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BEHAVIOURAL ANOMALIES IN *CYPRINUS CARPIO* L. INDUCED BY MONOCROTOPHOS INTOXICATION IN SUBLETHAL DOSES

***Sharmila, G.[1] and A.V.Kavitha[2]**

[1]P.G and Research Department of Zoology and Wildlife Biology
A.V.C. College (Autonomous), Mananmpandal – 609 305, Mayiladuthurai
Nagapattinam Distict, Tamil Nadu, India
*Corresponding author email id: viswanathansharmila@gmail.com
[2]P.G and Research Department of Zoology ,
Govt. College for Women (Autonomous), Kumbakonam - 612 001

ABSTRACT
Though plenty of research is available for short term impact of pesticides on fishes yet study on the quantification of behavioural implications were not carried out so far. In the present study, an attempt has been made to investigate the impact of chronic toxicity of Monocrotophos on the behavioural responses of freshwater fish, *Cyprinus carpio*. A static renewal bioassay was conducted to determine the acute toxicity of commercial grade organophosphorus pesticide, Monocrotophos (36% SL) to Common carp, *Cyprinus carpio*. The LC_{50} concentrations of monocrotophos to carp fingerlings for 96 hrs was found to be 15.53 ppm. The fishes were exposed to the sublethal concentrations $1/5^{th}$ and $1/10^{th}$ of LC_{50}96 hrs for 30 days. The impact of toxicity on the behaviour and movement of fishes were assessed at five sampling points viz. at 0, 7, 14, 21 and 28 days of the exposure period. Even though the mortality was insignificant in the sublethal concentrations, considerable variations in behavioural responses such as lethargy, increased mucous secretion, surfacing, gulping of air, skin discolouration, respiratory distress and lack of appetite were observed. The one way Anova results showed that treated fishes showed significant differences in the movement when compared to their movement prior to intoxication at the same interval of time (F value = 3.328, $p < 0.05$).

KEYWORDS: Bioassay, Behaviour, *Cyprinus carpio*, Monocrotophos, Sublethal concentration, Movement

INTRODUCTION

Application of synthetic fertilizers, insecticides and pesticides are increasing now-a-days with increasing demand of agricultural food materials throughout the world. There is a high chance of aquatic ecosystem to be contaminated, located in industrial or agricultural areas, through runoff or ground water leaching of a variety of chemicals (Todd and Leuwen, 2002), thereby affecting many other organisms away from the primary target. It is estimated that only 0.1% of the pesticide reaches the specific target (Agular, 2002). Exposure of aquatic ecosystems to these pesticides is difficult to assess because of their short persistence in the water due to low solubility and rapid degradation. Yet monitoring of these pesticides is important because they are highly toxic and pose greater hazards to the aquatic organisms especially fishes (Talebi, 1998; Uner *et al.*, 2006; Banaee *et al.*, 2008). Pesticides, upon entering into the organs and tissues of fishes significantly damage certain physiological and biochemical processes.

Monocrotophos (3-(dimethoxyphosphinyloxy)-N-methylisocrotonamide) is a synthetic organophosphate, nonsystemic, broad spectrum insecticide and acaricide, acting as a cholinesterase inhibitor with contact, stomach and respiratory action. The major use of monocrotophos in farming is to protect crops against a variety of insect pests from soft bodied insect to leaf eating beetles.

Fishes, as they occupy the top most trophic level of aquatic food chain are able to take up and retain chemicals dissolved in water not only via active or passive processes but also by biomagnification. They represent good model systems to detect and document pollutants released into their environment. The deterioration of water quality can be detected easily by any change in the behaviour and physiology of fishes, hence they are excellent biological indicators. Moreover, as the mammalian and piscine systems exhibit similar toxicological and adaptive responses to oxidative stress, there is a growing concern in understanding the physiological mechanisms associated with fish responding to environmental stress. A long term exposure to sublethal concentrations of pesticides in aquatic environments cause more prominent structural and functional changes in aquatic organisms rather than mortality. Since monorotophos has been banned in most of the countries except India, where it still remains as a benevolent pesticide, it is extensively applied in agriculture for pest eradication. Hence the present study holds greater promises and much significance to study the impact of its chronic toxicity.

The common carp, *Cyprinus carpio* L. is a highly palatable fish and preferred for culture due to its high growth rate and prolific breeding in confined water. Known as the Common carp, *Cyprinus carpio* is an economically important freshwater fish, native to china and has been introduced all over the world to form a significant part of freshwater fishery. This species is also a very important model organism for toxicity tests because of its availability throughout the year, higher survival rate compared to other fishes and easy acclimatization to laboratory condition. As the common carp, *Cyprinus carpio* L. (Cyprinidae), is a very important staple fish generally found in rivers, ponds and reservoirs and monocrotophos is extensively used organophosphate pesticide, an attempt has been made to evaluate the effect of sublethal concentrations of monocrotophos on the behavioural responses of carp.

