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STUDY OF BEHAVIOURAL AND MORPHOLOGICAL ANOMALIES OF FRY FISH OF FRESH WATER TELEOST, CHANNA PUNCTATUS UNDER CHLORPYRIFOS INTOXICATION

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ABSTRACT

The present study was conduct to assess the effect of Chlorpyrifos toxicity to behavioural and morphological manifestation of fry fish of *Channa punctatus*. The mortality data obtained through probit analysis was found to 0.365, 0.328, 0.269, and 0.253µl/l at 24^{th}, 48^{th}, 72^{nd}, and 96^{th} hrs respectively. The change in behaviour of fish such as convulsions, swimming erratically, vertical hanging, coughing, loss of balance, abnormal opercular movement, and lateral flexure, with tail beat were shown and finally fish became lethargic and settled at the bottom and their belly turned up before death. Opercular beat frequency (OBF/ min.), tail beat frequency (TBF/ min.), in gulping air or escaping attempt / min. and surfacing at sec. Increased with increasing concentration of CPF up to 24 hrs after that decreased following 96 hrs of exposure tenure. These quantal behaviour responses were significant at $p<0.001$.The prevalence of morphological deformities such as shedding of scales, discoloration, lesion of skin, split and necrosis of fins, eye deformities, scoliosis, damaged skull, lower lip extension and copious amount of mucus secretion all over the body were shown. The percentage of these deformities was dose and duration dependent.

KEY WARDS: **Chlorpyrifos, fry of *Channa punctatus*, LC_{50}, behaviour and morphology.**

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INTRODUCTION

Environmental pollution caused by pesticides, especially in aquatic ecosystems, has become a serious problem. These pesticides even when applied in restricted areas are washed and carried away by rains and floods to large water bodies like ponds and rivers and there by alter the physicochemical properties of water [1], this proved to the highly toxic, not only to the fishes but also to aquatic life forms and their environment [2, 3]. Contamination of water by pesticides, either directly or indirectly, can lead to fish kills, reduced fish productivity, or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans consuming these fish. Contamination of surface waters has been well documented worldwide and constitutes a major issue at local, regional, national, and global levels [4, 5]. Organophosphates (OP) are one of the most preferred pesticides due to their effectiveness and low persistence in the environment. OP pesticides directly inhibit acetylcholinesterase enzyme activity in fishes and invertebrates[6 - 8]. Chlorpyrifos (CPF) [O-O diethyl- O- (3, 5, 6, trichloro-2-pyridyl)-phosphorothiate], is one of the most wide used organophosphorous insecticide is used in both agricultural areas [9-11]. Toxicity studies have long played an important and modify the effects of his activities on the biota. The toxicity studies are especially useful sensitive species of an ecosystem that can be used as role in mans efforts to monitor in determining the indicator species, for a particular type of pollution. The results of toxicity are generally reported in terms of median lethal concentration LC_{50} and or median tolerance. Fishes come into contact with multiple contaminants in the aquatic environment as the pollutants. These pollutants built up in the food chain are responsible for adverse effects and death in the aquatic organisms[12]. Fish are largely being used for the assessment of the quality of aquatic environment and as such can serve as bioindicators of environmental pollution[13-15]. Variable concentrations of pesticides in aquatic environments cause structural and functional changes in aquatic organisms and this is more common than mortality. Any change in the behaviour and physiology of fishes indicates the deterioration of water quality, as fishes are the biological indicators. Behavioural changes in animals are indicative of internal disturbances of the body functions such as inhibition of enzyme activities, impairment in neural transmission, and disturbances in metabolic pathways[16]. The elimination of aquatic animals by small insidious physiological or behavioural changes has been reported to be more serious than a massive fish kill, since it is less likely to be observed and corrected[17]. The present work has been taken into consideration to evaluate LC_{50} values of CPF for freshwater stinging teleost, *Channa punctatus* (Bloch), a fish having nutritive value as well as which can serve as a better bioindicator of freshwater streams, lakes and ponds. Since the CPF demonstrate high levels of toxicity to fish, therefore it becomes necessary to study the effects of CPF on the behaviours (opercular beat frequency/min., OBF and tail beat frequency/min., TBF), morphology and mortality of the fish fry exposed to acute concentrations and duration.

