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A Study on Acute Toxicity of Triazophos, Profenofos, Carbofuran and Carbaryl Pesticides on *Cirrhinus mrigala*

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Abstract.- The aim of the present study was to evaluate the acute effects of commercial formulation of traiazophos, profenofos, carbofuran and carbaryl in *Cirrhinus mrigala* 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 1.05, 0.21, 0.49 and 4.75 mg/L for triazophos, profenofos, carbofuran and carbaryl, respectively. *Cirrhinus mrigala* mortality 100% mortality was observed at 1.6 mg/L, at 96 hours of carbofuran exposure of carbofuran. Median lethal concentrations of different insecticides at 24, 48, 72 and 96 h were observed in *Cirrhinus mrigala* as 1.05, 0.87, 0.75 and 1.05 mg/L, respectively for triazophos; 0.30, 0.25, 0.23 and 0.21 mg/L respectively for profenofos; 1.10, 0.86, 0.66 and 0.49 mg/L respectively for carbofuran and for 6.44, 5.19, 4.77 and 4.75 mg/L, respectively for carbyl. Acute toxic stress was noticed with subjects exhibiting behavioural intoxication, including suffocation, lying on the bottom, erratic swimming, lethargy and downward movements and gulping prior to mortality.

Keywords: *Labeo rohita,* acute toxicity, profenofos, triazophos, carbofuran, carbaryl.

INTRODUCTION

Fish, considered is to be the model animal, for understanding the deleterious effects of pesticides on aquatic life. Being core source of protein, it is also desirable to understand pesticide effect as well as accumulation in this prime source of protein and its aftereffects on man as end user.

Fish are sensitive to hormones and enzymatic disruptors. Chronic exposure to lower doses of pesticides may have more drastic effects as compared with acute poisoning. Pesticide doses that are not lethal for fish, may affect their physiology and behaviour, ultimately damage survival and reproduction (Kegley *et al*., 1999). Metabolic disturbances, growth retardation, enzymes inhibition, reduction in the longevity and fecundity of the organism are some biochemical changes caused by the pesticidal stress (Murty, 1986). Brain, kidney, gills and liver are the most susceptible fish organs exposed to poisons (Jana and Bandyopadhayaya, 1987). When exposed to pesticides fish show rapid movements in body, restlessness, convulsions, excess mucous secretion, respiratory problems, loss of balance and change in color. In several fish species exposed to different pesticides, similar behavioral changes have also been monitored (Haider and Inbaraj, 1986). Aquatic contamination by pesticides is a major problem in developing countries and is causing many health problems. Pesticides are applied for crop protection and mitigation of pests, but only 0.1 % of the applied pesticides reach the target pests, with the remaining amount persisting and spreading throughout the environment (Hart and Pimentel, 2002; Mahboob *et al*., 2011). Acute toxicity may be measured as oral, dermal and inhalation, acute inhalation toxicity is measured by LC_{50}. Acute toxicity is expressed not only by death, but also by various changes in behaviour and physical activities. The present studies were designed to investigate the acute toxicity and the median lethal concentration (LC_{50}) of four pesticides, two organophosphates and two carbamates, on *Cirrhinus mrigala* fingerlings.

The organophosphates and carbamates are group of synthetic insecticides and are potent

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neurotoxic molecules (Lundebye *et al*., 1997), which are frequently used in the developing countries to control different agricultural pests (Vioque-Fern´andez *et al*., 2007; Banni *et al*., 2005). Different pesticides, however, have different LC_{50} values in different organisms (Mathur and Singh, 2006). Acute toxicity of profenofos and triazophos in crucian carp was determined as 0.192 ppm and 8.4 ppm, respectively (Jin *et al*., 2010), These values were determined as 62.4 ppb and 1.00 ppm, respectively in common carp (Ismail *et al*., 2009). Assis *et al*. (2010) determined the LC_{50} values for carbaryl as 33.8 μmol/L in *Oncorhynchus mykiss*, while Boran *et al*. (2007) investigated acute toxicity of carbaryl and found it more toxic to rainbow trout then to guppies.

The present study was planned to determine the acute toxicity of commonly used triazophos and profenofos (OP) and carbofuran and carboryl (Cs) on fingerlings of *Cirrhinus mrigala*, which is one of the indigenous fish in the Pakistan.This study would supplement the current knowledge on toxicity of OP and carbamates and also help an effective management of fresh water reservoirs with respect to the input of these pesticides from agricultural fields.

MATERIALS AND METHODS

Cirrhinus mrigala fingerlings (L=2.65-3 inch, W= 18-21 g) were maintained in 70 litre glass aquaria, having been procured from the Fish Seed Hatchery, Satiana Road, Faisalabad, Pakistan. The fish were fed with commercial feed at 3% body weight during 15 days acclimatization period. Electrical conductivity, pH and temperature were analyzed and maintained at optimal conditions. The aquaria water temperature was maintained 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.

