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Acute Toxicity and Behavioral Responses of Common Carp *Cyprinus carpio* (Linn.) To an Organophosphate (Dimethoate)

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Abstract: Dimethoate, a broad spectrum OP insecticide is a potential toxic pollutant, adversely affecting the fauna of aquatic ecosystem. The aim of present study was to assess the acute toxicity of dimethoate on fingerlings of common carp, *Cyprinus carpio*. The fingerlings were exposed to dimethoate to determine LC_{50} values for 24, 48, 72 and 96 h and to study the behavioural alterations. The mortality data obtained was analyzed by LC_{50} software program of U.S. EPA based on Finney's Probit Analysis statistical method and was found to be 1.84, 1.78, 1.68 and 1.61 mg/L respectively. The test fish exhibited erratic swimming, increased surfacing, decreased rate of opercular movement, copious mucous secretion, reduced agility and inability to maintain normal posture and balance with increasing exposure time. Dissolved oxygen content of the troughs was measured at 24, 48, 72 and 96 h to assess the impact of toxicant exposure on oxygen consumption. Oxygen consumption of exposed fishes showed significant decline at all concentrations. It is concluded that dimethoate is highly toxic to fingerlings of common carp and severely affects their physiology and behaviour.

Key words: Dimethoate · Mortality · LC_{50} · *Cyprinus carpio* · Behavioral effects

INTRODUCTION

During past two decades the use of pesticides has increased considerably in agriculture and in 2000, roughly around 5400 million pounds of pesticides were applied throughout the world, of which 23% was accounted by US alone (URL1). Pesticides are employed routinely in the integrated farming practice to protect crops and animals from insects, weeds and diseases. Liberal use of pesticides at different stages of crop production, starting from seed processing to storage of agricultural produce, is posing great danger to aquatic environment.

These pesticides are carried into aquatic ecosystem by surface runoff from sites of application, where they enter the organisms through food webs and also through contact in water. Therefore, the health of aquatic ecosystem is being adversely affected because they serve as ultimate sink for these pesticides. These pesticides are found to be highly toxic not only to fish but also to other organisms which constitute food of the fishes. A number of pesticides currently in use are biocides that have high mammalian toxicity and necessitate considerable precaution in their application [1]. According to WHO estimate nearly three million cases of pesticide poisoning occur annually [2].

Among different classes of pesticides, organophosphates are more frequently used, because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment. Organophosphates accounted for 70% of the total insecticides used in US in the year 2001 (URL2). Dimethoate [IUPAC Name- 0, 0 dimethyl S- (N methyl carbamoylmethyl) phosphoro-dithioate], CAS No. 60-51-5, is an organophosphate available in the market by the trade name of Rogor. It is a systemic insecticide used for control of a wide variety of insect pests of fruits, vegetables and crop plants. Dimethoate is highly selective as insecticide because relative rate of degradation of toxicant by enzymes (esterases and amidases) are very low in insects as compared with those of mammals [3]. Like other organophosphates, rogor is also an acetylcholinesterase inhibitor (URL3), therefore, works primarily as nerve poison which is reflected in uncoordinated abnormal behavior of the fish soon

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after exposure to pesticide. Dimethoate is acutely toxic and is classified as a possible human carcinogen by USEPA based on occurrence of tumors in mice (URL4). In the WHO acute hazard ranking this is rated as moderately hazardous (URL5). USEPA has registered dimethoate as a systemic organophosphate insecticide but in 2006 it released Interim Re-registration Eligibility Decision (IRED) document for dimethoate in accordance with FQPA requirements (URL6).

Contamination of aquatic ecosystem by pesticide can cause acute and chronic poisoning of fish and other organism [4]. The pesticides are found to damage vital organs of fish [5,6], skeletal system and cause biochemical alterations in the exposed fishes [7-10]. Heavy contamination of water bodies by pesticides can lead to mass mortality of fish and other aquatic fauna. The common carp, *Cyprinus carpio* (Linn.), is a highly palatable fish and preferred for culture due to its high growth rate and prolific breeding in confined water. This exotic carp was introduced in India in the year 1957, now it is well adapted in culture ponds and even migrated to the river system of northern India. Since, there is scarcity of data of acute toxicity of dimethoate on carps; therefore, static bioassay was performed to determine LC_{50} values of dimethoate to common carp fingerlings.

