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Comparative studies on haemoglobin percentage of profenofos and carbofuran in *Channa* punctatus Bloch

Nakul Sah[1*], Rohit Kumar Verma[2], Dharmshila Kumari[1]
[1] Department of Zoology, T.M. Bhagalpur University, Bhagalpur, Bihar, India
[2] Department of Biotechnology, T.M. Bhagalpur University, Bhagalpur|, Bihar, India
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Abstract
Aquatic ecosystem is prone to be affected by agricultural pesticides used indiscriminately. The runoff water polluted the different fresh water bodies nearby the agricultural lands. Profenofos and Carbofuran are the two most commonly used pesticide worldwide including India used to minimize the agricultural loss. These pesticides produce threat to aquatic life including fish. In the present study fresh water fish *Channa punctatus* (Bloch.) was used to estimate the effect of Profenofos and Carbofuran on the amount of haemoglobin (g/dl) on a comparative account. The experiment was designed for three different doses for both Profenofos and Carbofuran as Lower, Middle and Higher doses *Viz*., P_1, P_2 & P_3 for Profenofos and CF_1, CF_2 and CF_3 for Carbofuran for and exposure period of 96 hours in acclimatized aquarium. Findings of the present piece of work suggest the concentration of Haemoglobin as 7.20, 6.80 and 5.96 g/dl respectively for experimental groups of Profenofos P_1, P_2 and P_3 respectively. In Carbofuran treatment group haemoglobin concentration was revealed as 6.99, 6.72 and 5.41 g/dl respectively with the control group having 7.72 (g/dl) haemoglobin concentrations. The results suggest the Carbofuran is more deleterious for haemoglobin amount in all the three experimental groups in comparison to Profenofos.

Keywords: Aquatic ecosystem, Channa punctatus Bloch., Profenofos, Carbofuran, Haemoglobin.

Introduction
The haematological characteristics of fish are seen as a crucial biological element that explains how fish survive illnesses, such as pathogenic infections. The hematological percent of fish (Summarwar, 2012) [11] may be negatively impacted by the contamination of insecticides to the aquatic environment. The haemoglobin proteins in red blood cells are essential for carrying oxygen to every area of the body, thereby ensuring its life-saving function. Variations in haemoglobin levels might serve as a primary predictor of physiological state and reveal shifts in health.

The hematology parameter can be used as a marker of health and to assess the physiological condition of fish and other vertebrates (Chandra and Chandra, 2013; [3] Blahova *et al*., 2014). A blood's biochemical profile is extremely valuable because it may be used to quickly and efficiently evaluate the health state of an organism (Hrubec *et al*., 2000) [6]. Patho-physiological circumstances in fish may be assessed using hematological variables as a sensitive indicator (Kori-Siakpere *et al*., 2005) [7].

When the experimental fish (rainbow trout) were exposed to Diazinon, there was a significant decrease in the quantity of haemoglobin (Far *et al*., 2012) [4]. In the fish, *Cyprinus carpio*, a noteworthy decrease in haemoglobin concentration was observed (Adedeji *et al*., 2009) [1]. Gautam *et al*. (2014) [5] looked at the poisonous impact of the organophosphate Nuvam on the blood chemistry of the freshwater fish *Claris batrachus*. Using the Indian major carp *Labeo rohita* as a bioassay, Tripathi and Yadav (2015) [15] investigated the acute toxicity of the organophosphate insecticide phenthoate at concentrations ranging from 0.4 to 4.0 mg/L, with a 0.4 mg/L interval, after exposure for 24, 48, 72 and 96 hours, respectively.

Pesticide infiltration into freshwater ecosystems through agricultural runoff, leaching and spray drift causes devastating physiological, biochemical and behavioral disruptions in non-target fish. Because fish are continuously exposed to these dissolved toxins, their essential biological mechanisms fail at highly sub-lethal (Tang *et al*., 2021) [13].

Materials and Methods
Treatment Protocol
We gathered mature *Channa punctatus* (Bloch) specimens, averaging 18.0±5.0 cm in size and 70±5.0 g in weight, from a nearby market in Bhagalpur. To acclimatize them for laboratory settings and give them a balanced diet, they were maintained for 10 to15 days in a muddy water aquarium (Pandey *et al*., 2011) [8].

