type II synthetic pyrethroid deltamethrin was used as a test chemical and other analytic grade chemicals were procured from the agro-chemical market of Daliganj, Lucknow.

The acclimatization of fish: About 60 well grown-up and live fish of *Channa punctatus* (weighing 25-35 g, length 10-12 cm) were collected from unpolluted freshwater ponds nearby Lucknow (26° 55' N, 80° 59' E), India. After the assortment of fishes, they were treated with $KMnO_4$ for the elimination of dermal infections, if any, and were accustomed under standard laboratory conditions for 10 days before the beginning of the experiments. Also, the physicochemical qualities of each aquarium water were estimated on every day. The fish were acclimatized for 10 d under the given laboratory conditions by following standard methods [7] (Table 1). Fishes were kept in glass aquaria containing faucet water. During acclimatization period, fishes were fed with fish food and water of each aquaria was likewise changed once in a day.

Median sub-lethal concentration (96 h-LC_{50}) of deltamethrin for fish *Channa punctatus:* Acclimatized fishes were initially exposed to different concentrations (0.001, 0.01, 0.1, 1.0 and 10.0 ppm) of deltamethrin, on logarithmic scale, for estimating its range. Again, fishes were separately exposed to definitive concentrations like 0.15, 0.25, 0.35, 0.45, 0.55, 0.65, 0.75, 0.85, 0.95 and 1.05 ppm of deltamethrin along with a control. Also, fish mortality was recorded after 24, 48, 72 and 96 h. So, the 50% mortality of fish was regarded as median sub-lethal concentration (LC_{50}) after 96 h [8].

Experimental set up: Fish *Channa punctatus* were exposed to 10% of 96 h-LC_{50} (0.026 ppm) of deltamethrin for 7, 14 and 28 days, to determine the sub-lethal effects in terms of haematological and histopathological variations. The fishes were kept in 4 different aquaria. Out of 4 aquaria, 3 aquaria were used as triplicates of single aforesaid concentration of deltamethrin and one as control. The entire experiment was conducted for 28 days of exposure period.

Assessment of physicochemical parameters of test water: The water testing assays were done to check the water quality during the entire study. Also, the physicochemical parameters (Dissolved oxygen, hardness, alkalinity, chloride, pH and temperature)

were quantified by following the standard methods [7] in both control and triplicates of treated group after 7, 14 and 28 days of exposure intervals.

Haematological parameters in deltamethrin exposed fish: The blood samples were collected through heart puncture with heparinized syringe for the estimation of hemoglobin (Hb%), white blood corpuscles (WBCs), red blood corpuscles (RBCs) and hematocrit (Hct) by using the standard methods [9,10].

Histopathological abnormalities in gills and muscle of deltamethrin exposed fish: On the end of exposure intervals of 7, 14 and 28 days, the gills and muscle were immediately taken out from the control and treated fishes, arbitrarily. Collected gills and muscle washed thoroughly and put them into saline water for few minutes. After, the tissues were fixed in Bouin's fluid for 48 h. The tissues were dehydrated in 70% ethanol and hardened in cedar wood oil for 24 h. Then, the tissues were embedded in paraffin wax and sectioned in 3 μm thickness by using a rotary microtome. Further, the slides containing tissue sections were stained with hematoxylin (H) and eosin (E) for 1 min and 2 min, respectively. Next, the slides were mounted in DPX (distyrene, plasticizer and xylene) and examined under 10/40X magnification of objective lenses of light microscope.

Statistical analyses: The values were expressed as mean ± standard error mean (S.E.M.). The data were tested at the significant ($p < 0.05$) level by using one-way analysis of variance (ANOVA) with Tukey's post hoc test by Statistical Package for the Social Sciences (SPSS) software (version 20.0, Chicago, IL, USA).

3. RESULTS AND DISCUSSION

Median sub-lethal concentration (LC_{50}) of deltamethrin for fish, *C. punctatus:* The semi-static bioassays were conducted to estimate the median sub-lethal concentration for 96 h **(**96 h-LC_{50}) of deltamethrin for *C. punctatus.* Its calculated value was found to be 0.26 ppm with 95% lower and upper confidence limits as 0.21 ppm and 0.32 ppm, respectively.