MATERIALS AND METHODS

Maintenance of test animal

Fry of fish *Channa punctatus* were purchased from the Prakash hatchery, Barabanki. Fish fry were brought to the laboratory in wide mouth plastic container having oxygenated water. After testing with disinfectant (0.1% $KMnO_4$ for 3 minutes) they were transferred to the glass aquaria. The water quality of media in which fish fry (70) acclimatized was characteristics: temperature, 23.01±1.22ºC; dissolved oxygen, 4.41±1.42 mg/l; pH, 7.61±1.31;

total alkalinity, 16.9±1.92 mg/l as CaCO3; total hardness, 16.58±1.21 mg/l) for one week. The water in the aquaria was renewed daily and the fish were fed twice a day on a laboratory prepared artificial fish feed, 1% body weight. Fish were not fed 24 hrs before the commencement of and during the experiment to minimize contamination of the test media.

Experimental design

Chlorpyrifos (CPF 20 % EC) was purchased from the market under the trade name Hilban®. In this experiment the toxicity of chlorpyrifos were carried out in glass aquaria. A range finding test was conducted to determine the concentrations to be used in the actual experiment. A stock solution of CPF concentration 1ml/l in water was prepared and 20 days old, acclimatized with average weight & a length of (17 - 20 mg & 1.5 - 2.0 cm respectively) fries of *Channa punctatus* were used for 96 h experiment and the test compound concentration were 0.025, 0.05, 0.10, 0.20, 0.35, 0.55, and 0.80 µl/l prepared in different small aquaria which maintained in 2 liter of test medium and 10 numbers of healthy fish fry were released in each concentration. Acute toxicity test were carried out for period of a 96 hrs the mortality rate was determined at 24th, 48th, 72nd and 96th hrs. The static renewal bioassay method was adopted to determine the 96 h LC_{50}[18].

Ten fish was randomly introduced into each of the aquaria. Test solutions and water in the control were renewed daily. Acute toxicity test were carried out for a period of 96 hrs the mortality rate was determined at 24th, 48th, 72nd and 96th hrs. The behavioral changes of healthy fish and fish subjected to various dose of CPF and evaluated as regard to behaviour anomalies, during experimental period with control group and the frequency of changes was recorded per minute.

Statistical analysis

50% mortality concentration is the statically derived single dose of a chemical that can be expected to cause death in 50% of a given population of organism under a defined set of experimental conditions. In the present study opercular beat frequency (OBF), tail beat frequency (TBF), air ingulping, and surfacing phenomenon with other abnormal behaviour were recorded at the initial (0 - 6), 24th, 48th, 72nd and 96th hrs in all concentration of adult and fry fish. Data obtained from the experiments (OBF min^{-1}, TBF min^{-1}, air ingulping min^{-1}, and surfacing at sec., and cumulative mortality) were subjected to one way ANOVA and differences among means were separated by New man's Kuel multiple rang test[19]. The mortality was regressed on concentration of the CPF and correlation coefficient recorded and the analyses of lethal concentration with associated confidence interval were done with probit analysis[20].

RESULTS

1. Acute toxicity (LC_{50})

Acute toxicity (up to 96 h LC_{50}) of chlorpyrifos for the fresh water fry fish of *Channa punctatus* was found to be 0.365, 0.328, 0.269, and 0.253 for 24th, 48th, 72nd, and 96th h respectively. The fry fish exposed with the presumably harmless concentration was 0.025µl/l and at 0.80µl/l, there was 100 percent mortality found. The mortality of fish occurred steadily with passes of time in each of the CPF concentration. The probit 50 % mortality and the upper and lower 95 % confidence limit are presented in (table 1).

2. Behavioural Manifestation

In the present study the test fish exposed to different graded concentrations of chlorpyrifos exhibited a number of abnormalities in their behavioural responses. Fish swim faster than control fish around the aquarium without being provoked, faster opercular activity, surfacing and gulping of air in all concentration at initial an exposure time. However at higher concentration within 30 minutes of exposure up to 24 hrs the fish appeared hyper excitable that away from the stimuli and swimming become erratic from

0.55 to 0.88 µl/l concentration with jerky movements and the schooling was disturbed followed by hyperactivity, lateral flexure, pectoral fin forwarded, air ingulping, spiralling convulsions and tendency of escaping from the aquaria and loss of balance by vertically hanging in the water or test media (Table 2). Convulsive behaviour was reduced just after 30 minutes and the swimming speed was also decreased with increasing duration and concentration of CPF, when fish became lethargic. The obf in fish exposed to chlorpyrifos increased up to 24 h after that it was decreased significantly (fig.1), tbf increased initially which was least variable at 72 h decreased (fig. 2). The surfacing behaviour of fish increased up to 72 h which was decreased later in all concentration (fig. 3) and air ingulping (escaping attempts) of fish was very variable in all concentration and duration of exposure. It was increased and less variable at 24 but was more variable at 48 h with a peak at 72 h (fig 4). The chlorpyrifos produced marked change ($p<0.001$) in the value of obf, tbf, surfacing and air ingulping (not significant) of fry of *Channa punctatus* at various exposure duration and concentration of the chemical.

