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RESEARCH ARTICLE

ACUTE EFFECTS OF CHLORPYRIFOS ON OXYGEN CONSUMPTION AND FOOD CONSUMPTION OF FRESHWATER FISH, *OREOCHROMIS MOSSAMBICUS* (PETERS)

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ABSTRACT

Aquatic toxicity risks of agricultural pesticides to non-target organisms especially fishes are pivotal. These pesticides influence metabolism at very low concentrations by disrupting physiological balances. An attempt has been made in the present investigation to determine the acute toxicity of chlorpyrifos on oxygen consumption and food consumption of freshwater fish, *Oreochromis* mossambicus (Peters). Short-term acute toxicity tests were performed adopting renewal bioassay technique over a period of 96 hr, using different concentrations of chlorpyrifos to the fish and the 96 hr LC_{50} value was found to be 0.022ppm. For oxygen consumption and food consumption study, the fish were exposed to two sublethal concentrations viz., $1/5^{th}$ of LC_{50} (0.0044 ppm) and $1/10^{th}$ of LC_{50} (0.0022 ppm) along with lethal concentration (0.022 ppm) as reference for 48 hr indicated that lethal concentrations had profound effect than sublethal concentrations. The fish exhibited respiratory distress (such as gasping in air), loss of balance and erratic swimming prior to death. Chlorpyrifos had a significant effect on functional activity of experimental fish by altering respiration rate and impairing feeding behavior.

INTRODUCTION

The aquatic environment is continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activity (Begum, 2004). The pollution of aquatic environment with wide array of xenobiotic compounds has become a menace to the aquatic flora and fauna and is a problem of immediate concern. Use of pesticides have become a necessary evil in developing countries like India where it is estimated that approximately 30% of its crop yield are lost due to pest attack each year. Unfortunately, these pesticides lack target specificity and cause severe and long lasting effects on terrestrial and aquatic non-target organisms, especially the fish. A large amount of the pesticides used, never reaches the intended targets and enter the aquatic environment which is currently under threat of the indiscriminate use of pesticides. Pesticides can cause acute and chronic poisoning of fish and may damage their vital organs (Joshi *et al*., 2007).

In the aquatic environment one of the most important manifestation of the toxic action of chemical is the over stimulation or depression of respiratory activity. Pesticides are indicated to cause respiratory distress or even failure by affecting respiratory centres of brain or the tissue involved in breathing. The changes in the respiratory activity of fish have been used by several investigators as indicators of response to environmental stress. The effect of toxicants on the respiration of fishes and invertebrates has received wide spread attention and were reviewed by (Hughes, 1976) and (Wright, 1978). The respiratory potential or oxygen consumption of an animal is the important physiological parameters to assess the toxic stress, because it is a valuable indicator of energy expenditure in particular and metabolism in general (Prosser *et al*., 1977).

Pesticides in sublethal concentrations present in aquatic environment are too low to cause rapid death directly but may affect the functioning of the organisms, disrupt normal behavior, and reduce the food consumption (Susan et al., 2010). Fish behavior under stress conditions provides important information for aquaculturists (Kristiansen *et al* 2004). Methods of monitoring and quantifying the behavioural response have become potential alternatives for assessing stress, disease, water pollution and toxic material in water (Kane *et al* 2004). Food intake is one of the most important factors regulating the level of metabolism. Detection of derangements in metabolism is most sensitive parameters of stress, since it integrates many processes, including enzyme activity and modulation, substrate pools and physiological response (Soucek, 2007). Fish experiencing acute exposures to sublethal concentrations of pesticide exhibit significant feeding impairment, with potentially severe consequences for their ecological fitness (Floyd, 2008). Among different classes of

