

# A study on behavioural responses and haematological effects of synthetic pyrethroid deltamethrin on fresh water teleost *Channa punctatus* (Bloch)

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## Abstract

Deltamethrin is a synthetic pyrethroid Pesticide widely used in agriculture. Pesticides in agricultural runoff can adversely affect any aquatic ecosystem and be detrimental to the aquatic fauna. Fishes are known to be widely affected by the toxic effects of these harmful chemicals, of which deltamethrin can be regarded as the most potent one. In the present study, the effect of deltamethrin on some behavioural and haematological parameters of the freshwater teleost *Channa punctatus* was investigated. Experimental fish were exposed to 0.075 mg/L and 0.15 mg/L sub-lethal concentrations of deltamethrin (LC<sub>50</sub>- 0.5 mg/L) for 7 consecutive days. After 7 days of exposure, the alternation in various behavioural responses and haematological effects on fish was evaluated. The study revealed that Deltamethrin has toxic effects on behavioural as well as haematological aspects of *Channa punctatus*. The treated fish showed an increased number of surfacing and opercular movements, fast swimming, increased mucous secretion, and jumping to get rid of the treated water. Significant decrease in Red Blood Cells (RBCs) count, and Haemoglobin (Hb) concentration was observed. An increased number of White blood cells (WBC) in comparison with the control was also observed in the treated groups. From this present investigation, it can be substantiated that deltamethrin induces diverse negative impacts on fish.

**Keywords:** Deltamethrin, Pyrethroid, Haematology, Haemoglobin

## Introduction

Chemical pesticides are well recognized as an economic approach to control pests. At the same time, these pesticides finally find their way into water bodies through surface runoff, sediment transport from treated soil and direct application as a spray to water, and this alters the physicochemical properties of water and causes harm to aquatic flora and fauna. Pandey *et al.*, (1993) <sup>[9]</sup> reported that long-term exposure to pesticides induces physiochemical disturbances, behavioural changes, histopathological changes, haematological alteration, and biochemical changes in the fish germplasm resource. Ayas *et al.* (2007) suggest that the comparative toxicity of various pesticides on aquatic organisms under controlled laboratory conditions has been observed, such that at lower concentrations, the toxic materials may not cause the death of the organism. It may change the function and physiochemical disturbances in the tissue at the cellular level, and have adverse effects on fish behaviour and morphology. Among all other tissues, blood is a suitable biomarker where damage can be detected, and changes can be observed when fish are stressed, and environmental quality is altered Tierney *et al.*, (2004). Haematological parameters are potential biomarkers. It reflects the state of fish under stress more quickly as they respond quickly to changes in the environmental conditions. Behavioural changes are the most sensitive indicator of toxic effects. Changes in the behaviour and physiology indicate the deterioration of the water quality. Fish are one of the main

inhabitants of aquatic ecosystems. They are a good bio indicator of aquatic environment as they are sensitive to any change in a short-term acute or long-term chronic environment Deka and Datta, (2016) <sup>[4]</sup>. *Channa punctatus*, the spotted snakehead belonging to the family Channidae, is regarded as one of the most important teleosts in India. It is a fish of high food value and has little value as an aquarium fish. The fish is hardly easily available throughout the year and has a great capacity to survive in laboratory conditions due to its air-breathing habit.

Therefore, keeping these in view, the present study was undertaken to analyse the toxic effects of sub-lethal concentrations of deltamethrin on fresh water teleost *Channa punctatus* (Bloch). To fulfil this aim, the following objectives were chosen.

- To study the effects of deltamethrin on behavioural responses and morphology of *Channa punctatus*.
- To study the effects of deltamethrin on haematological aspects like RBC, WBC, and haemoglobin concentration.

## Materials and Methods

### Experimental animal

The fish selected for the study was *Channa punctatus*. Healthy, fresh water air-breathing fish *Channa punctatus* (Family channidae, order channiformes) was procured from the local market of Dhing, Nagaon, Assam. The specimen had an average weight and length of 40 to 50g and 16 to 18 cm, respectively. The specimen was acclimatised for 2 days under

laboratory conditions in a large plastic bucket containing 15 litres of tap water, and the fishes were fed daily with commercial fish food.

