

Original Research Article

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Effect of Profenofos on Rohu Fish (*Labio rohita*): A Fish Widely Cultivated In Rural Areas of India

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ABSTRACT

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The rohu, *Labio rohita*, is an important freshwater fish. The rohu occurs throughout in South Asia and is an important aquaculture freshwater species. Rohu is one of the major carp, being cultivated in small aquatic water bodies as well as in artificial tanks in rural parts of Jabalpur. On the other hand, profenofos, an organophosphate insecticide, is being used on a variety of crops including wheat, cotton, maize, potato, soybean, and sugar beet. It is primarily used against lepidopteran insects as well as against wheat and cabbage aphids. Extensive use of this pesticide has increased its content in the soil and also in aquatic bodies in rural areas of India. The present study is focused on lethal effects of profenofos on rohu fish, being cultivated in and around Jabalpur and changes thereof in the fish hematology. The *in vitro* study will come up with knowledge of toxic values of the pesticide and will help the fish cultivators of the rural area of Jabalpur.

Introduction

Fisheries in India are a very important economic activity and a flourishing sector with varied resources and potentials. Only after the Indian Independence, has fisheries together with agriculture been recognized as an important sector. The vibrancy of the sector can be visualized by the 11-fold increase that India achieved in fish production in just six decades. As the second largest country in aquaculture production, the share of inland fisheries and aquaculture has gone up from 46 percent in the 1980s to over 85 percent in recent years in total fish production. Freshwater aquaculture showed an overwhelming ten-fold growth from 0.37

million tonnes in 1980 to 4.03 million tonnes in 2010; with a mean annual growth rate of over 6 percent. Freshwater aquaculture contributes to over 95 percent of the total aquaculture production. The freshwater aquaculture comprises of the culture of carp fishes, culture of catfishes, freshwater prawns, pangasius, and tilapia. Thus, the production of carp in freshwater from the bulk of major areas of aquaculture activity. The three Indian major carps, namely catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) contribute the bulk of production to the extent of 70 to 75 percent of the total fresh water fish production (Ayyappan, 2014).

In rural areas, the carp culture is mainly done in natural ponds as well as in artificial ponds made in the agricultural fields. Intensive uses of pesticides and insecticides in agriculture has shown its side effects as the agricultural runoff or the water from the fields, that drains into the water bodies or aquaculture ponds is polluting the water with excessive amounts of pesticides and insecticides. The higher levels of such pollutants make the water unsuitable for aquaculture as well as for other recreational purposes (Dunier and Siwicki, 1993). Profenofos is an organophosphate insecticide, largely used against Cotton MealyBug, cabbage caterpillar, *Plutella xylostella* and asparagus caterpillars, as well as against wheat and cabbage aphids (US EPA, 2015). Like other organophosphates, the profenofos mechanism of action is via the inhibition of the acetyl cholinesterase enzyme.

The organophosphate insecticides have been shown to exert lethal effects on some species of fishes and other aquatic fauna (Bacchetta *et al.*, 2014). Since rohu is one of the major carp and economical backbone of the aquaculture industry in India, the present paper is oriented towards studying the lethal effects of propenofos on vital organs of the rohu fish (*Labeo rohita*) in laboratory conditions in order to understand the deleterious effects of the propenofos.

Materials and Methods

Collection and preparation of experimental fishes

The test fishes (*L. rohita*) were collected from local aquaculture pond in the city of Jabalpur (India). Living and healthy *Labeo rohita* of body size of 10 ± 1 cm and body weight of 30 ± 2 g were chosen for the study. The fishes were kept in glass aquaria containing 25 L of ground water, with continuous aeration through aquaria pumps. Fishes were treated

with 0.01% potassium permanganate solution to obviate dermal infections. The fishes were fed with commercially available fish food and acclimatized for 15 days before starting the experiment.

