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ACUTE TOXICITY II: EFFECT OF ORGANOPHOSPHATES AND CARBMATES TO *CATLA CATLA* FINGERLINGS

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ABSTRACT

Pesticides are widely used in modern agriculture to aid in the production of high quality food. However, some pesticides have the potential to cause serious health and/or environmental damage. Organophosphates (OCP'S) and carbamates can cause pollution in freshwater ecosystem, as well as having a significant effect on the health of fish. The acute effects of commercial formulation of triazophos, profenofos, carbofuran and carbaryl were determined in one of the indigenous fish species, *Catla catla* fingerlings. Pesticides were applied to fingerlings that had been grown under optimised standard conditions under a maintained static bioassay system. Probit analysis was used for the estimation of LC_{50} values, which were ascertained as 4.84, 0.19, 0.99 and 7.89 mg/L for triazophos, profenofos, carbofuran and carbaryl, respectively. 100% mortality of *Catla catla* was observed with a 2.8 mg/L dose of carbofuran at 96 hours with a significant difference. Acute toxic stress was noticed with subjects exhibiting behavioral intoxication, including suffocation, lying on the bottom, erratic swimming, lethargy and downward movements and gulping prior to mortality.

Key words: *Catla catla, Acute toxicity, Profenofos, Triazophos, Carbofuran, Carbaryl.*

INTRODUCTION

The living world is heavily dependent on chemicals (natural and synthetic), growing demand of food for increasing population has led to substantial production and application of various agro-chemicals (pesticides and fertilizers). Increased use of chemicals, particularly affecting man and environment and increased the burden of these chemicals in the environment due to non biodegradability of some of these compounds (Tripathy *et al*., 2002; Kumar al., 2005). The living world is heavily dependent on chemicals (natural and synthetic), growing demand of food for increasing population has led to substantial production and application of various agrochemicals (pesticides and fertilizers). Among anthropogenic contaminants, pesticides are widely detected in freshwater and marine ecosystems. These chemicals are spread on terrestrial cultures and enter waterways from agricultural and urban runoff (Var´o *et al*., 2008). It can produce adverse effects on non target aquatic organisms living in areas near agricultural fields. It often ends up in aquatic habitats carried up by the wind, runoff, or through uncontrolled waste disposal. Fish and aquatic animals, as non- target species, are exposed to pesticides in three primary ways (i) dermally (direct absorption through the skin) (ii) breathing in contaminated water (uptake through the gills) (iii) orally (drinking or feeding on pesticide-contaminated water or pesticide contaminated prey) (Mathur and Singh, 2006).

The organophosphates (OP) and carbamates (Cs) are modern synthetic insecticides and are potent neurotoxic molecules (Lundebye *et al*., 1997), which are commonly used in the Mediterranean area to treat a variety of agricultural pests (Vioque-Fern´andez *et al*., 2007; Banni *et al*., 2005; Ghazala *et al*., 2014).The risk that a pesticide poses to the surrounding environment depends on its toxicity to fish and other organisms, and their exposure to the pesticide. With high effect, wide in variety rapid degradation and low toxic residues, such pesticides are widely used as pesticides in Pakistan. Poisoning/toxicity is categorised as either acute or chronic and the determination of the median lethal concentration (LC50) is considered to be the preliminary step for studies into the extent of acute or chronic toxicity The short term toxicity of a chemical either natural or man-made, is measured using the LC50 (lethal concentration) value. An LC50 is a measure of how much product is required to kill 50% of the test population over a period of time.

