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Original Article

Effects of Chlorpyrifos on proximal characteristics of *Labeo rohita fish* in acute and chronic exposure

Adeeba Syed[1], Sumaira Mazhar[1*], Uzma Rafi[1*] and Dilawar Hussain [2]

[1] Department of Biology, Lahore Garrison University, Phase-VI, DHA, Lahore, Pakistan
[2]Department of Zoology, Government College University, Lahore, Pakistan

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***Corresponding Author:**
Sumaira Mazhar
smz.mmg@gmail.com,
Uzma Rafi
uzmazeeshan@lgu.edu.pk

ABSTRACT

Objective: The aim of present was to observe the proximal characteristics of a very famous fresh water fish commonly called as rohu(*Labeorohita*) with acute and chronic exposure of pesticide,Chlorpyrifos (CPF).Method: To check acute exposure, concentrations of CPF used include, 0, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03, 0.04 and 0.05 mg/L for 96 hrs in glass aquaria. For *Labeorohita*, 96 hrs LC_{50} value of CPF was found 0.01 mg/L. To study chronic exposure on *Labeorohita*, 1/3rd, 1/5th, 1/7th and 1/9th of LC_{50} for 30 days concentrations were used. At the end of the experiment, samples were collected for proximal analysis. The parameters included moisture, ash, lipids and protein **Results:** The exposure of CPF reduced the protein content. Moreover, there was reduction in ash and moisture contents. **Conclusions:** It is therefore concluded that CPF adversely affects the major organs of the fish *Labeorohita*.

INTRODUCTION

Chemical burden has been increased on agricultural land throughout the world due industrialization[1]. Pesticides are the destructive chemicals which are released in the environment. Contamination caused b the pesticides in water reservoirs is a serious predicament [2]. Usually, pesticides are genotoxic [3] and their genotoxicity is a worldwide problem [4].The result of agricultural and industrial activities causes increase in pollution specially affecting the aquatic environment. This is due to the fact that toxic chemicals are discharged with waste waters and agricultural drainage[5].Majority of the waste becomes a part of ocean currents, bottom topography, open cage net pens and fish farms, wild fish tends to feed on this waste [6].In extreme conditions, the pesticide containing runoff can kill fish to a larger number [7]. The effects of pesticides are manifested in reduced inland fisheries [8]. Organophosphates were previously introduced as alternatives of organochlorines.Hundreds of organophosphates are being used as pesticide [9].Chlorpyrifos (O,O diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) commonly known as CPF is a non-systemic and broad spectrum pesticide. It is one of the earliest developed organophosphate[10].Non persistence of compounds like CPF in aquatic reservoirs have shownserious concerns about their potential drastic affects on non-targeted organisms found in water like fish[11].It has been reported earlier that Chlorpyrifos is highly toxic to fish [12].Fish are good examples of test animals which are to be used to test the effects of water born pollutants because these organisms can store, metabolize and concentrate these compounds. The effects of Chlorpyrifos has been studied earlier on many types of fish such

METHODS

Maintenance of Experimental Fish:For the experiment, healthy fingerlings of Rohu fish (*Labeorohita*) were obtained from Fish Hatchery, Manawa, Lahore. It was kept in clean glass aquaria having capacity of 110 L each in fish experimental rooms, animal house of Department of Zoology, Government College University, Lahore. Fifteen days before the start of experiment, the specimens were disinfected with table salt and Potassium Permanganate to prevent fungal infection. A good quality commercial food was used to feed the fish. Tap water was changed on daily basis with physiological parameters maintained (pH 7.3 and temperature 32 C). Chlorpyrifos Acute Exposure: To study the effects of acute exposure of Chlorpyrifos, four different concentration were used(0.008, 0.009, 0.01 and 0.02 mg/L). These concentrations were prepared in equal sized aquaria. One aquaria was used as control. The aquaria contained 10 litres of water and 15 individual fish were added containing CPF. Fish were exposed for 96 hours. Dead fish were removed from aquaria and were observed up to fourth day. Profit analysis was used to determine mortality percentage. The experiment was performed in triplicate. Chlorpyrifos Chronic **Exposure:** To study chronic exposure, fish samples were randomly divided in five groups, where first group was used a control. Control group was labeled as T1 and experimental groups were labeled as T2, T3, T4 and T5 respectively. Each aquarium contained 40 litres water and 20 individual fish specimens. $LC_{50}$was found to be 0.01 mg/L, 1/3rd, 1/5th, 1/7th and 1/9th of LC_{50} were used.The exposure was continuous for 30 days. Samples were collected at the end of experiment. Sampling:Proximate Analysis of Whole Body of Fish (Protien, Lipids, Ash and Dry Matter):50 gram of sample was collected (containing 6 t0 7 fish individuals) and sent to the Quality Control Lab of Manawa Fish Hatchery, Lahore for the complete Proximate analysis of whole body of fish. **Proximate Composition:** Proximate analysis of *Labeorohita*under various treatments was assessed by following AOAC (1990). **Estimation of Moisture Contents:** To determine the moisture content on less than 100 mm/Hg, fresh fish sample (0.5 g for each treatment) was crushed and immediately dried in oven at 67-70°C for 24 hour. Moisture contents were calculatedby the following formula:

$$\text{Moisture \%} = \frac{\text{Loss in weight of sample} \times 100}{\text{Weight of sample}}$$

