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MONOCROTOPHOS: TOXICITY EVALUATION AND RESPIRATORY RESPONSES OF *CYPRINUS CARPIO* (LINNAEUS)

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Abstract

Static rencval bioassay method was used to determine the 96 hr LC_{50} of monocrotophos on *Cyprinus carpio*. Oxygen consumption and behavioral responses were recorded at lethal and at two sublethal (1/10th and 1/15th) concentrations. Oxygen consumption responses were concentration dependent. The data indicated maximum decrease in oxygen consumption at lethal exposure (86.4 µg/L) over control on 4th day followed by 2nd, 3rd, 1st days. At 1/10th of lethal concentration (8.64 µg/ L) continuous decrease on 1st, 10th and 20th day but decrease was reduced on 30th day. Similar variations were observed at 1/15th of lethal exposure (5.76 µg/ L) from day 1 to day 10. The decrease continued on 30th day which was witnessed to be very less amongst all. Behavioral responses were significant in lethal exposures due to intoxication. Fish showed hyper excitation, erratic swimming, jumping, and lethargy due to low breathing frequency followed by muscular rigidity and abundant mucous secretion over the gills.

Keywords: Monocrotophos, Toxicity, Respiration, *Cyprinus carpio*, Behavior

Introduction

Monocrotophos (MCP) is one of the organophosphorus insecticides extensively used in agriculture and animal husbandry (Rao, 2004). Monocrotophos has been withdrawn from use in developed countries due to its high toxicity against beneficial and non-target insects such as honey bees, fish and birds (available from http://www.pan-uk.org/pestnews/actives/monocrot.htm). But its usage for the control of major insect pests in agriculture is being continued in developing countries like India primarily due to lack of alternative replacements (Banerjee *et al.,* 2000).

Monocrotophos is commonly used organophosphate pesticide for pest control of crops in India (Ray *et al.,* 1985). MCP (dimethyl (E)-1-2-ethylcarbamoylvinylphosphate), is widely used to control a variety of insects on crops such as cotton, sugar cane, peanuts, ornamentals, and tobacco. MCP is highly toxic and has contact, systemic and residual activity (Ray, 1989). Today, MCP occupies a prime position in pest management in India, and its consumption in India is estimated at 6000 metric tons per annum (Anon, 2001). It is systemic insecticide and acaricide of the vinyl phosphate group. Their wide uses provide many routes of entry into aquatic environment and adversely affect many non-target species. Fishes form an important class of organisms on the basis of their use as nutritive food and are also a useful indicator of pollution.

MCP is soluble in water and easily gains entry into the wastewater generated during its manufacture. Removal of MCP from industrial effluent is essential, since MCP is a human health hazard and may cause irritation of eyes and skin on contact, inhibition of acetyl cholinesterase, sweating, muscular weakness, blurred vision, and a risk of death owing to respiratory failure (Ray, 1989).

The assessment of the ecotoxicological risks caused by pesticides to ecosystems is based on data on the toxicity and effects of pesticide preparations to non-target organisms. Fish are among the group of non-target aquatic organisms. The present paper is a contribution to the assessment of toxicity, respiratory responses, and effects of a monocrotophos-based pesticide to fish behaviour.

Materials and Methods

Healthy and active *Cyprinus carpio* (3±0.5 g, 6 cm) fingerlings were procured from the State Fisheries Department, Dharwad, India. Carps were acclimatized to laboratory conditions for 15 d at 24±1 ^{0}C and held in large glass aquaria containing dechlorinated tap water of the quality used in the test. The physico-chemical characteristics of water were analyzed following the methods mentioned in APHA (2005) and found as follows, Temperature: 24±2 ^{0}C, pH: 7.1±0.2 at 24 ^{0}C, Dissolved oxygen: 9.3±0.8 mg/L, Carbon dioxide: 6.3±0.4 mg/L, Total hardness: 23.4±3.4 mg as $CaCO_3$/L, Phosphate: 0.39±0.002 µg/L, Salinity–nil, Specific gravity: 1.0030 and conductivity less than 10

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µScm^{-1}. Water was renewed everyday and 12-12 h of photoperiod was maintained daily during acclimation and test periods. Fish were fed regularly with oil cake and rice bran during acclimation and feeding was stopped three days prior exposure to test medium.

