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Note

Investigations on the acute toxicity and behavioural alterations induced by the organophosphate pesticide, chlorpyrifos on *Puntius chola* (Hamilton-Buchanan)

VINOD KUMAR VERMA AND AMITA SAXENA*

Regional Centre of the Central Inland Fisheries Research Institute, Guwahati - 781 006, Assam, India
**G. B. Pant University of Agriculture and Technology, Pantnagar - 263 145,Uttarakhand, India*
e-mail: agnesv2008@gmail.com

ABSTRACT

Chlorpyrifos, a broad spectrum organophosphate insecticide which is a common contaminant of urban streams, is highly toxic to fish causing fish mortality in waterways near treated fields and buildings. The acute toxicity of chlorpyrifos was investigated on the freshwater fish *Puntius chola* in a 96 h static laboratory bioassay. The 96 h LC_{50} of chlorpyrifos is 0.219 ppm. Analysis of variance showed that there was significant difference ($p<0.01$) in the mortality of *P. chola* exposed to different concentrations of chlorpyrifos at 4, 8, 24, 48, 72 and 96 h of exposure.

Keywords: Chlorpyrifos, Fish, *Puntius chola*, Toxicity

Introduction

Indiscriminate use of pesticides in agriculture has resulted in the contamination of environment causing hazard to terrestrial as well as aquatic life. New chemical formulations are widely being used to control pests of agricultural crops. Pesticide runoff from agricultural fields may easily find their way into natural water bodies and adversely affect the quality of water and create hazards for aquatic life resulting in serious damage to non-target species, including fish (Bondarenko *et al.*, 2004; Ayoola, 2008). Open water resources like rivers and reservoirs are more vulnerable to pesticide contamination through agricultural, domestic and industrial run-off, accidental spillage and unintentional dumping or deliberate contamination of water resources. Chlorpyrifos is a toxic crystalline broad spectrum chlorinated organophosphate insecticide, acaricide and nematicide which is used extensively in households and farms to control pests including mosquitoes, cockroaches, grubs, flea beetles, fire ants, and others. Chlorpyrifos is an inhibitor of acetylcholinesterase (ACh-ase) enzyme vital to the nervous system of animals and humans.

The fish *Puntius chola* (Hamilton-Buchanan), commonly known as swamp barb, is an indigenous omnivorous freshwater fish exhibiting prolific breeding and high growth rate. It belongs to the order Cypriniformes and is distributed throughout the tropical and sub-tropical zones of the world. The present study was undertaken to determine the acute toxicity of chlorpyrifos and its effect on the behavioural response of *P. chola* with a view to investigate the effect of indiscriminate use of this pesticide on aquatic life.

Live fingerlings of *P. chola*, of mean total length 5.75±0.75 cm and mean weight 3.5±0.5 g were obtained from the fish farm of College of Fisheries, Pantnagar. They were acclimatised in 1000 l capacity fibre glass tank for 21 days. The water (temperature 22±0.5 ^{0}C, pH 7.3±0.04, dissolved oxygen 6.9±0.5 mg l^{-1}) used for acclimatisation and experimentation was the same as used in the toxicity studies. During the acclimtisation period, fishes were fed with conventional fish feed (rice bran and soya cake in 1:1 ratio) at the rate of 4% body weight. Unconsumed feed and faecal matter were siphoned out and water was replenished, on alternate days to prevent accumulation of organic wastes.

Commercial grade chlorpyrifos (O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) having 20% EC was used for the study.

Static renewal bioassay technique was adopted in which the test medium was renewed at the same concentration once every 24 h (ASTM, 1991). Preliminary screening was carried out to determine the appropriate concentration range for testing chemical as described by Solbe (1995). After proper acclimatisation, the fingerlings of *P. chola* were exposed to five different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 ppm) of chlorpyrifos (20% EC) insecticide in triplicates (R_1, R_2 and R_3) along with control groups for 96 h. Fish mortality was recorded at 4, 8, 24,

48, 72 and 96 h post-exposure. Dead fishes observed were removed immediately.

