



Changes in the hematology of the freshwater fish, *Channa punctatus* (Bloch) exposed to the toxicity of deltamethrin

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ABSTRACT

The freshwater fishes are an important source of animal protein to human population. They are adversely affected by aquatic pollutants such as pesticides used in agriculture, which are easily washed off into the water bodies. The effect may be direct when the pesticide is absorbed into the body through the skin, gill, intestine, wound, etc. It may be indirect when the pesticide alters the quality of the water, such as depletion of dissolved oxygen. The aim of this study was to evaluate the changes in some hematological parameters of the fresh water fish *Channa punctatus* exposed to lower (0.075 mg/l) and higher (0.15 mg/l) sub lethal concentrations of LC_{50} (0.75mg/l) of the synthetic pyrethroid pesticide deltamethrin for 15, 30 and 45 days. In the fish exposed to both the concentrations for 45 days there was a significant decrease in the hemoglobin content, total erythrocyte count, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin concentration, as compared to the respective control fish. On the other hand, there was a significant increase in the total leukocyte count, mean corpuscular volume, erythrocyte sedimentation rate, and clotting time values during 30 and 45 days of exposure in lower and higher concentrations of deltamethrin. The differential leukocyte count in fish of 30 and 45 day test period showed a significant increase in the populations of lymphocytes, neutrophils and eosinophils, and significant decrease in monocyte and basophil populations. There was significant reduction in the mean length, breadth and area of erythrocytes in these deltamethrin exposed fish. The result of this study reveals that the synthetic pyrethroid pesticide deltamethrin adversely affects the hematology of the freshwater fish *Channa punctatus*, and calls for the limited and cautious use of highly toxic pesticides like deltamethrin.

Key words: *Channa punctatus*, Deltamethrin, sub lethal, hematological parameters.

INTRODUCTION

The pesticides used in agriculture are posing a great threat to aquatic fauna especially to fishes, which constitute one of the major sources of protein rich food for mankind [21]. Injudicious and indiscriminate use of agrochemicals such as fertilizers, pesticides, insecticides and fungicides to boost crop production with the sole aim of getting more yield, water bodies like ponds, lakes, river and low lying water areas are continuously getting polluted. Normally these pesticides reach the aquatic environment through surface runoff, sediment transport from treated soil and direct application as a spray to water bodies to control the inhabiting pests [9].

Pyrethroid insecticides are commonly used to control insect pests and represent about 30% [1] of world insecticide consumption. Their mean life in water is two weeks but they get rapidly absorbed by aquatic organisms with a significant toxicity [17]. Clark *et al.*, (1985) suggest that the pyrethroids can be absorbed by fish gills even at very low concentration in water. Saxena and Seth (2002) showed a significant change in the hematological parameters of the common freshwater fish *Channa punctatus* on exposure to cypermethrin could be used as potential biomarkers of pyrethroid insecticides.

Deltamethrin [(S)-a-cyano-3 phenoxybenzyl (R1-R2)-3-(2,2 dibromovinyl)-2,2 dimethylcyclo-propanocarboxylate] is one of the most important widely used pyrethroid pesticide and insecticides, since the application of pyrethroid as insecticide and antiparasitary preparations has been accepted on a large scale for agricultural purposes and very markedly increased during last 10-15 years; even though it is already known that this insecticide is highly toxic to fish and various other aquatic organisms [12]. The presence of low deltamethrin concentration in water has sub lethal effects such as altered hematological profile, energy metabolism and ionic regulation. Since the variations of hematological parameters have been demonstrated to be sensitive to sub lethal concentration of different toxic agents, they can be used for detecting pollutant exposure in the environment [13].

The present study was designed to assess the effect of two (lower and higher) sub lethal doses of deltamethrin on several hematological parameters of the freshwater fish *Channa punctatus*. The objective of the present study was to demonstrate the hematological changes in the fish exposed to different toxic levels of deltamethrin.

