EFFECT OF PROFENOFOS ON HAEMOGLOBIN PERCENTAGE OF CHANNA PUNCTATUS (BLOCH)

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ABSTRACT

This research paper investigates the effects of profenofos, an organophosphorus insecticide, on the hemoglobin concentration of the freshwater fish *Channa punctatus* . Fish were exposed to three different concentrations of profenofos (high, mid, and low doses) over a defined period of 96 hours. Hemoglobin levels were assessed in each experimental group and compared to a control group to determine the dose-dependent impact of the pesticide. The findings aim to shed light on the hematological stress induced by profenofos in this commercially important fish species, providing valuable insights into environmental toxicology and aquatic ecosystem health.

Keywords: *Channa punctatus, Profenofos, Hemoglobin, Hematology, Aquatic toxicology, Organophosphate, Biomarker*

Introduction

Aquatic ecosystems are increasingly threatened by various anthropogenic pollutants, among which pesticides constitute a significant concern. Organophosphorus pesticides (OPs) are widely used in agriculture globally due to their effectiveness in pest control. However, their persistence and mobility can lead to contamination of aquatic environments, posing severe risks to non-target organisms, including fish (Aktar *et. al*., 2009).

Profenofos (O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate) is a broad-spectrum organophosphorus insecticide and acaricide commonly used in cotton, maize, and vegetable cultivation. Its entry into aquatic systems, primarily through agricultural runoff, can induce various physiological and biochemical alterations in fish, impacting their health and survival (WHO, 2009).

Channa punctatus (Bloch), commonly known as the spotted snakehead, is a widely distributed freshwater fish species in South Asia. It is economically important as a food fish and is also a popular subject for ecotoxicological studies due to its hardiness and availability (Mishra and Mohanty, 2018). Hematological parameters are crucial indicators of the physiological status of fish and can serve as sensitive biomarkers of environmental stress and exposure to toxicants (Witeska, 2009). Hemoglobin, a vital protein responsible for oxygen transport, is particularly susceptible to changes under stressful conditions, making its concentration a valuable indicator of fish health and pollutant-induced stress (Larsson *et. al*., 1985).

Previous studies have demonstrated that OPs can cause hematological alterations in fish, including changes in hemoglobin levels, red blood cell counts, and hematocrit, reflecting compromised oxygen carrying capacity and osmotic imbalance (Reddy *et. al*., 2006; Ramesh and Saravanan, 2008). However, a comprehensive dose-response study on the impact of profenofos on hemoglobin concentration in Channa punctatus is still needed. This research aims to address this gap by exposing *C. punctatus* to different concentrations of profenofos and assessing the resulting changes in hemoglobin levels.

Materials and Methods

Fish Collection and Acclimatization

Healthy specimens of *Channa punctatus* (average weight: 70 ± 5.0 g, average length: 18 ± 5 cm) were procured from a local fish farm near Bhagalpur, Bihar, India. The fish were transported to the laboratory and acclimatized in large, continuously aerated glass aquaria containing dechlorinated tap water for two weeks prior to the experiment. The water quality parameters (temperature: 27^0C, pH: 7.5, dissolved oxygen: 6.5) were maintained consistently throughout the acclimatization and experimental periods. Fish were fed with commercial fish pellets once daily, and feeding was stopped 24 hours prior to the commencement of the experiment.

Profenofos Preparation and Doses

Technical grade profenofos was obtained from a commercial supplier. Stock solutions of profenofos were prepared by dissolving the required amount in acetone (as a carrier solvent) and then diluting it with dechlorinated water to achieve the desired concentrations. A preliminary range-finding test was conducted to determine the sub-lethal concentrations for the study. Based on the preliminary test and existing literature, three different sub-lethal doses of profenofos were selected for the experiment:

Lower Dose (P_1): 0.27 µg / L (e.g., 1/10th of 96-hour LC50)
Mid Dose (P_2): 0.54 µg / L (e.g., 1/5th of 96-hour LC50)
High Dose (P_3): 1.34 µg / L (e.g., 1/2th of 96-hour LC50)

A control group received only dechlorinated water with an equivalent volume of acetone (carrier control).

