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ORIGINAL ARTICLE

Dimethoate Toxicity and Biochemical Changes in the Freshwater Fish *Channa punctatus* (Bloch)

Ranjan Kumar
Jai Prakash University, Chapra (Bihar)
Email: ranjankumar.1955@rediffmail.com

ABSTRACT

The fresh water fish Channa punctatus was exposed to Dimethoate in the laboratory to study its toxicity. The acute toxicity tests were conducted during certain intervals in various concentrations of Dimethoate. The physical and chemical analyses of water were carried out by following APHA methods. The lethal and sub-lethal concentration of Dimethoate were found to be LC100 (25 mg/L) and LC0 (5 mg/L), respectively. The antioxidant enzyme activity in the liver, muscle and gill, respectively increased during the accumulation of Dimethoate, whereas it decreased respectively during depuration period. The effects of Dimethoate resulted in the gradual decrease of nucleic acids, protein, free amino acids (FAA) and glycogen. During recovery period, the levels of biochemical components progressively increased indicating a probable recovery from the disruption of internal organ. Hence, the pesticide intoxication has made defective consequences in the normal metabolic pathways which led increasing the rate of mortality in fish population.

Key words*: Channa punctatus, Dimethoate, Protein, Nucleic acids and Antioxidant enzymes*

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INTRODUCTION

The Dimethoate contamination of ponds is a potential problem for aquaculture in tropical countries. The pesticide, on reaching to aquatic systems, greatly influences the non-target organisms such as fish and birds. Histological studies on fish have revealed that various toxicants have produced pathological changes in the tissues such as macrobiotic changes in the liver, tubular damage of kidneys, gill and lamellar abnormalities (Ramalingam, 2000). Due to growth of agriculture in and around fresh water bodies the pesticides are used abundantly during the cultivation season and found their way into water bodies.

The degree of toxicity produced by the poisonous substance is dose independent upon environmental conditions such as temperature, pH, oxygen content and presence of residue molecules (Singh and Mishra, 2009). It is well known that protein, carbohydrates and lipid play a major role as energy precursors in fish under stress conditions. Enzymes play significant role in food utilization and metabolism. The proteolytic enzymes participate in the breakdown of protein molecules into amino acids and these amino acids are in turn oxidized to give energy for body function (Saravanan et *al*., 2000). Pollutants can produce metabolic changes at cellular levels by a way of influencing enzyme systems.

The present study has been made to investigate the biochemical changes followed by mortality in the fresh water fish *Channa punctatus* induced by sub lethal dosages of the pesticide.

MATERIALS AND METHODS

The collected Fishes were fed daily and acclimatized in laboratory for 30 days. The physical and chemical analyses of the water were carried out (APHA, 2005). Fish were divided into seven groups (each containing 10 fish) where six were experimental and one group as control. Acute toxicity study was carried out using the standard guidelines to determine the lethal (LC100), median (LC50) and safe sub lethal (LC0) levels of Dimethoate in various concentrations (5, 10, 15, 20, 25 & 30 mg/L). The mortality of fish (%) was assessed during the interval of 24, 48, 72 and 96 hours. The 1/3rd of median lethal concentration (5 mg/L) was taken to study the effect of Dimethoate on the biochemical constituents and detoxifying ability of fish.

The water was renewed freshly every day to produce constant effect of Dimethoate on fish. At the end of 15 days exposure, the tissues such as liver, muscle and gill were collected by dissecting the animal and stored at -20ºC for biochemical parameters studies. The remaining fish released into freshwater for 15 days to know the detoxifying ability of the fish. At the end of 30 days, tissues were collected again and one gram of muscle, liver and gill samples were suspended in 5mL of 0.1 M phosphate buffer of pH=7 and homogenized. These homogenates were stored for further studies at -20ºC. The Catalase activity assay was performed according to Beaumont et *al* (1990) by following the H_2O_2 dismutation at 240 nm in a reaction mixture composed of 0.1 M phosphate buffer, pH=7, 50–100 mg protein and 18 mM H_2O_2. GST activity was measured at 37°C using 1 mM l-chloro-2,4 dinitrobenzene (CDNB) as substrate.

The activity of acid phosphatase and alkaline phosphatase were assayed with the method of TennisWood et *al* (1976). Proteins levels were estimated by the method of Lowry, *et al.,* (1951) using bovine serum albumine as standard. Homogenates 2 ml (w/v) cold distilled water was prepared in 30% TCA; values are expressed as mg/100 mg wet wt of tissue. Free amino acids (FAA) were estimated using the ninhydrin method (Moore and Stein, 1954). FAA was expressed as mg/100 mg wet wt of the tissue.

The values were expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by LSD tests using the computer package SPSS 18.0 v and the significance of difference was set up at ($p< 0.05$).

RESULTS AND OBSERVATIONS

The percentage mortality of *Channa punctatus* exposed to Dimethoate in 5, 10, 15, 20, 25 and 30 mg/L for 24h, 48h, 72h and 96h was assessed (Table 1).