EXPERIMENTAL SECTION

2.1 Animal collection

Healthy, fresh water fish *Channa punctatus*, measuring 850 to 970 mm and weighing 18.56 ± 2.4 g (mean \pm SD, n = 300), were collected from Retteri Lake, near Chennai, India. The fish were properly washed in tap water and treated with 0.02% KMNO₄ and 0.004% formalin solution to remove external infections of fungi, algae, etc. Fish were acclimatized and maintained in the laboratory conditions for 6 weeks in 100 l tanks containing dechlorinated and aerated tap water. The levels (mean \pm SD) of physical and chemical parameters of the tank water were kept nearly constant: temperature, 25.0 ± 1.6 ; pH, 6.9 ± 1.4 ; dissolved oxygen 7.2 ± 0.8 mg; O₂ l⁻¹; hardness 38 mg CaCO₃ l⁻¹. During the acclimation period, the fish were fed at 48 hour intervals with a commercial fish feed containing 45% of protein.

2.2 Experimental design

The experimental sub lethal concentration [(96h., LC₅₀ = 0.75 mg l⁻¹] of *Channa punctatus* was determined by Probit analysis method (Finney, 1971). A concentration of 0.075mg l⁻¹ and 0.15 mg l⁻¹ of deltamethrin (1/10th and 1/5th LC₅₀) and the exposure time of 15, 30 and 45 days was selected for sub lethal test.

The fish were divided into fifteen groups, each group consisting of 20 fish. The first six groups were exposed to 0.075mg l⁻¹ and the second six groups were exposed to 0.15 mg l⁻¹ of deltamethrin for 15, 30 and 45 days, while the remaining three groups were maintained as control in deltamethrin free tap water. The pesticide exposed groups and the control groups were fed with pelleted commercial fish feed once daily. Water was exchanged every 48 h to maintain the concentration of deltamethrin during the period of exposure. Aeration was provided to each tank. No mortality occurred in any group during the experimental period.

Seven fish from each group were anaesthetized on the 15th, 30th and 45th days prior to collecting blood. Blood was collected by cardiac puncture, using 2 ml glass syringe rinsed with an anticoagulant (1% EDTA) for estimation of hemoglobin (Hb%), Packed cell volume (PCV), Total erythrocyte count (TEC), Total leukocyte count (TLC), Differential leukocyte count (DLC), Erythrocyte sedimentation rate (ESR), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and Clotting time (CT). The hemoglobin concentration was estimated by the Acid - hematin method [25]. TEC and TLC were determined using Neubauer's haemocytometer and Neubauer's counting chamber with RBC diluting fluid and Turke's solution, respectively [9]. PCV was estimated by micro hematocrit method [20]. DLC, ESR, MCV, MCH, MCHC and clotting time were examined following the procedures of Wintrobe (1957) and Sood (1996). Blood was collected from another ten fish from each group by caudal vein puncture to prepare smear slides. The thin blood smears were fixed in methanol and stained with Leishman's stain. Prepared slides were used for measuring cell size and surface area of the erythrocytes adopting the method of Singh and Singh (1982).

The data were subjected to standard statistical analysis. The values reported are mean \pm standard error of mean (SEM) of n =7. One way ANOVA and Duncan multiple range tests ($\alpha = 0.01$) were used for group wise comparisons.

RESULTS AND DISCUSSION

In the present study, exposure of fish to sub lethal concentrations of deltamethrin (0.075 mg l⁻¹ and 0.15mg l⁻¹) for 15, 30, 45 days caused significant alterations in hematological parameters of freshwater fish *Channa punctatus* (Table 1). The alterations observed in hematological parameters such as TEC, Hb, PCV, MCH, MCHC values were

decreased significantly and MCV, ESR and CT values increased significantly after 15, 30 and 45 days of exposure periods, respectively, in comparison with control.

Table 1: Mean hematological parameters of *Channa punctatus* exposed to sub lethal concentrations (0.075 mg/l and 0.15 mg/l) of deltamethrin in 15, 30 and 45 days