### Experimental chemical

The synthetic class II pyrethroid insecticide deltamethrin ( $C_{22}H_{19}Br_2NO_3$ ) with trade name DECIS (28% EC) was purchased from the local market store of Dhing and used as an experimental chemical.

### Determination of sub-lethal concentration

Determining the 96-hour  $LC_{50}$  value of deltamethrin. A four-day static acute toxicity test was performed in which five different concentrations (0.2, 0.3, 0.4, 0.5, 0.6) mg/L of deltamethrin were used in the test series (10 fish for each). A control group (10 fish) was maintained in a pyrethroid-free container throughout the entire experimental period. At 24, 48, 72 and 96 hours, the mortality rate was assessed, and the 96h  $LC_{50}$  value of the test chemical for the specimen was 96h. ( $LC_{50} = 0.5$  mg/L) Based on the 96h  $LC_{50}$  value, to test concentrations of Deltamethrin, sub-lethal concentration 1 (SL-1:  $1/15^{th}$  of  $LC_{50} = 0.075$  mg/L) and sub-lethal concentration 2 (SL -2:  $1/3^{rd}$  of  $LC_{50} = 0.15$  mg/L) were estimated and used for the experimental study. For the determination of the  $LC_{50}$  value of deltamethrin, the method of Karber, as reported by Dede and Kaglo (2001) [3], was considered.

### Experimental design

A concentration of 0.075 mg/L and 0.15 mg/L of deltamethrin ( $1/15^{th}$  and  $1/3^{rd}$  of  $LC_{50}$ ) and an exposure time of 7 days were selected for the sub-lethal test of experimental parameters.

The collected fish were randomly divided into 3 groups, each group consisting of 10 fish.

- Group A, treated as control/standard
- Group B, treated as sub-lethal concentration of deltametharin 0.075 mg/L
- Group C, treated as a sub-lethal concentration of deltamethrin 0.15 mg/L

After 7 days of exposure, the following parameters were observed.

#### 1. Behavioural studies

Behavioural responses and morphological changes in fish exposed to deltamethrin and in control fish were observed at 24, 48, 72, and 96h. One randomly selected fish from each of the groups was transferred to an aquarium providing the same environment as in their respective groups. Morphological changes like skin lesions, skin colouration, and changes in gills were also observed in treated fish groups. Surfacing, opercular movements, and jumping of those fishes were recorded at two-minute intervals for each fish.

#### 2. Haematological studies

For haematological studies, blood was collected from control and deltamethrin-treated fish by severance of the caudal peduncle using a 2ml disposable syringe rinsed with anticoagulant (1% EDTA) for estimation of haemoglobin (Hb), Total Erythrocyte Count (TEC), and Total Leukocyte Count (TLC).

#### 2.1. Total Erythrocyte Count (TEC)

Total RBCS were counted using Neubauer's haemocytometer. Blood was drawn up to 0.5 mark of RBC diluting pipette from both control and treated group of fish and diluted with RBC diluting fluid (Hayem's fluid) up to 101 mark to get dilution and mixed thoroughly by rotating the bulb of the pipette several times. After that, a drop of diluted blood was then placed on the counting slide with a coverslip and allowed to spread equally. After a few minutes, erythrocytes were counted under observation of the microscope and followed the routine procedure (Wintrobe, 1967) [16].

#### 2.2. Total Leucocyte Count (TLC)

Total leucocyte count was done using Neubauer's haemocytometer. Blood was drawn up to the 0.5 mark of the WBC diluting pipette, diluted with WBC diluting fluid (Turk's fluid) up to the 11 mark and mixed thoroughly by rotation. A drop of diluted blood was then placed on the counting slide, allowed to settle for some time, and WBC counting was done.

#### 2.3. Estimation of Haemoglobin (Hb)

Haemoglobin was determined by Sahli's haemoglobinometer. At first, dilute HCL was placed in a diluting tube up to the 20 cubic mm mark. Then blood was drawn into the haemoglobin pipette up to the 20 cubic mm mark, and the blood was blown into the diluting tube and mixed well. After 10 minutes, distilled water was added in drops to mix the blood in the tube until it had the same colour as the comparison standard. Then the reading was recorded, which indicates the % of haemoglobin.