Poisoning/toxicity is categorised as either acute or chronic and the determination of the median lethal concentration (LC_{50}) is considered to be the preliminary step for studies into the extent of acute or chronic toxicity. Different pesticides, however, have different LC_{50} values in different organisms (Mathur *et al*. 2006). Thus, Pentachlorophenol (PCP) has an LC_{50} value of 0.58 ppm in *H. fossilis* (Farah *et al*. 2004), while, in *Lepomis macrochirus*, the LC_{50} values of diazinon, chlorfevinifos and profenofos were calculated as 2.5 ppm, 2.9 ppm and

300 ppb respectively (Dembele *et al*. 2000; Tomlin 1994). Likewise, acute toxicity of profenofos and triazophos in crucian carp was determined as occurring at 0.192 ppm and 8.4 ppm respectively (Jin *et al*. 2010), whereas, in common carp, acute toxicity of profenofos (LC_{50}) and triazophos (LC_{100}) was determined as occurring at 62.4 ppb and 1.00 ppm, respectively in two different studies (Ismail *et al*. 2009). Assis *et al*. (2010) determined the LC_{50} values for carbaryl and carbofuran as 33.8 µmol/L and 0.92 µmol/L, respectively, while Boran *et al*. (2007) investigated acute toxicity of carbaryl in *Oncorhynchus mykiss and Poecilia reticulate* and Beauvais *et al*. (2000), meanwhile, showed that acute malathion intoxication led to swimming and locomotory dysfunction in rainbow trout larvae. *Catla catla* is one of the indigenous and fast growing freshwater fish. This fish is facing serious threats due to indiscrimninate use of pesticides in the country. *Catla catla* is surface feeder and is more vulnerable to the all kind of pollutants in freshwater ecosystem in the country.This fish has been selected for this because of its commercial importance. The present study was undertaken to estimate the acute toxicity of commonly used organophosphates and carbamates in Pakistan for fingerlings of *Catla catla*, which is one of the indigenous fish in the Indo-Pak regions.

MATERIALS AND METHODS

Live fingerlings (L=2.65-3 inch, W= 18-21 g) of *Catla catla* were maintained in 70 liter glass aquaria at the Department of Zoology, GC University, Faisalabad, Pakistan, having been transferred from the Fish Seed Hatchery, Satiana road, Faisalabad, Pakistan. During acclimatization (15 days) the fish were fed with commercial feed at 3% body weight. Water parameters (electrical conductivity, pH and temperature) were analyzed and maintained at optimal conditions. The temperature of the water was regulated at 27 ± 1°C.Aquaria were continuously aerated, except at the time of feeding, so as the level of dissolved oxygen did not drop below 4.0 mg/L. The electric conductivity and the pH of water were 2.70–2.80 mS and 8.85–9.40, respectively.

Test chemicals: Technical grades of triazophos 90% [diethyl o-(1-phenyl-1h-1,2,4-triazol-3-yl) phosphorothioate)], profenofos 98% [O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate], carbaryl 97%(1-naphthyl methylcarbamate)] and Carbofuran 90% (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) were obtained from Ali Akbar Enterprises, Lahore, Pakistan. Triazophos was dissolved in Methanol (Analytical grade, Merck), profenofos was dissolved in Acetone (Analytical grade, Merck), carbofuran and carbaryl were dissolved in ethanol (Analytical grade, Merck). To confirm the solubility of pesticides in water, 1ppm concentration of each pesticide dissolved in relevant solvent in the test water sample was prepared and was confirmed with HPLC (Model L7400). The Solid phase extraction technique was used for the extraction of pesticide residues from water sample (TOTOLIN, 2003). Since toxicity test fish were divided into six groups, each with 10 individuals following random selection to test 6 dilution (for each pesticide) in triplicates with negative control receiving no pesticide but maximum solvent that any dosing solution contain. Before acute toxicity tests (LC50) preliminary tests were done with 1ppm and 10ppm concentration of all pesticides to determine the minimum and maximum limits of viability and mortality of fish fingerlings.