Parameter	Concentration (mg/l)	Control	Treated		
			15 days	30 days	45 days
TEC ($\times 10^6 \text{mm}^{-3}$)	0.075	3.165 \pm 0.023	2.833 \pm 0.023*	2.577 \pm 0.019*	2.272 \pm 0.027*
	0.15	3.139 \pm 0.019	2.753 \pm 0.028*	2.341 \pm 0.018*	1.873 \pm 0.028*
TLC ($\times 10^3 \text{mm}^{-3}$)	0.075	18.808 \pm 0.556	20.564 \pm 0.861*	19.784 \pm 0.520	17.639 \pm 0.542
	0.15	18.084 \pm 0.670	19.991 \pm 0.838*	17.259 \pm 0.420	14.793 \pm 0.596*
Hb (g/100ml)	0.075	12.537 \pm 0.309	10.536 \pm 0.280*	7.486 \pm 0.378*	5.724 \pm 0.296*
	0.15	12.603 \pm 0.273	9.447 \pm 0.280*	5.441 \pm 0.304*	2.691 \pm 0.127*
PCV (%)	0.075	34.366 \pm 0.236	31.143 \pm 0.038*	28.669 \pm 0.183*	25.757 \pm 0.205*
	0.15	34.343 \pm 0.213	30.669 \pm 0.280*	26.534 \pm 0.288*	21.699 \pm 0.175*
MCV (10^{-4}fl)	0.075	1.086 \pm 0.010	1.100 \pm 0.009	1.113 \pm 0.012	1.134 \pm 0.009*
	0.15	1.094 \pm 0.011	1.115 \pm 0.016	1.113 \pm 0.012	1.159 \pm 0.018*
MCH (10^5pg)	0.075	3.962 \pm 0.101	3.719 \pm 0.087*	2.906 \pm 0.150*	2.521 \pm 0.146*
	0.15	4.015 \pm 0.088	3.432 \pm 0.102*	2.324 \pm 0.129*	1.434 \pm 0.068*
MCHC (g%)	0.075	36.482 \pm 0.8800	33.831 \pm 0.918*	26.117 \pm 1.432*	22.227 \pm 1.201*
	0.15	36.700 \pm 0.913	30.781 \pm 1.139*	20.505 \pm 1.080*	12.480 \pm 0.671*
ESR (mm/hr)	0.075	5.071 \pm 0.189	7.000 \pm 5.000*	8.786 \pm 0.567	10.286 \pm 0.267*
	0.15	5.214 \pm 0.267	7.429 \pm 0.189*	10.429 \pm 0.534*	13.000 \pm 0.289*
CT (Sec.)	0.075	26.714 \pm 0.488	30.000 \pm 0.577*	35.000 \pm 0.577*	41.571 \pm 0.976*
	0.15	26.571 \pm 0.787	31.714 \pm 0.756*	40.571 \pm 0.787*	50.857 \pm 0.690*

Values are mean \pm standard error of mean, n=7, * = Significant at p<0.01 level, TEC=Total erythrocytes count, TLC=Total leucocytes count, Hb=Haemoglobin, PCV=Packed cell volume, MCV=Mean corpuscular volume, MCH=Mean corpuscular Haemoglobin, MCHC= Mean corpuscular Haemoglobin concentration, ESR=Erythrocyte sedimentation rate, CT=Clotting time.

Table 2: Changes in mean size and surface area of erythrocytes (μm) of *Channa punctatus* exposed to sub lethal concentration (0.075 mg/l and 0.15 mg/l) of deltamethrin in 15, 30 and 45 days

Parameter	Concentration (mg/l)	Control	Treated		
			15 days	30 days	45 days
Length (μm)	0.075	8.732 \pm 0.183	8.571 \pm 0.142	8.036 \pm 0.200*	7.661 \pm 0.200*
	0.15	8.786 \pm 0.200	8.464 \pm 0.200*	7.661 \pm 0.200*	7.018 \pm 0.183*
Breadth (μm)	0.075	6.375 \pm 0.306	6.000 \pm 0.375	5.786 \pm 0.200**	5.304 \pm 0.142*
	0.15	6.375 \pm 0.217	6.000 \pm 0.217*	5.250 \pm 0.306*	4.661 \pm 0.200*
Area (μm^2)	0.075	55.667 \pm 2.887	51.408 \pm 2.950*	46.527 \pm 2.781*	40.641 \pm 1.860*
	0.15	56.029 \pm 2.814	50.786 \pm 2.221*	40.179 \pm 1.689*	32.705 \pm 1.578*

Values are mean \pm standard error of mean, n=7, * = Significant at p<0.01 level

The TLC value was significantly higher after 15 days of exposure, and decreased after 30 and 45 days of exposure in both the concentrations (Table 1). Percentage of lymphocytes, neutrophils and eosinophils was registered an increasing trend over and above the control. The maximum decrease in percentage of monocytes and basophils was recorded after 45 days of exposure (Figure 1a to 1e).