### Experimental findings

#### 1. Behavioural studies

In the present study, fish in the control group were found to be active and healthy with normal swimming behaviour. When fishes were exposed to sub-lethal concentrations of deltamethrin, they slowly moved to the bottom of the aquarium tank and found swimming independently, fish increased their visit to the surface of the water. Surface visit was found to be very rapid for the first 24 hours and moderate for 48 hours, and finally, surface visit was decreased by 72 and 96 hours. Treated fishes tend to move faster quadratically, and they were under stress. The tendency to jump out of the tank and fast swimming were observed in the first few hours. Treated fishes were having mucous secretion all over the body, more profusely in the gill region. The opercular movement was found to be mild for 24 and 48 hours and moderate to rapid for 72 and 96 hours. In comparison to the control group, the treated fish group showed more surfacing and jumping behaviour and showed more quadrate-to-quadrate movement. The treated fish exhibit more opercular movement than the control group. Though in the result it was observed that with the increase of duration of exposure, the surface visit and fast swimming decreased significantly, and fishes were seemed to be inactive and fatigued. On the other hand, mucous secretion and opercular movement were increased. With the increase in concentration, all behavioural responses become stronger than usual.

**Table 1:** Behavioural responses of Deltamethrin-exposed *Channa punctatus* at two sub-lethal concentrations and the control group

Concentration of Deltamethrin	Surface visit	Jumping	Fast swimming	Mucous secretion	Opercular movement
0mg/L (Control)	-	-	+	-	+
0.075mg/L	+	+	++	+	++
0.15mg/L	+++	++	+++	+++	+++

Notes: None: -, Mild: +, Moderate: ++, Rapid: +++

**Table 1(A):** Behavioural responses of deltamethrin-exposed *Channa punctatus* at exposure periods of (0.075mg/L) concentrations

Exposure periods (0.075mg/L)	Behavioural response			
	Surface visit	Fast swimming	Mucous secretion	Opercular movement
24 hrs	++	++	-	+
48 hrs	+	++	+	+
72 hrs	+	+	+	++
96 hrs	-	-	++	++

Notes: None: -, Mild: +, Moderate: ++, Rapid: +++

**Table 1(B):** Behavioural responses of deltamethrin-exposed *Channa punctatus* at exposure periods of (0.15mg/L) concentrations

Exposure periods (0.15mg/L)	Behavioural response			
	Surface visit	Fast swimming	Mucous secretion	Opercular movement
24 hrs	+++	+++	-	+
48 hrs	++	++	+	++
72 hrs	+	+	++	++
96 hrs	-	-	+++	++

Notes: None: -, Mild: +, Moderate: ++, Rapid: +++

## 2. Haematological studies

### 2.1. Total erythrocyte count

The total erythrocyte count of the healthy control group showed a mean value of  $3.103 \pm 0.075 \times 10^6/\text{mm}^3$ . The fishes exposed to sublethal concentrations of 0.075mg/L and 0.15mg/L of deltamethrin showed mean values of RBCs as  $2.606 \pm 0.173 \times 10^6/\text{mm}^3$  and  $2.535 \pm 0.176 \times 10^6/\text{mm}^3$  respectively. The treatment with deltamethrin was found to cause a drastic reduction in the total count of RBCs. The reduction was dose-dependent because as the concentration of deltamethrin increased, the RBC levels were found to decrease (Table 2). The values mentioned above showed significant decreases when compared to the control ( $p < 0.05$ ).

### 2.2. Total leucocyte count

The results of the total count of WBC revealed that the blood

of the control group showed a mean value of  $12.673 \pm 0.494 \times 10^3/\text{mm}^3$  of blood. The fishes exposed to sub-lethal concentrations of 0.075 mg/L and 0.15 mg/L showed a mean value of  $17.116 \pm 0.423 \times 10^3/\text{mm}^3$  and  $18.066 \pm 0.253 \times 10^3/\text{mm}^3$  of blood, respectively. The value showed a significant increase when compared to the control ( $P < 0.05$ ).

### 2.3. Haemoglobin content

The control group showed a mean value of  $9.036 \pm 0.314 \text{ gm}\%$  for haemoglobin. The fishes exposed to sub-lethal concentrations of 0.075 mg/L and 0.15 mg/L of deltamethrin showed a mean value of Haemoglobin as  $6.5 \pm 0.171 \text{ gm}\%$  and  $6.5 \pm 0.149 \text{ gm}\%$ . The values showed a significant decrease when compared to the control group ( $p < 0.05$ ).