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pesticide, organophosphorous insecticides represent one of the most widely used classes of pesticide with high potential for human exposure in both rural and residential environments (Ngoula *et al*., 2007). Chlorpyrifos (O, O-diethyl-O-3, 5, 6-trichlor-2-pyridyl phosphorothioate; CPF) is a broad spectrum organophosphate insecticide widely used to control foliar insects in agricultural crops (Rusyniak and Nanagas, 2004). It is the second highest selling organophosphate insecticide and is more toxic to fish than organochlorine compounds (Tilak *et al*., 2001). Chlorpyrifos is a non-systemic insecticide designed to be effective by direct contact, ingestion and inhalation (Tomlin, 2006). Fishes have greater sensitivity to changes in the aquatic environment (Vinodhini and Narayanan, 2008). Capacity to accumulate large quantity of pollutants and important link in the food chain, fishes are often used as indicator organisms to monitor quality of aquatic systems the world over (Rajkowska and Protasowicki, 2011). Fish sensitivity to different pesticides could be explained by their relatively slow metabolism and elimination of these compounds (David, 2003). The fish *Oreochromis mossambicus* was selected as experimental model because of its low-cost culture and management and has been proposed for use as a test organism in toxicological assays for its suitability for toxicity testing, wide geographical distribution and availability throughout the year. The purpose of this study is to determine the acute effects of chlorpyrifos (20% EC: emulsifiable concentrate) on the oxygen consumption and food consumption in tilapia exposed to lethal and sublethal concentrations of chlorpyrifos.

MATERIALS AND METHODS

Animal collection and maintenance

Oreochromis mossambicus fry (2-3 cm) acquired from Chintamani fish farm, Chickaballapur district, Karnataka were transported to the FRIC Hebbal, Bangalore in well oxygenated polythene bags containing clean pond water. The fish were reared to fingerling size (9-10 cm) with artificial feeding. Later, the fishes were released into the freshwater aquariums of 50 liter capacity (10 No's each) for proper acclimation in the laboratory and were fed every 24 hr with commercial feed. The walls of the tank were periodically cleaned to avoid algal growth. The excreta were siphoned off on a daily basis to prevent the buildup of ammonia in the medium. Fishes were conditioned for 10 days prior to use them for the experiments. The water temperature, dissolved oxygen level and pH are monitored regularly. Individual fishes measuring 9±0.5 cm in total length and weighing 13±0.5 g were selected for the present study.

Bioassay test

Toxicity study was carried out by following the standard guidelines (APHA, 2005) to determine the lethal (LC_{50}) level of chlorpyrifos using static renewal method. Ten fish each were accommodated in 45 liters of test solution in the aquarium. The experiment was conducted in triplicate. Dead fishes were removed immediately from the test medium to avoid deterioration. Three set of replicates were performed for each concentration. The 96 hr LC_{50} value of the mortality in each exposure concentration of chlorpyrifos were recorded and tested by probit analysis program as described by (Finney, 1971).

Estimation of oxygen consumption

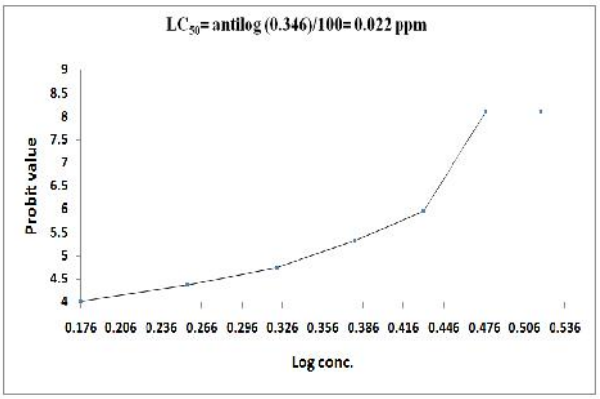
The experiments on the oxygen consumption of fish were carried out in a glass aquarium of 50 liter capacity. The lethal (LC_{50} at 96 hr) and two sub lethal concentrations ($1/5^{th}$ of LC_{50} and $1/10^{th}$ LC_{50} at 96 hr) were selected to study the oxygen consumption rate for 48 hr in static system with 12 hr interval. Ten fish each were accommodated in 45 liter of test solution. The surface water of the control and test chamber was covered with a thin film of liquid paraffin, to prevent diffusion of atmospheric air into test medium. The amount of dissolved oxygen in water for every 12 hr was estimated by Winkler method (Golterman and Clymo, 1969). The difference in dissolved oxygen content between initial and final water samples represents the amount of oxygen consumed by the fish. The oxygen consumption examination was based on the method described by (Chinni *et al*., 2000).