The mean length, breadth and surface area of erythrocytes are shown in Table 2. Mean length, breadth and area of erythrocytes in high concentration (0.15 mg⁻¹) deltamethrin exposed fishes for 30 and 45 day test periods were significant when compared to the respective control values (Figure 2d).

Hematological indices are very important parameters for the evaluation of the physiological status of the fish. The changes depend on fish species, age, the cycle of the sexual maturity of spawners and diseases ([27]; [6] and [10]). Pollutants generally produce relatively rapid changes in blood characteristics of fish [7]. The red blood cells count, PCV and hemoglobin concentration vary with diet and strain as well as temperature, season of the year and nutritional status of the fish [2].

The exposure of *Channa punctatus* to sub lethal concentrations of deltamethrin led to anemia, as indicated by the significant decrease in Hb, TEC, and PCV values leading to anemia. The anemia might have led to a fall in the red blood cell count, hemoglobin concentration and haematocrit volume. The anemic condition in fish results from an unusually low number of red blood cells or too little hemoglobin in the red blood cells. According to Pamila *et al.*, (1991) the pesticide induced anemia in fish may be due to the inhibitory effect of the toxic substance on the enzyme system responsible for the synthesis of haemoglobin. It may also be due to impaired intestinal absorption of iron, as suggested by Joshi *et al.*, (2002).