Determination of sub lethal concentration and acute toxicity test: Four day static toxicity tests were performed to determine the LC_{50} values (OECD 1992). Stock solutions of the pesticides were made by dissolving profenofos in acetone, triazophos in methanol and both carbofuran and carbaryl in ethanol. Six dosing solutions were prepared from the stock prepared by mixing different proportions of stock solution in acetone (profenofos), triazophos (methanol) and ethanol (carbofuran and carbaryl) to get the desired concentrations. The nominal concentrations of active tested ingredients for all fish fingerlings were determined by following OECD (1992). Nominal concentrations of active ingredient tested were: triazophos 4, 5, 6, 7, 8, 8.5, 9; profenofos 0.098, 0.196, 0.284, 0.392, 0.49, 0.588, 0.686; carbofuran 0.25, 0.50, 1, 2, 4, 8 and carbaryl 6, 7, 8, 9, 10, 11, 12 mgL-1. The specimens were fed with commercial feed @ 3 % of their body weight once in a day and the feeding was stopped 24 h prior to the pesticide exposure to till the end of the experiment and water exchange was stopped. Mortality and behavioral changes of the specimens were recorded after 24, 48, 72 and 96 hours. Median lethal concentrations based on 96 hours acute toxicity were determined according to the guidelines of OECD (1992). LC50 of triazophos, profenfos, carbofuran and carbaryl and the 95% confidence limits were calculated by a computer program, [TSK (Trimmed Spearman–Karber) program (1991) Version 1.5] as described by Finney's Probit Analysis LC50 Determination Method (1978).Data for mortality and a live fish was analyzed using Probit analysis (Finney 1971). The behavioral changes in the specimens were also noted right after the application of testing dose till the end of an experiment. The behavioural changes of the healthy fish and the fish subjected to various doses of tested pesticides were evaluated as regard to behaviour anomalies. A guide covering some general information on methods for qualitative and quantitative assessment of the behavioral responses of fish (locomotory activity, feeding, and social responses) during standard laboratory toxicity tests to measure the sublethal effects of exposure to chemical substances were determined as described by ASTM (2008). The negative control group was also monitored in the same way for mortalities and change in behaviour, including loss of balance, moving in a spiral fashion with jerks, lying laterally and opened mouth with rapid opercular movements. In addition, LC50 values were compared by the method of APHA (1995). Quality-assurance measures applied in the laboratory included rigorous contamination-control procedures (strict washing and cleaning procedures), monitoring of blank levels of solvents, equipment and other materials, analysis of procedural blanks, recovery of spiked standards, monitoring of detector response and linearity, and analysis of a reference material. Recoveries of pesticides in the reference material were between 80 and 110 % of certified concentrations.

RESULTS AND DISCUSSION

Effect of pesticides on behavior of the fish: In the present study, the control fish were active for feeding and alert to slightest of the disturbance with their well-synchronized movements. The behavior did not significantly vary between the control groups; therefore, these results were taken as standards for the entire experimentation. The effects of pesticide intoxication were observed as suffocation, restlessness, loss of equilibrium and erratic swimming on prodding with all the tested pesticides. This followed loss of co-ordination and occupancy of twice the area to that of control group were the early responses of the fish following exposure to sodium cyanide in both the sublethal concentrations. Subsequently, fish moved to the corners of the test chambers, which can be viewed as an avoidance behavior of the fish to profenofos, triazophos, carbofuran and carbaryl. Further, fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium followed by hanging vertically in water. Fish often remained at the bottom with mouth opened before dying (Table 1). Behavioral responses meet the criteria as rapid tool for bioassay testing and could be easily standardized using pesticides as reference toxicant. The development of behavioral methods in fish is an important tool in aquatic toxicology. Behavioral responses represent an integrated response of fish species to toxicant stress (Kane *et al.* 2005). Changes in spontaneous locomotor activity and respiratory responses are sensitive behavioral indicators of sublethal exposure in fish (Scherer 1992). Behavior provides a unique perspective linking the physiology and ecology of an organism and its environment (Little and Brewer, 2001). Avoidance response and locomotor activity are referred to the same category of behavioral responses as described by Scherer (1992). However, avoidance response can be initiated through chemosensory irritation, since it was established that the fish olfactory system is involved in the formation of an avoidance response to heavy metals (Sveceviceius 1991). Locomotor activity may reflect a more non-specific stress response resulting in changes in blood cortisol and glucose levels.

