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Effect of chlorpyrifos on protein and carbohydrate content of *Heteropneustes fossilis* (Bloch, 1794)

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Abstract
Organophosphorus pesticides are used in the agricultural area due to their rapid biodegradability and non-persistent nature to control pests but their broad spectrum of harmful effects extends far beyond the pest. The objective of this research was to investigate the effect of sub-lethal chlorpyrifos effect on ovarian protein and carbohydrate content of the freshwater catfish "*Heteropneustes fossilis*". LC_{10}, LC_{25} and LC_{50} value of chlorpyrifos were calculated for 24, 48, 72 and 96 hr by probit analysis (Finney, 1971). The LC_{10}, LC_{25} and LC_{50} value for 24 hr were 9.79, 21.59 and 51.95µl/l, and 3.3, 7.54 and 18.91µl/l for 48 hr, and for 72 hr: 0.85, 2.39 and7.47µl/l. The 96 hr LC_{10}, LC_{25} and LC_{50} value were 0.12, 0.55 and 4.64µl/l respectively. The result showed that CPF significantly (p<0.05) decreased the ovarian protein and carbohydrate concentration. Therefore, it may be concluded that CPF is highly toxic to aquatic organism.

Keywords: Chlorpyrifos, *Heteropneustes fossilis*, protein, carbohydrate

1. Introduction
Pesticides are commonly used in agricultural activities. Because of drift, atmospheric transport, agricultural and residential runoff, individual misuse, and improper disposal, pesticides are also found in aquatic habitats [1, 2] where it act as a toxicant for aquatic organisms. These toxicants produce several biochemical and physiological responses. Thus, it is important that toxic effects be determined and interpreted in biochemical terms.
Chlorpyrifos (CPF), O, O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate is an organophosphorus pesticide used extensively for the management of domestic and agricultural pests. It impairs toxic effects on aquatic non-target organisms [3]. CPF are powerful neurotoxic compound as they inhibit acetylcholinesterase (AChE) [4-9]. A number of reports are investigated the toxicity of CPF in different aquatic models viz., *Vibrio fischeri*, *Thamnocephalus platyurus* and *Daphnia magna* [3], *Gambusia affinis* [10], *Channa punctatus* [11], *Oreochromis mossambicus* [12], aquatic invertebrates and fish [13], yellow perch and rainbow trout [14], *Labeo rohita* [15]. Biochemical constituents are serving as potential biomarkers for a variety of different organisms [16]. The pesticide effect on biochemical properties has been studied by many workers [17, 21].
Fish acts as bio-indicators of aquatic contaminants [22]. Fish play an important and significant role in the food chain, thus, study of pesticide effect on fish has a diagnostic significance in evaluation of adverse effect of pesticides to human health [23]. *Heteropneustes* fossilis is a valuable high priced freshwater food fish and therefore often chronically exposed to insecticides. Several investigation were reported on the toxicity of various pesticides to fish, viz. Shukla *et al.* [24], Sahai *et al.* [25], Kumari [26] and Kumari [27]. The aim of the present study was to investigate the impact of chlorpyrifos on ovarian biochemical parameters of *H. fossilis*.

2. Materials and Methods
2.1 Chemicals
All the chemicals and reagents used in this study were of analytical grade and were purchased locally. The test compound chlorpyrifos was purchase from local market (Hindustan insecticide limited, India) as trade name Hilban®.

2.2 Animal collection and their maintenance
An experiment was performed in accordance with local/national guidelines of ethical committee for experimentation in animals.

The fresh water *H. fossilis* of relatively same size and weight (160±10gm) were collected from commercial fisherman of Lucknow, Uttar Pradesh, India. After formal quarantine treatment with 0.05% $KMnO_4$ solutions, fish were acclimatized in laboratory conditions under normal photoperiod (12:12 light: dark) and temperature (22±2ºC) for three weeks in 120 L glass aquaria containing water. The physico-chemical characteristics were analyzed by APHA [28] (Table 1). Water was renewed daily to remove faecal matter and waste metabolite of fish during acclimatization. During this period, fish were fed regularly with commercial fish food pellets and goat liver.

Table 1: The physico-chemical characteristics of water used for experiment

S. No.	Water parameters	Mean Values
1	pH	7.6-7.9
2	Temperature (ºC)	23.5-26.5
3	DO (mg/l/)	6.3-7.5
4	Free CO_2 (mg/l)	36-38
5	Total hardness (mg/l)	215-220
6	Alkalinity (mg/l)	184-190

2.3 Acute toxicity and biochemical studies

Pilot experiments were conducted to determine the LC_{10}, LC_{25} and LC_{50} concentrations for acute toxicity. Fish were exposed to different concentrations of chlorpyrifos ranged from 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 15, 20 and 25µl/L for 24, 48, 72 and 96 hr in groups of 10 individuals in five replicates. During the whole process a control group (without toxicant) was maintained side by side. The lethal concentration values were obtained and analyzed by Finney's probit analysis method [29]. The fish were not fed on the day before the beginning of the experiment.