After that, the fish were acclimatized for 10 to 15 days in a glass aquarium filled with room-temperature tap water before being moved to the Cytogenetics lab of the University Department of Zoology at T.M. Bhagalpur University in Bhagalpur. Dry prawn meal and hard-boiled chicken eggs were fed to the fish three times daily throughout the acclimatization period. For pesticide therapy, including Carbofuran and Profenofos, these experimental fish were divided into four groups fifteen days later. Ten fish are included in each group, along with twenty liters of tap water.

The initial group was treated as a control (no pesticide treatment) in the experimental protocol for the pesticide Profenofos. The second, third, and fourth groups were treated for pesticides in proportion to 20 liters of tap water (Table-1) using Profenofos at a low dose (P_1), a middle dose (P_2), and a higher dose (P_3) respectively Similarly, three experimental groups of Carbofuran were made as per the treatment protocol and designated as CF_1, CF_2 and CF_3 for Lower, Middle and Higher doses (Table 1). The experiment is repeated for five times (n=5) to statistical analysis.

Pesticides Under Investigation

Profenofos

Profenofos is a broad-spectrum organophosphate insecticide and acaricide used heavily in commercial agriculture. It was first registered in 1982 by the EPA and belongs to the IRAC Group 1B (Acetylcholinesterase inhibitors). It is primarily applied to non-food crops like cotton, but it is also utilized globally on vegetables and grains to control both chewing and sucking pests.

There are two main ways that Profenofos affects the insect's central nervous system. Acetylcholinesterase (AChE) Inhibition as it prevents the AChE enzyme from binding to it. This results in an accumulation of acetylcholine at nerve synapses, which causes continuous nerve stimulation, paralysis, and a quick demise for pests. The present study is based on the Profenofos pesticide purchase from the local market of Bhagalpur, Bihar, India with a trade name Carina (50% E.C.).

Carbofuran

Carbofuran is a broad-spectrum, systemic carbamate pesticide chemically designed to control insects, mites, and nematodes. Unlike organophosphates like Profenofos, Carbofuran belongs to the N-methylcarbamate family, which is derived from carbamic acid. It is notorious for being one of the most toxic and fast-acting commercial pesticides ever synthesized. For Carbofuran pesticide the Furadon™ (3% CG) was purchased from the local agricultural pesticide seller of Bhagalpur, Bihar, India.

The use of Carbofuran has been severely restricted or completely banned in many nations, including India, the United States, and the European Union, due to its high toxicity to birds, mammals, and humans. With acute median lethal concentration values often far below 1.0 mg/L, Carbofuran is highly harmful to fish as per the earlier investigations. Carbofuran, an unintentional and harmful aquatic poison, is quickly transported into freshwater environments by agricultural runoff due to its highwater solubility. It has a significant impact on fish health in a variety of physiological systems.

Table 1: Experimental design for Profenofos and Carbofuran pesticide treatments

Group	Treatment Protocol	Symbol	Dose	LC_{50}
1	Control	C	No Treatment of Profenofos	-
2	Lower dose of Profenofos	P_1	$0.27\ \mu g/L$ ($\approx \frac{1}{10}$ of LC_{50})	$2.68\ \mu g/L$ (Pandey *et al*., 2011) [8]
3	Middle dose of Profenofos	P_2	$0.54\ \mu g/L$ ($\approx \frac{1}{5}$ of LC_{50})	
4	Higher dose of Profenofos	P_3	$1.34\ \mu g/L$ ($\approx \frac{1}{2}$ of LC_{50})	
5	Lower dose of Carbofuran	CF_1	0.09 mg/L (≈1/10 of LC50)	0.9 mg/L (Tiwari *et al*., 2016) [14]
6	Middle dose of Carbofuran	CF_2	0.18 mg/L (≈ 1/2 of LC50)	
7	Higher dose of Carbofuran	CF_3	0.45 mg/L (≈1/2 of LC50)	

Calculating Haemoglobin

Estimation of Haemoglobin

The haemoglobin concentration (g/dl) was calculated using Sahli's techniques (Acid Haematin method), taking the Wintrobe, 1967 [16] approach.

Sahli's Approach

The normal acid haematin solution in a sealed tube is made in order to compare it to the blood acid haematin solution that was created in graded tubes. The haemoglobin in the red blood cells is transformed into brown acid haematin when diluted hydrochloric acid (HCl) is introduced to the blood. The acid haematin solution is then diluted until its color is comparable to the brown glass permanent standard using direct vision.