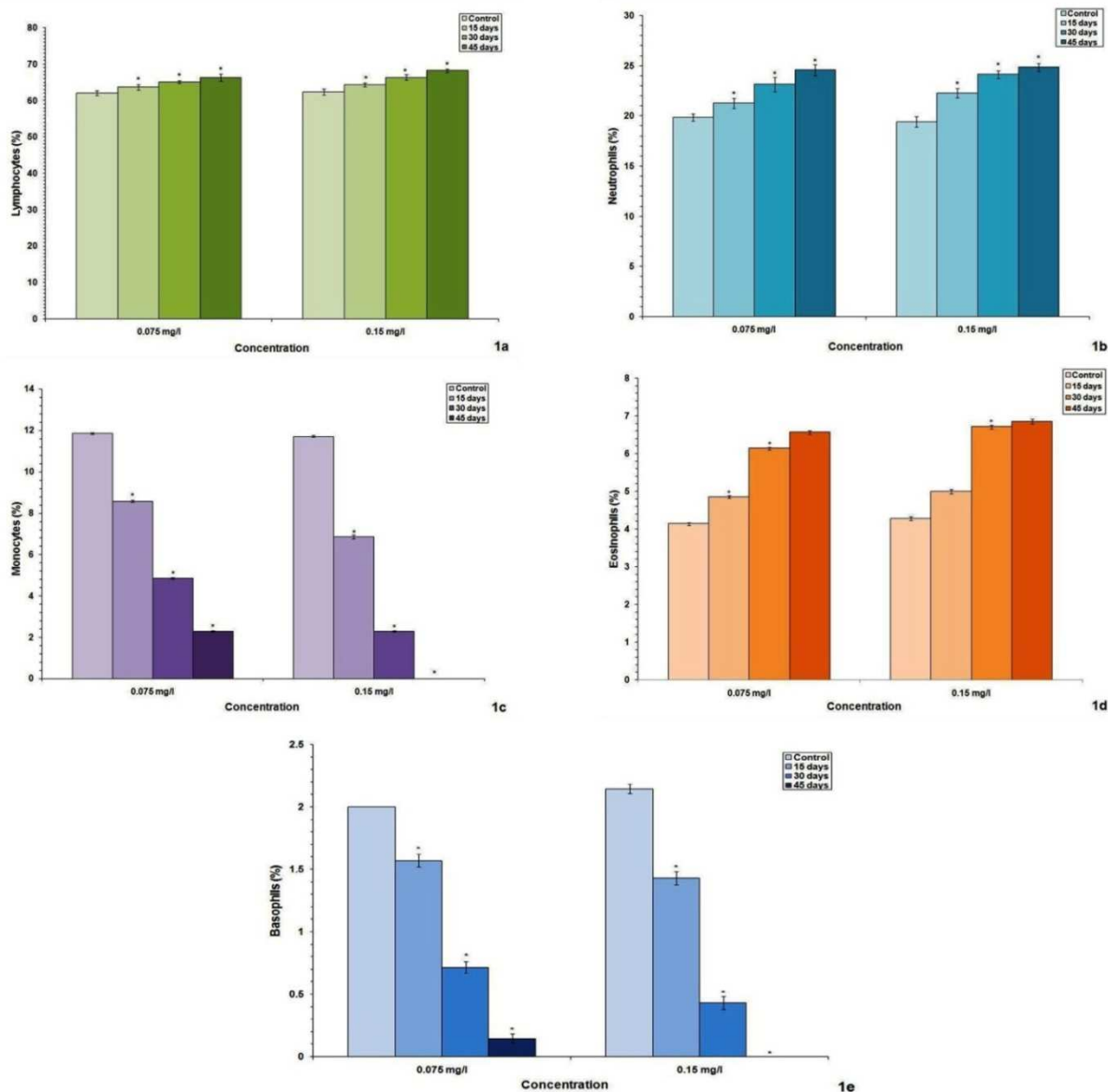


Figure 1a to 1e: Changes in percentage of differential leucocytes count (DLC) of *Channa punctatus* exposed to sub-lethal concentration (0.075 mg/l and 0.15 mg/l) of deltamethrin in 15, 30 and 45 days

Values are mean \pm standard error of mean, $n=7$, * = Significant at $p < 0.01$ level.

Decrease in MCH and MCHC values and increase in MCV, ESR and CT values and total leucocytes count suggested that the anemia was of macrocytic type [24]. The total leukocyte count (TLC) variation was reported in case of organophosphorous pesticides in the haemoglobin percentage (Hb%) in pesticide stress was reported in *Channa punctatus* ([16]; [18]). The lymphocytes are more responsible for immune response. Lymphocyte contains a large nucleus leaving less boundary of cytoplasm. Increase of neutrophils in the blood causing a disease neutrophilia leads to non-specific response to a variety of stress stimuli in fishes. Eosinophils are considered to play a major role in phagocytosing antigen / antibody complexes and implicated in inflammation [22].

The clot is formed under normal conditions undergoes contraction, when serum is expressed from the clot, and finally the clot becomes denser. The high percentage of prothrombin, the blood clotting substance is responsible for blood clotting and a substance called thromboplastin, released by the platelet also responsible for blood clotting reaction ([3].; Pandey [15]). Due to the increased concentration of toxicant, the production of these substances is decreased, so, the blood takes more time to clot.

The surface area reduction of erythrocytes suggests the hypoxic effect prevailing over the body tissues of fishes due to damaging effect on the gill tissue. The erythrocytes in the exposed fish were shrunk, with cell wall distortion (Figure 2d) showing crenated margin and the nuclei were hypertrophied [11].