Exposure to profenofos

For the experiments on exposure of profenofos, the method of Jhingran (2007) was adopted. Profenofos 98% [O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] was purchased from the local market, manufactured by Excel Crop Care Ltd, Mumbai. The fishes were divided into groups, having 10 fishes in each group. The first group served as a control and received no insecticide. The other groups received different concentrations of propenofos. The fingerlings of *Labeo rohita* were exposed to the 6 concentrations of profenofos i.e., 0.2, 0.4, 0.6, 0.8, 1.0 and $1.2 \mu\text{g L}^{-1}$ (Ghazala *et al.*, 2016, Singh and Alam, 2016). Fish were fed daily with commercial diet at the rate of 3 % of their body weight in two fractions at an interval of 8 hours. The fish blood was collected at 96 hours for the haematological experiments.

Haematological tests

The fish collected every 24 hours was immediately processed for the determination of vital haematological parameters such as haemoglobin (Hb), RBC count, WBC count and haemtocrit (Hct) using standard haematological procedures.

Determination of LC₅₀ values

The 96 hour 50% lethal concentration (LC₅₀) was calculated using log of the concentration verses mortality rate at different time intervals and fitting a non-linear regression curve using Sigma Graphpad Prism[®] software, version 6.0.

Results and Discussion

The *in vitro* experiments for exposure of *Labio rohita* with different concentrations of profenofos showed higher mortality rates after 96 hours in higher concentrations. The LC50 values obtained by fitting a non-linear regression dose response curve (Fig 1) was found to be 0.62 µg L⁻¹ with a range of 0.58 to 0.67 µg L⁻¹ (Table 1).

The haematological parameters were tested for the fishes survived 72 hours or more under influence of various dosages of profenofos. At least 3 fishes were used for the haematological analysis using standard methods. Table 2 shows that the haemoglobin

percentage decreased from 10.2 ± 1.10 to 7.9 ± 0.56. The dosage of 0.4 µg L⁻¹ and higher showed significant differences in haemoglobin concentration in test fishes. Similar effects were shown by RBC count as well as haematocrit (packed cell volume). The RBC count reduced from 3.41 ± 0.56 to 2.01 ± 0.31 x 10⁶ cells µl⁻¹ while haematocrit was reduced to 27.6 ± 1.10 to 22.1 ± 0.31 %. The WBC count was also affected by increasing concentrations of profenofos. The WBC count increased from 4.73 ± 0.32 to 6.24 ± 0.61 x 10³ cells µl⁻¹. Again, the profenofos concentration higher than 0.4 µg l⁻¹ produced significant increases in WBC count as shown by one way ANOVA test in comparison to control.

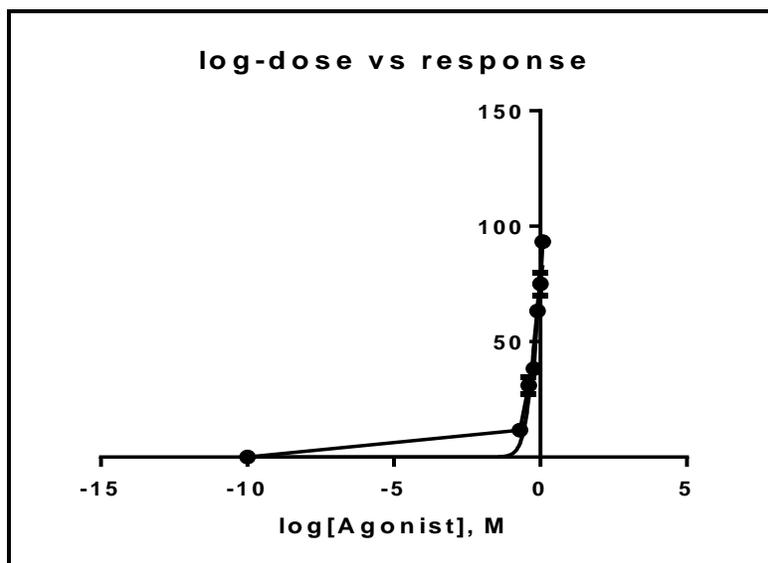
Table.1 Analysis of LC50 values of profenofos on *Labio rohita* under in vitro conditions