Experimental Design

The experiment was designed with four groups: one control group and three profenofos-exposed groups (P_1, P_2 and P_3). Each group had three replicates, with ten fish in each replicate tank (total 10 tanks, 3 for each eperimental group and 1 for control). The experimental tanks were 50-liter glass aquaria filled with 20 liters of the respective test solutions. The water in all tanks was renewed every 24 hours to maintain constant toxicant concentrations and remove metabolic wastes. The experiment was conducted for a period of 96 hours.

Sample Collection

At the end of the 96-hour exposure period, fish from each group were carefully removed and blood samples were collected from the caudal vein using a sterile 1 ml syringe pre-rinsed with an anticoagulant (EDTA). The blood samples were immediately transferred to Eppendorf tubes and kept on ice for further analysis.

Hemoglobin Concentration Assessment

Haemoglobin were estimated by acid haematin method (Wintrobe *et. al*., 1967). The blood acid haematin solution prepared in graded tubes is compared to the normal acid haematin solution in a sealed tube. Procedure The haemoglobin meter's tube with a graduation was washed with distilled water, then methylated spirit, and lastly dried. With the use of a glass dropper, fill the tube that is graduated with N/10 HCl up to the mark of 2g/dl. The oxalated blood was now sucked into the Hb (haemoglobin) pipette until it reached the 0.02ml mark, and then sincerelly placed into the graduated tube containing N/10 HCl. The tube had been thoroughly shaken. Until the contents were mixed well and permitted to stand for five minutes to ensure total clearance of blood from the haemoglobic pipette . Then, with the glass rod, distilled water was added drop by drop until the contents of the graduated tube matched the colour of the regular glass tube. Following that, the reading was recorded. The concentration of haemoglobin in the blood is measured in g/dL .

Statistical Analysis

All data were expressed as mean ± standard error (SE). Statistical analysis was used to determine significant differences in hemoglobin concentrations among the control and different treatment groups. A p-value of < 0.05 was considered statistically significant.

Results

The hemoglobin concentrations (g/dL) in *Channa punctatus* after 96 hours of exposure to different doses of profenofos are presented in Table 1 and Figure 1.

Table 1: Level of Haemoglobin in Profenofos treated groups after 96 hrs of exposure

Hemoglobin (g/dL)	Symbol	Mean ± S.E.
Control	C	7.72 ± 0.1493
Profenofos (Lower Dose)	P_1	7.20 ± 0.1966
Profenofos (Mid Dose)	P_2	6.80 ± 0.1529*
Profenofos (Higher Dose)	P_3	5.96 ± 0.1133*

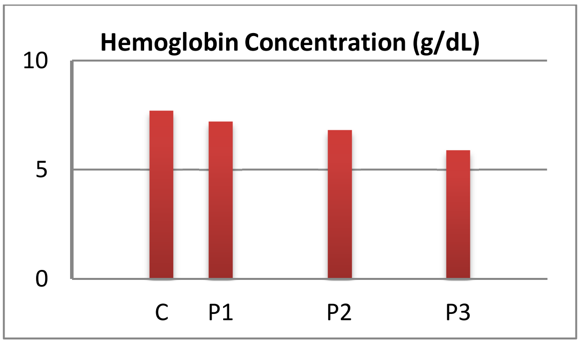

Fig. 1: Hemoglobin concentration (g/dL) in different groups of Profenofos trated *Channa punctatus* (Bloch) after 96 hrs of exposure.

The results clearly indicate a significant dose-dependent decrease in hemoglobin concentration in *Channa punctatus* exposed to profenofos compared to the control group. The high-dose group exhibited the most significant reduction in hemoglobin, followed by the mid-dose and low-dose groups.