2.4 Biochemical studies

Fish were exposed to different concentrations of CPF (0.01 to 25µl/l) in batches of ten with five replicates. Control groups of fish were maintained concurrently. Water was renewed daily with CPF concentration up to 96 hr of study period. After 96 hr study, fish from each group were removed and sacrificed for ovarian tissue sampling for protein and carbohydrate analysis study. The total protein was determined by Lowry's *et al.* [30] methods and estimation of total carbohydrates by Roe [31] method and respectively.

2.5. Statistical analysis

The data was subjected to one-way analysis of variance (ANOVA) and the significance difference was set up at $p<0.05$. These values were expressed as Mean ± SD.

3. Results and Discussion

The catfish *H. fossilis* showed varied degrees of mortality with different concentration of CPF. The mortality% was significantly increased in concentration and duration dependent manner of CPF ($F=52.27$, $p<0.05$). The calculated LC_{10}, LC_{25} and LC_{50} values were shown in table 2.

The protein and carbohydrate concentration of ovary tissue was varied with the concentrations of pesticide, chlorpyrifos. Control fish showed maximum protein and carbohydrate content in ovary. As the concentration of CPF was increased, the protein and carbohydrate content was decreased significantly ($F=5415$, $F=19203$; $p<0.05$). The minimum protein and carbohydrate concentration were noticed in the fish tissue exposed to highest concentration of CPF i.e., 25µl/l and maximum content were observed in lowest concentration of CPF (0.01µl/l) after 96 h of exposure (Fig. 1). Shinde *et al.* [32] have also been reported the decrease of protein content in ovary of *Notopterus notopteus* treated with heavy metals. Same findings were observed in other fish viz., *Colisa fasciatus* [33], *Catla catla* [34], *Labeo rohita* [35, 36], *Cyprinis carpio* [37]. Tilak [38] reported reduction of protein content of liver, brain and ovary of *C. punctatus* exposed to fenvalerate. CPF cause hyper activity which results in breakdown of food reserves to meet the energy demand [39]. Total protein content decreased due to CPF exposure which suggests the possible breakdown of proteins in the fabrication of some amount of energy for organism [40]. Neff [41] stated that under toxicant exposure, animal required more energy hence protein level was depleted. Proteins are mainly involved in the architecture of the cell which is chief source of nitrogenous metabolism and during toxicant stress they are also source of energy [42]. It serves as a source of energy for fish and it will be used when insufficient energy is available from other sources like carbohydrate. It regulates the process of interaction between intra and extra cellular media. The carbohydrate comprised mainly glycogen and total free sugars and the fluctuations in this content may be due to accumulation and utilization of glycogen and total free sugars at different phases of life like growth, gametogenesis and spawning. In fish, generally the carbohydrate reserves may be rapidly utilized under unfavorable conditions like under toxicant exposure. The decrease in total carbohydrate signifies its utility possibly to meet the higher energy demands of fish reeling under pesticide stress [43].

Table 2: Estimation LC_{10}, LC_{25} and LC_{50} values along with 95% lower and upper confidence limits of *H. fossilis* exposed to CPF for 24, 48, 72 and 96 hr.

Probit value µl/L)	24 h	48 h	72 h	96 h
LC_{10}	9.79 (5.37-14.31)	3.3 (1.13-5.84)	0.85 (0.16-1.95	0.12 (0.02-0.59)
LC_{25}	21.59 (14.91-27.73)	7.54 (3.74-11.27)	2.39 (0.78-4.34)	0.55 (0.05-2.84)
LC_{50}	51.98 (42.79-62.62)	18.91 (13.06-25.25)	7.47 (4.04-11.69)	4.64 (1.82-9.02)

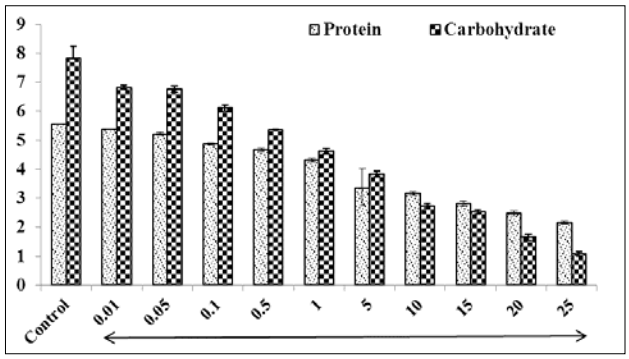

Fig 1: Effect of Chlorpyrifos on protein (mg /ml/100mg tissue wt) and carbohydrate content (mg /ml/100mg tissue wt) of freshwater catfish, *H. fossilis.*

4. Conclusion

Frequent use of this pesticide in paddy fields and its disposal in water bodies is very hazardous and unwarranted to the fresh water ecosystem. On the basis of above mentioned findings, it may be suggested that indiscriminate use of this pesticides in water bodies should not be encouraged by the farmers particularly those who are planning for farming catfishes in their paddy fields.

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