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). *Cirrhinus mrigala* were divided into six groups each with ten individuals for toxicity test to test 6 dilution in triplicates with negative control receiving no pesticide but maximum solvent that any dosing solution contain. Before acute toxicity tests (LC_{50}) preliminary tests were done with 1ppm and 10ppm concentration of all pesticides to determine the minimum and maximum limits for 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). Six dosing solutions were prepared from the stock prepared by mixing different proportions of stock solution in acetone (profenofos), methanol (methanol) and ethanol (carbofuran and carbaryl) to prepare the experimental concentrations. Nominal concentrations of active ingredient tested were: triazophos 4.0-9.0; profenofos 0.098-0.686; carbofuran 0.25-8.0 and carbaryl 6.0-12.0 mgL^{-1} The specimens were fed 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 obsereved and noted after 24, 48, 72 and 96 h. The data for mortality and alive fish was analyzed using Probit analysis (Finney, 1971). 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 (2005). Recoveries of pesticides in the reference material were between 80 and 110 % of certified concentrations.

RESULTS AND DISCUSSION

The effects of pesticide intoxication were observed as suffocation, restlessness, loss of equilibrium and erratic swimming on prodding with all the tested pesticides. Fish often remained at the bottom with mouth opened before dying (Table I). Although different fish species may manifest different behavioral responses, these observations observed in *Cirrhinus mrigala* are similar to those observed by Da Silva *et al*. (1993) on *Callichthys callichthys* (Pisces: Teleostei) 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 OP and Cs that were observed in this study could be linked to a failure in energy production or release of stored metabolic energy in severe stress, ultimately leading to fish death, as reported by Chakraborty *et al*. (1989). The effects of intoxication with profenofos presented as erratic swimming, hyperexcitability, discoloration of the skin and secretion of mucus on the body and gills, leading eventually to death.

Mortality response and relationship of selected fish to various concentrations of pesticides are presented in Table II and Figure 1. An increase in the number of mortalities was observed as the concentration of insecticide was increased. There was no mortality in the control group. There was a 10% mortality at 0.2 mg/L (the lowest exposure concentration) of triazophos. LC_{50} of triazophos at 24, 48, 72 and 96 h were observed in *Cirrhinus mrigala* as 1.05, 0.87, 0.75 and 1.05 mg/L, respectively (Table II). 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 h; *i.e.* LC_{50} was reduced. There was a significant difference ($P < 0.05$) among LC_{50} values obtained at different times of exposure. 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; Ghazala *et al*., 2014 a,b). At 96 h the LC_{50} was recorded as 0.21 mg/L. The 100% mortality of *Cirrhinus mrigala* was observed with a 1.6 mg/ L dose of carbofuran at 96 h with a significant difference. LC_{50} values of carbofuran at 24, 48, 72 and 96 h were estimated as 1.10, 0.86, 0.66 and 0.49 mg/L, respectively. Median lethal concentrations of carbaryl at 24, 48, 72 and 96 h were observed in *Cirrhinus mrigala* as being as 6.44, 5.19, 4.77 and 4.75 mg/L, respectively, at 95% confidence intervals (Table II). 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 *Cirrhinus mrigala.* The least toxic compound was found to be carbaryl as compared to other tested compounds. In general, acute susceptibility of the tested pesticides was as follows: profenofos > carbofuran > triazophos > carbaryl. 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) and Ghazala *et al.* (2014 a, b) where acute toxicity of profenofos to *Channa punctuates* was observed as 2.68 µg/L. Kamanyire and Karalliedde (2004) reported that in addition to acute symptoms, some OPs can cause other symptoms usually appear 1–4 days after exposure or poisoning with OPs, such as weakness in flesh 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.

