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Research article

Behavioral abnormalities in Labeo Rohita Under the Acute Exposure of Organophosphate Insecticide, Chlorpyrifos

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Abstract

Chlorpyrifos (CPF) is a widely used organophosphate pesticide which has an unfavorable impact on the aquatic ecosystem. This work was designed to compute the LC_{50} and lethal concentration (96-hr) of chlorpyrifos for *Labeo rohita*. The LC_{50} and lethal concentration (96-hr) of chlorpyrifos for *Labeo rohita* was determined as 10.39±0.03 and 15.31±0.05 μgL^{-1}, respectively. During acute toxicity test, behavioral changes in *Labeo rohita* were also observed i.e. rapid opercular movement, profuse mucus secretions, imbalance swimming, increased surface activity, loss of equilibrium, convulsion, body discoloration, and decreased hyperactivity. Jumping of fish out of exposed medium proved the avoidance behavior against pesticide toxicity.

Keywords: Acute toxicity, behavior, fish, pesticides

1. Introduction

Environmental pollution by different compounds including pesticides is a basic concern which may have an unfavorable impact on the sensitive ecosystem [1, 2]. In numerous zones, these delicate ecosystems are at a greater risk due to release of pesticides from agricultural and urban sources to waterbodies influencing the aquatic life [3, 4]. An organophosphate pesticide like chlorpyrifos (CPF) is widely applied due to its short term persistence and low amassing ability in the environment.

This insecticide, extensively supplied to kill pests but also has serious hazardous impact on non-target aquatic fauna [5]. Acute tests such asLC_{50} and lethal concentration are commonly applied to determine the tolerance limits of fish against different toxicants[6]. In these assays, data on mortality are use to measure the duration dependent response of different species of fish to chemicals that might be provide useful information regarding to sustainable conservation of major carps in Pakistan. These assays also determined the toxic impact of any test chemical on aquatic fauna in a short time of their life span[7-9] and permit us to evaluate the impacts of different toxicants on the biology of organisms [10] to check their capacities to adjust beneath certain toxicity levels and to estimate conceivable impacts of harmfulness on them [11, 12]. The sudden behavioral changes seen in fish make them perfect subjects for observation, and examination of fish behavior has been a wellknown approach to identify changes in the aquatic ecosystem[13]. Fish are perfect animal for behavioral tests of different stress factors and toxicants (like metals and insecticides) exposure due to their ecological relevance in various natural water bodies[14, 15].

Exposure of chlorpyrifos caused change in the behavior of fish such as erects swimming, vertical hanging, convulsions, equilibrium loss, convulsions [16].fish showed rapid gulping of water and increased opercular movement under acute exposure of chlorpyrifos.

Therefore, this work was performed to observe the behavioral abnormalities in *Labeo rohita* under the acute exposure of chlorpyrifos. Fish behavior is often a sensitive indicator of environmental stress, and alterations in behavior could indicate

shifts in the ecosystem's functioning and stability. This information could prompt policymakers and environmentalists to take action to prevent further degradation and promote ecosystem restoration.

2. Material and Methods

2.1 Fish and acclimatization

The experiment was conducted in limnology laboratory at Fisheries Research Farms, University of Agriculture, and Faisalabad. *Labeo rohita* (Average weight 8.12±0.11) were housed in tank to acclimatized laboratory environment for couple of weeks. No fish were died during this period. In acclimatization, 12-hr light and 12-hr dark photoperiod was maintained.

2.2 Acute Toxicity Assay

Technical grade chlorpyrifos was dissolved in methanol to prepare stock solutions while further dilutions were made by dissolving an appropriate volume of stock solution in deionized water. *Labeo rohita* was exposed to each of the 21 different concentrations (0.75-15 mg/L) of chlorpyrifos to estimate the LC_{50} and lethal concentration for 96-hr. The whole experiment was carried out with triplet in 100-liter water capacity glass aquaria each holding 10 juveniles. All the test and control mediums were supplied with continuous air with an air pump through capillary system.

2.3 Physico-chemistry of water

The total hardness, temperature and pH of water were maintained as 225 mL^{-1}, 30ºC and 7.0, respectively. Other physico-chemical variables such as electrical conductivity, total ammonia, calcium, sodium, magnesium, potassium and carbon dioxide were also determined (A.P.H.A., 2005).

3.3 Behavioral study

The behavioral changes like jumping, equilibrium status, opercula movement, fin movement, erratic swimming, convulsion, skin discoloration, hyperactivity, surfacing activity, mucus secretion, caudal bending in control and treated fish were observed.

3.4 Statistical Analyses

Probit Analysis method (at 95% confidence interval) was applied on mortality data to compute the LC_{50} and lethal concentrations (96-hr) of chlorpyrifos for *Labeo rohita*.

3. Results and Discussion

3.1 Acute toxicity Assay

LC_{50} and lethal concentration (96-hr) of chlorpyrifos for *Labeo rohita* was estimated as 10.39±0.03and 15.31±0.05μgL^{-1}, respectively (Table 1). The fish mortality was increased with increasing concentrations of chlorpyrifos and exposure duration. The chlorpyrifos-methyl toxicity (96-hr LC_{50}) to the tilapia was computed as 1.57 mg/L [18]. The tolerance limit (96-hr LC_{50}) of chlorpyrifos was estimated at 0.160 mg/L [19]. Chlorpyrifos toxicity to *Gambusia affinis* and *Oreochromis moss ambicus*was found to be 0.297 and 0.0259mg/L[20, 21]. The 96-hr LC_{50} of chlorpyrifos for *Puntius chola* was estimated as 0.219 ppm [22]. The 96-hr LC_{50} value of chlorpyrifos for *Labeo rohita* was found to be 0.01 mg/L [23].