3. Morphological Manifestation

The prevalence of morphological deformities such as shedding of scales, discoloration, lesion of skin, split and necrosis of fins, eye deformities, scoliosis, damaged skull, lower lip extension and copious amount of mucus secretion all over the body were shown. The percentage of these deformities was higher at higher concentration (0.35, 0.55, and 0.80µl/l) and duration (72 and 96 hrs.) of treatment (table – 3 and fig.-5). The extent of caudal bending (scoliosis) was pronounced in the highest concentration of chlorpyrifos. It may be due to the inhibition of muscular AChE activity resulting in blockage of neuraltransmission. At higher chemical concentration, scale depletion start, mild skin lesion observed from dorsal to lateral side of the body of fish, copious mucous, clumping of gills increases with the increasing of concentration of toxicant. The fishes lost their natural coloration and become almost pale yellow in colour.

Table 1
LC_{50} and 95 % confidence intervals for fry of C. punctatus exposed to CPF.

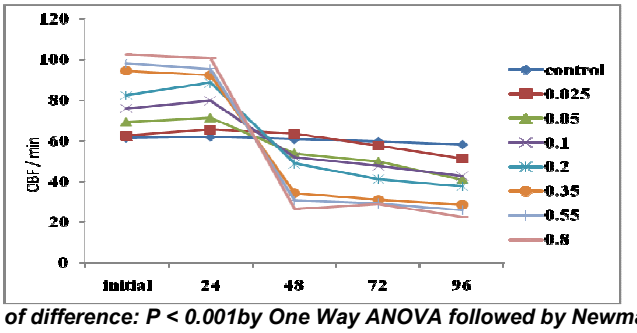
S. No.	Probit value	24 h	48 h	72 h	96 h
1.	LC_{50}	0.365	0.328	0.269	0.253
2.	Regression value ($Yi = \alpha + \beta Xi$)	Y = - 1.1+ 139.86	Y = 9.4 + 123	Y = 18.67 + 116.08 X	Y = 21.49 + 112.68 X
	95 % confidence intervals				
3.	Lower limit (µl/l)	0.157	0.117	0.040	0.022
	Upper limit (µl/l)	0.573	0.539	0.489	0.484
4.	r^2 value	0.9368	0.9560	0.9545	0.9236

Table 2
Behavioural response of fry fish of C. punctatus in various concentration of CPF up to 24 h.

Quantal variables		Concentration of CPF (µl/l)							
		control	0.025	0.05	0.10	0.20	0.35	0.55	0.80
Swimming pattern	convulsions	-	-	+	+	+	-	-	-
	Locomotion speed	-	-	-	-	- -	- -	- -	- - -
	Hanging	-	-	-	+	++	+++	+++	+++
	Loss of balance	-	-	-	-	+	++	+++	+++
	Lateral flexure (TBF)	-	-	-	+	++	++	+++	+++
	Pectoral fin (forward)	-	-	-	+	+	++	++	++
Respiratory pattern	Opercular movement	-	-	++	++	+++	+++	+++	+++
	Air ingulping	-	-	+	+	++	++	+++	+++
	Coughing	-	-	++	++	++	+++	+++	+++
	Surfacing	-	-	+	+	++	++	++	++

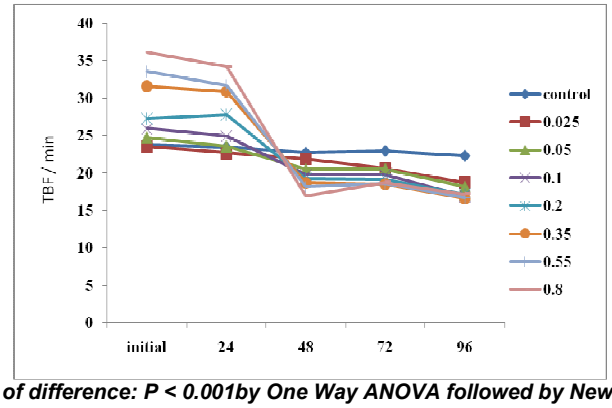
Note: Normal → (-), (+) → mild, (++) → moderate, and (+++) → maximum behaviour sign of minus (-, - -, - - -) denoting decreased behaviour, as compared to control.