Determination of acute toxicity: Mortality response and relationship of selected fish to various concentrations of pesticides are presented in Table (2) and Figures (1-4). An increase in the number of mortalities was observed as the concentration of insecticide was increased. There was no mortality in the control group as well as in the group receiving 4 mg/L and 2 mg/L (lowest dose) of triazophos. *Catla catla* fingerlings died after 2-3 hours of exposure at 8 mg/L. In the case of profenofos, a dose dependent increase and time dependent decrease were observed in the mortality rate at the exposure time increased from 24 to 96 hours; i.e. the median concentration was reduced. There was a significant difference ($P < 0.05$) among LC50 values obtained at different times of exposure. At 96 hours median lethal concentrations were recorded as 0.19 mg/L (0.14- 0.24). The 100% mortality of *Catla catla* was observed with a 2.8 mg/l dose of carbofuran at 96 hours with a significant difference. LC50 values of carbofuran at 24 and 96 hours were estimated as 2.40 mg/L (1.76-3.30) and 0.99 mg/L (0.73-1.35), respectively. Median lethal concentrations of carbaryl at 24 hours, 48 hours, 72 hours and 96 hours were observed in *Catla catla* as being as 9.49 mg/L (8.91-10.08), 9.10 mg/L (8.50-9.76), 8.42 mg/L (7.85-9.09) and 7.89 mg/L (7.31-8.67) mg/l (7.65-8.90) respectively, at 95% confidence intervals (Table 2). An overall comparison of all the tested pesticides from toxicity point of revealed profenofos as highly toxic, at its very low concentrations and caused mortality in *Catla catla.* The least toxic compound was found to be carbaryl as compared to other tested compounds. Median lethal concentration at 24 hours, 48 hours, 72 hours and 96 hours, were observed in *Catla catla* as 9.49 mg/L (8.91-10.08), 9.10 mg/L (8.50-9.76), 8.42 mg/L (7.85-9.09) and 7.89 mg/L (7.31-8.67). In general, acute susceptibility of the tested pesticides was as follows: profenofos > carbofuran > triazophos > carbaryl.

At 96 hours, median lethal concentrations of profenophos and triazophos were 0.19 mg/L (0.14- 0.24) and 4.84 mg/L (4.31- 5.42) in *Catla catla* respectively (Table 2). The results of the present study showed a higher median lethal concentration but nearly similar effects on the behaviour of fish compared to the findings of Pandey *et al.* (2011) where acute toxicity of profenofos to *Channa punctuates* was observed as 2.68 µg/L. The effects of intoxication with profenofos presented as erratic swimming, hyperexcitability, discoloration of the skin and secretion of mucus in the body and the gills, leading eventually to death.

In the current study, 96 hours median lethal concentrations of carbofuran was estimated at 0.99 mg/L (0.73- 1.35) while median lethal concentrations of carbaryl at 24 hours, 48 hours, 72 hours and 96 hours were observed in *Catla catla* as 9.49 mg/L (8.91 - 10.08), 9.10 mg/L (8.50 - 9.76), 8.42 mg/L (7.85- 9.09) and 7.89 mg/L (7.31 - 8.67) at 95% confidence intervals, respectively (Table 2). Assis *et al*. (2010) determined the LC_{50} values for carbofuran and carbaryl as 0.92 µmol/L and 33.8 µmol/L respectively in *Arapaima gigas*. In this present study, there was also a higher median lethal concentration of carbaryl compared to carbofuran. Acute toxicity of carbaryl was also investigated by Boran *et al*. (2010) in *Oncorhynchus mykiss* and *Poecilia reticulate.* Hernandez-Moreno *et al*. (2011) investigated the acute effects of carbofuran on sea bass (*Dicentrarchus labrax)*. The observed values of LC_{50} of carbofuran and carbaryl in current studies are in agreement with those calculated with cabofuran and carbaryl for common prawn (Assis *et al*. 2010).

When fish were exposed to pesticides they displayed the signs of intoxication in behavioral alterations such as loss of equilibrium and agitation. These symptoms were followed by increased respiratory rhythm and increased opercular movement and ended with the intermediate period, where fish lay at the bottom of the aquaria exhibiting muscular weakness and erratic movement (Table 1). Although different fish species may manifest different behavioral responses, these observations observed in *Catla catla* are similar to those observed by Da Silva *et al*. (1993) on *Callichtys callichtys* exposed to Folidol 600 (pesticide) and those observed by Fernandez-Vega *et al.* (1999) and Farah *et al.* (2004). The behavioral effects on fish of intoxication with organophosphates and carbamates that were observed in this study could be linked to a failure in energy production or release of stored metabolic energy of severe stress, ultimately leading to fish death, as reported by Chakraborty *et al*. (1989).

