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Toxic Impact of Dimethoate on Fish, *Heteropneustes fossilis*

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ABSTRACT
The freshwater fish, Heteropneustes fossilis was exposed to sublethal concentrations of Dimethoate for a period of 45 days and the changes in the carbohydrate metabolism of vital organs such as muscle, liver and gill were studied. It was observed that the liver is vital organ of carbohydrate metabolism was drastically affected. Increasing concentration and exposure period the carbohydrate concentration was reduced.
Keywords: *Sublethal concentrations, carbohydrate, aquatic toxicology*

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INTRODUCTION

Indiscriminate uses of pesticides are very common in India as well as in several Asian, American and African countries. Pesticides are used by farmers for spraying directly into the crops in the fields and storage etc. These chemicals are capable of killing pests and insects but on the other hand, they are highly toxic to animals as well as human beings [1]. As considerable amount of pesticides and their by products enter the fish body, through the food chain, where they are distributed and metabolised depending upon the detoxifying ability of the fish, and elicit some responses in fish which depends on the nature and concentration of pesticides as well as on the duration of detainment of these pesticides in fish body, and the ability of fish to metabolise the pesticides observed [2-3]. The introduction of pesticides in agriculture created a major shift in work activities, reduced costs, and increased productivity. Unfortunately, in spite of these advantages, the use of pesticides has caused numerous problems stemming from their use and release in to the environment, as well as problems related to potential adverse effects on human health. A number of investigations have been reported that most of the synthetic organic pesticides of organochlorine, organophosphates and carbamates are extremely toxic to non-target freshwater fauna, adversely [4]. Once absorbed, contaminants may interact with endogenous substances, causing biological effects that may impair the life quality, not only of the exposed organisms but also of the whole ecosystem [5]. Fishes are particularly sensitive to the environmental contamination of water. Hence, pollutants such as insecticides may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes [6]. These investigations linked the pesticides to

number of biochemical reactions, which could explain their adverse effects on the morphology and physiology of a number of freshwater organisms. Hence the present investigation is aimed to evaluate the toxicity risk of Dimethoate on the carbohydrate metabolism of a Indian catfish, *Heteropneustes fossilis.*

MATERIAL AND METHODS

Experimental set up

The freshwater fish *Heteropneustes fossilis* (size . 8 ±1cm and 5-8 g weight) were brought from a local fish farm and acclimatized in the laboratory for one week. The water used for acclimatization and conducting experiments was clear dechlorinated ground water. They were exposed to different concentrations of Dimethoate individually for the static bioassay test. Then 24, 48, 72, 96 and 120 hrs LC_{50} values of Dimethoate were computed . LC50 values were calculated by Finney's [7]. From the 96 hours LC_{50} values two sub-lethal concentrations, viz 0.166 mg/l ($1/4^{th}$) and 0.083 mg/l ($1/8^{th}$) were chosen to expose the fish for biochemical studies. After 15, 30, and 45 days exposure, the fishes were taken out and the following tissues, *viz.,* liver, muscle and gill were dissected out under aseptic condition. The selected wet sample, were used for total carbohydrate estimation by the methods of Carrol et al., [8].

Estimation of carbohydrate

About 10 mg sample was grained by using mortar and pestle, with 2 ml of 80% ethanol, after grain the sample was transferred into the centrifuge tube and was centrifuged for 100 minutes at 3000 rpm. The supernatant was taken and 1 ml of 10% TCA was added. Again the content was centrifuged at 3000 rpm for 10 minutes. 1 ml of the supernatant was taken and 5 ml of 0.2 % anthrone reagent was added. Test tubes were covered and kept in boiling water bath at 60 ˚C for 10 minutes. They were cooled to room temperature and optical density was measured at 620 nm using spectrophotometer. The total carbohydrate content was expressed in mg/100 mg wet tissues.

Statistical analysis

All the obtained data were subjected two 2 way ANOVA.

Result and Discussion

The physic chemical parameter of the exposure medium, was pH 7.21, hardness 188 mg/L, dissolved oxygen 78% and temperature 24-25.5 °C. The entire test period the pH of the medium was not deviated more than 1 unit (pH 7.21 -8.10).