Figure 1
Opercular beat frequency (OBF) / min of fish fry.



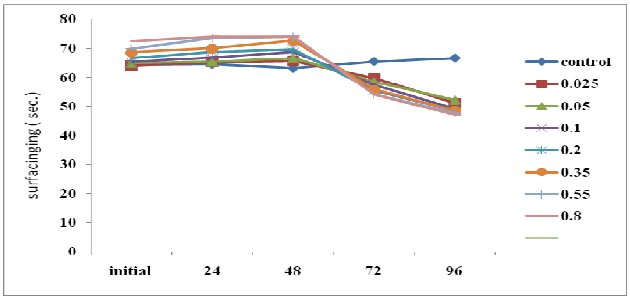
Significant of difference: P < 0.001by One Way ANOVA followed by Newman's Kuel.

Figure 2
Tail beat frequency (TBF) / min of fish fry.



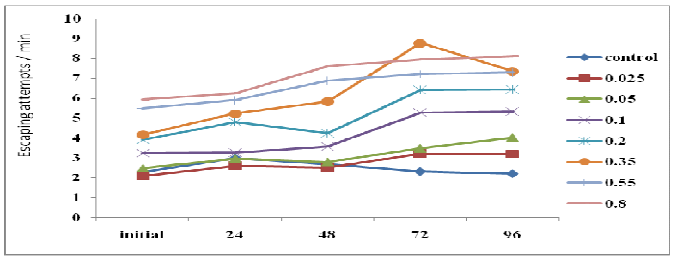
Significant of difference: P < 0.001by One Way ANOVA followed by Newman's Kuel.

Figure 3
Surfacing (sec.) of fish fry



Significant of difference: P < 0.001by One Way ANOVA followed by Newman's Kuel.

Figure 4
Air - ingulping frequency / min of fish fry.



Significant of difference: P < 0.05 by One Way ANOVA followed by Newman's Kuel.

Table 3
Morphological deformities of fry fish of C. punctatus in various concentration of CPF (20 % EC) up to 24h.

S. No.	Deformities		Concentration of CPF (µl/l)							
			control	0.025	0.05	0.10	0.20	0.35	0.55	0.80
1.	Shedding of scales		0	0	0	0	0	0	16.6 ± 0.69	16.6 ± 0.69
2.	Discoloration		0	0	0	0	0	0	16.6 ± 0.69	26.6 ± 0.82
3.	Lesion of skin		0	0	0	0	0	0	0	16.6 ± 0.69
4.	Eye	Micropthalmia	0	0	16.6 ± 0.69	16.6 ± 0.69	53.3 ± 0.92	53.3 ± 0.92	53.3 ± 0.92	53.3 ± 0.92
		Exophthalmia	0	0	10 ± 0.55	16.6 ± 0.69	16.6 ± 0.69	26.6 ± 0.82	26.6 ± 0.82	26.6 ± 0.82
		Unilateral anapthalmia	0	0	0	0	0	0	26.6 ± 0.82	36.6 ± 0.89
5.	Fin	Necrosis	0	0	0	0	0	0	26.6 ± 0.82	26.6 ± 0.82
		Split fins	0	0	0	36.6 ± 0.89	36.6 ± 0.89	53.3 ± 0.92	83.3 ± 0.69	83.3 ± 0.69
6.	Skeletal	Scoliosis	0	0	26.6 ± 0.82	30.3 ± 0.84	30.3 ± 0.84	36.6 ± 0.89	53.3 ± 0.92	60.0 ± 0.91
		Damaged Skull	0	0	36.6 ± 0.89	60.0 ± 0.91	73.3 ± 0.82	73.3 ± 0.82	96.6 ± 0.3	96.6 ± 0.3
7.	Lower lip extension		0	0	0	0	0	10 ± 0.55	16.6 ± 0.69	30.3 ± 0.84
8.	Mucus secretion		0	0	36.6 ± 0.89	53.3 ± 0.92	60.0 ± 0.91	60.0 ± 0.91	70 ± 0.85	96.6 ± 0.3

Data are means ± SEM (N = 30). The value of all morphological deformities expressed as percentage.

