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Sublethal effect of Ethephon pesticide on carcass composition of rosy barb, *Puntius conchonius* (Hamilton, 1822)

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Abstract
Experiment on bioassay was undertaken to find out the toxic effect of ethephon on proximate composition of rosy barb (*Puntius conchonius*). The 96 hrs LC_{50} of ethephon for rosy barb fish was found to be 64.034 mgl^{-1}. During sublethal exposures for a period of 28 days, two concentrations were selected i.e. $1/10^{th}$ and $1/5^{th}$ of LC_{50} such as of 12.81 and 6.40 mgl^{-1}. At the end of exposure periods of 7^{th}, 14^{th}, 21^{st} and 28^{th} days i.e., after every seven days, ten fingerlings were sacrificed for carcass biochemical analysis such as moisture content, crude protein, crude fat, total ash content and carbohydrate. Crude protein significantly decreased from 60.08 ±0.05 on 1^{st} day to 53.38±0.05 on 28^{th} day; crude fat significantly decreased from 10.07 ± 0.05 on 1^{st} day to 8.08±0.02 on 28^{th} day of the study. Whereas, carbohydrate increased from 10.06±0.02 on 1^{st} day to 12.02±0.02 on 28^{th} day, ash content increased significantly from 9.94 ±0.05 on 1^{st} day to 11.08±0.01 on 28^{th} day and moisture content also increased from 9.79 ± 0.03 to on 1^{st} day to 12.30±0.02 on 28^{th} day respectively. All the estimated parameters showed significant alteration during exposure period indicated toxicity of ethephon pesticide to the rosy barb fish.

Keywords: Ethephon, rosy barb, carcass composition, toxicity

1. Introduction
Ethephon [(2-chloroethyl) phosphonic acid] (ETF) is a major plant growth regulator (PGR) that promotes fruit ripening, abscission, flower induction, and other responses by releasing ethylene gas, a natural plant hormone [20, 7, 26, 9] which spontaneously decomposes at physiological pH [27]. In India, it is being extensively used to accelerate the post-harvest ripening of bananas, mangoes, pineapples etc. Unlike, other four classes of plant hormones, ethylene diffuses easily through the air from one plant to another and its residual effect in soils remains and come to the aquatic environment during rainy period [22].
Cyprinids are commonly bred as aquarium fish, but also as model fish for scientific research. These include the zebra fish (*Danio rerio*), rosy barb (*Puntius conchonius*), Indian medaka (*Oryzias melastigma*), Gambusia (*Gambusia affinis*), Guppy (*Poecilia reticulata*) etc. [12, 21, 5]. The rosy barb is found in natural conditions in the tropical waters of south-east Asia, including: Afghanistan, Pakistan, Nepal, India and Bangladesh. The popularity of domestic aquarium fish, including the rosy barb, is caused both by its attractive colouring and ease of breeding [4, 5, 10, 11, 17]. In addition rosy barb proved to have a high sensitivity to agrochemicals and therefore selected as a potential model fish for present study.

2. Materials and Method
2.1 Chemicals
Ethephon used in the experiments was purchased from local agriculture fertilizer shop in Ratnagiri of Vallabh pesticide Ltd (Gujrat, India). All other chemicals were analytical reagents.

2.2 Animals and treatments
Adult rosy barbs *P. conchonius* (Average body weight 2.55 ± 0.03 g) obtained from a local fish dealer were maintained in dechlorinated water at 25±1ºC. The fishes were fed twice in every 24 hrs with commercial feed and acclimatized for two weeks before exposure to the test pesticide.

A stock solution of the chemical was prepared having the strength of 1000 mgl^{-1} by adding the known quantity of pesticide in 1000 ml distilled water. Calculated amount of stock solution was added to the water and mixed thoroughly to arrive at a required level of working concentrations.

2.3 Lethal toxicity (LC_{50}) study

Lethal toxicity (LC_{50}) study was carried out by following the standard guidelines [7]. To determine the LC_{50} of ethephon in rosy barb for 96 hrs by probit analysis method [8]. A total of 20 rosy barbs per group were exposed to the concentrations of ethephon in 40 litre glass tanks having dechlorinated water for 96 hrs. ethephon was always freshly dissolved in appropriate volume of water as a stock solution, and added immediately into the test solutions. After every 24 hrs, the dead animals were removed and the surviving ones were recorded.

2.4 Sublethal Experiment

During sublethal exposures for a period of 28 days two concentrations were selected i.e. $1/10^{th}$ and $1/5^{th}$ of LC_{50}, such as 6.40 and 12.81 mgl^{-1}. At the end of every seven days exposure periods i.e. 7^{th}, 14^{th}, 21^{st} and 28^{th} days, ten fingerlings were sacrificed for carcass biochemical analysis such as moisture content, crude protein, crude fat, total ash content and carbohydrate content.