MATERIAL AND METHODS

Live fingerlings of common Carp, *Cyprinus carpio communis* (Teleostei; Cypriniformes; cyprini) were collected from local ponds during the month of August. They were brought to laboratory carefully in plastic bags to avoid any injury and disinfected by giving a bath for two minutes in 0.05% $KMnO_4$ solution. Thereafter, they were transferred to plastic tank of 500 liter capacity for two weeks acclimatization to laboratory conditions. During acclimatization fish were fed daily with rice bran mixed with mustard oil cake in the ratio of 2:1. Leftover food in the tank was removed daily when water of the tank was changed. Dead fishes, whenever located were removed immediately to avoid fouling of the tank water.

After two weeks of acclimatization fingerlings were starved 24 h prior to exposing them to dimethoate (Rogor 30% EC, Rallis India Ltd., Mumbai). Stock solution of 1 mg/ml dimethoate was prepared in absolute alcohol. Variable quantities of stock solution were added to same volume of water in different glass troughs. The pesticide was mixed thoroughly by stirring with a glass rod before adding fishes in to glass troughs.

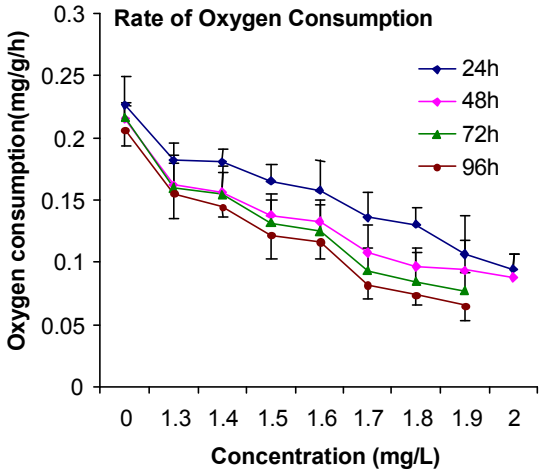

Fig 1: Two way ANOVA result of rate of Oxygen Consumption: Concentration Effect and Duration Effect, both Significant ($p < 0.0005$)

Fishes of similar size (5±1.5 cm) and weight (9±1.5 g) were sorted out and separated in to eight groups of four fish each. They were exposed to 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4 mg/L concentration of dimethoate in the range finding test. Each group was placed in a glass trough containing 10 liters of water. After determining the range, fish were exposed to final concentration of 1.30, 1.40, 1.50, 1.60, 1.70, 1.80, 1.90, 2.0 and 2.10 mg/L of dimethoate to determine LC_{50} values for 24, 48, 72 and 96 h. Four replicates were taken for each concentration. A control was run simultaneously containing 2 ml. of absolute alcohol in 10 liter water. During assay no food was administered to fishes [11]. Mortality in each group was recorded and dead fishes were immediately removed. Fish mortality data obtained with respect to time was analyzed by EPA Probit Analysis, Version 1.5, statistical software (URL7) based on Finney's Probit Analysis method for determination of LC_{50} values and 95% lower and upper confidence limits. Behavioral responses of fishes were noted during first 6 h and at 24, 48, 72 and 96 h of exposure.

Experiment was conducted under natural photoperiod and temperature in the month of August and September. Physicochemical characteristics of water were as follows: temperature 28 ± 2°C, pH 7.4 ± 0.5, Dissolved oxygen 7.2 ± 0.5 mg/L and total hardness as $CaCO_3$, 115.24 ± 1.3 mg/L measured after APHA [12]. During experiment, oxygen consumption was measured in mg/g/h (Winkler's method) at 24, 48, 72 and 96 h and the values are plotted in Figure 1. Opercular movement (OCM) per minute was also recoded at same interval of exposure

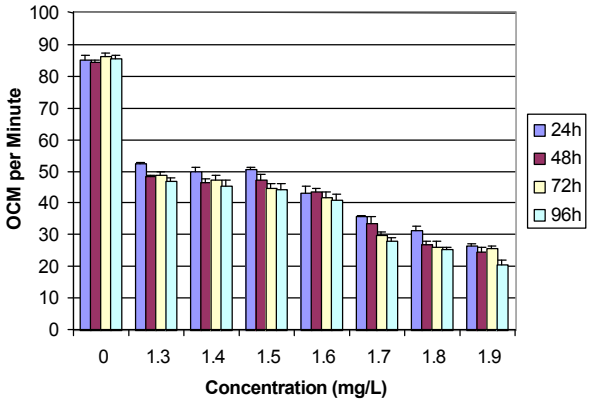

Fig. 2: Two Way ANOVA Results of OCM: Duration Effect - insignificant (p value=0.605); Concentration Effect - significant

and the values were plotted in Fig. 2. The observed values of oxygen consumption and OCM were analyzed by two way ANOVA for assessing statistical significance.