In the present study dimethoate showed LC_{50} value of 1.023, 0.911, 0.866, 0.711, 0.666 and 0. 610 mg/l for different exposures period of 12, 24, 48, 72, 96 and 120 hrs respectively against *H. fossilis*. The LC_{50} values differed from species to species for single pesticide, due to their mode of action. In common carp, acute toxicity of profenofos (LC_{50}) and triazophos (LC_{100}) was determined as 62.4 ppb and 1.00 ppm, respectively by Ismail *et al.* [9]. Likewise, acute toxicity of profenofos and triazophos in crucian carp was determined as 0.192 ppm and 8.4 ppm respectively [10]. Assis *et al.* [11] determined the LC_{50} values for carbaryl and carbofuran as 33.8 µmol/L and 0.92 µmol/L, respectively, Somaiah Karra [12] studied the LC_{50} values of Phenthoate were determined to be 3.0, 2.6, 2.3 and 2.1 mg/L for 24, 48, 72 and 96 hrs respectively.

The LC_{50} values are useful measure of acute toxicity of tested pesticide used under certain environmental conditions, but do not really represent concentration may be safe or harmless to fish habitats subjected to pollutant discharges. The concentration which was harmless to the fish within 96 hrs may be very toxic under condition of continuous exposure. The toxicity of a pesticide can be modified by various factors including the physico-chemical characteristics of the medium, biological behaviours and status of test animal [13]. The status of test fishes includes size, weight, age, sex and life cycle stage. The physico-chemical factors such as temperature, pH, alkalinity and hardness also influence the toxicity [14] .

Sub-lethal concentration of Dimethoate caused a significant decrease in the carbohydrates content in all the tissues like liver, muscle and gill of *H. fossilis* (Table 1-3a) and Figure 1-3. The changes in the carbohydrate content in the liver and the muscle of *H. fossilis* observed in the present study. The results of the present finding showed a significant decrease in carbohydrate content in all the tissues studied. The decrease in carbohydrate indicated the

altered carbohydrate metabolism which might have resulted from the enhanced breakdown through glycogenolysis to meet the high energy demand, due to stress [15-16].

Amali [17] reported that the total loss of carbohydrate content in different tissues of *Labeo rohita* due to the reason that the fish has adopted a compensatory mechanism so as to derive energy during pyrethroid (cypermethrin) and organophosphate (quinolphos and padan). The fish exposed to a pesticide showed heavy physical exercise in the form of erratic and rapid movement, which would have increased the utilization of the reserve carbohydrate of liver and resulted with loss of glycogenesis [18-21]. Lutherdas *et al.*, [22] reported that the reduction in carbohydrate content in the muscle and other tissues of cypermethrin exposed *Oreochromis mossambicus* was due to the differing prolonged muscular activity.

David et al., [23] suggested that carbohydrate metabolism disturbed when fish *Labeo rohita* exposed to pesticide fenvalerate. Neeraja and Giridhar [24] reported decline glycogen content followed by elevation in blood glucose level in the fresh water fish *Labeo rohita* on exposure to deltamethrin. Bhattacharjee and Das [25] reported decline in glycogen content followed by elevation in blood glucose level in fish *Cyprinus carpio* on exposure to lindane.

Gopalarao [26] had reported that reduction in glycogen level in the muscle and other tissues of *C. punctatus* exposed to cypermethrin was due to the differential prolonged nuclear activity. Sreeya [27] reported that the liver is vital organ of carbohydrate metabolism and were drastically affected by the pesticides. Sagar [28] reported toxic stress imposes an increased energy requirement from the animal adopt to the changed metabolic condition and this achieved through utilization of reserve stores of carbohydrate in fish tissues under toxic stress is due to increased glycogenolysis.

Table 1. Changes in the level of carbohydrate content (mg / 100 mg wet tissue) in the muscle tissue of *Heteropneustes fossilis* exposed to sub lethal concentrations of Dimethoate

Days	**Control**	**Concentrations of Dimethoate (mg/l)**	
		0.083	**0.166**
15	4.17±0.02	3.48±0.01 (16.54)	3.13±0.03 (24.94)
30	4.62±0.05	2.73±0.02 (40.90)	2.55±0.03 (44.80)
45	5.02±0.01	2.41±0.04 (51.99)	2.17±0.02 (56.77)

Table 1a.Two- way ANOVA of carbohydrate content (mg / 100 mg wet tissue) in the muscle tissue in *Heteropneustes fossilis* exposed to different concentrations of Dimethoate