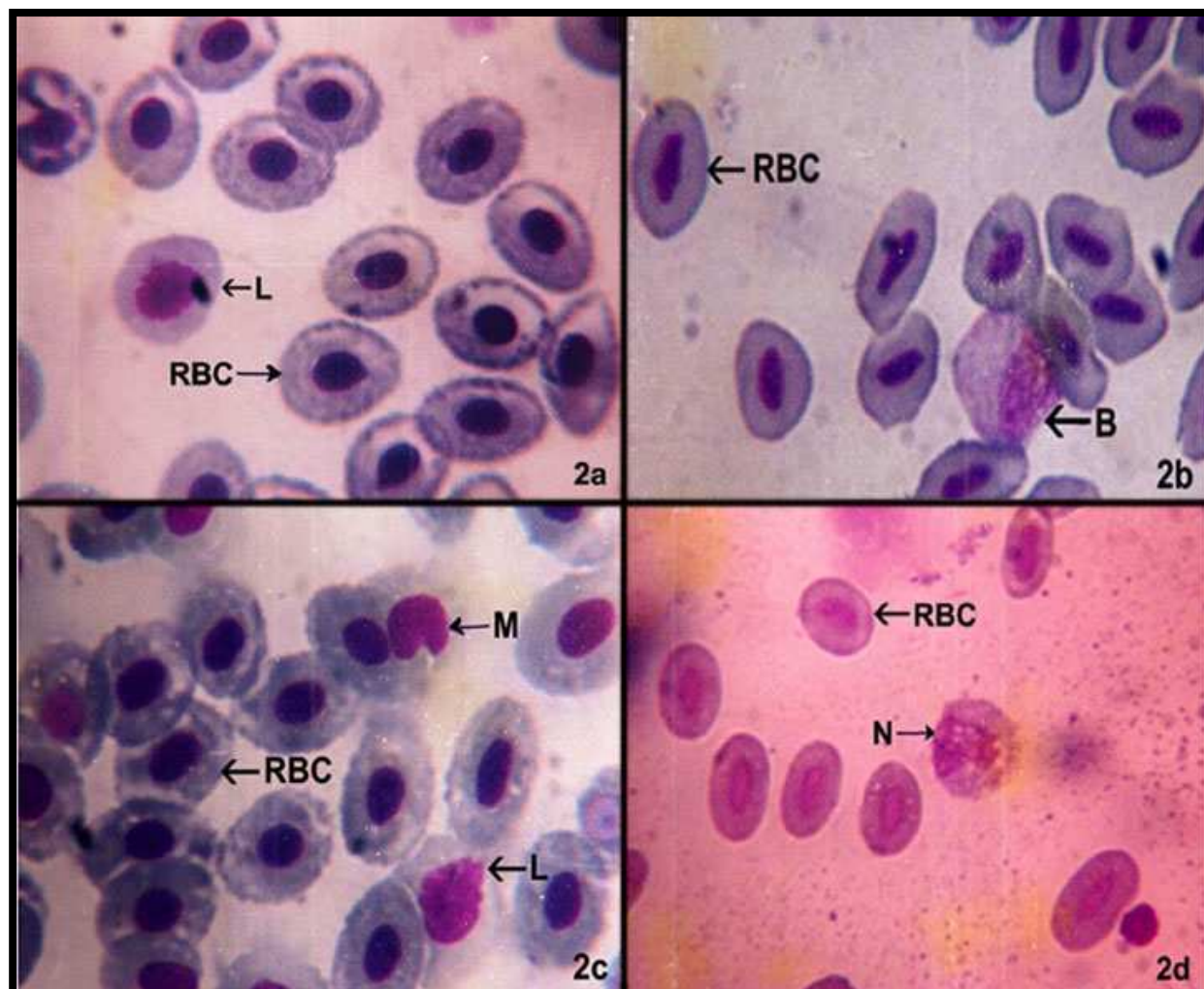


Figure 2a: Blood smear-Leishman's stain of control fish showing mature normal Red blood cells (RBC) and Lymphocyte (L). X 1000

Figure 2b: Blood smear-Leishman's stain of 15 days exposure of fish at 0.15 mg/l concentration of deltamethrin showing Basophil (B) and irregular shape of Red Blood Cells (RBC). X 1000

Figure 2c: Blood smear-Leishman's stain of 30 days exposure fish at 0.15 mg/l concentration of deltamethrin showing Monocyte (M), Lymphocyte (L) and the cleaved cytoplasm of the Red blood cells (RBC). X 1000

Figure 2d: Blood smear-Leishman's stain of 45 days exposure fish at 0.15 mg/l concentration of deltamethrin showing Neutrophil (N) and the reduction in the size of the Red blood cells (RBC). X 1000

CONCLUSION

The hematological parameters are the most sensitive indices in monitoring the toxicity of deltamethrin at sub lethal concentrations. Since the fish are the most sensitive aquatic fauna, any little change that occurs in their living media might immediately influence their physiology. Deltamethrin is highly toxic to *Channa punctatus* even in very low concentration (0.075mg l^{-1}). Therefore, it is suggested that the use of these types of pyrethroids to control agriculture pests should be judicious and controlled.

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