Extraction of Protein:

By automatic analyzer (Kjeldahl'smethod), total nitrogen contents were observed. In concentrated H_2SO_4, K_2SO_4 and $CuSO_4$ fresh fish samples (0.5 g for each treatment)were digested in digestion tube, temperature

Proximate Composition (PC): Moisture Contents:Moisture contents were recorded as 81.327±2.003, 84.363±0.425, 86.863±0.210, 80.660±0.43 and 84.483±0.285 in T_1, T_2, T_3, T_4 and T_5 experimental groups, respectively (Table 1 and Figure 1A). Maximum and minimum moisture contents were gradually increased (50-374°C), the end result of digested samples were dark or bluish greenish in colour that is Ammonium Sulphate, and then distilled the sample at Kjeldahl's apparatus. After that the sample was titrated with boric acid as indicator with greenish end point.

Fatanalysis: By using Soxlet apparatus, fats were extracted. Each sample (0.5g) was taken in extraction thimble. In 250 ml of round bottom flask, 12 ml of n-hexane was taken, after that thimble putted in extractor and whole assembly was fitted for extraction of oils. The extraction was preceded for 16 cycles to collect Crude oil.By using rotary evaporatorhexane was separated from the oil. In a round bottle flask, fats and hexane oil were heated in bath tub at 65°C, round flask rotating at the speed of 40 cycles per minute and ultimately hexane was distilled away, by using glass pipette oil and fats were sucked. Remaining hexane removed by Nitrogen generator and pure oil was obtained. **Estimation of Ash Contents:** For the estimation of ash contents 0.5g of samples were collected into weighed Crucibles. In electric furnace (at 450°C) these crucibles were placed for 12 hours until white ash was formed.Following formula were used to calculate the Ash Contents.

$$\text{Ash \%} = \frac{\text{Loss in weight of sample} \times 100}{\text{Weight of sample}}$$

Statistical analysis: The data of the present study has been given in the table form as mean ± Standard Deviation. One-way ANOVA test was applied to the data. It was followed by least significant difference test, the Tuckey Test to find out significant variations between dietary levels and mean values.

RESULTS

observed in T_3 and in T_4 experimental groups, respectively. Analysis of Variance (ANOVA) showed that the differences were remained statistically significant ($P< 0.05$) for moisture contents in T_1 with remaining experimental groups. Whereas, remaining four groups T_2 to T_5 have non-significant difference.**Total Ash Contents:**Ash contents were recorded as in T_1, T_2, T_3, T_4 and T_5 experimental groups, respectively (Table 1 and Figure 1B). Analysis of Variance (ANOVA) showed that the difference remained statistically highly significant ($P< 0.01$) for ash contents in all experimental groups. Comparison of means through Least Significant difference (Tuckey test) revealed that T_1, T_2 and T_5 were statistically similar ($P>0.05$), whereas, there was a significant difference Protein Contents:Protein contents were recorded as 12.717±0.448, 10.873±0.535, 9.7600±0.494, 10.413±1.023 and 9.017±0.416 in T_1, T_2, T_3, T_4 and T_5 experimental groups, respectively (Table 1 and Figure 1C). Maximum and minimum protein contents were observed in T_1 and T_5 experimental

groups, respectively. Analysis of Variance (ANOVA) showed that the differences were remained statistically non-significant (P> 0.01) for protein contents in all experimental groups. Comparison of means through Least Significant difference (Tuckey test) revealed that T_2 and T_3, T_3 and T_4 were statistically similar (P>0.05), whereas, there was a significant difference between T_1, T_2 and T_5.

Total Fat/Lipids Contents: Total fat contents were recorded as 3.636±0.030, 2.586±0.290, 2.960±0.170, 3.413±0.265 and 2.210±0.08 in T_1, T_2, T_3, T_4 and T_5 experimental groups respectively (Table 1 and Figure1D). Maximum and minimum fat contents were observed in T_1 and T_5 experimental groups, respectively. Analysis of Variance (ANOVA) showed that T_1 and T_5 different significantly (P< 0.01) for fat contents from other dietary treatments (T_2, T_3 and T_4). Comparison of means through Least Significant difference (Tuckey test) revealed that T_1 and T_5 showed significant differences (P>0.05) with T_2, T_3 and T_4.