Source of Variation	**Sum of squares**	**Degrees of freedom**	**Mean sum squares**	**F-Value**	**P-value**
Total Variance	8.438022	8			
Variance due to exposure duration	0.250756	2	0.125378	0.424418	(P>0.05)
Variance due to concentration	7.005622	2	3.502811	11.85741	* (P<=0.05)
Error variance	1.181644	4	0.295411		

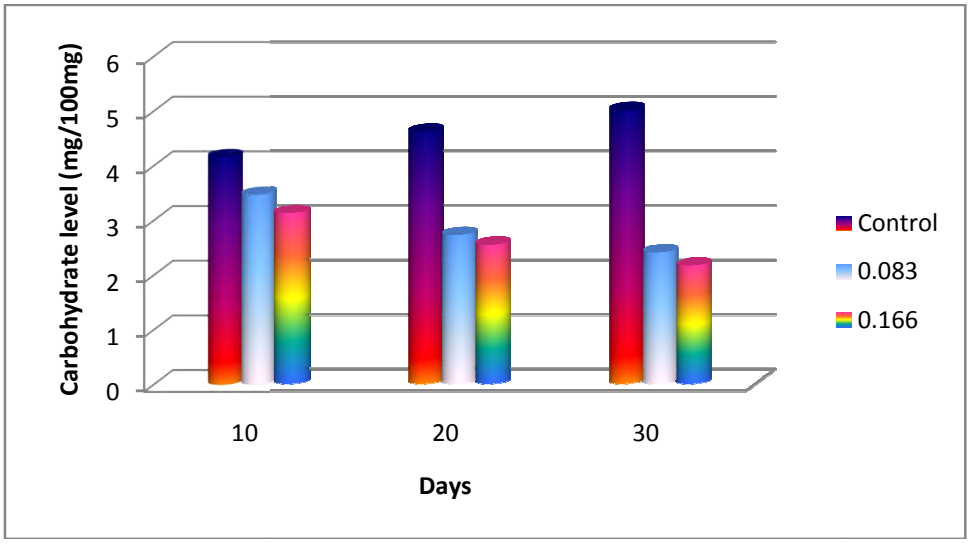

Fig. 1 Changes in the level of Carbohydrate content in the muscle tissue of *Heteropneustes fossilis* exposed to Dimethoate

Table 2. Changes in the level of carbohydrate content (mg / 100 mg wet tissue) in the liver tissue of *Heteropneustes fossilis* exposed to sub lethal concentrations of Dimethoate

Days	Control	Concentrations of Dimethoate (mg/l)	
		0.083	0.166
15	4.81± 0.03	3.61±0.01 (24.74)	3.17±0.03 (34.09)
30	5.34± 0.02	2.73±0.02 (48.87)	2.51±0.01 (52.99)
45	5.98±0.04	2.23±0.02 (62.70)	2.05±0.04 (65.71)

Table 2 a.Two- way ANOVA of carbohydrate content (mg / 100 mg wet tissue) in the liver tissue in Heteropneustes fossilis exposed to different concentrations of Dimethoate

Source of Variation	Sum of squares	Degrees of freedom	Mean sum squares	F-Value	P-value
Total Variance	16.5654	8			
Variance due to exposure duration	0.321267	2	0.160633	0.325278	(P>0.05)
Variance due to concentration	14.2688	2	7.1344	14.44698	* (P<=0.05)
Error variance	1.975333	4	0.493833		