Table 1: Effect of triazophos, profenofos, carbofuran and carbaryl on the behavior of *Catla catla*

Visual effects	Suffocation	Laying on the bottom	Erratic swimming	Opening of mouth and gills	Lethargic movements	Downward movement	Gulping before death
Triazophos	+++++	+++++	++++	+++	_	_	+++
Profenofos	+	++	+++	++++	+++++	+++++	+++++
Carbofuran	++	_	++	_	+++	_	_
Carbaryl	++	_	_	_	++++	_	++

The increase or decrease in the level of behavioral parameters is shown by numbers of (+) sign. The (-) sign indicates normal behavioral conditions.

Table 2: Median lethal concentration (LC_{50}) of triazophos, profenofos, carbofuran and carbaryl in *Catla catla* at different time intervals.

Pesticides	Points	24Hours	48Hours	72hours	96Hours
Triazophos (mg/L)	LC_{50}	6.64	5.83	5.64	4.84
	Lower and upper confidence limits (95%)	6.15- 7.13	5.35- 6.32	4.98- 7.85	4.31- 5.42
Profenofos (mg/L)	LC_{50}	0.33	0.29	0.25	0.19
	Lower and upper confidence limits (95%)	0.26- 0.39	0.23- 0.37	0.20-0.30	0.14- 0.24
Carbofuran (mg/L)	LC_{50}	2.40	1.81	1.31	0.99
	Lower and upper confidence limits (95%)	1.76- 3.30	1.26- 3.31	0.94- 1.92	0.73- 1.35
Carbaryl (mg/L)	LC_{50}	9.49	9.10	8.42	7.89
	Lower and upper confidence limits (95%)	8.91 - 10.08	8.50 - 9.76	7.85- 9.09	7.31 - 8.67

Kamanyire and Karalliedde (2004) reported that in addition to acute symptoms, some organophosphates can cause other symptoms usually appear 1 – 4 days after exposure or poisoning with organophosphates, such as weakness in the muscle and breathing difficulties and the observations in the present study are in line with these findings. Symptoms of acute pesticide poisoning can be divided according to the site of acetylcholine accumulation in the organism. Acetylcholinesterase (Ach) remains active throughout the nervous system in the fish, but contaminants may interfere with the cholinergic neural transmission, even in the case of sublethal exposure, intoxication may be in the form of interference with carbohydrate metabolism, reproduction and behaviour (Banerjee *et al*. 1999). Neurotoxicants may also impair cholinergic neural transmission, primarily through cholinesterase (ChE) inhibition in acute toxicity (Pavlov *et al*. 1992; Marrs 1993).

This current study shows that profenofos was highly toxic as compared to the other pesticides studied in the context of LC_{50} values, in that only low concentrations caused death of *Catla Catla.* If we compare the toxicity of other pesticides then it would be apparent that carbaryl was less toxic in *Catla catla*, and also caused the least effects on behaviour. Owing to the highly toxic effects of these pesticides, they must be properly monitored in the environment so that their toxic effects on non-target organisms can be reduced.

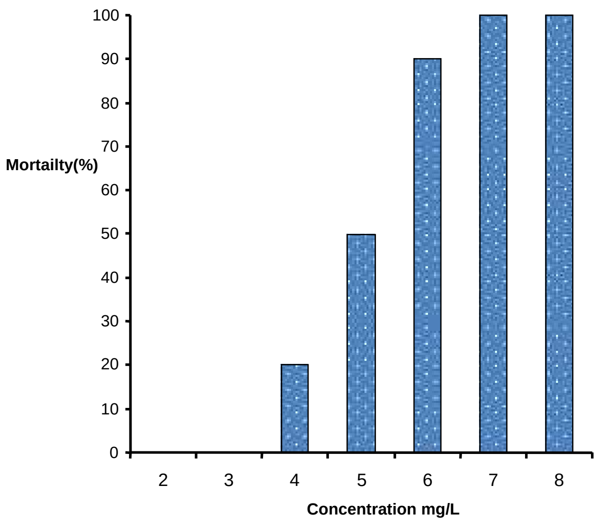

Figure 1: Effect of different concentrations (mg/L) of triazophos on mortality (%) of *C. catla* after 96 hours exposure