Physico-chemical parameters such as temperatue, pH, dissolved oxygen, free carbondioxide and ammonia in the test media were recorded at 4 h, 32 h and 96 h post-exposure. All the parameters were analysed following standard methods as per APHA (1992).

One way analysis of variance (ANOVA) was used to test the significance level in the mean mortality response of *P. chola*. SPSS 11 for windows was used to calculate the LC_{50} values of chlorpyrifos. The data were subjected to Probit analysis as suggested by Finney (1971).

During the experimental period, temperature (Fig. 1) and pH (Fig. 2) in the test media did not differ much between various concentrations of the pesticide as well as with control. Temperature ranged between 25.3±0.043 ^{0}C and 25.4±0.082 ^{0}C while pH ranged between 7.3±0.035 and 7.4±0.082. Dissolved oxygen (Fig. 3), free carbon dioxide

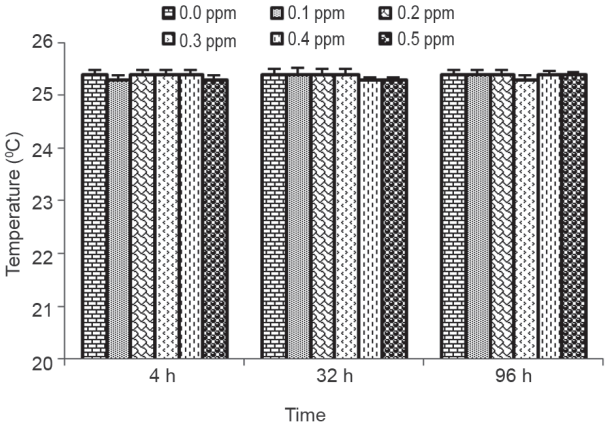

Fig. 1. Variation in water temperature (°C) in the experimental tanks during the short term experiment

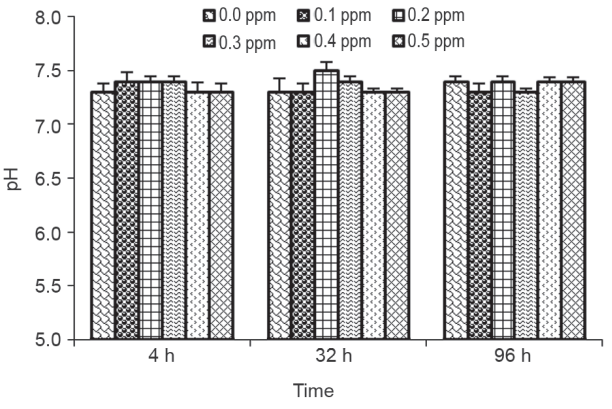

Fig. 2. Variation in water pH in the experimental tanks

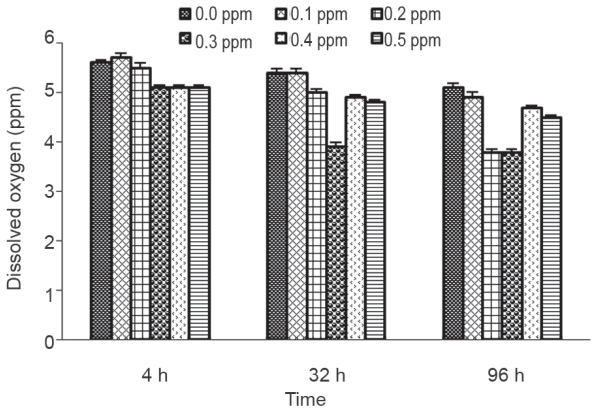

Fig. 3. Variation in dissolved oxygen (ppm) in the experimental tanks

(Fig. 4) and ammonia (Fig. 5) of the chlorpyrifos treated media showed significant variations ($p<0.01$). In control group, maximum dissolved oxygen (5.6±0.047 ppm) was recorded at 4 h and minimum (5.1±0.081 ppm) at 96 h. Among the treated groups, maximum dissolved oxygen (5.4±0.081 ppm) was recorded in 0.1ppm treatment at 4 h and minimum (3.6±0.041ppm) in 0.2 ppm treatment at 96 h.