Treatment	Moisture	Ash	Protein	Lipids
T_1	81.327±2.003B	2.0100±1.225B	12.717±0.448A	3.636±0.030A
T_2	84.363±0.425A	2.0000±0.01B	10.873±0.535B	2.586±0.290CD
T_3	86.863±0.210A	0.2400±0.053C	9.7600±0.494BC	2.960±0.170BC
T_4	80.660±0.43A	5.0000±0.458A	10.413±1.023BC	3.413±0.265AB
T_5	84.483±0.285A	3.560±0.505AB	9.017±0.416C	2.210±0.08D

Table 1: Proximate composition of *Labeorohita* under various treatments of Chlorpyrifos
Values sharing similar letters in rows are statistically non-significant (P>0.05).

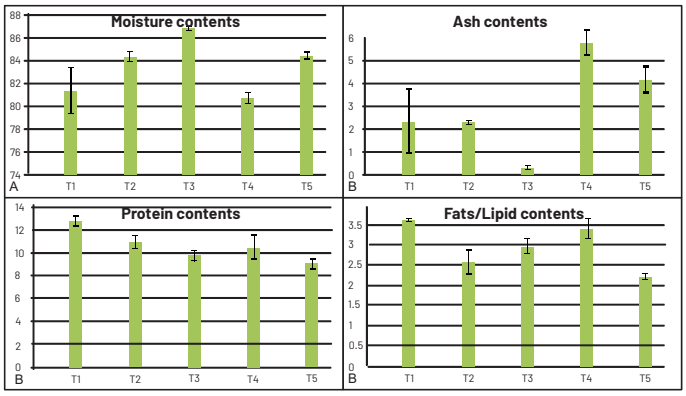

Figure 1A: Graph showing Moisture Contents in different experimental groups with Chlorpyrifos, 1B: Ash Contents in different experimental groups with Chlorpyrifos, 2C: Protein Contents in different experimental groups with Chlorpyrifos, 2D: Fats/Lipids Contents in different experimental groups with Chlorpyrifos

DISCUSSION

Proximate composition (protein, lipid, moisture and ash) of muscle tissue was determined according to the procedures of AOAC (1990). A number of factors including food, space, chemicals, season, temperature, salinity and physical activity are known to affect fish growth. Proximate analysis of parameters indicating nutritional quality and energy content are good indicators of physiological condition of fish [14]. Protein, lipids, moister and ash (proximate composition) was determined as reported by AOAC (1990). Fish growth is dependent upon several factors like temperature, salinity, weather, physical activity, space, chemicals and food. Nutritional quality and energy content, as shown by proximate analysis of these parameters, are good indicators of the physiological condition of a fish [15]. In the results of proximal composition, moisture contents in T_1 81.327%, T_2 84.363%, T_3 86.863%, T_4 80.660% and T_5 84.483%. In dry Ash content T_1 2.0100%, T_2 2.0000%, T_3 0.2400%, T_4 5.0000% and T_5 3.560%. In protein content T_1 12.717%, T_2 10.873%, T_3 9.7600%, T_4 10.413% and T_5 9.017%. In lipid/fat contents T_1 3.636, T_2 2.586, T_3 2.960, T_4 3.413 and T_5 2.210%. The result showed that the moisture contents were increased as the concentration of CPF increased. In case of dry ash T_4 (5.0000%) has highest value of ash and T_3 (0.2400%) has the lowest value of ash. Protein contents are high in T_1(12.717%) and decreased in T_5(9.017%). T_1(3.636) has high value of lipids and T_5(2.210) has lowest value of lipids. In the improvement of health of fish, its growth, development and resistance to diseases, protein play a key role [16,17]. Reduction of protein content my possibly be due to necrosis which results in the impairment of protein synthesis [18] and increased proteolytic activity as a result of pesticides stress in muscles. Skeletal muscle of diploid *Clariasgariepinus*, exposed to 28 µg l–1 CPF decreased protein content significantly, compared to control group. In agreement to our findings, *C. batrachus* when exposed to sub-lethal concentration of CPF for a period of 28 days a decline in protein synthesis was found in different tissues was observed [19]. Food consumption and food conversion efficiency reduced the moister and ash content [20]. The result of the present study shows that CPF has toxic effects on *Labeorohita*. In acute exposure, it affected the protein, moister and ash contents. Chronic exposure has shown drastic effects on nutritional value of *Labeorohita*.

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