METHODOLOGY

Collection and Acclimatization of the Specimen:

Live and healthy *C. carpio* (n = 80) were collected from a commercial fish farm at Maruthur village of Mayiladuthurai taluk and brought to the laboratory. Fishes were stocked and maintained in aquaria under a natural photo-regime and at a constant water temperature of 23 ± 5°C and a pH of 7-8 to acclimatize for a period of 20 days before starting the experiment. Mean length of the fish was 6.78 cm (range 5.0 to 8.5) and weight was 5.73 gm (range 3.8 to 7.3). Feeding was stopped prior to the experimentation to reduce additive and contamination effects of the animal excreta in the test medium, as suggested by Arora *et al.*, (1984).

Selection of toxicant:

Organophosphorous pesticide, Monocrotophos (36% SL), commonly used in agricultural fields was used for the present study. Predominantly used in pest control, it is less persistent in the body as well as in the environment, usually rapidly degraded and eliminated, hence needs to be more frequently applied in the fields to have effective pest management. The pesticide was obtained from a retailer pesticide shop.

Physico-Chemical Parameters of the Test medium:

Dechlorinated tap water was used in the test and its physico-chemical characteristics (temperature, dissolved oxygen (DO), salinity and pH) were analyzed following the standard methods of APHA (1998).

Range finding tests:

The fishes were exposed in batches of 10 to varying concentrations of monocrotophos with 20 lit. of water in 3 replicates for each concentration along with control sets in range finding test. Concentrations of the test compound used in short term definitive tests were between the highest concentration at which there was 0% mortality and the lowest concentration at which there was 100% mortality. Replacement of the water medium was followed by the addition of the desired dose of the test compound. For the LC_{50} calculation, mortality was recorded every 24 h and the dead fishes were removed when observed, every time noting the number of fish deaths at each concentration up to 96 h. The LC_{50} values with 95% confidence limit for monocrotophos were determined for 96 h by probit analysis (Finney, 1971).

Chronic toxicity tests:

One fifth and one tenth of the acute toxicity value (LC_{50}) were selected as sublethal concentrations for assessing chronic toxicity. Fishes were exposed to both the sublethal concentrations for 30 days along with the control sets. Behavioural responses and movement were studied during the experimental period. The control (toxicant free medium) and monocrotophos exposed fishes were kept under continuous observation during experimental periods. Five sampling points were set during a period of 30 days in the experiment (0, 7, 14, 21, and 28 days post treatment). At each sampling point, the modifications of behavioural characteristics were recorded with respect to activity, movement, mucous secretion, skin colouration etc. The wall of a glass aquaria on all four sides were divided into squares of 2 cm and the movement were determined by the number of squares crossed by each fish per minute at each sampling point.

Statistical analysis:

Data correspond to the average of three replicates. The data obtained were recorded as Mean $\pm$ S.E and subjected to one way Anova ($p < 0.05$).

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RESULTS AND DISCUSSION

Acute toxicity:

Acute toxicity of monocrotophos for *C. carpio* (LC_{50} 96hrs) was found to be 15.53 ppm. The upper and lower 95% confidence limits were found to be 18.93 and 15.94 ppm respectively. It is evident from the results that the monocrotophos can be rated as highly toxic to fish. No significant mortality was observed during the experimental period in both the sublethal concentrations.

Behavioural responses:

Experiments were done to assess the behaviour of carp without any toxicant and with two sublethal concentrations of monorotophos. Behavioural responses were assessed at 0 , 7 , 14 , 21 and 28 days in all the samples. The degree of intensity of each behavioural characteristic during exposure period was recorded in the Table – 1.

Table – 1: Behavioural alterations induced by Monocrotophos in *Cyprinus carpio* during the experimental period

Behavioural responses	Control					Treated 1					Treated 2				
	0 d	7 d	14 d	21 d	28 d	0 d	7 d	14 d	21 d	28 d	0 d	7 d	14 d	21 d	28 d
lethargy	-	-	-	-	-	+	+	++	++	+++	-	-	+	+	++
Increased mucus	-	-	-	-	-	+	++	++	+++	+++	-	-	+	++	+++
Skin discolouration	-	-	-	-	-	-	+	++	++	+++	-	-	-	-	+
Respiratory distress	-	-	-	-	-	++	++	++	+++	+++	-	-	+	++	+++
Feeding behaviour	N	N	N	N	N	N	N	LA	LA	LA	-	-	-	-	LA

**N denotes normal behaviour; LA indicates loss of appetite; + indicates above normal;
++ indicates moderate; +++ indicates severe.**