**Table 2:** Total count of erythrocytes, leucocytes and haemoglobin content in the control group and deltamethrin-treated *Channa punctatus*

Concentration of deltamethrin	No. of erythrocytes 106/mm <sup>3</sup>	No. of leucocyte 103/mm <sup>3</sup>	Haemoglobin gm (%)
Control	$3.103 \pm 0.075$	$12.673 \pm 0.494$	$9.036 \pm 0.314$
0.075mg/L	$2.606 \pm 0.173^*$	$17.116 \pm 0.423^*$	$6.5 \pm 0.171^*$
0.15mg/L	$2.535 \pm 0.176^*$	$18.066 \pm 0.253^*$	$6.5 \pm 0.149^*$

Each Value is the mean  $\pm$  SD of six observations. (+) indicates an increase, (-) indicates a decrease over control, \* indicates significant ( $p < 0.05$ ).

## Discussion

The present study reveals the effects of deltamethrin on various parameters of the experimental fish *Channa punctatus*. The observed behavioural changes of the fish include surfacing, a change in opercular movement rate, fast swimming, and mucous secretion, which could be considered to be toxicity of deltamethrin. The frequent surfacing of the fish can be considered as a shifting adaptation towards the aerial mode of respiration due to the toxic effect of deltamethrin. Similar

behavioural phenomenon of the fish due to toxicity was also reported by Kumar *et al.*, (2007) [6] in *Channa punctatus*. Increased Opercular movement observed in the present study was an indication of an oxygen-depleted environment where the fish was under stress and needed oxygen for survival. Depleted dissolved oxygen in fish causes intense opercular movement. (Olufayo and Aidde, 2012) [8] reported that increased opercular movement in *Heterobranchis bidasalis* exposed to cypermethrin might be hyperexcitability, which

involved considerable energy expenditure and there by mode of greater oxygen demand. Excessive secretion of mucous all over the body, in general and more pronounced in the gill region, might be the hyperactivity of the mucous gland of the fish observed in the present investigation, a defensive mechanism of the fish to protect the body from an adverse environment. A similar phenomenon was reported by Prasant *et al.*, (2005) and Veni and Veeriah (2014) <sup>[15]</sup> in *Cirrhinus mrigala* exposed to cypermethrin.

The study of haematology of *Channa punctatus* subjected to sub-lethal concentration of deltamethrin showed significant changes with respect to total erythrocyte count, total leucocyte count and haemoglobin % in the blood. Treated fish might have impairment of respiratory activity and might be prone to rapid haemolysis of the circulating red blood cells. A decrease in haemoglobin level in the study may be due to the release of immature cells from haemopoietic tissue into the bloodstream, as well as disruption of iron metabolism that leads to defective haemoglobin synthesis, reported by Khatun N. and Kalita. E (2018) <sup>[5]</sup>. On the other hand, a significant increase in total leucocytes indicates an adjustment in the defensive and immunological status of the fish. Similar observations are also reported by Abdul *et al.*, (2010) <sup>[1]</sup> in *Channa punctatus* exposed to triazophos, Jayaprakas and Shettu (2013) <sup>[2]</sup> in *Channa punctatus* exposed to deltamethrin, Saxena and Seth (2012) <sup>[11]</sup>, in *Channa punctatus* treated with cypermethrin. Thus, it is clear that deltamethrin causes various alterations in the treated fish, ranging from behavioural patterns to haematological parameters. From the present experimental work, it is evident that deltamethrin is highly toxic to the freshwater teleost *Channa punctatus*. This insecticide causes alteration in behavioural and haematological aspects of the fish.

### Conclusion

The present study clearly revealed the toxic nature of deltamethrin and its effects on the experimental fish *Channa punctatus*. It provides valuable information in the assessment of fish health and in monitoring stress and responses. From the basis of present experimental findings, it may be concluded that deltamethrin contamination is dangerous to the aquatic ecosystem. It can also affect other organisms, including human beings, through the food chain. Therefore, judicious use of recommended pesticides such as deltamethrin should be done as per the prescribed dose and method of application, thereby avoiding their indiscriminate use. Possible alternative measures, such as the use of biological methods, could be used instead of deltamethrin in order to protect the natural environment.

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