Blood Collection

After being exposed to the pesticides for 96 hours, blood samples were taken. To determine the percentage of haemoglobin, ten fish were sacrificed from each experimental group. The blood was collected in a clean, pre-chilled test tube after using a scissor to cut off the live fish's caudal peduncle.

Statistical analysis

Statistical analysis was carried out using a t-test at a $P < 0.05$ level, and the results were presented as percent plus or minus standard error.

Results

Effect of Profenofos

In acclimatized *Channa punctatus* (Bloch) test groups, the current study found changes in haemoglobin concentration in control and treated groups, and it calculated the concentrations of Profenofos and Carbofuran after 96 hours of exposure.

In the control group, the mean value of haemoglobin was determined to be 7.72 (g/dl). Mean haemoglobin concentrations (g/dl) in the treatment group of Profenofos at three concentrations (P_1, P_2, and P_3) were 7.20, 6.80, and 5.96 respectively. In middle and higher doses of Profenofos, the mean value of haemoglobin decreased considerably gradually but in lower doses of Profenofos, the mean value of haemoglobin decreased insignificantly (Table 2 and Figure -1).

Table 2: Level of Haemoglobin (g/dl) in control and treated with three different doses of Profenofos

Group	Experimental Protocol	Symbol	Mean±S.E. {Haemoglobin (g/dl)}
1	Control	C	7.72±0.1493
2	Lower Dose	P_1	7.20±0.1966
3	Middle Dose	P_2	6.80±0.1529*
4	Higher Dose	P_3	5.96±0.1133*

*Indicate significant difference at $p < 0.05$

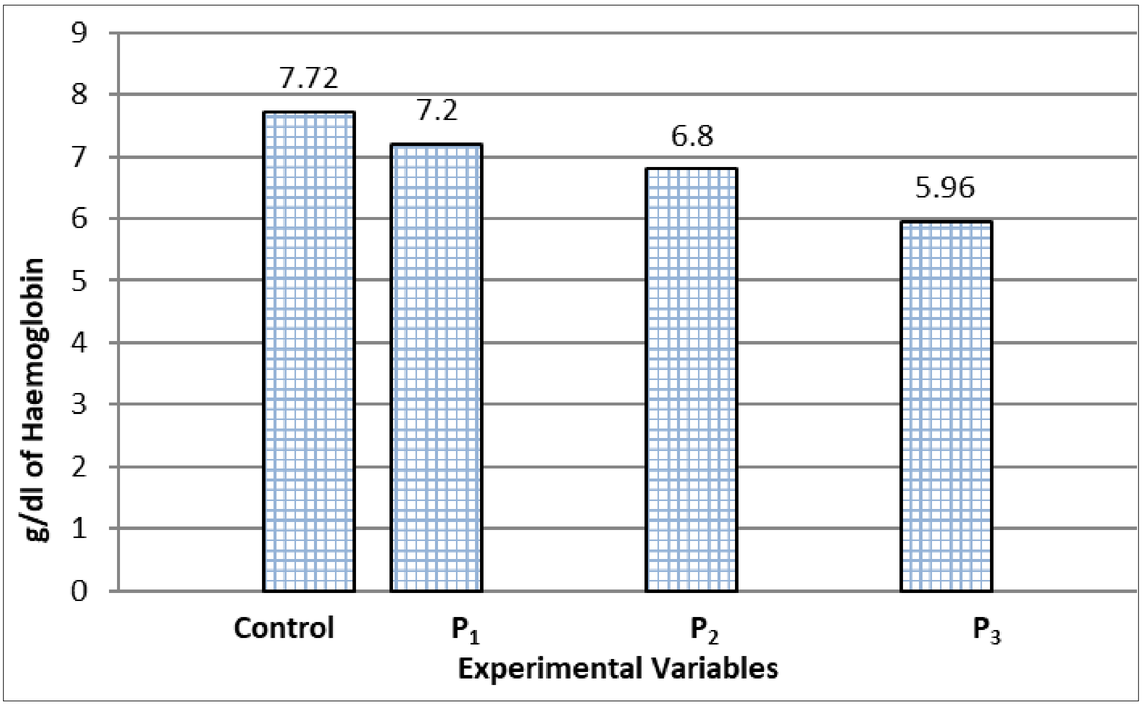

Fig 1: Showing Haemoglobin value (g/dl) in control and different treated group of Profenofos

In the Profenofos treated group the mean value of haemoglobin was 5.96±0.1133 g/dl for the higher dose (P_3) but 5.41±0.1899 in higher dose. Similarly, the amount of haemoglobin was 6.80±0.1529 g/dl for middle dose of Profenofos (P_2). Likewise, the amount of haemoglobin was estimated as 7.20±0.1966 g/dl for the lower dose (P_1) group of Profenofos.