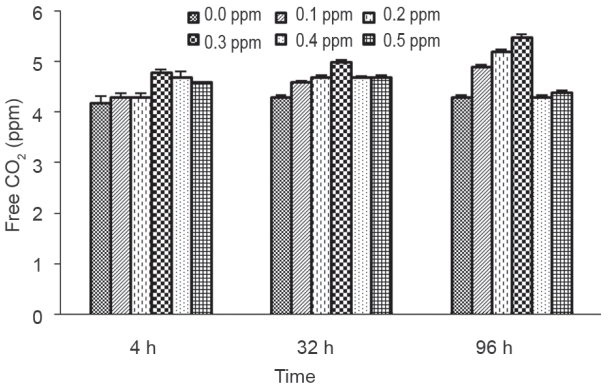

Fig. 4. Variation in free CO_2 (ppm) in the experimental tanks

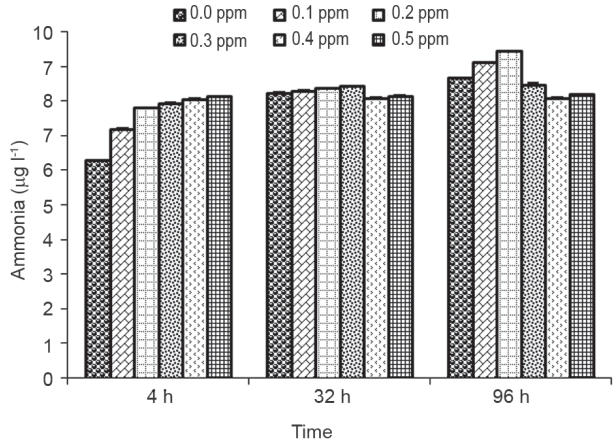

Fig. 5. Variation in ammonia (µg l^{-1}) in the experimental tanks

Effect of time and treatment interaction on dissolved oxygen showed significant difference ($p<0.01$) between control and treated groups.

Increase in oxygen consumption is an indication of stress. Fishes in treated group were likely to have consumed oxygen more rapidly than in the control group to tolerate the stress. Oxygen consumption rate decreased significantly with time for Zn exposed *Phascolosoma esculenta* and also with the interaction between concentration of Cd and time in Cd exposed fish (Chen *et al.*, 2009).

Increase in carbondioxide production was more pronounced in chlorpyrifos treated fishes than fishes from the control group (Fig. 4); which could be attributed to increased metabolic activity and respiration rate in treated groups than in control group fishes. In control group, free carbondioxide recorded was 4.2±0.124 ppm. Among the treated groups, minimum free carbondioxide (4.6±0.081 ppm) was recorded in 0.1 ppm treatment at 4 h and maximum (6.2±0.042 ppm) in 0.2 ppm treatment at 96 h. During the exposure period, significant increase ($p<0.01$) in ammonia was also observed in treated media. The values ranged from 7.21±0.032 μl^{-1} in 0.1 ppm treatment at 4 h to 9.45±0.016 μl^{-1} in 0.2 ppm treatment at 96 h,while in control group the ammonia concentration recorded was 6.29±1.832 μl^{-1} (Fig. 5). This may be due to the increased metabolic activity in the fishes to tolerate the stress induced. Our findings are similar to the results of Boeck*et al.* (1995). Wood *et al.* (2010) observed that when water flow is restricted, ammoniotelic teleost fish may create their own extreme external and internal environments high in ammonia. Wilkie *et al.* (2010) studied the neural mechanism of ammonia and anoxia tolerance in gold fish (*Carassius auratus*) and reported ammonia and anoxia cause neurotoxicity by overactivating N-methyl-D-aspartate receptors in the central nervous system.

