Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 98, No. 1, January 1989, pp. 51–55.

Sublethal effects of organochlorine insecticide (endosulfan) on protein, carbohydrate and lipid contents in liver tissues of *Oreochromis mossambicus*

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MS received 25 May 1988

Abstract. Endosulfan, a chlorinated hydrocarbon insecticide was tested against the fish *Oreochromis mossambicus*. The median lethal concentrations for 24, 48, 72 and 96 h were 0·02, 0·012, 0·007 and 0·006 ppm respectively. The fish were exposed to sublethal concentrations for about 10 days and the protein, carbohydrate and lipid contents in the liver tissue during this period was estimated. There is a general decline in the level of protein, carbohydrate and lipid which is due to the increasing length of exposure to endosulfan.

Keywords. Sublethal effects; endosulfan; liver tissues; *Oreochromis mossambicus*.

1. Introduction

Pesticides have been one of the most effective weapons discovered by man to protect agricultural products from the attack of pests. But the extensive use of pesticides pose a constant threat to the aquatic life by altering the habitat, behaviour pattern, growth and reproductive potential (Jarvinen *et al* 1977; Anderson and Peterson 1969). Although there are considerable research activities in the field of pesticides, there is wide variation in the amount of information available concerning the effect of particular pesticides on selected non-target organisms. Among the organisms studied, fishes have drawn more attention due to their economic importance. *Oreochromis mossambicus*, an economically important fish, abundantly available in local water bodies and is a valuable protein-rich source of food for humans. Lack of information on the toxicity and sublethal effects of endosulfan on the biochemical composition of the species mentioned has induced the authors to conduct the present study.

2. Materials and methods

Oreochromis mossambicus weighing approximately 10 g were collected from ponds in and around Madurai Kamaraj University. They were maintained in large cement tanks at 30 ± 2°C for atleast two weeks before use, during which they were regularly fed with minced goat liver. The water used was clear, unchlorinated having 5·6–8·4 ppm of dissolved oxygen and pH range from 7·6–7·8. During the period of experiment the fishes were not fed.

Lethal toxicity of endosulfan (thiodan, 94% active ingradient) was calculated by conducting static method (APHA 1971). The pesticide formulation in acetone was serially diluted with water to obtain the desired concentrations of 0·005, 0·007, 0·009, 0·01, 0·02, 0·04, 0·05 and 0·1 ppm endosulfan. Healthy fish were chosen at random from the acclimation tanks and 10 numbers were introduced into 15 L experimental glass troughs. A control was kept without endosulfan. Mortality was recorded at every 30 min intervals up to 24 h, 3 h intervals up to 48 h and 6 h intervals up to 96 h. LC_{50} values were calculated for the stipulated time (24, 48, 72 and 96 h) by plotting percentage mortality against the concentration of the pesticide. As there was no mortality in the control there was no need for any correction.

For biochemical studies, *O. mossambicus* were exposed to sub-lethal concentrations of 0·003, 0·004 and 0·005 ppm endosulfan. Ten animals were kept at each concentration for the period of 4, 7 and 10 days. Atleast two replicate troughs were maintained for each concentration. Control fish were reared in pesticide-free water. All the fish were regularly fed with minced goat liver during the experimental period. After exposure, fish from each group were sacrificed and the liver were pooled. Carbohydrate, protein and lipid contents of the liver were then estimated by the methods of Dubois *et al* (1956), Lowry *et al* (1951) and Bligh and Dyer (1959) respectively.

3. Results and Discussion

The median lethal concentrations calculated for 24, 48, 72 and 96 h were 0·02, 0·012, 0·007 and 0·006 ppm respectively (figure 1). Fish survived at 0·005 ppm and below, indicating that 0·005 ppm is the sub-lethal level. The 96 h LC_{50} values of endosulfan noted for *Barbus stigma* was 0·0043 ppm (Manoharan and Subbiah 1982). Using endosulfan, Amminikutty and Rege (1977) observed that 0·016 ppm is the LC_{50} value for the fish *Gymnocorymbus ternetzi*. Singh and Narain (1982) noticed variations in 96 h LC_{50} on the cat fish *Heteropneustes fossilis* in relation to season, size and weight of the fish. Rao and Murty (1982) demonstrated in 3 species of cat fish that the relative toxicity between species could not be determined using LC_{50} values alone, the slopes of endosulfan toxicity curves were different for different species. The data obtained in the present work indicates that different fishes have different tolerance range against the toxic effects of the same pesticide. In this way *O. mossambicus* seems to be more tolerant to endosulfan than *B. stigma*. The higher tolerance of this fish to this pesticide is basically due to species and size variations.