2.5 Chemical Analysis

The protein content in the sample was estimated by the method of Lowry *et al.* (1951), the crude fat was determined by using the method described by Sadasivam and Manickam (1997). The AOAC (1995) method was adopted for determination of carbohydrate, moisture and ash contents.

2.6 Statistical Analysis:

The LC_{50} value was estimated by probit analysis method [8]. In the sublethal testing, significance of differences was tested using one way ANOVA (Analysis of variance). Duncan multiple range test was used to check the significant differences ($P< 0.05$) among the control and treatments.

3. Results

3.1 Experiment 1: Lethal toxicity (LC_{50}) experiment

Initially, a range finding test was conducted to ascertain the range of ethephon pesticides to be selected in the definitive test. During the trial, the test animals were exposed to a broad range of concentration on logarithmic scales (0, 0.001, 0.01, 0.1, 1.0, 10.0, and 100 mgl^{-1}). In narrow-range finding test of ethephon on rosy barb, the mortality percentage between 10% to 100% was considered. The results of 96 hrs median lethal concentration value for rosy barb was calculated and presented in Figure 1. The LC_{50} value of ethephon for rosy barb was found to be 64.034 mgl^{-1} at 96 hrs.

3.2 Experiment 2: Sublethal experiment:

In Sublethal experiment, two concentrations such as 6.40 and 12.81 mgl^{-1} was selected i.e. $1/10^{th}$ and $1/5^{th}$ of LC_{50} for a total period of 28 days. Sublethal effect of ethephon pesticide on carcass composition of rosy barb, *Puntius conchonius* is given in Table 1and the details are as follows;

3.2.1 Crude protein

The crude protein percentage in ethephon exposed rosy barb showed decreasing trend. Control groups did not showed any significant change in crude protein i.e. 59.53±0.02, 59.62±0.01, 59.62±0.02 and 60.21±0.004 on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively. In 6.40 mgl^{-1} ($1/10^{th}$ concentration of LC_{50}), the protein percentage significantly decreased from 60.08 ±0.05 on 1^{st} day to 58.50±0.01 on 7^{th}, 57.39±0.01 on 14^{th} day, 57.04±0.01on 21^{st} and 56.10±0.03 on 28^{th} day. In higher concentration of 12.81 mgl^{-1} ($1/5^{th}$ concentration of LC_{50}), protein percentage significantly ($P< 0.05$) decreased to 56.37±0.02 on 7^{th}, 55.75±0.02 14^{th}, 54.06±0.01 on 21^{st} and 53.38±0.05 on 28^{th} day. Fishes exposed to 12.81 mgl^{-1} concentrations showed significantly reduction in protein percentage than fishes exposed to 6.40 mgl^{-1} concentration during the study period.

3.2.2 Crude Fat

The crude fat content significantly decreased from 10.07 ± 0.05 on 1^{st} day regard to increased concentration i.e. 10.04±0.01 (control), 9.85±0.012 (6.40 mgl^{-1}) and 9.11±0.027 (12.81 mgl^{-1}) on 7 day; 10.07±0.02 (control), 9.64±0.01 (6.40 mgl^{-1}) and 8.87±0.18 (12.81 mgl^{-1}) on 14 day and 10.09±0.021 (control), 9.35±0.010 6.40 mgl^{-1}) and 8.75±0.01 (12.81 mgl^{-1}) on 21 day exposure and 10.11±0.02(control), 8.86±0.012 (6.40 mgl^{-1}) and 8.08±0.02 (12.81 mgl^{-1}) on 28 day exposure. Fishes exposed to 12.81 mgl^{-1} concentration showed significantly ($P<0.05$) lower fat percentage than fishes exposed to 6.40 mgl^{-1} concentration during the study period.

3.2.3 Carbohydrate

The exposed fishes to ethephon showed significant ($P<0.05$) increase in the carbohydrate as compared to initial value (10.03±0.01). The carbohydrate was recorded lower in control groups throughout the study period i.e. 10.06±0.02, 10.09±0.02, 10.09±0.01 and 10.18±0.03 on 7^{th}, 14^{th}, 21^{st} and 28^{th} days respectively. In 6.40 mgl^{-1} ($1/10^{th}$ concentration of LC_{50}), the carbohydrate increased from 10.24±0.01 on 7^{th} day, 10.28±0.01 on 14^{th} day, 10.85±0.01 on 21^{st} and 11.01±0.02 on 28^{th} day. In higher concentration of 12.81 mgl^{-1} ($1/5^{th}$ concentration of LC_{50}), the carbohydrate further increased from 10.54±0.02 on 7^{th} day, 10.96±0.01 on 14^{th} day, 11.15±0.01 on 21^{st} day and 12.02±0.02 on 28^{th} day.