Toxicity of chlorpyrifos was time and dose dependent. There was a significant negative correlation between LC values and exposure periods. The analysis of dose response data of chlorpyrifos when tested in *P. chola* revealed that LC_{50} values ranged from 0.219 ppm at 96 h to 0.557 ppm at 4 h. Thus with increase in exposure periods, the LC_{50} values decreased (Table 1). The analysis of variance (ANOVA) showed that there was significant difference ($p<0.05$) in the quantal response (mortality) of *P. chola* to different treatments (concentrations) of chlorpyrifos at 4, 8, 24, 48. 72 and 96 h of exposure. The highest concentration of the toxicant resulted in the highest mortality rate which is in agreement with the study of FAO (1977) and Ayoola (2008), who reported that in all toxicants a threshold is reached, above which there is no drastic survival of animal and below the threshold, the animal is in a tolerance zone, while below the tolerance zone is the zone of resistance.

In control group fishes, throughout the 96 h experimental period normal behaviour was observed. Fishes were observed to breath and swim freely, with normal operculum opening and normal mucus production and the skin colour remained silvery white. In 0.1 ppm treatment till 8h post-exposure, movement was normal; after which movement became slow. At 72 and 96 h, settling of the fishes at the bottom of the tanks and increase in mucus production were observed. In 0.2 ppm treatment, slightly fast movement was observed in comparison to control, which became slow as time elapsed. Skin colour changed slightly due to mucus secretion and mortality was observed at 48 to 96 h. In 0.3 ppm treatment, fast and jerking movement with mortality and increased mucus production was observed. As time elapsed, fish exhibited vertical hanging and settling atthe bottom. In 0.4 ppm and 0.5 ppm treatments, fishes exhibited stress symptoms, vertical hanging in the water and mortality. Skin colour became brownish with a red spot in the head region, and slimy body was noticed. Fishes of all treated groups showed hyper activity and frequent surfacing to gulp air. It was also noticed that as time passed, they continued to swim near the water surface and tried to jump out from the holding tanks. Once the fishes were exhausted, they sank to the bottom of the tanks with no opercular movement and finally succumbed with their mouth opened. The nature and rapidity of onset of fish behavioural responses indicated that these compounds act on the neuromuscular system of fish. Saxena (1995) noted mortality of fishes in polluted water owing to changes at the molecular level. Animal behaviour is a neurotropically regulated phenomenon, mediated by neurotransmitters. The stressful breathing behaviour exhibited by fish may be the result of respiratory

Table 1. Relative acute toxicity (LC_{50}) of chlorpyrifos on *Puntius chola,* with lower (LCL) and upper (UCL) 95% confidence limit

Exposure period (h)	LC_{50} values (ppm)	LCL	UCL
4	0.557	0.460	0.995
8	0.514	0.424	0.927
24	0.339	0.287	0.391
48	0.265	0.240	0.304
72	0.236	0.218	0.256
96	0.219	0.201	0.237

impairment due to effect of toxicant on the gills. The above symptoms could be due to inhibition of acetylcholinesterase activity leading to accumulation of acetylcholine in cholinergic synapses ensuing hyper stimulation. Inhibition of acetylcholinesterase activity is a typical characteristic of organophosphate compounds (Rao*et al*., 2005; Siang *et al*., 2007). The behavioural observations made were similar to the observations made by Hülya *et al*. (2006) in *Oreochromis niloticus* following sublethal exposure to diazinon. It was also observed during the experiment that the fishes in treatment groups were unable to take as much food in comparison to control fishes as observed by Sandahl *et al*. (2005). According to Lawal and Samuel (2010), exposure of *Poecila reticulata* to actellic resulted in aggressive behaviour, rapid gulping of water, increased opercular movement and abnormal swimming movements.

Shahi and Singh (2010) observed various forms of abnormal behaviour in *Channa punctatus* when exposed to different concentrations of rutin, taraxerol and apigenin. Susan and Sobha (2010) studied the toxic effect of fenvalerate on Indian major carps, *Labeo rohita, Catla catla* and *Cirrhinus mrigala* and observed behavioural changes such as swimming at the water surface, hyper excitation, loss of equilibrium, flaring of gills and increased mucus production. Kochhann *et al.* (2010) studied the effect of different crude oil fractions on the swimming performance of tambaqui (*Clossoma macropomum*) and observed decreased swimming activity in fish exposed to insoluble crude oil. The findings of this study revealed that chlorpyrifos is highly toxic to fish and hence indiscriminate use of this insecticide on/near fish farms or water bodies should be discouraged.

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