RESULTS

No mortality and 100% mortality of *Cyprinus carpio* was recorded at 1.30 and 2.10 mg/L of dimethoate respectively. LC_{50} values of dimethoate for 24, 48, 72 and 96 h and their respective 95% confidence limits, slope function and intercepts are given in the table 1 as calculated by EPA Probit Analysis, Version 1.5, statistical software (URL7) and the plot of adjusted probits and predicted regression line for 96 h dimethoate toxicity to common carp is depicted in Figure 3.

The carp fingerlings exhibit a number of abnormalities in their behavior when exposed to dimethoate. Within a few minutes of exposure to higher concentrations (1.60 mg/L onwards), the fishes appear excited, the swimming becomes erratic and the schooling is disrupted. However, within 1-2 h of exposure they calm down and start swimming slowly. Surfacing frequency and gulping of surface water with occasional coughing is increased remarkably in exposed fishes, as a result, in very short duration the surface water in test troughs exhibits more air bubbles than control. The OCM is decreased with

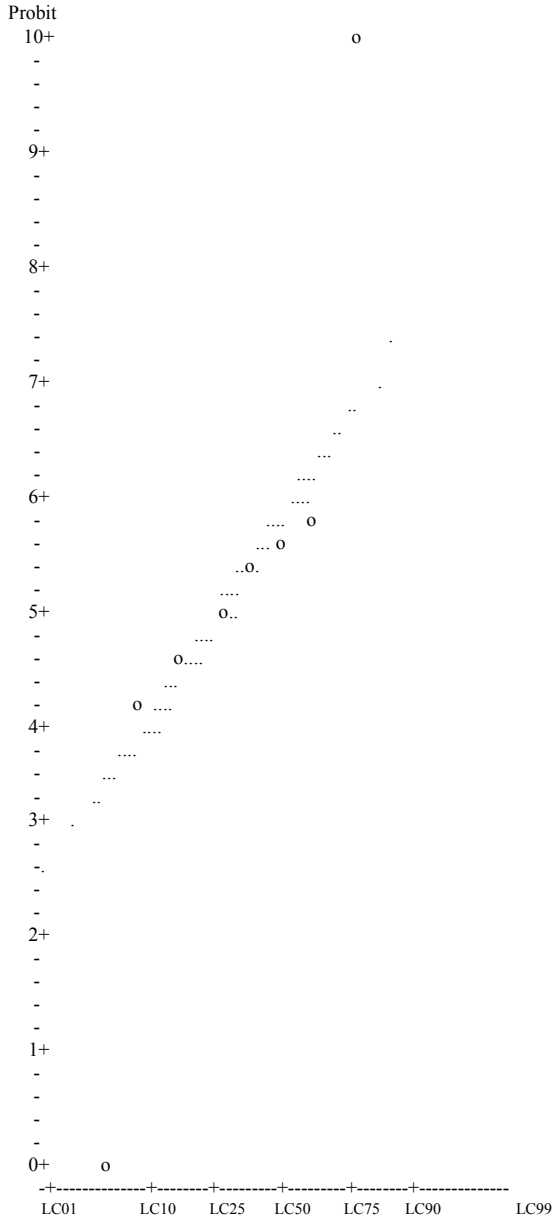

Fig. 3: Plot of adjusted probits and predicted regression line for dimethoate to common carp

rising toxicant concentration in the exposed fishes. The results are statistically significant (ANOVA test, $p < 0.0063$).