3.2.4 Ash

The total ash content showed an increasing trend with regard to increase concentrations (Table 1) i.e. from 9.94 ±0.05 on 1^{st} day to 10.06±0.01 (control), 10.24±0.01 (6.40 mgl^{-1}) and 10.42±0.01 (12.81 mgl^{-1}) on 7^{th} day; 10.07±0.01 (control), 10.33±0.01 (6.40 mgl^{-1}) and 10.62±0.02 (12.81 mgl^{-1}) on 14^{th} day and 10.06±0.00 (control), 10.48±0.01 (6.40 mgl^{-1}) and 10.90±0.02 (12.81 mgl^{-1}) on 21^{st} day exposure and 10.09±0.01 (control), 10.68±0.01 (6.40 mgl^{-1}) and 11.08±0.01 (12.81 mgl^{-1}) on 28^{th} days exposure.

3.2.5 Moisture

The moisture content showed an increasing trend with regard to increased concentration (Table 1) i.e. from 9.79 ± 0.03 on 1^{st} day to 10.32±0.02 (control), 10.51±0.18 (6.40 mgl^{-1}) and 9.93±0.023 12.81 mgl^{-1}) on 7^{th} days; 10.13±0.02 (control), 11.35±0.01 (6.40 mgl^{-1}) and 11.09±0.20 12.81 mgl^{-1}) on 14^{th} day and 10.12±0.03 (control), 11.50±0.03 (6.40 mgl^{-1}) and 11.81±0.02 12.81 mgl^{-1}) on 21^{st} day and 9.40±0.043 (control), 12.04±0.02 (6.40 mgl^{-1}) and 12.30±0.02 (12.81 mgl^{-1}) on 28^{th} day exposure.

4. Discussion

In present study, LC_{50} value for rosy barb exposed to ethephon estimated was 64.034 mgl^{-1} at 96 hrs while the LC_{50}

value for rosy barb exposed to nonylphenol was 1.72 mgl $^{-1}$ [3] and for copper and zinc were 0.5mgl^{-1} and 9 mgl^{-1} to *Puntius parrah* [6]. The results of proximate composition of different experimental groups of fish rosy barb exposed to two sublethal concentrations of ethephon for a period upto 28 days (12.81 and 6.40 mgl^{-1}) showed significant reduction in crude protein and crude fat contents of muscle. It can also be seen that the increasing value of carbohydrate, ash and moisture was proportional to the concentration of ethephon and exposed period. The present investigation indicated the utilization of all these energy components when fish is under stress. The carcass composition at the end of the 28 days exposure period between control fish and those of exposed to various sublethal concentrations was significantly differed.

Behavioural responses of fish exposed to sublethal concentration of ethephon showed that they were under stress conditions. During stress condition, fish needed more energy to detoxify the toxicants and to overcome stress. Similar results were reported by Palanichamy *et al.* (1986). They have reported that the moisture and ash levels were significantly increased and the crude protein and crude fat contents were significantly decreased at various sublethal concentrations of monocrotophos. Other such worker as Malla Reddy and Bashamohideen (1995); Singh *et al*. (1996) have also reported the decline in protein constituent in fish muscle exposed to sublethal concentrations of insecticides. Singh and Singh (2011) revealed about the depletion of protein content when *Trichogaster fasciatus* exposed to an organophosphate dipterex. Palaniappan *et al.* (2009) observed a decrease in the nutritive value of muscle of *Catla catla* fingerlings with an increase in ash and moisture content at sublethal concentrations of lead. Present study revealed that significant increase in carbohydrate content compared to initial value and control group. Similar results were observed by Amin and Indulkar (2017) when common carp exposed to imidacloprid pesticide. Stoner and Livingston (1978) reported significant decrease in protein, fat with an increase in carbohydrate is direct result of significantly reduced food consumption and food conversion efficiency as was observed in pinfish (*Lagodon rhomoides*) exposed to sublethal concentrations of bleached Kraft mil effluent and in rohu juveniles exposed to sublethal concentration of phenol [1].

