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Monocrotophos induced toxicity and physiological stress on fish *Puntius filamentosus*. (Val, 1844)

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Abstract: *The effect of lethal (26.5µl/l) and sub-lethal (2.65µl/l) concentrations of monocrotophos (36% SL) on oxygen consumption and behavior of the fish Puntius filamentosus were studied in the laboratory. The oxygen consumption level was drastically reduced in lethal concentration and no much difference was seen at sub-lethal concentration. Similarly, in lethal concentration, the fish behaved abnormally with abnormal swimming pattern and eventually died. In sub-lethal concentration a little difference was seen in the behavior of the fish for the first 4 days but later on the fish adapted to the surrounding environment. The study reveals that the fish P. filamentosus can adapt to low concentration of monocrotophos toxicity during long-term exposure periods but the increase in concentration is toxic to the fish.*
Keywords: *Organophosphate, insecticide, Monocrotophos, Puntius filamentosus, behavioural studies, oxygen consumption*

I. Introduction

Monocrotophos (MCP) is one of the highly toxic organophosphorus pesticide 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Monocrotophos has been withdrawn from use in developed countries due to its high toxicity against beneficial and non-target insects such as honeybees fish and birds.
The Food and Agriculture Organization (FAO) and WHO have encouraged countries to phase out highly hazardous pesticides. Leading Asian countries have banned the use of monocrotophos because of unacceptable health risks, but in India, monocrotophos continues to be produced, used and exported. Urgent action is therefore needed to reduce the availability of and the demand for highly hazardous pesticides, as recommended by WHO and FAO.

In India Monocrotophos is banned for use on vegetables since 2005 and is under 'restricted use' category. But the perception that monocrotophos is cheap and necessary, have prevented the product from being taken off the market and they are widely available in the market.
The main problem in India is that the government's extension services to monitor pesticide usage have failed completely in reaching out to farmers. Farmers say they have no choice but to depend on pesticide dealers for advice. Recommendations from agriculture universities don't reach them. They end up using any pesticide indiscriminately no matter how toxic they are.
Monocrotophos is soluble in water and easily gains entry into the wastewater generated during its manufacture. The surface run-off from agricultural fields plus their seepage into groundwater pollutes natural waters and finally enters the aquatic food chain.
The present study is a contribution to the assessment of toxicity (bioassay) and its effects on some physiological indices of freshwater/brakishwater fish *Puntius filamentosus* (blackspot barb).

II. Materials and Methodology

2.1) Experimental fish

Healthy fish of *Puntius filamentosus* (of average weight 4.5 gms. and standard length of 5cms) of mixed sexes were obtained from Kali estuary (Karwar- Karnataka, India). In the laboratory the fish were transported in glass tanks with dechlorinated tap water. The fish were acclimatized to laboratory conditions for 10 days. During acclimatization the fish were fed with pelleted feed at 5% body weight twice per day.

2.2) Lethal (LC_{50}) Bioassy

Ten fish were randomly selected and exposed to different concentrations of monocrotophos (36%SL) for 96 hours and the data obtained were subjected to probit kill (Finney1971) and Dragstedt-Behrens equation (Carpenter1982) as mentioned by Bhargava and Rawat (1991) to determine LC_{50} value.

2.3) Behavioral study

Fish were exposed to lethal and sub lethal concentrations were observed for the behavioral changes. Behavioral patterns were studied at the interval of 3, 6, 24 hr. followed by 48, 72 and 96 hrs. from the exposure to the monocrotophos. In the sub lethal concentrations fish behavior was monitored initial for 6hrs and continued for 15 days of the exposure period.

2.4) Determination of whole animal oxygen consumption

For determining the oxygen consumption Winkler's lodometric method as described by Welsh and smith (1953) was employed. The apparatus set up was created as described by Saroja (1962).

2.5) Statistical Analysis

The data were subjected to analysis of variance (ANOVA) and the means were compared by Duncan multiple range to test at the 0.05 % level (Duncan 1955) to draw the mean comparison among the results.

III. Results

3.1) Toxicity Evaluation

The mortality data of *Puntius filamentosus* in different concentrations of monocrotophos (36% SL) is presented (table 1).