The physicochemical factors of test water: Water was fundamental for survival of all the organism. No living being on the Earth can survive without it. The significant part of water on earth was marine water which can't be utilized without handling by people. The main accessible freshwater which could be utilized for the purpose of drinking emerges starting from the earliest stage. The percent volume of freshwater was not adequate to cater the needs of the living creatures, if it would have not been used properly. Also, the water quality played a significant role in the body of living organisms since supporting the physiological pathways in them. The pesticides entered into freshwater bodies through surface overflow or through draining [11]. The water characteristics were estimated and their numerical values were given in Table 1.

Hematological parameters in blood of fish: The significantly ($p < 0.05$) increased values of WBC count ($\times 10^3/mm^3$) as well as declined values of Hb (g%), Hct (%) and RBC count ($\times 10^6/mm^3$) were observed in exposed group (0.026 ppm; 10% of 96 h-LC_{50} of deltamethrin) as compared to control after 7, 14 and 28 days (**Table 2**). These parameters altered in a time-dependent manner. The highest reduction in RBC count, hemoglobin (Hb) level and hematocrit (Hct) were found in the blood of treated fish after the completion of exposure period (28 days). Strongly, the changed blood parameters engendered the physiological manifestations in animals against exposure of xenobiotic [12]. The highly variable values of erythrocytes count and total leucocytes count have been reported in fishes [13, 14]. These blood parameters can be indicators to evaluate the physiological status of fish under pesticides stress [15, 16]. Further, similar findings were reported in dichlorvos exposed *Ctenopharyngodon idella* [17] and in chlorpyrifos induced *Oreochromis mossambicus* [18].

Table 1. Physicochemical parameters of test water of control and treated group after 7, 14 and 28 days of exposure intervals

Parameters	Control	7 Days	14 Days	28 Days
Dissolved oxygen (DO; mg/L)	6.21 ± 0.02	6.33 ± 0.04	6.11 ± 0.08	6.17 ± 0.02
Hardness (mg/L)	127.32 ± 0.03	125.63 ± 0.02	126.3 ± 0.04	124.98 ± 0.05
Alkalinity (mg/L)	106.63 ± 0.03	105.3 ± 0.01	107.3 ± 0.02	106.25 ± 0.12
Chloride (mg/L)	43.21 ± 0.02	40.91 ± 0.21	42.39 ± 0.34	41.24 ± 0.22
pH	7.1 ± 0.2	6.8 ± 0.1	7.2 ± 0.1	6.9 ± 0.3
Temperature (℃)	29.8 ± 5.4 °C	26.1 ± 2.2 °C	25.21 ± 3.1 °C	27.11 ± 2.5 °C

(Values displayed as mean ± S.E.M.)

However, the findings showed the maximum augmentation in WBC count in exposed fish as compared to control after 28 days. In fact, the increased WBC count indicate an activation of immune system to protect the pesticides exposed workers by producing antibodies and chemical substances working as defense against the physiological perturbations [19, 20].

Table 2. The hematological factors altered in exposed group (0.026 ppm; 10% of 96 h-LC_{50} of deltamethrin) as compared to control after 7, 14 and 28 days

Groups	Periods (days)	RBC (x 10^6/mm^3)	Hb (g%)	WBC (x 10^3/mm^3)	Hct (%)
Control	7	3.01 ± 0.09	11.09 ± 0.29	17.03 ± 0.25	31.24 ± 0.73
	14	2.98 ± 0.14	11.21 ± 0.28	17.12 ± 0.17	30.36 ± 0.54
	28	2.87 ± 0.12	10.95 ± 0.14	16.78 ± 0.28	31.21 ± 0.22
10% of 96 h-LC_{50} of deltamethrin	7	2.23 ± 0.21*	8.87 ± 0.26*	18.54 ± 0.34*	26.95 ± 0.56*
	14	2.17 ± 0.22*	8.26 ± 0.24*	19.78 ± 0.63*	24.36 ± 0.22*
	28	1.89 ± 0.12*	7.65 ± 0.11*	19.88 ± 0.74*	23.83 ± 0.46*