Concentrations of the test compounds used in short term definitive tests were between (38.4 and 110.4 µg/L) the concentration at which there was 0% mortality and the concentration at which there was 100% mortality in the range finding tests. Replacement of the water medium was followed by the addition of the desired dose of the test compound. Fishes were exposed in batches of ten to varying concentrations of monocrotophos with 20 L of water in six replicates for each concentration along with a control. Monocrotophos (96%) was procured from the Sudarshan chemicals, limited, Puna, India. Required quantity of monocrotophos was drawn directly from this emulsified concentrate using variable micropipette.

For LC_{50} calculation, mortality was recorded every 24 h and dead fish were removed when observed, every time noting the number of fish deaths at each concentration up to 96 h. Duncan's Multiple Range Test (DMRT) was employed for comparing mean mortality values after estimating the residual variance by repeated measures ANOVA (Winner, 1971) for arc sine transformed mortality data (dead individuals/initial number of individuals). Time of exposure was the repeated measure factor while treatment (concentration and control) was the second factor. In addition, LC_{50} were compared by the method of APHA (2005). The LC_{50} with 95% confidence limit for monocrotophos were determined/estimated for 96 h by probit analysis (Finney, 1971).

One tenth and one fifteenth of the LC_{50} (8.64 and 5.76 µg/L respectively) were selected as sub lethal concentrations for sub acute study (1, 10, 20 and 30 d). The control and exposed fishes were kept under continuous observation during experimental periods. The whole animal oxygen consumption was measured for lethal and sublethal concentrations besides controls by following the method of Welsh and Smith (1953) as described by Saroja (1959).

Statistics

The data for in sublethal toxicity experiments were shown as the mean of five individuals. Statistical analyses were performed with one-way analysis of variance (ANOVA). The differences are significant if $p < 0.05$ and extremely significant if $p < 0.01$, which were indicated by $*$ and $**$ respectively.

Results

Acute toxicity of monocrotophos

Acute toxicity of *monocrotophos* for the fish *Cyprinus carpio* was found to be 86.4 µg/L. The upper and lower 95% confidence limits were found to be 91.3 µg/L and 83.6 µg/L respectively.

Table 1. 96 h LC_{50}, slope and 95% confidence limits of monocrotophos to the freshwater carp, *Cyprinus carpio*

Pesticide	96 h LC_{50} value (µg/L)	Slope	95% Confidence limits	
			Upper limit	Lower limit
Monocrotophos	86.40 ± 0.33	1.19	91.3	83.6

No mortality or morphological changes were observed in the control groups during 96 h acute toxicity tests. It is evident from the results that the monocrotophos can be rated as toxic to *Cyprinus carpio* (Table 1). Fishes in the control group appeared active and healthy throughout the test tenures.

Whole animal oxygen consumption

The data indicates that, the fish exposed to lethal and two sublethal concentrations (8.64 and 5.76 µg/L) of monocrotophos significantly decreased over control (Table.2;fig.1).

Fig.1. Percent change of whole animal oxygen consumption in *Cyprinus carpio* on exposure to lethal and sublethal concentrations of monocrotophos

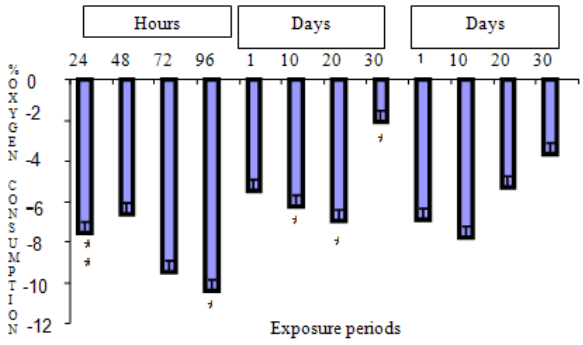

Table 2: Whole animal oxygen consumption in *Cyprinus carpio* on exposure to lethal and sublethal concentrations of monocrotophos

Estimations	Exposure periods in days											
	Lethal				Sub lethal One tenth				Sub lethal One fifteenth			
	24	48	72	96	1	10	20	30	1	10	20	30
Control	68.62^{A}	64.52^{C}	64.32^{D}	63.98^{E}	64.89^{B}	62.34^{G}	63.68^{F}	61.48^{H}	64.89^{B}	62.34^{G}	63.68^{F}	61.48^{H}
±SE	0.5477	0.4619	0.6741	0.6766	0.7265	0.6386	0.6928	0.6200	0.6692	0.5486	0.7099	0.4712
±SD	0.948	0.800	1.167	1.171	1.258	1.106	1.200	1.300	1.159	0.722	1.229	0.816
O_2 C	63.42^{A}	60.23^{E}	58.21^{I}	57.32^{K}	61.34^{B}	58.44^{H}	59.23^{F}	60.21^{E}	60.42^{C}	57.48^{J}	60.28^{D}	59.22^{G}
±SE	0.4933	0.7816	0.5568	0.4410	0.6173	0.7506	0.4096	0.7506	0.4933	0.4173	0.5576	0.4595
±SD	1.069	1.300	0.709	1.073	0.854	0.950	0.965	0.795	.854	1.353	0.964	0.763
% Change	7.57	6.64	9.49	10.40	5.47	6.25	6.98	2.06	6.88	7.79	5.33	3.67

OC: Oxygen consumption; ±SE: Standard error; ±SD; Standard deviation; the same letters indicated at the appendices are not significantly different from each; All the values are significant at P>0.05 level.