Table 1: LC50 Values and 95% Lower & Upper Confidence Limits, Slope and Intercept for Dimethoate at different intervals for the fish Cyprinus carpio

Duration (h)	LC50 Values (in mg/L)	95% lower confidence limit	95% upper confidence limit	Slope	Intercept
24	1.84	1.78	1.99	18.31±2.92	0.14±o.76
48	1.78	1.72	1.84	18.84±2.85	0.25±0.72
72	1.68	1.63	1.74	18.80±2.81	0.73±0.65
96	1.61	1.55	1.67	17.52±2.60	1.35±0.56

The mucous secretion increases considerably in the exposed fishes and turbidity of the water of the test troughs increases gradually during static exposure. Defecation is increased and more fecal matter was found in the bottom of the test troughs than control at all observations (viz. 24, 48, 72 and 96 h) of acute exposure. The exposed fishes exhibit tremors and gradual weakening of reflexes leading to imbalance in posture and loss of equilibrium. In due course of time a few fishes start drowning but by sudden somersaulting, regain normal posture and balance temporarily. Finally, however, they succumb to poison with mouth and operculum wide open and body, slime covered. Body color changes from silvery white to pale white. At lower concentration, however, the changes in behavior are not as conspicuous. The rate of oxygen consumption decreased significantly at all durations of exposure (Fig. 1).

DISCUSSION

Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxicant, its concentration and duration of exposure. The LC_{50} values of Dimethoate for certain air-breathing teleosts are reported to be very high, as in *Clarias batrachus* [13], it is 65 mg/L for 96 h, in *Channa punctatus* [14] it is 17.9 mg/L for 96 h, whereas in *Heteropneustes fossilis* [15] very low LC_{50} value for24, 48, 72 and 96 h dimethoate exposure is recorded as 3.38, 3.23, 3.08 and 2.98 mg/L. In contrast, the carps are very sensitive to dimethoate and record very low LC_{50} values. In *Catla catla* [16] the LC_{50} value for 96 h is reported as 0.007 ppm and in the present study the 24, 48, 72 and 96 h LC_{50} value of dimethoate for *Cyprinus carpio* is found to be 1.84, 1.78, 1.68 and 1.61mg/L respectively. Surprisingly, however, De Mel, *et al*. [17] has reported very high LC_{50} value (26.11mg/L) of dimethoate for 96 h in *Cyprinus carpio* fry (size 20 – 34 mm). Thus in air breathing fishes LC_{50} values are much higher than in carps for the same pesticide, probably because the fishes with accessory respiratory organs can adaptively shift towards aerial breathing when the water is contaminated. Even in carps, LC_{50} values show considerable variation reflecting different tolerance limit of different species. This is in agreement with Sprague [18] who observed variation in LC_{50} values for the same species and toxicant depending on size, age and condition of test species along with experimental factors.

Behavioral changes observed in the exposed carp fingerlings, appear to be the manifestation of dimethoate toxicity. Upon exposure to the pesticide, increase in surfacing and gulping of surface waters appears to be an attempt by the fish to avoid breathing in the poisoned water. Similar observation has been reported in *Anabas testudineus* after exposure to monocrotophos [19]. Moreover, hypoxic condition also contributes to increase surfacing as reported by Radhaia *et al.* [20]. Hypoxic condition arises primarily due to damage of gills of pesticide exposed fish which hampers oxygen uptake [6].

Decreased opercular movement probably helps in reducing absorption of poison through gills. This results in reduced rate of oxygen consumption as observed in the present study. Reduction in oxygen consumption in *Cyprinus carpio* has also been reported after sublethal exposure of copper [21] and antimony chloride [22]. Ganeshwade *et al.* [23] however, have reported increased opercular rate and coughing in the common carp exposed to industrial effluents. Infact, coughing response is shown to have direct relationship with concentration of pollutant in water [24]. Erratic movements and abnormal swimming are triggered by deficiency in nervous and muscular coordination which may be due to accumulation of acetylcholine in synaptic and neuromuscular junctions [25]. Tremors, gradual loss of equilibrium and drowning are caused by adverse effects of organophosphate on central nervous system. Increased mucus secretion after dimethoate exposure is probably an adaptive response to counter the irritating effect of the pesticide on body surface and mucous membrane.

Changes in body color from silvery white to pale white may be caused by impairment of pituitary functions reflected by reduction in number and size of chromatophores and their pigment content [26]. Body color changes have also been observed in *Cyprinus carpio* after exposure to $HgCl_2$ [27]. Defecation is considerably increased in the exposed fishes in comparison to control group. This is in accordance with typical organophosphate toxicity involving hyper stimulation of muscarinic receptors in the smooth muscles of the end organs viz., gastrointestinal tract and secretory glands [28].

It is concluded that carps are more sensitive to dimethoate as compared to air-breathing teleosts. They exhibit behavioral changes which reflect its direct effect on nervous system of exposed fishes. Further studies on toxicity of dimethoate and its combinations with other pesticides on mortality and behavior of fish in the laboratory and field conditions are required.

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