(Values expressed as mean ± S.E.M. (n = 5) and superscript shows the statistical significance at p < 0.05 in comparison to control)*

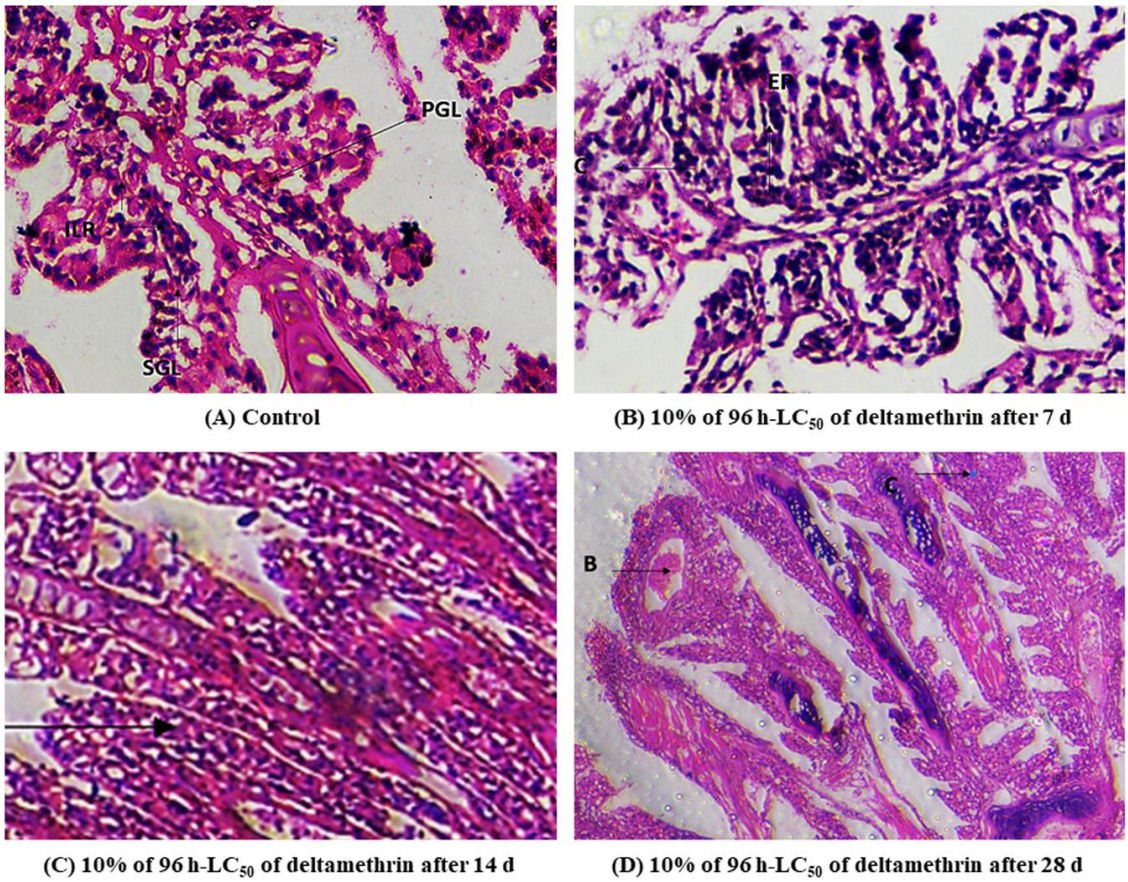
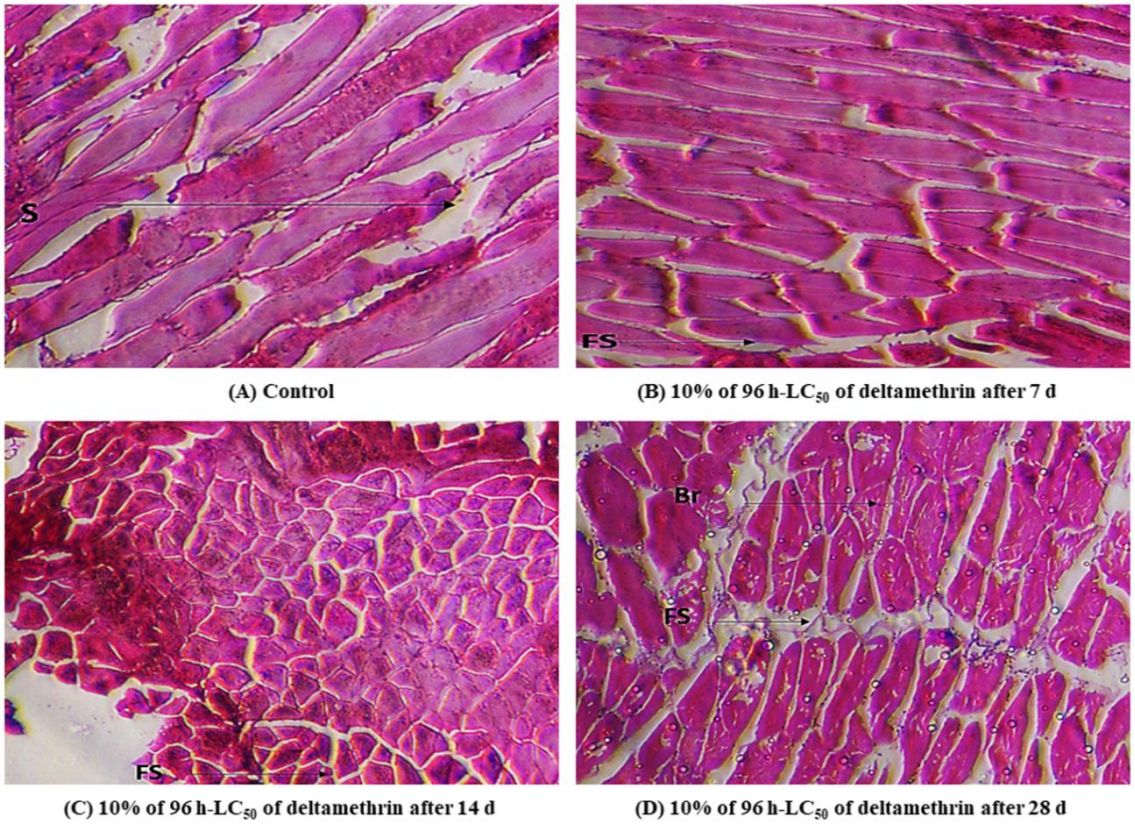