Oxygen consumption showed continuous abrupt variations over control in all the three concentrations. At lethal concentrations maximum (10.40%) decrease was recorded at 96 h and minimum (6.64%) at 48 h, while at 72 h increased (9.49%) over 24 (7.57%) and 48 h. One tenth of lethal concentration i.e. 8.64 µg/L treatment showed continuous decrease right from day 1 to day 20, however decrease was reduced on 30 days of exposure, maximum(6.98%) at day 20 and minimum (2.06%) at day 30. Similar variations were observed in 5.76 µg/L treated fishes, initially OC was decreased from day1 to day 10, however it decreased but day 30 witnessed very low (3.67%) among the group. Overall OC responses were concentration dependent, interestingly in $1/10^{th}$ of LC_{50} on day 30 was recorded most lowest among the treated fishes indicating recovery to normalcy, however at 5.76 µg/L it was not the case though the concentration was very low.

Discussion

The acute test for a long time has been a major component in toxicity testing in which acute chemical toxicity is determined as a 96 h LC_{50} value. Values of LC_{50} confirmed the high degree of monocrotophos toxicity in the present study. It is difficult to make comparisons with other species due to the great range in acute toxicity levels for organophosphorus insecticides in any species as suggested by *Hughes et al.,* 1997.

Acute toxicity of *monocrotophos* for the fish *Cyprinus carpio* was found to be 8.64 µg/L for 96 h. Similarly, earlier studies on toxicity values 6.7 mg L^{-1}96 h-LC_{50} of monocrotophos to red drum (Shaoguo *et al.,*2003), 4.9 mg L^{-1} for *Oreochromis niloticus* (Thangnipon *et al*., 1995) and 7.0 mg L^{-1} for *Prgrosomus major* (Jia *et al*., 1999). Toxicity expression in *Cyprinus carpio* seems to be more resistant in comparison to above expressed values of toxicity in different experimental animals. Monocrotophos toxicity is due to inhition of brain AchE since; MCP contains oxon structure, which can inhibit enzymes *in vitro and in vivo.*

It is usually accepted that organophosphorus LC_{50} for 96 h values are associated to inhibition of cholinesterase activity and that would be probably associated with behavioral effects as reported by Heath, 1987. Intoxication symptoms associated with toxicity of monocrotophos observed in the present study includes hyper excitation, erratic swimming, and jumps followed by lethargy and low breathing frequency. In dying fish it was observed a great muscular rigidity and an abundant mucus secretion which were directly related to the concentrations and are in agreement with the recent reports (Patil and David, 2008: Ramesh and David, 2010).

The depletion in oxygen consumption may be due to the disorganization of the epithelium of the gill and also due to the disturbance in mitochondrial integrity and decreased activities of some mitochondrial enzymes as reported by Ravindra (1988). Decrease can also be attributed to the induction of hypoxic conditions within the animal due to the intimate contact of the respiratory surface with toxic water resulting in the alteration of normal respiratory area of the animal. The secretion of mucus layer over the gill lamellae has been observed during monocrotophos stress. The coagulation of mucus on the gill caused annihilation of various important processes such as gas exchange, nitrogen excretion, salt balance and circulation of blood (Skidmore, 1964). The decrease in oxygen consumption in the sublethal concentrations of monocrotophos appears to be mainly due to lowering down of energy requirements and if so, such lowering of maintenance is to be considered as adaptive and even strategic. In addition the fish might have

overcome the pesticide toxicity by triggering the process of detoxification (Biotransformation).

The analysis of data from the present investigation evidenced that monocrotophos primarily induces high physiological stress on the freshwater fish, *Cyprinus carpio* even at sublethal concentrations resulting differences in the oxygen consumption. Also, provides an easy diagnosis for monocrotophos contamination and may become helpful in evaluating the ecotoxicological effects in aquatic environment close to agricultural fields and possible effects on fish populations.

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