Table 1: Mortality of *Channa punctatus* exposed to Dimethoate

S No.	Concentration (mg/l)	Exposure period (hours)				LC_{50}
		24	48	72	96	
1	5	N	N	N	N	15 mg/l
2	10	N	10%	10%	10%	
3	15	10%	20%	30%	50%	
4	20	20%	50%	70%	90%	
5	25	60%	100%	N	N	
6	30	10%	N	N	N	

The median lethal concentration was observed as 15mg/L since it is caused 50% mortality in 96 h using the "Maximum likelihood method" (Finney, 1971). 1/3rd of median lethal concentration (5 mg/L) was taken to study the effect of Dimethoate on the biochemical constituents and detoxifying ability of fish.

The activity of antioxidant enzymes in the liver, muscle and gill of *Channa punctatus* exposed to LC0 concentration of 5 mg/L Dimethoate during accumulation were observed as showed in Table 2. Depletion on biochemical parameters like Protein, Glycogen and Free amino acid were evaluated during various periods of exposure (Table 3).

Table 2: Anti-oxidant enzyme activity in the tissues of *Channa punctatus* during accumulation and de-purination periods

Accumulation study (µmole of Phenol liberated/min/100 mg Protein)							
S No.	Anti-Oxidant enzyme	Liver		Muscle		Gill	
		Control	Day 15	Control	Day 15	Control	Day 15
1	Catalase	13.6±1.13	42.8±2.1	6.8±0.10	16.2±0.54	6.7±0.13	23.4±0.15
2	Glutathione 5-transferase	19.5±1.12	269.8±0.14	92.9±0.12	142.9±1.04	119±0.52	214.8±0.70
Accumulation study (µmole of Phenol liberated/min/100 mg Protein)							
1	Catalase	38.03±1.03	16±1.42	10.3±0.72	7.9±0.23	21.9±0.84	9.7±0.64
2	Glutathione 5-transferase	251.9±1.40	218.8±1.10	134.9±0.16	107.8±0.32	17.9±0.27	158.2±0.44

Table 3: Sub-lethal effects to Dimethoate on Protein, Glycogen and Free amino acid in the tissues of *Channa punctatus*

S No.	Organs	Biochemical parameters	Control	Sub-lethal Concentrations			
				24hr	48 hr	72 hr	96 hr
1	**Liver**	Protein	225.3±1.42	206.68±1.20	180.2±0.44	178.86±0.42	142.2±0.78
		Glycogen	11.2±0.32	10.4±0.16	9.6±0.15	8.7±0.22	6.2±0.16
		FAA	28.4±0.15	27.4±0.15	35.4±0.26	33.1±0.22	28.2±0.42
2	**Muscle**	Protein	189.3±0.3	172.4±0.42	140.24±0.26	134.8±0.12	127.3±0.14
		Glycogen	9.6±0.32	9.2±0.32	7.4±0.16	6.7±0.26	5.2±0.16
		FAA	17.4±1.13	13.4±0.52	28.4±0.52	24.4±0.10	20.4±0.24
3	**Gill**	Protein	198.3±0.32	162.6±0.54	125.3±0.63	96.9±0.12	86.3±0.15
		Glycogen	11.2±0.23	9.46±0.02	9.2±0.04	6.8±0.11	5.2±0.17
		FAA	22.4±0.15	22.2±0.48	21.6±0.46	20.4±0.12	19.8±0.25

Reduction on macro and micromolecules are directly proportional to the concentration of Dimethoate and exposure periods. The values were expressed as mean ± SEM and the significance of difference was set up at ($p< 0.05$).

DISCUSSIONS

The fish were seen to exhibit several behavioural responses, such as fast jerking, frequently jumping, erratic swimming, spiraling, convulsions and tendency to escape from the aquaria during study. Rao et *al* (2005) reported that abnormal changes in behavior in mosquito fish *Gambusia affinis* in response to the sub-lethal exposure to chlorpyrifos.

The fish exhibited unrest and a peculiar tumbling motion before they died. Moreover, the herbicide Dimethoate persists in the aquatic system for a long period of time. The liver, muscle and gill tissues showed decreased level of acid phosphatase (ACP) and Alkaline Phosphatase (ALP) activities. Shakoori et *al* (1992) have suggested the decrease (or) inhibition of ACP and ALP activities are due to increased necrosis in the tissues like hepatocytes.

The protein, glycogen and free amino acids were decreased gradually compared to control, when the period of exposure increased. The depletion of protein may also be attributed to spontaneous utilization of amino acids in various catabolic reactions inside the organism in order to combat the stress condition (Borah, 1996). Increase of total free amino acids (TFAA) is an induction of stepped up proteolysis or fixation of ammonia into keto acids resulting in amino acid synthesis. Generally, these two processes contribute to the amino acid pool (Mohapatra and Noble, 1992). The carbohydrate reduction suggests the possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress condition (Reddy et *al* (1993).

The present investigation shows biochemical changes due to sub lethal concentration of Dimethoate in total proteins, free amino acids (FAA) and glycogen in target organs and tissues significantly. Thus the pesticides toxicity has disturbed the normal functioning of

cells with the resultant alterations in the fundamental biochemical mechanisms in fish. This would in turn result in the mortality of fish on chronic exposure to the pesticide.

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