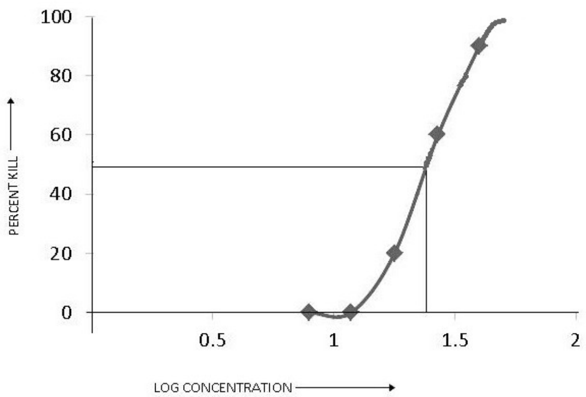
Table 1:

Sr. No.	Conc. of Monocrotophos µl/l	Log conc. of monocrotophos	No. of fish exposed	No. of fish alive	No. of fish dead	Percent mortality	Probit mortality
01	08	0.90	10	10	0	0	0
02	12	1.07	10	10	0	0	0
03	18	1.25	10	08	02	20	4.16
04	27	1.43	10	04	06	60	5.25
05	40	1.60	10	01	09	90	6.28

Fish exposed to 96 hour, showed nil mortality at 8µl/l and 12 µl/l. The mortality rate increased with an increase in the concentration of monocrotophos and 100% mortality was observed at40 µl/l. When percent mortality was plotted against the log concentration of monocrotophos a sigmoid curve was obtained (Fig. 1).

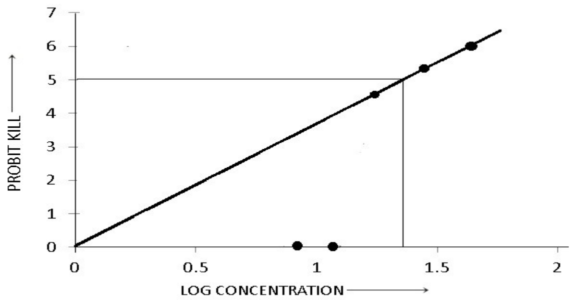
Fig.1

Toxicity evaluation of monocrotophos (36% SL) to *Puntius filamentosus.* The graph shows a sigmoid curve between percent mortality of fish against log concentration.



The LC_{50}value obtained from fig.1 is 24.54µl/l. The LC50 96 hour value was also determined by Probit Method (Finney, 1971). The percent mortality after transforming to probit mortality was plotted against the log concentration of monocrotophos. From this, a straight line was obtained (Fig. 2).

Fig. 2
Toxicity evaluation of monocrotophos (36% SL) to *Puntius filamentosus.* The graph shows a linear curve between the probit mortality of fish against log concentration.



The LC50 value obtained from the graph was 24.65μl/l. The LC50 96 hour value was further verified by the method of Dragsted - Beheren's equation (Carpenter, 1982) and the value calculated by this method was found to be 25.87μl/l. Thus, the average LC50 96 hr. value determined by the above three methods was 25.02μl/l.

3.2) Behavioral Studies

3.2.1) Normal fish

Control fishes maintained a fairly compact school, covering approximately one third of the bottom swimming position was horizontal and normal. The fishes rarely visited the surface (mostly when food provided).When startled, they instantly formed a tight school that was maintained briefly.

3.2.2) Exposed fish

When exposed to lethal concentration the fish immediately migrated at the bottom of the tank. They were spread out and appeared to be swimming independently of one another. The swimming movement was very rapid for the first day but drastically reduced and at the end of the second day and the fish appeared to be slow. They showed irregular, erratic, jumpy swimming movement .On the 4th day the fish appeared to be swimming at the surface area with ventral side facing up. They completely lost their balance and eventually died with operculum wide open.

In sub-lethal treatment, the swimming activity was a little bit rapid with the fishes frequently visiting the surface for first two days. From day fifth onwards the swimming behavior came to normal. Further, the fish at 15 days of exposure exhibited balanced swimming and active feeding.

3.3) **Whole animal oxygen consumption**

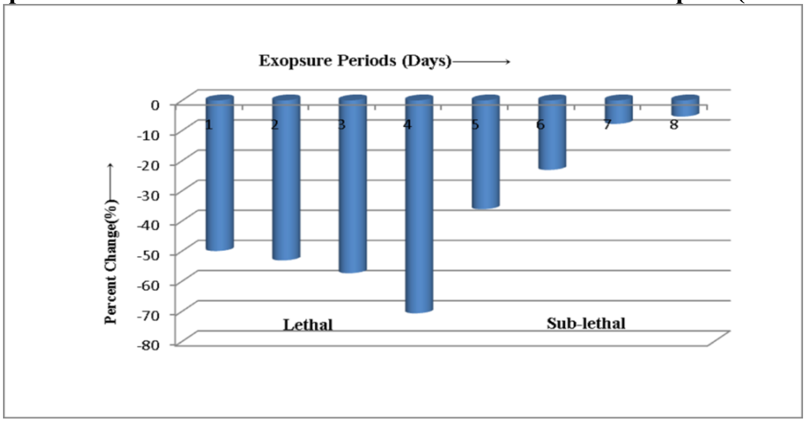
The rates of whole animal oxygen consumption of control and monocrotophos treated fishes were presented in table 2.