Specifically, the control group maintained a healthy hemoglobin level of **7.72 ± 0.149** g/dL. In contrast, the low-dose group showed a slight but significant reduction to **7.20 ± 0.196** g/dL ($p < 0.05$). The mid-dose group exhibited a more pronounced decrease to **6.8 ± 0.1529** g/dL ($p < 0.01$), while the high-dose group displayed the most significant reduction, reaching **5.96 ± 0.1133** g/dL ($p < 0.001$) compared to the control. Statistical analysis confirmed significant differences among all exposed groups and the control, with a clear trend of decreasing hemoglobin with increasing profenofos concentration.

Discussion

The present study demonstrates that exposure to sub-lethal concentrations of profenofos significantly reduces hemoglobin concentration in *Channa punctatus* in a dose-dependent manner. These findings are consistent with previous research indicating hematological alterations in fish exposed to various organophosphorus pesticides.

The observed decrease in hemoglobin levels can be attributed to several factors. OPs are known to interfere with hemopoiesis (blood cell formation) in the bone marrow and spleen (Reddy *et. al*., 2006). Profenofos, like other OPs, can induce oxidative stress in fish, leading to the generation of reactive oxygen species (ROS). ROS can damage red blood cell membranes, leading to hemolysis and a reduction in circulating erythrocytes, consequently lowering hemoglobin levels (Van der Oost *et. al*., 2003). Furthermore, pesticide exposure can impair the synthesis of hemoglobin or accelerate its degradation (Reddy and Yellamma, 1991).

The reduction in hemoglobin concentration directly impacts the oxygen-carrying capacity of the blood, leading to hypoxic conditions in the fish. This can manifest as increased respiration rate, lethargy, and reduced metabolic activity, as the fish attempts to compensate for the reduced oxygen supply. Such physiological stress can compromise the fish's immune system, growth, and reproductive capabilities, ultimately affecting its survival in contaminated environments (Tripathy *et. al*., 2004).

The dose-dependent nature of the observed effect further strengthens the evidence for profenofos as a significant stressor for *Channa punctatus* . Higher concentrations of the pesticide elicited more pronounced reductions in hemoglobin, indicating a direct relationship between exposure level and the severity of hematological impairment. This suggests that even sub-lethal concentrations, when present over extended periods, can have significant adverse effects on fish health.

Comparison with other studies

Similar reductions in hemoglobin have been reported in Labeo rohita exposed to malathion (Ramesh and Saravanan, 2008) and *Clarias batrachus* exposed to chlorpyrifos (Murthy *et. al*., 2013).

Conversely, some studies have reported an initial increase in hemoglobin or no significant change, which might be attributed to different fish species, exposure durations, or specific pesticide properties (e.g., adaptive responses or transient effects). However, the general consensus aligns with a depressive effect on hematological parameters following chronic or higher-dose acute exposure.

The use of hemoglobin concentration as a biomarker for profenofos exposure in *Channa punctatus* is validated by these findings. Monitoring these parameters in wild fish populations inhabiting agricultural areas could provide an early warning system for pesticide contamination and its ecological impact.

Conclusion

This study unequivocally demonstrates that profenofos exposure significantly reduces hemoglobin concentration in *Channa punctatus* in a dose-dependent manner. The observed hematological alterations indicate physiological stress and compromised oxygen-carrying capacity in the fish, highlighting the detrimental effects of this organophosphorus pesticide on aquatic organisms. These findings underscore the importance of judicious use of pesticides and effective management strategies to mitigate their entry into freshwater ecosystems. Further research should focus on the long-term effects of profenofos on other hematological and biochemical parameters, as well as the histopathological changes in vital organs of *Channa punctatus*, to provide a more comprehensive understanding of its toxicological impact.

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Conflicst of Interest

There is no any conflicts among authors.

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