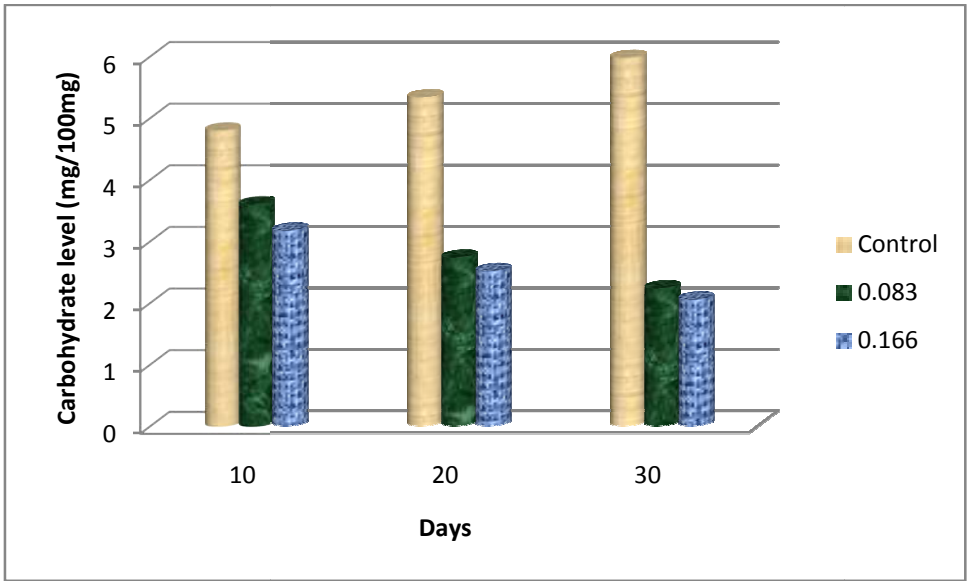

Fig. 2. Changes in the level of Carbohydrate content in the liver tissue of *Heteropneustes fossilis* exposed to Dimethoate

Table 3. Changes in the level of carbohydrate content (mg / 100 mg wet tissue) in the gill tissue of *Heteropneustes fossilis* exposed to sub lethal concentrations of Dimethoate

Days	Control	Concentrations of Dimethoate (mg/l)	
		0.083	0.166
15	3.25±0.01	2.56±0.02 (21.23)	2.15±0.01 (33.84)
30	3.56±0.04	2.20±0.04 (38.20)	2.04±0.08 (42.69)
45	3.97±0.02	2.02±0.02 (49.11)	1.77±0.01 (55.41)

Table 3a.Two- way ANOVA of carbohydrate content (mg / 100 mg wet tissue) in the gill tissue in *Heteropneustes fossilis* exposed to different concentrations of Dimethoate

Source of Variation	Sum of squares	Degrees of freedom	Mean sum squares	F-Value	P-value
Total Variance	4.9224	8			
Variance due to exposure duration	0.007467	2	0.003733	0.031042	(P>0.05)
Variance due to concentration	4.433867	2	2.216933	18.43348	** (P<=0.01)
Error variance	0.481067	4	0.120267		

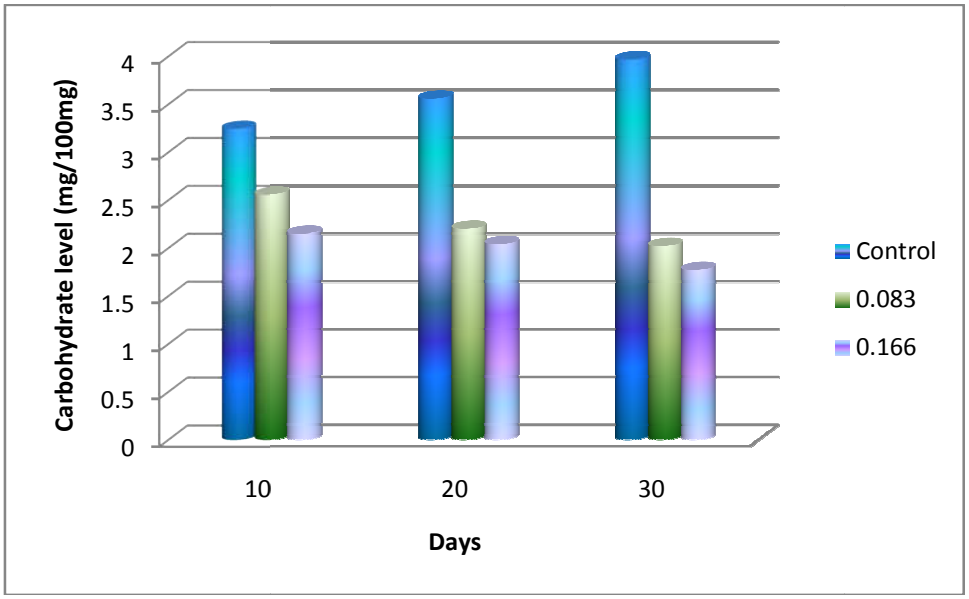

Fig.3. Changes in the level of Carbohydrate content in the gill tissue of *Heteropneustes fossilis* exposed to Dimethoate

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