Estimation of food consumption

The food consumption rate of fish was estimated according to (Broeck *et al*., 1997). The experiments were carried out in a glass aquarium of 50 liter capacity. The lethal and two sub lethal concentrations were selected to study the food consumption rate for 48 hr in static system with 12 hr interval. Ten fish each were accommodated in 45 liter of test solution. Fishes were fed once in 12 hr with "TAIYO Grow" floating type dry feed pellet. After 30 min, the remaining food was removed. It was dried overnight at 60°C and weighed to compare mean food consumption.

Table 1 Determination of 96 hr LC_{50} of Chlorpyrifos in *Oreochromis mossambicus*

Conc.(ppm)	No. of fishes used	Mean % Mortality
0.015	10	16
0.018	10	26
0.021	10	40
0.024	10	63
0.027	10	83
0.030	10	100
0.033	10	100



Graph 1 Graphical derivation (based on Probit analysis) of log concentration of pesticide, Chlorpyrifos Vs Percent mortality of *Oreochromis mossambicus* for 96 hours.

Table 2 Oxygen consumption (mg l^{-1} gm^{-1} h^{-1}) and Food consumption (gm feed gm^{-1} body weight) of *Oreochromis mossambicus* at different concentration of Chlorpyrifos

Conc.(ppm)/Hour	12	24	36	48
Oxygen consumption (mg l^{-1} gm^{-1} h^{-1})				
Control	0.10873 ± 0.0009	0.10989 ± 0.0018	0.11037 ± 0.0007	0.11446 ± 0.0026
0.0022	0.17113 ± 0.0015	0.17066 ± 0.0009	0.17005 ± 0.0006	0.16008 ± 0.0014
0.0044	0.18024 ± 0.0008	0.17538 ± 0.0012	0.16816 ± 0.0004	0.16273 ± 0.0002
0.022	0.26399 ± 0.0014	0.25641 ± 0.0006	0.23027 ± 0.0018	0.23809 ± 0.0025
Food consumption (gm feed gm^{-1} body weight)				
Control	0.01315 ± 0.0002	0.01306 ± 0.0004	0.01321 ± 0.0006	0.01339 ± 0.0007
0.0022	0.01052 ± 0.0003	0.01019 ± 0.0005	0.01005 ± 0.0002	0.01034 ± 0.0004
0.0044	0.00941 ± 0.0002	0.00918 ± 0.0001	0.00902 ± 0.0001	0.00926 ± 0.0003
0.022	0.00657 ± 0.0005	0.00582 ± 0.0004	0.00509 ± 0.0003	0.00361 ± 0.0009

P<0.05 significant difference

Statistical analysis

The data were subjected to statistical analysis employing ANOVA and Duncan's multiple range test at P<0.05 (Duncan, 1995; Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

Fishes exposed to lethal and sublethal concentrations of an organophosphorous pesticide, chlorpyrifos for a short-term exposure were studied in terms of general behavior, rate of survival, mortality, oxygen consumption and food consumption. The LC_{50} value of chlorpyrifos for *Oreochromis mossambicus* was 0.022 ppm at 96 hr (Graph 1). Fish mortality increased with increase in concentration of chlorpyrifos. The 16%, 40%, 83% and 100% of fish death were observed at 0.015 ppm, 0.021 ppm, 0.027 ppm and 0.030 ppm respectively for 96 hr of exposure to chlorpyrifos (Table 1), however no deaths of fish were observed in control.