Fig. 1. Microphotographs of gills of fish showing well defined primary gill lamella (PGL), secondary gill lamella (SGL) and inter lamellar region (ILR) in control (panel A), along with the treated gill sections (10% of 96 h-LC_{50} of deltamethrin) illustrating epithelial lifting (EL) and congestion (C) after 7 days (panel B), the increased extent of congestion (C) after 14 days (panel C) and higher congestion (C) and bulging (B) after 28 days (panel D). The gill sections stained with hematoxylin (H) and eosin (E) and examined under 40X magnification of objective lenses of light microscope



(A) Control

(B) 10% of 96 h-LC_{50} of deltamethrin after 7 d

(C) 10% of 96 h-LC_{50} of deltamethrin after 14 d

(D) 10% of 96 h-LC_{50} of deltamethrin after 28 d

Fig. 2. Photographic sections of muscle of fish displaying compact and well defined showing sarcoplasm (S) in control (panel A). The treated muscle sections (10% of 96 h-LC_{50} of deltamethrin) exemplifying the least fragmentation (FS) after 7 days (panel B), the higher extent of fragmentation after 14 days (panel C) and the maximum fragmentation and bursting after 28 days (panel D). The muscle sections stained with hematoxylin (H) and eosin (E) and examined under 40X magnification of objective lenses of light microscope