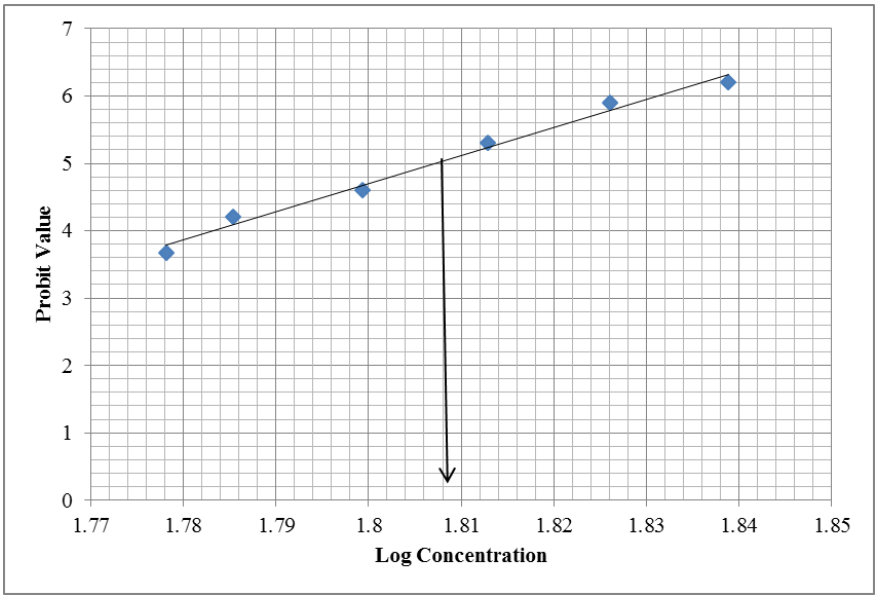

Fig 1: Graphical representation of 96h LC_{50} of Ethephon concentration in rosy barb

Table 1: Proximate composition of rosy barb (*Puntius Conchonius*) exposed to sublethal concentration of ethephon

	Initial	Conc. (mgL^{-1})	Days			
			7th Day	14th Day	21st Day	28th Day
Crude Protein	60.08 ±0.05^{a}	Control	59.53±0.02^{a}	59.62±0.01^{a}	59.62±0.02^{a}	60.21±0.004^{a}
		1/10th	58.50±0.01^{b}	57.39±0.01^{c}	57.04±0.01^{cd}	56.10±0.03^{d}
		1/5th	56.37±0.02^{b}	55.75±0.02^{c}	54.06 ±0.01^{d}	53.38±0.05^{e}
Crude Fat	10.07 ± 0.05^{a}	Control	10.04±0.01^{a}	10.07±0.02^{a}	10.09±0.021^{a}	10.11±0.02^{a}
		1/10th	9.85±0.012^{b}	9.64±0.01^{c}	9.35±0.010^{d}	8.86±0.012^{e}
		1/5th	9.11±0.027^{b}	8.87±0.18^{c}	8.75±0.01^{d}	8.08±0.02^{e}
Carbohydrate Content	10.03±0.01^{a}	Control	10.06±0.02^{a}	10.09±0.02^{a}	10.09±0.01^{a}	10.18±0.03^{a}
		1/10th	10.24±0.01^{b}	10.28±0.01^{bc}	10.85±0.01^{c}	11.01±0.02^{d}
		1/5th	10.54±0.02^{b}	10.96±0.01^{c}	11.15±0.01^{d}	12.02±0.02^{e}
Ash Content	9.94 ±0.05^{a}	Control	10.06±0.01^{a}	10.07±0.01^{a}	10.06±0.00^{a}	10.09±0.01^{a}
		1/10th	10.24±0.01^{b}	10.33±0.01^{c}	10.48±0.01^{d}	10.68±0.01^{e}
		1/5th	10.42±0.01^{b}	10.62±0.02^{c}	10.90±0.02^{d}	11.08±0.01^{e}
Moisture Content	9.79 ± 0.03^{a}	Control	10.32±0.02^{a}	10.13±0.02^{a}	10.12±0.03^{a}	9.40±0.043^{a}
		1/10th	10.51±0.18^{b}	11.35±0.01^{c}	11.50±0.03^{d}	12.04±0.02^{e}
		1/5th	9.93±0.023^{b}	11.09±0.20^{c}	11.81±0.02^{d}	12.30±0.02^{e}

The value expressed as mean ± S.E. (n=7). #Different superscripts (a, b, c, d, e) in the same row indicate significant difference (P<0.05).

5. Conclusion

The results of the present investigation revealed that ethephon has the harmful impact on the proximate composition of rosy barb in both sublethal concentrations. crude protein content, crude fat content, moisture content and total ash content showed sensitivity to the sublethal exposure of ethephon over a period of 7th days. The crude protein and crude fat content decreased while moisture and total ash contents increased with increased concentration and exposure period. It, thus, evident from the present studies that increase in concentrations and exposure period of ethephon has adversely affected the carcass composition of the rosy barb (*Puntius conchonius*).

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