DISCUSSION

Chlorpyrifos poses toxic effects on the snakehead fish *Channa punctatus*. The LC_{50} of chlorpyrifos was found to be 0.365, 0.328, 0.269, 0.253 µl/l for 24th h, 48th h, 72nd and 96th h exposure period respectively. The relationship of mortality of fish fry was calculated by regression equation to be probit which were recorded for 24, 48, 72, and 96 hrs that is, y = - 1.1 + 139.86 x, y = 9.4 + 123.0 x, y = 18.67 + 116.08 x, y = 21.49 + 112.68 x and R square value (r^2) and positive correlation between mortality and concentration of toxicant shows that the mortality rate of the fish increased with increase in the concentration of CPF (table 1). The differences in the 96 h LC_{50} value between the fry of *Channa punctatus* in present study and other reports of adult fish[21] may be attributed to the fact that chlorpyrifos induced changes differ from, species to species and from one experimental condition to other. The 96 h LC_{50} of chlorpyrifos to channel catfish, *Ictalurus punctatus* and sheepshead minnow, *Cyprinodon variegatus* as 0.280 mg/L and 0.136 mg/L, respectively[22, 23]. Chlorpyrifos toxicity reported by[24] to euryhaline and mosquito fish, and for *Catla catla, Labeo rohita* and *Cirrhinus mrigala*, *Oreochromis mossambicus* (Tilapia) and *Gambusia affinis*[25] by semi-static method is 0.0259 mg/L and 0.297 mg/L, for *Cyprinous carpio* was 0.160 mg/l through static renewal[26] respectively.

Behavioural manifestation of acute toxicity in fry fish of *Channa punctatus* were more or less similar to those reported in other fishes exposed to chlorpyrifos[24-26]. Behavioural changes have been established as sensitive indicator of chemically induced stress in aquatic organisms[27]. The frequency of lateral flexures was counted through tail beat cycle. It was counted by number of tail beat frequency (TBF) / minutes. The TBFs was significantly ($p<0.001$) increased with increasing concentration at initial period fish, after 24 hrs it was decreased up to 96 hrs. The overall increase in opercular beats as observed in present study is in good conformity with earlier reports on different fishes in relation to various toxicants[28-29]. Similar observations as the present study was reported in juvenile of *Tillapia guineensis* exposed to different concentration of chlorpyrifos[30]. The increased opercular activity may be due to shock received by the fish in new toxic environment along with sensory stimulus to increase the opercular movement for proper ventilation of the gills to cope with hypoxia[31]. The increase in TBF and OBF may be associated with sudden response of the fish to the shock of exposure to the Agro-chemical[32]. This behaviour may be an adjustment of the internal homeostatic of the fish to the stress imposed by the toxicant. The pattern of OBF in the species at the various concentrations which was raised, peaked and then declined with exposure duration in this study may reflect the trend in available energy[33, 34] Surfacing phenomenon i.e., significant preference of upper layers in exposed group might be a demand for higher oxygen level and gulping of surface water appears to avoid breathing of fish in the poisoned water during the exposure period[35]. Increase in surfacing Moreover, thehypoxic condition also contributes to increase surfacing as reported by[36]. This situation further continued intensely throughout the test tenures, which is in accordance with the observations through[37] made.

In morphological deformities fish showed: fusion of fins, dullness in body colour, eye deformities, shedding of scales, decalcified skull from which slightly visible brain, caudal bending (scoliosis) and mucous secretion. The percentage of these deformities was increased with increasing concentration and duration. And fish body becoming lean towards abdominal position, and being under stress in later exposure periods was observed. Similar observations

were reported by various studies on fish morphology[38, 26]. There was a slight swelling of the abdominal region that remained so throughout the test tenures. Caudal bending (left side) was noticed in both the toxicant concentrations with time, which greatly retarded the normal swimming pattern. The extent of caudal bending was pronounced in higher toxicant concentration. Caudal bending may be a sort of paralysis, which might be due to the inhibition of muscular AChE resulting in blockage of neural transmissions. Accumulation of mucus also was observed on the gill filaments and body surface of the dead fish in all concentration which was maximum at higher concentration of chlorpyrifos. Increase in production of mucus over the body as a result of toxicant may interfere with the gaseous exchange, secretion and waste products and osmoregulation[39]. Malformation of eye included micropthalmia, anapthalmia, exophthalmia, such abnormalities were found which may be due to the apoptosis of cells in theeye or reduce in the diameter of eye socket in present study are similar with reported by[40] in *Cyprinous carpio* larvae exposed to paper mill effluents.

CONCLUSION

Behavioral and morphological alterations in fry of *Channa punctatus* under the influence of pesticide can be used as a sensitive model to monitor the aquatic pollution. The current result indicates that the pesticide contamination definitely affects the central and peripheral nervous system of fish due to the accumulation of chlorpyrifos on the synaptic cleft of neurotransmitters. The present research work served as an experimental tools and bioindicators for the first line evaluation of environmental pollution.

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