Table 2:

Estimations	Control	Exposure periods in days							
		Lethal				Sub-lethal			
		1	2	3	4	1	5	10	15
Mean	0.706^{A}	0.353^{E}	0.331^{F}	0.301^{G}	0.207^{H}	0.451^{D}	0.543^{C}	0.651^{B}	0.668^{B}
SD $\pm$	0.0004	0.0002	0.0003	0.0005	0.0002	0.0003	0.0003	0.0002	0.0003
% Change	------	-50.0	-53.11	-57.36	-70.67	-36.11	-23.08	-7.79	-5.38

Values are Means $\pm$ SD (N=3) for oxygen consumption in a column followed by the same letters are not significantly different ($P \leq 0.05$) from each other according to Duncan's Multiple Range (DMR) test.
Further, a graph was plotted from above data and is presented (fig.3).

Fig.3:
Percent decrease over control in whole animal oxygen consumption of the fish (ml/gm/wet wt/hr) *Puntius filamentosus* on exposure to lethal and sub-lethal concentration of monocrotophos (36%SL)



The data indicate that in fish exposed to lethal concentrations of monocrotophos oxygen consumption was reduced greatly. At the end of day 4, the fall was drastic and irreversible.
In sub-lethal the oxygen uptake rate was not much disturbed and found to be very close to normal. From10th day onwards the fish showed normal oxygen consumption rate which remained constant till day 15. Further Statistical results also justified the fact that the 10 and 15 day oxygen consumptions were non-significant at the P< 0.05level.

IV. Discussion

From the results arrived during the study it can be concluded that Monocrotophos36%SL at very low concentration caused mortality. Thus, it is necessary to select behaviour and other factors that indices the organism's natural behaviour pattern in the field in order to derive a more accurate assessment of the hazards that a contamination may pose in natural systems

The migration of the fish to the bottom of the tank following the addition of monocrotophos, clearly indicates the avoidance behavior of the fish as observed in the carps which was reported by David et al., (2002); Murthy, (1987). Nisar Ahmed (1989) has also observed the avoidance nature by *Labeo rohita* on exposure to three pollutants viz., endosulfan, Malathion and Sevin. The decreased mobility is may be due to on the effect of the organophosphate on the activity of acetyl cholinesterase (Bretaud et al., 2000). The hyper excitability of the fish invariably in the lethal and sub lethal exposure of monocrotophos may probably be due to stress.
The depletion of the oxygen consumption is due to the disorganization of the respiratory action caused by a rupture in the respiratory epithelium of the gill tissue (K. Tilak, R. Kumari, 2009).

As per the data, oxygen uptake is decreased when the time of exposure to toxicant is increased. The respiratory potential and the oxygen consumption of an animal are the important physiological parameters to assess the toxic stress because it is a valuable indicator of energy expenditure during metabolism (Proser and Brown, 1973). Bradbury et al. (1986) stated that the greater decrease in the rate of oxygen consumption in the fish *Cirrhinus mrigala* may be due to the internal action of the pesticide, as toxicant altering the metabolic cycle at the subcellular level. Similar observations were also reported by Tilak and Kumari (2009).

V. Conclusion

From the above assessment pertinent to physiological and behavioral response of the fish, *Puntius filamentosus* to monocrotophos (36%SL), the conclusion could be drawn that the changes arrived at are dependent on the concentration of pesticide and the duration of exposure. Irreparable damage was caused to the physiological and behavioral activities of the fish at higher concentration. The damage increased and prevailed over time of exposure. Under low concentration, i.e., sub lethal concentration stress in fish was observed only for short period (1 to 5 days) and on later days of exposure the stress appeared to lessen and the fish seemed to adapt the toxic environment. The recovery tendency shown by the fish, perhaps, could be due to physiological resistance developed by the animal, which also be reasoned as a possible enhancement of detoxification mechanism and monocrotophos elimination processes. Thus, the above statement suggests that the fish can adapt to low concentration of monocrotophos toxicity during long-term exposure periods.

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