Histopathological abnormalities in deltamethrin exposed gills of fish: Chronically, the exposure of deltamethrin posed a variety of histopathological changes in gills of fish in a time-dependent manner (**Fig. 1**). In the fishes of control, there were nominal/no histological changes. In gills of exposed fish, the histological changes consisted a breakdown of the epithelial cell framework with numerous changes in their design. Also, the progressions in their deterioration incorporate epithelial lifting (EL) and congestion (C). In this context, the synthetic pesticides like deltamethrin posed the histopathological anomalies in the fishes. Similarly, the pesticide chlorpyrifos induced histological manifestations recorded in freshwater fish *Channa punctatus* [21]. The lifting of gill lamellar, oedema, melded hypertrophy and hyperplasia of lamellar epithelial cells with desquamation were observed in fish gills. The impact of pesticide was reported by analysing the damage and other manifestations in gills of *Oreochromis niloticus* and *Cyprinus carpio* [22]. Also, the pesticides altered histopathology of gills were described as the decay of primary and secondary gill lamellae in fish *Channa punctatus* [23]. The vascular degeneration, necrotic and hydrops changes were recorded in the exposed primary gill of *Labeo rohita* [24]. Several previous studies reported the different malformations in gill lamellae of *Guppy poecilia* [25] and in mosquito fish *Gambusia affine* [26]. Moreover, the histopathological perturbations such as putrefaction, aneurysm in secondary lamellae, desquamation, lifting of the lamellar epithelium, epithelial hyperplasia, oedema and combination of the auxiliary lamellae were described in deltamethrin exposed gills of normal carp [27]. Additionally, the deltamethrin prompted gill irritation observed in *Tamaqua* [28]. Overall, the persistent pesticide exposure influenced the histopathology of gills which could weaken the crucial components of animal's breath.

Histological alterations in deltamethrin induced muscle of fish: In fish muscle, the histological alterations were remarkably observed. Usually, the

construction of fish muscle is made up of two filaments which are partitioned into upper mass and lower mass and again into many longitudinal fibre blocks isolated by connective tissue sheets. In present study, muscles notably showed muscle fragmentation of sarcoplasm and bursting in deltamethrin exposed fish in time-dependent manner (Fig. 2).

Usually, the higher destruction and anomalies in muscle tissue reported in animal because of its more activeness and also required larger amount of energy for it. Muscles also illustrated vigorous struggling during muscle activity which may probably contribute to protein degradation or proteolysis. Remarkably, the muscle necrosis with fragmentation of sarcoplasm and mononuclear cell infiltration were recorded in pendimethlain exposed muscle of *Tilapia nilotica* [30]. Several investigations described the histopathological alterations in fish muscles under the effects of pollutants [31, 32, 33]. Likewise, the separation of muscle fibres and oedema were notably observed. Initially, the stimulus of deltamethrin engendered the excitability and hyperactivity in animals, leading to posed muscular fatigue due to more release and accumulation of lactic acid. Clearly, the abnormalities reflected through histopathological changes in muscle and brain tissues [34]. Also, the histological alterations and the physiological disturbances were reported in the muscle tissue of freshwater fish *Hoplias alabaricus* procured from Ponta Lake of Brazil contaminated with pesticides [35]. Moreover, the morphological damages observed in the form of histopathological anomalies and necrosis in *Tilapia zilli* and *Solea vulgaris* [36].

4. CONCLUSION

The present study recorded the deleterious effects of deltamethrin on blood, gills and muscle of fish *Channa punctatus*. Notably, the significant ($p < 0.05$) reduction in RBCs count, hemoglobin and hematocrit found in fish blood in time-dependent manner. However, the significant ($p < 0.05$) increase in WBCs count was reported in blood of fish. Besides, the prominent histopathological changes were observed in gills and muscle of fish. The extent of tissues abnormalities enhanced in a time-dependent manner. These analyzed parameters can be used as tools or indicators for monitoring of aquatic contaminations and they will also be pertinent for the fish diversity conservation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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