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 *Cirrhinus*

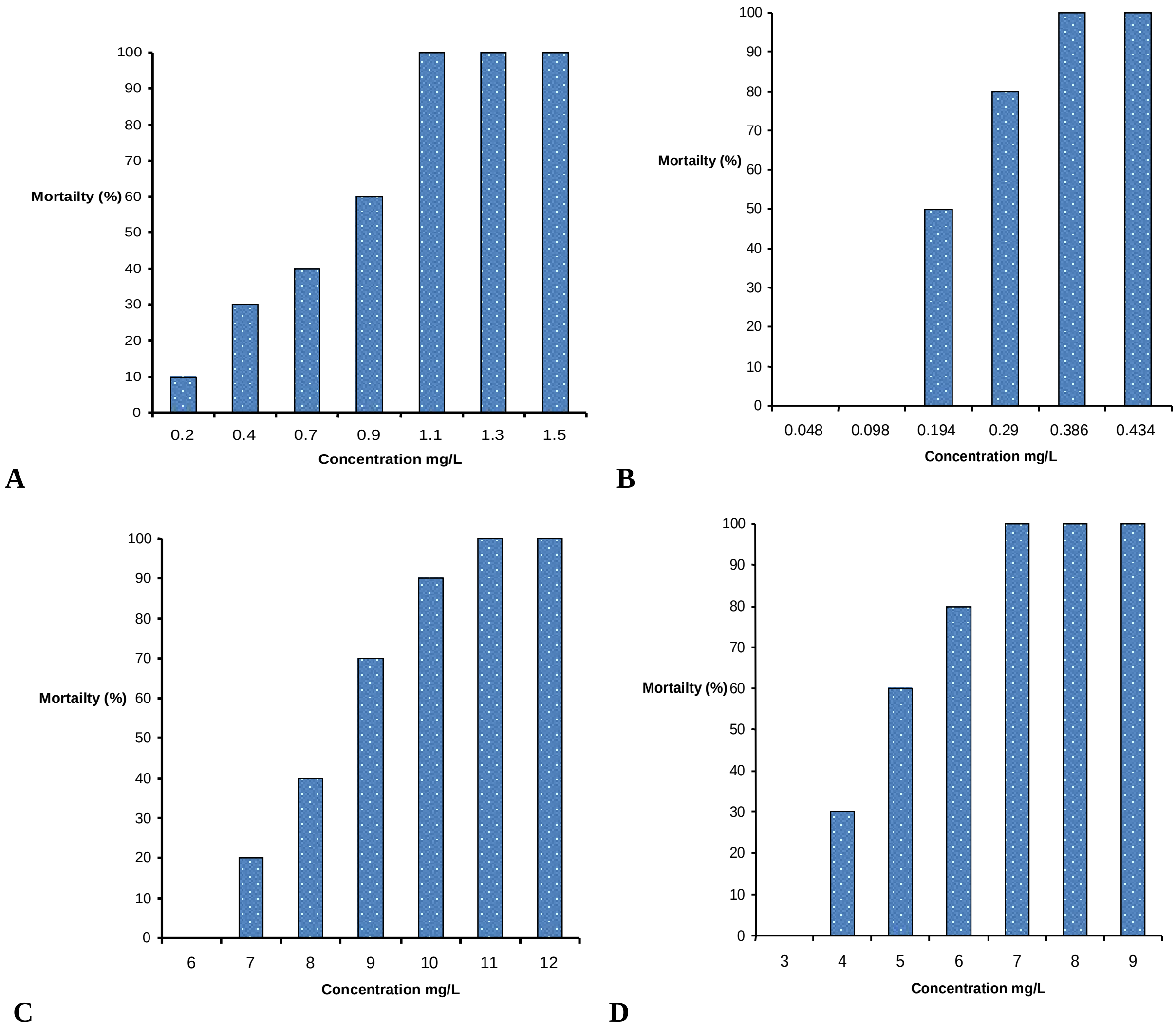

Fig. 1. Effect of different concentrations (mg/L) of triazophos (A), profenofos (B), carbufuran (C) and carbaryl (D) on mortality (%) of *C. mrigala* after 96 hours exposure.

Table I.- Effect of triazophos, profenofos, carbofuran and carbaryl on the behavior of C*irrhinus mrigala*

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. Increase (+) indicate the severity of change in the behavior of fish with pesticidal stress.The (-) sign indicates normal behavioral conditions.

Table II.- LC_{50} and range of toxicity of triazophos, profenofos, carbofuran and carbaryl in *Cirrhinus mrigala* at different time intervals.

Pesticides	24 h	48 h	72 h	96 h
Triazophos (mg/L)	1.05 (0.91-1.20)	0.87 (0.71-1.00)	0.75 (0.59-0.91)	1.05 (0.43-0.84)
Profenofos (mg/L)	0.30 (0.24-0.36)	0.25 (0.20-0.30)	0.23 (0.18-0.28)	0.21 (0.16-0.26)
Carbofuran (mg/L)	1.10 (0.84-1.38)	0.86 (0.62-1.23)	0.66 (0.47-0.96)	0.49 (0.35-0.70)
Carbaryl (mg/L)	6.44 (5.70-7.33)	5.19 (4.56-5.78)	4.77 (4.16-5.37)	4.75 (4.17-5.47)

mrigala . If we compare the toxicity of other pesticides then it would be apparent that carbaryl was less toxic in *Cirrhinus mrigala*, 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.

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