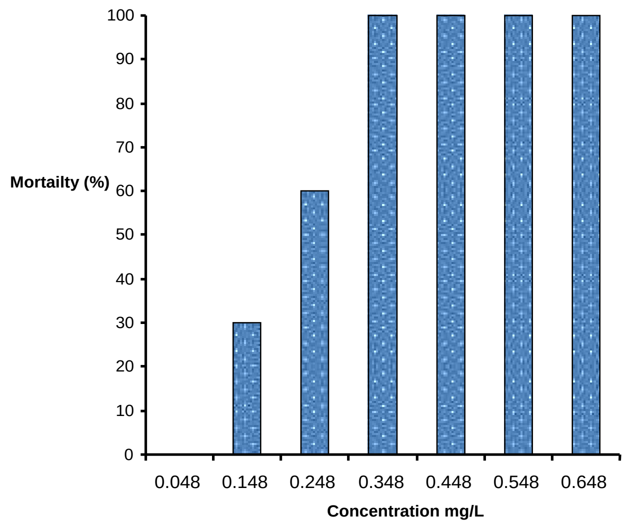

Figure 2: Effect of different concentrations (mg/L) of profenofos on mortality (%) of *C. catla* after 96 hours exposure.

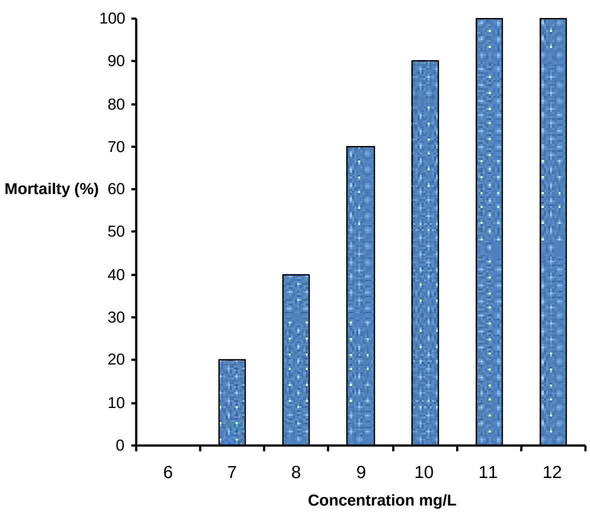

Figure 3: Effect of different concentrations mg/L) of carbofuran on mortality (%) of *C.catla* after 96 hours exposure

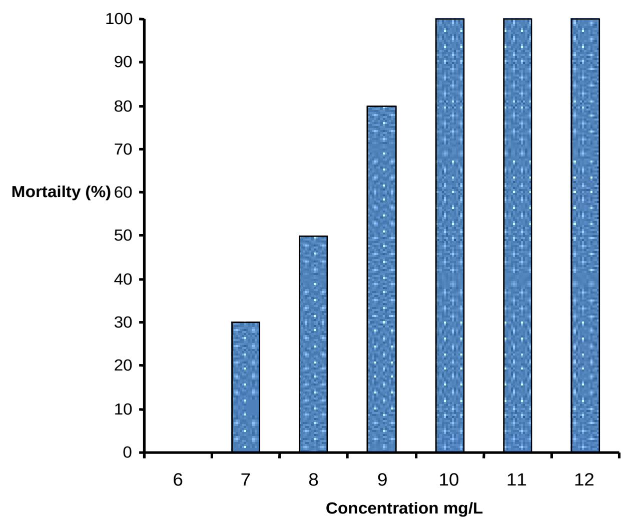

Figure 4: Effect of different concentrations (mg/L) of carbaryl on mortality (%) of *C. catla* after 96 hours exposure

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