Effect of Carbofuran

The mean value of haemoglobin was 7.72 (g/dl) in control group. In treated group of Carbofuran at three Concentrations (CF_1, CF_2 and CF_3) mean value of haemoglobin (g/dl) concentration were 6.99, 6.72 and 5.41 respectively. It showed significantly gradual decrease in haemoglobin with the increasing doses of Carbofuran (Table - 3 and Figure - 2).

Table 3: Level of Haemoglobin (g/dl) in control and treated with three different doses of Carbofuran

Group	Experimental Protocol	Symbol	Mean±S.E. {Haemoglobin (g/dl)}
1	Control	C	7.72±0.1493*
2	Lower Dose	CF_1	6.99±0.1785*
3	Middle Dose	CF_2	6.72±0.1629*
4	Higher Dose	CF_3	5.41±0.1899*

* Indicate significant difference at $p<0.05$

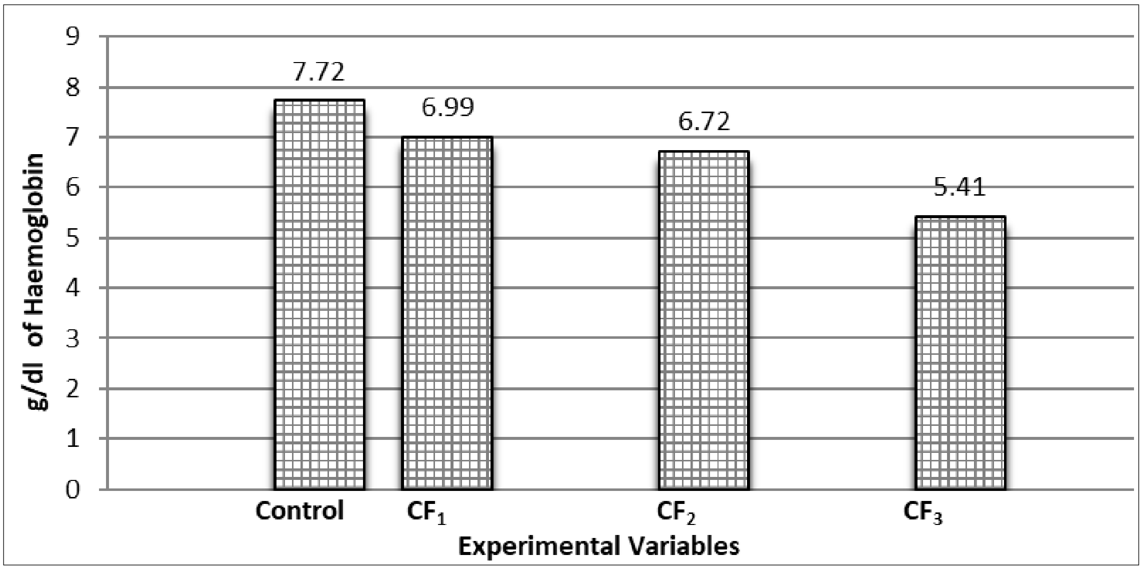

Fig 2: Showing Haemoglobin value (g/dl) in control and different treated group of Carbofuran.