In the present study the control fish behaved in a natural manner, i.e., they were active for feeding and alert to slightest of the disturbance with their well synchronized movements. The behaviour did not vary significantly between the control groups. Therefore, the results of these nonexposure series were taken as standards for the whole test period. In toxic media, carp exhibited disrupted shoaling behaviour, localization to the bottom of the test chamber, and independency (spreading out) in swimming. The above symptoms followed the loss of co-ordination among individuals and occupancy of twice the area of that of the control group were the early symptoms of monocrotophos exposure in both the sublethal concentrations. Subsequently, fishes moved to the corners of the test chambers, which can be viewed as avoidance behaviour of the fish to the monocrotophos. In the toxic environment, fishes exhibited irregular, erratic, and darting swimming movements and loss of equilibrium followed by hanging vertically in water. The above symptoms are due to inhibition of acetylcholine esterase (AChE) activity leading to accumulation of acetylcholine in cholinergic synapses ensuring hyperstimulation. Inhibition of AChE activity is a typical characteristic of organophosphate compounds (Timchalk *et al.*, 2002). The fishes slowly became lethargic, restless, and secreted excess mucus all over the body. Intermittently some of the carp were hyperexcited resulting in erratic movements. An excess secretion of mucous in fish is a non-specific response against toxicants, forming a barrier between the body and the toxic medium, so as to minimize its irritating effect, or to scavenge it through epidermal mucus. Similar observations were made by Rao (2006) following RPR-V exposure to euryhaline fish, *Oreochromis mossambicus*.

Disrupted shoaling behaviour, easy predation, gulping air, and swimming at the water surface (surfacing phenomenon) were observed on the first day in both the sublethal exposure periods. This continued throughout the test tenures, which is in accordance with the observations made by Ural and Simsek (2006). Gulping of air may help to avoid contact of

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toxic medium and to ease respiratory stress. Surfacing phenomenon in the exposed group might be a demand for higher oxygen level. This reflects the catastrophic impact posed by the toxicant which is followed by other behavioural anomalies such as lethargy, skin discolouration and respiratory distress during the subsequent days of exposure. Of all, easy predation phenomenon is one of the most critical damages caused by a pollutant on sensitive species like fish, which ultimately decide the species survival in a given ecosystem.

Alterations in movement:

The movement (No. of squares covered/min.) were recorded for control and treated fishes at different sampling periods during the study period as shown in Table – 2 and Fig. 1.

Table – 2: Movement (No. of squares covered/min.) of control and treated fishes during the study period (Mean ± S.E)

Sampling points	Control	Treated 1	Treated 2
0 day	36.67 ± 2.9	123.92 ± 3.2	114.58 ± 3.5
7 day	65.75 ± 2.2	110.77 ± 2.4	95.43 ± 2.9
14 day	71.08 ± 2.2	92.1 ± 2.2	80.45 ± 2.3
21 day	84.67 ± 2.8	76.9 ± 2.1	71.28 ± 2.8
28 day	85.33 ± 2.3	64.33 ± 2.8	62.1 ± 2.4

At the initial sampling point (0 day), the movement of treated fishes was found to be greater than that of the control in both the sublethal concentrations. But subsequently, the movement of treated fishes gradually decreased when compared to the control fishes on 7th, 14th, 21st and 28th day.

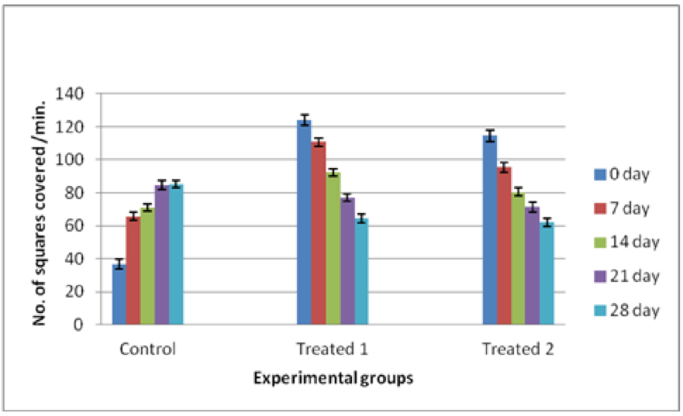

Figure 1. Movement (No. of squares covered/min.) of untreated and treated fishes during the study period

The one way ANOVA results showed a significant difference between the means of groups (f (4) = 3.328, $p < 0.05$). The increased movement in treated fishes on 0 and 7 days reflects the avoidance behaviour of fishes to the toxicant medium initially. Later, the fishes may have developed certain mechanism like mucus secretion, surfacing phenomenon and gulping of air either to get rid of the toxicant or to escape from the toxicant, which are possible strategies adopted to sustain their survival in the toxic environment. This has been indicated by the movement of treated fishes on the 14th, 21st and 28th days.

CONCLUSION

Since fishes are important sources of protein and lipids for human, so due consideration must be given to health of fishes. In the present study it is evidenced that monocrotophos is toxic even in sublethal concentration and has a profound impact on the behaviour and movement to *C. carpio*. Deviation from the normal behaviour is due to altered metabolism and physiological response to monocrotophos stress. Monocrotophos decreases the ability of animals to adapt to its environment by increasing the respiratory distress, lethargy and loss of appetite in varied degree of intensities during the exposure period. The severe degree of behavioural changes towards the end of exposure period indicates the enormous stress induced by long term exposure to the toxicant. The reason may be due to slow release of sequestered monocrotophos from the storage tissues. Hence it is clear that the prolonged exposure to monocrotophos would cause drastic effect on the survival of fishes and due steps must be taken to encourage the farmers to use the pesticides as per the guidelines.

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