Fish constitutes one of the major sources of cheap nutrition for the human beings (Bhagavathi and Rath 1982). The nutritional value of different fishes depends on their biochemical compositions like protein, amino acids, vitamins, mineral contents etc. yet biochemical changes induced in the different tissues of fish by pollutant have not been studied in significant details (Natarajan 1981; Murty and Devi 1982). In the present investigation, liver protein content of the fish exposed to 0·003, 0·004 and 0·005 ppm of endosulfan showed a general increase over the control on the 4th day. The magnitude of increase was directly proportional to the concentration of the pesticide. Then it started declining from the initial level towards the control level on the 7th day. Liver protein content of the treated fish on the 10th day showed a steady decline below the control level. Here the decrease in the protein content was directly proportional to the length of exposure (table 1). This result stands in good agreement with the observation reported by Manoharan and Subbiah (1982). Similar observations were also noted when fish were exposed to pollutants (Rath and Misra 1980). The decline in protein suggests an intensive

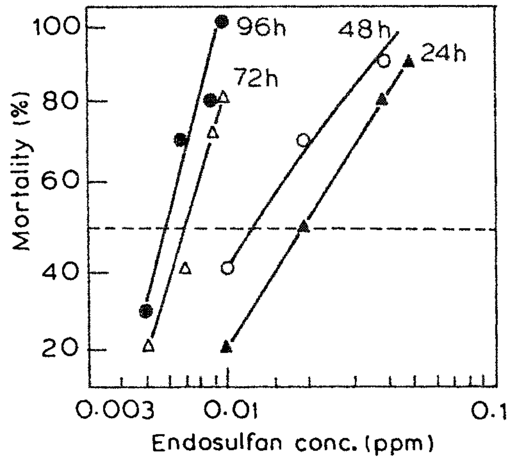

Figure 1. LC_{50} of *O. mossambicus* exposed to endosulfan for different durations at 30±2°C.

Table 1. Effect of sub-lethal concentrations of endosulfan on liver protein content of *O. mossambicus*.

Duration of exposure (days)	Pesticide concentration (ppm)	Liver protein content (mg/g liver dry wt)
4	Pesticide free water	9·9±0·1
	0·003	11·2±0·09
	0·004	13·2±0·08
	0·005	14·6±0·1
7	Pesticide free water	10·6±0·12
	0·003	10·8±0·1
	0·004	12·4±0·007
	0·005	14·4±0·09
10	Pesticide free water	11·4±0·1
	0·003	4·7±0·08
	0·004	5·2±0·09
	0·005	8·2±0·1

proteolysis which in turn could contribute to the increase of free amino acids to be fed into the tricarboxylic acid cycle as keto acids, thus supporting the hypothesis of Kabeer (1979).

The lipid content of liver decreased in the pesticide treated fish as in the case of protein (table 2). Decrease in the lipid content in the present study might be due to the utilization of lipids for the energy demand associated with the situation of stress (Rao and Rao 1981; Harpert *et al* 1977).

There was a general decline in the liver carbohydrate content of fish exposed to sublethal concentrations of endosulfan. Here the carbohydrate content dropped significantly below the control level with an increase in the pesticide concentration after 4, 7 and 10 days of exposure (table 3). This confirms the findings of Murthy and Devi (1982). Similar trend was also observed in endosulfan treated *Barbus*

Table 2. Effect of sub-lethal concentrations of endosulfan on liver lipid content of *O. mossambicus.*

Duration of exposure (days)	Pesticide concentration (ppm)	Liver lipid content (mg/g liver wet wt)
4	Pesticide free water	30·0 ± 0·18
	0·003	35·0 ± 0·37
	0·004	40·0 ± 0·21
	0·005	50·0 ± 0·31
7	Pesticide free water	33·0 ± 0·32
	0·003	30·0 ± 0·41
	0·004	32·0 ± 0·28
	0·005	34·0 ± 0·19
10	Pesticide free water	36·0 ± 0·26
	0·003	20·2 ± 0·21
	0·004	29·5 ± 0·34
	0·005	32·5 ± 0·29

Table 3. Effect of sub-lethal concentrations of endosulfan on liver carbohydrate content *O. mossambicus.*

Duration of exposure (days)	Pesticide concentration (ppm)	Liver carbohydrate control (mg/g liver dry wt)
4	Pesticide free water	22·0 ± 0·21
	0·003	23·0 ± 0·18
	0·004	24·0 ± 0·19
	0·005	24·0 ± 0·2
7	Pesticide free water	45·0 ± 0·26
	0·003	42·0 ± 0·24
	0·004	41·0 ± 0·21
	0·005	37·0 ± 0·19
10	Pesticide free water	50·0 ± 0·3
	0·003	39·0 ± 0·28
	0·004	36·0 ± 0·19
	0·005	27·0 ± 0·2

stigma (Manoharan and Subbiah 1982). Umminger (1970) found that carbohydrates represent the principal and immediate energy precursors for fishes exposed to stress conditions while proteins being the energy source to spare during chronic periods of stress.

Generally more energy is needed to mitigate any stress condition. This energy may be obtained from carbohydrates, proteins and/or lipids.

Acknowledgements

RMG and SRDJ are grateful to the Council of Scientific and Industrial Research and Department of Science and Technology, New Delhi respectively for financial support.

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