3.2 Behavioral study

From both exposed and control groups behavior of fish was observed. Exposed fish showed rapid opercular movement, profuse mucus secretions and imbalance swimming, increased surface activity, loss of equilibrium, body discoloration and decreased hyperactivity and fin movement before the death were observed. Jumping of fish out of exposed medium, which proves the evasion behavior of fish against pesticide toxicity. In the control conditions, no behavioral changes were observed in fish (Table 2). Alterations in the behavior of organisms are the most important sensitive indicator of stress caused by toxicants [24]. In present observation, exposed fish showed rapid opercular movement, profuse mucus secretions and imbalance swimming, increased surface activity, loss of equilibrium, convulsion, body discoloration, caudal bending, increased hyperactivity, jumping and fin movement were observed. The fast opercular movements may be due to increase in mucous over gill surface due to the pollutants[25]. Loss of balance and erratic swimming in *Oreochromis mossambicus* exposed to chlorpyrifos was observed [26, 17] Recorded abnormal swimming, aggressive behaviour,

Table 1 96-hr acute toxicity of chlorpyrifos (μgL^{-1}) for *Labeo rohita*

Fish specie	Pesticide	LC_{50}	95% Confidence Interval	Lethal conc.	95% C.I LCL- UCL	Pearson Goodness of Fit Tests		
						χ^2	DF	p-value
Labeo rohita	CPF	10.39	9.63-11.07	15.31	14.27-16.87	5.16	18	0.999

CPF=Chlorpyrifos; Lethal Concentrations=Lethal Conc. (mgL^{-1}); Chi-Square=χ^2; Degree of Freedom =DF.

Table 2Effect of chlorpyrifos on behavior of *Labeo rohita*under the acute exposure

Concentrations μgL^{-1}	Erratic swimming	Jumping	Opercula movement	Fin Movement	Mucous secretion	Loss of Equilibrium	Skin discoloration	Hyper-activity	Surface activity
0.00	-	-	-	-	-	-	-	-	-
0.75	-	-	-	-	-	-	-	-	-
1.50	-	-	-	-	-	-	-	-	-
2.25	-	-	-	-	-	-	-	-	-
3.00	-	-	-	-	-	-	-	-	-
3.75	-	-	-	-	-	-	-	-	-
4.50	+	-	-	-	-	-	-	-	-
5.25	+	-	+	+	+	+	+	+	+
6.00	+	-	+	+	+	+	+	+	+
6.75	++	+	++	++	++	++	++	++	++
7.50	++	+	++	++	++	++	++	++	++
8.25	++	+	++	++	++	++	++	++	++
9.00	++	++	++	++	++	++	++	++	++
9.75	+++	++	+++	+++	+++	+++	+++	+++	+++
10.50	+++	++	+++	+++	+++	+++	+++	+++	+++
11.25	+++	+++	+++	+++	+++	+++	+++	+++	+++
12.00	++	+++	+++	+++	+++	+++	+++	+++	+++
12.75	+	+++	+++	+++	+++	+++	+++	++	+++
13.50	+	++	++	+	+++	+++	+++	+	+
14.25	-	-	+	+	+++	+++	+++	+	-
15.00	-	-	-	-	+++	+++	+++	-	-

Note: Normal (-); Mild(+); Moderate (++); Strong (+++)

increased opercular movement and hyperactivity in *Poecila reticulata* as a response to chlorpyrifos exposure. *Channa punctatus* exposed to chlorpyrifos showed loss of balance, reduction in swimming rate, reduced feed intake, and convulsions [27].

Exposure to chlorpyrifos increased secretion of mucusin [19]. Fast swimming activity, loss of equilibrium, jerky movement, profuse secretion of mucus and hypersensitivity in chlorpyrifos exposed fish was observed by Ramesh et al. [28]. Banaee et al. [29] reported unbalanced swimming and increased surface swimming in *Cyprinus carpio* exposed to chlorpyrifos. Behavioural changes such as swimming erratically, convulsions, vertical hanging, coughing, loss of balance and abnormal opercular movement in chlorpyrifos exposed *punctatus* was observed by Yogita et al. [16]. Similar behavioral changes were also observed by Verma et al. [22]. Prashanth et al. [30] recorded the increased opercular movement, secretion of mucus and surface activity, body discoloration, irregular swimming and rapid jerk movement of *C. mirgala* exposed to lethal level of cyper menthrin. Common carp exposed to chlorpyrifos showed irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium, sinking to the bottom and caudal bending [19, 31]. Irregular swimming of *Rutilus frisiiKutum* and *Rutilus caspicus*exposed to pesticide (Hinsosen) was observed by Naserabad et al. [32]. *Clarias gariepinus*showed erratic and

jerky swimming, attempt to jump out of water, increased surfacing and gulping of air, reduced opercula movement and secretion of mucus on the body and gills followed by exhaustion and death after acute exposure to chlorpyrifos[31].

4. Conclusions

Behavioural changes can be used as a beneficial approach in biomonitoring programme to check ecotoxicity threat of pesticides to the test animals. During this study, acute exposure of chlorpyrifos had clear negative effects on the behavior of *Labeo rohita*. Chlorpyrifos reduced instinctive behavioural response and also cause the morphological changes. So, to mitigate the effects of pesticides pollution on fish, it is crucial to implement strict regulations and practices to minimize the release of these contaminants into water bodies

Data Availability statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

All authors declare that, they have no conflict of interest.

Author Contributions

Mubashra Ikram executed the research work; Sajid Abdullah planed this work. Huma Naz and Khalid Abbas help in writing article. Tanveer Ahmed assist in lab work. Iqra Zulfiqar and Nimra Zahid help in statistical analysis.

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