Gulping air, abnormal swimming, loss of equilibrium, rapid opercular movements, excess secretion of mucus, disrupted shoaling behavior was observed during exposure period. When exposed to lethal concentration, body surface acquired dark colour before their death which is one of the symptoms of toxicity. While in control fish, swimming and opercular movements were normal. Similar observations were made by (Ural and Simsek, 2006) and (Chebbi and David, 2010) when they exposed fingerlings of European catfish to dichloro-vos and common carp to quinalphos respectively. (Ramesh and Munniswamy, 2009) also reported an excess secretion of mucus in *Cyprinus carpio* when exposed to chlorpyrifos.

The respiratory potential or oxygen consumption of an animal is the important physiological parameter to assess the toxic stress. As aquatic organisms have their outer bodies and important organs such as gills almost entirely exposed to water, the effect of toxicants on the respiration is more pronounced. Pesticides enter into the fish mainly through gills and with the onset of symptoms of poisoning, the rate of oxygen consumption increases. (Holden, 1973) observed that one of the earliest symptoms of acute pesticide poisoning is respiratory distress. This serves not only as a tool in evaluating the susceptibility or resistance potentiality of the animal, but also useful to correlate the behaviour of the animal. It is clearly evident from the results that chlorpyrifos affected the oxygen consumption rate of fish under both lethal and sub lethal concentrations (Table 2).

Highest oxygen consumption rate was attained in lethal concentration 0.022 ppm (0.26399) during 12^{th} hr and lowest observed in control (0.10873) during 12^{th} hr. The rate of oxygen consumption was higher than the control. The metabolic rate in relation to respiration of fish could be increased under chemical stress (Chebbi and David, 2010). There was a significant decrease in oxygen consumption in fish exposed to sub lethal concentration 0.0022 ppm (0.17113-0.16008) and 0.0044 ppm (0.18024- 0.16273) and lethal concentration 0.022 ppm (0.26399- 0.23809) during 12 hr to 48 hr. A decrease in the respiratory rate in both the lethal and sub lethal concentrations due to toxicant induced stress, avoidance and biotransformation. If gills or membrane functions are destroyed due to xenobiotic chemicals or the membrane functions are disturbed by a change in permeability the oxygen uptake rate would rapidly decrease (Grinwis *et al*., 1998, Hartl *et al*., 2001).

Change in feeding behaviour is considered to be a sensitive indicator to detect pollution due to pesticides. The data on food consumption, calculated per gram body weight in sublethal and lethal concentrations of chlorpyrifos for *Oreochromis mossambicus* is given in Table 2. There was a significant decrease in food consumption of fish exposed to both lethal 0.022 ppm (0.00657- 0.00361) and sub lethal concentration 0.0022 ppm (0.01052- 0.01034) and 0.0044 ppm (0.00941-0.00926) as compared to control (0.01315- 0.01339). The highest food consumption was attained during 48^{th} hr in control (0.01339) and lower food consumption was attained during 48th hr in lethal concentration (0.00361). The control fish had significantly higher average feed intake than that of treated fish. Reduction in feeding behavior under toxic environmental condition might be profitable to lower the energetic costs of digestion (Halappa and David, 2009).

Chlorpyrifos had a significant effect on functional activity of tilapia since an increase in chlorpyrifos concentration induced a decrease in food consumption rate in all the concentration when compared to control. *Oreochromis mossambicus* exposed to sub lethal level of phosphamidon and methyl parathion significantly affected the rates of feeding, absorption, metabolism and conversion (Singh *et al*., 2010). Fish experiencing acute exposure to sub lethal concentrations of the insecticide exhibited significant feeding impairment with potentially severe consequences for their ecological fitness (Floyd *et al*., 2008) presumably, the decrease in food intake may be due to increase in metabolic rate associated with tissue repair and development of defense and copper excreting

metabolisms (Broeck *et al*., 1997). From the present study, it is evident that chlorpyrifos (20% EC) was highly toxic and had a profound impact on the oxygen consumption and food consumption of *Oreochromis mossambicus* exposed to lethal and sublethal concentrations.

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