S.No.	Particulars	Values
1.	LogLC50	-0.2049
2.	Slope	2.445
3.	LC ₅₀	0.62
4.	Upper limit	0.6747
5.	Lower limit	0.5768
6.	Degrees of Freedom	19
7.	R square	0.9561

Table.2 Haematological parameters of *Labio rohita* exposed to different concentrations of profenofos under in vitro conditions. The data are presented as mean ± standard deviation (n=3). The values marked with asterisk are significantly different from control using one way ANOVA (p>0.05)

Concentration of profenofos (µg L ⁻¹)	Haematological parameters (mean ± SD)			
	Haemoglobin (%)	RBC count (x10 ⁶ cells µl ⁻¹)	Haematocrit (%)	WBC Count (x10 ³ cells µl ⁻¹)
(Control)	10.2 ± 1.10	3.41 ± 0.56	27.6 ± 1.10	4.73 ± 0.32
0.2	10.1 ± 0.82	3.20 ± 0.67	26.7 ± 0.98	4.81 ± 0.34
0.4	9.6 ± 0.75*	3.05 ± 0.23*	25.9 ± 0.76*	4.90 ± 0.29*
0.6	9.2 ± 1.02*	2.82 ± 0.34*	25.8 ± 0.67*	5.21 ± 0.39*
0.8	8.7 ± 0.92*	2.39 ± 0.39*	24.8 ± 0.52*	5.34 ± 0.54*
1	8.1 ± 0.45*	2.34 ± 0.29*	23.7 ± 0.43*	5.78 ± 0.67*
1.2	7.9 ± 0.56*	2.01 ± 0.31*	22.1 ± 0.31*	6.24 ± 0.61*

Fig.1 Dose response curve showing mortality rate of *Labio rohita* against various doses of profenofos under in vitro conditions



The organophosphates, i.e. profenofos are modern synthetic insecticide and are potent neurotoxic molecules (Lundbaya *et al.*, 1997). The environmental risk assessment of any given pesticide/insecticide depends on its toxicity to the fish and other organisms. Profenofos is one of the organophosphates that are widely used in India for the agricultural purposes. The 50% lethal concentration (LC50) is the parameter that shows the toxicity and is considered to be the preliminary step for studies into the extent of acute or chronic toxicity. Our results showed a 96 hour LC50 value of $0.62 \mu\text{g L}^{-1}$ for *Labio rohita* fingerlings. Singh and Ansari (2016) showed a 96-h LC50 value of profenofos for zebrafish as 0.388 L^{-1} respectively. The results are close and the differences could be due to the greater body size of *L. rohita* and *in vitro* conditions of the experiments. At 96 h, median lethal concentrations of profenofos were 0.31 mg/L (0.26-0.38) in another major carp *Catla catla* (Ghazala *et al.*, 2014). The present study showed remarkable toxic effects of profenofos on *L. rohita*, which may be one of the reasons of fish mortality due to higher concentrations of the insecticide. Shrafeldin *et al.*, (2015) showed highly significant increase in WBCs counts during both the acute and chronic exposure to

profenofos on Nile tilapia *Oreochromis niloticus*. Highly significant decrease in RBCs counts, Hb content and Hct % was noticed during experimental periods on Nile tilapia. Similar observations were mentioned after exposure of Nile tilapia to organophosphate pesticides (Ibrahim *et al.*, 2005; El-Sayed *et al.*, 2007; El-Sayed and Saad, 2008). Such increase in leucocyte counts is believed to be the alteration in defence mechanism. Oppositely, leucopenia was observed in common carp, *Cyprinus carpio* exposed to sublethal concentration of profenofos which was explained as the malfunction of the hematopoietic system caused by toxicant stress (Marie *et al.*, 1998). It seems that tilapia may be more resistant than carp. It can be concluded that profenofos is highly toxic to the aquacultured carps and exert severe haematological changes in fishes.

In conclusion, the rural area of Jabalpur, which regularly culture rohu, as an edible carp should be aware of the uses of profenofos in their fields as well as the fields nearby the aquaculture ponds. Awareness on restricting the uses of organophosphate insecticide will be helpful in aquaculture practices.

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