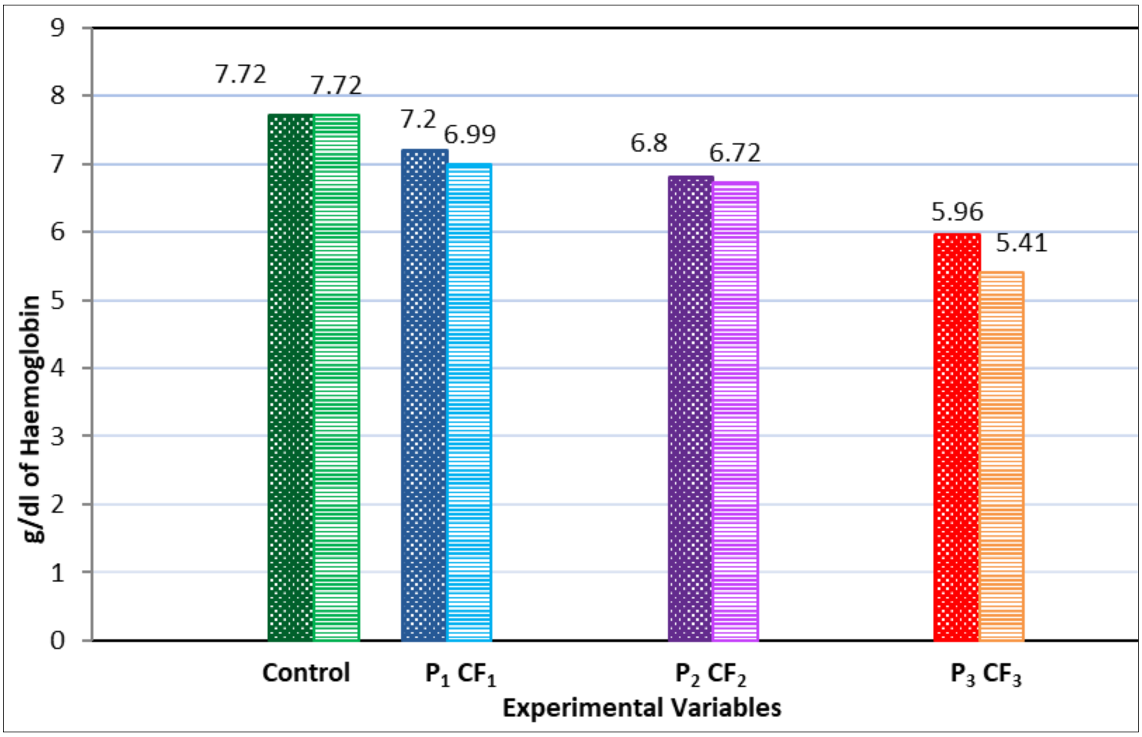

Fig 3: Showing comparison of Haemoglobin (g/dl) in control and different treated group of Profenofos and Carbofuran

With increasing dosage of either pesticide (Profenofos and Carbofuran), the average value of haemoglobin (g/dl) falls in both. However, compared to Profenofos, Carbofuran had a greater diminishing effect on haemoglobin (Figure -3).

The mean haemoglobin level in the group treated with Profenofos was 5.96±0.1133 g/dl for the higher dose (P_3), while it was 5.41±0.1899 g/dl for the higher dose of Carbofuran (CF_3). Similarly, the middle dosage of Carbofuran (CF_2) had a haemoglobin level of 6.72 0.1629 g/dl, while the middle dose of Profenofos (P_2) had a haemoglobin content of 6.80 0.1529 g/dl. In a similar vein, the lower dose (P_1) group of Profenofos had an estimated haemoglobin level of 7.20±0.1966 g/dl, while the lower dose of Carbofuran (CF_1) had an estimated haemoglobin level of 6.99±0.1785 g/dl.

Consequently, it can be deduced that, at three different dosages, Carbofuran was more hazardous than the Profenofos pesticide after a 96-hour exposure.

Discussion

In this study, the haematological parameters were investigated in fresh water fish *Channa punctatus* in relation to the dose of Profenofos and Carbofuran pesticides. The results show a direct correlation between the degree of nuclear damage and the dosage of pesticide examined. The biological system alters its survival tactics in response to haematological factors like the proportion of haemoglobin and the degree of pesticide exposure.

After being exposed to various organophosphorus pesticides, Svoboda *et al*. (2001) [12] and Ramesh and Saravanam (2008) [9] observed a drop in haemoglobin percentage across various fish species. An increase in the rate of haemoglobin breakdown or a decrease in the rate of haemoglobin biosynthesis could also be the cause of the relevant drop in the percentage of haemoglobin (Reddy and Bashamohideen, 1989) [10]. The dramatic and apparent decline in the haemoglobin percentage is thus a clear indicator of the severe anemia caused by the exposure of pesticides such as Profenofos and Carbofuran to the experimental fish *Channa punctatus* (Bloch).

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Conflict of Interest

It is to declare that there is no conflict of interest among the authors.

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