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Thiram a fungicide induced toxicity on glycogen and blood glucose level of freshwater fish *Cyprinus carpio* (Hamilton)

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Abstract
The present study was investigated on Freshwater fish, C*yprinus carpio* exposed with technical grade di-thiocarbamate a fungicide, Thiram for 96h and the LC_{50} value were determined at 0.66 mg/l. One–tenth of LC_{50} was selected a sub-lethal dose for studies on liver, muscle glycogen and blood glucose levels for a period of 1, 7, 15 and 30th days. The present study revealed that, the LC_{50} value gradually decreased with the increase of exposure period and the mortality rate increased with increase in concentration with fungicide exposure and the depletion of glycogen levels in liver, muscle and elevation of blood glucose levels were observed.

Keywords: Thiram, LC_{50}, glycogen, Blood glucose, *Cyprinus carpio*

1. Introduction
Thiram is moderately toxic by ingestion, but it is highly toxic if inhaled. Hepatotoxicity is one of the many toxic effects of thiram exposed to workers and test animals. Typical symptoms include as liver enlargement and dysfunction, hepatitis, degenerative changes, and focal necrosis [1, 2]. Clinical signs in thiram poisoning areanorexia, listless behaviour, dyspnoea, convulsions, and death due to cardiac arrest [3]. It is metabolized in the body to toxic metabolites di-methyldithiocarbamate and carbon disulfide. Although these compounds have been shown to inhibit hepatic microsomal enzymes [4]. Levels of thiram ranging from 100-500 ppm in the food rations of hens, quail and partridges inhibit egg laying [5]. Thiram-contaminated poultry feed caused soft egg shells, depressed growth and leg abnormalities in about 1million birds. Thiram is also used as a seed protestant and to protect fruits, vegetables, ornamental and turf crops from a variety of fungal diseases [6].It has been reported that thiram degrades faster in the field than in the greenhouse, and as a vettable powder than as a emulsion [7]. Thiram was rapidly degraded in a water solution when irradiated with UV light. More than 94% of the pesticide was destroyed within 20min [8].
Dethiocarbamate fungicides form a large group of chemical that have numerous uses in agriculture and medicine. It may be applied to the foliage of plants, seed treatment, almonds, and fruits and also used as a bird and rodent repellent. Thiram, zinc dimethyl bisdithiocarbamate belongs to a class of fungicides used worldwide in agriculture. They are generally used because of low cost, good efficacy and broad spectrum of antifungal activity. The fungicides have caused extensive damage to various tissues of fish as reported by many workers [9, 10], Toxicity to a chemical refers to an individual organism's response at a particular dose for a specific period of time. Lethal effects are rare in nature because the organisms are exposed to low concentration, which are normally sub lethal [11].The fungicides usage is desirable for the control of pests on the one hand and on the other hand, these are causing environmental pollution [12, 13]. The contamination of water by pesticides may effect on non - target organisms like fish [14, 15, 16]. So an attempt was made on sub lethal impact of deltamethrin on some aspects of carbohydrate metabolism in selected fish.

2. Materials and Methods
The freshwater fish, *cyprinus carpio* were procured from the Department of Zoology &Aquaculture, Acharya Nagarjuna University, Guntur, Andhra Pradesh. during the period of 20/2/2017 to 27/8/2017. They were acclimated to laboratory condition for fifteen days prior to the experiment. During acclimatization, fish were fed daily with rice bran and oil cake in the ratio of 2:1.

Cyprinus carpio weighing 10±2 gm was selected from the stock. Technical grade thiram (90%) was obtained from Rallis India ltd., Bangalore. Fish were exposed to different concentration of thiram and mortatlity rate was recorded. to determine LC_{50} values(table1). In the present study,1/10th of 96 hours LC_{50} value of thiram (0.66 mg/l) was selected as sub lethal concentration for chronic studies (1,7,and 15days) to observe the liver, muscle glycogen and blood glucose levels of various organs of fish. The concentration of glycogen was determined by the method of Caroll [17].The values were expressed as mg/gm wet weight. Blood was collected by cutting the caudal peduncle dissection method Reichenbach [18]. Using heparin as anticoagulant and the blood glucose was determined by method of Mendel [19]. The values were expressed as mg of glucose/100ml of blood.

3. Results and Discussion

3.1 Determination of median lethal concentration (LC_{50})

The LC_{50} value was calculated as the concentration of test chemical which caused 50% mortality of total fish during the respective periods of exposure i.e., 24, 48, 72 and 96 hr. Then, the LC_{50} values of thiram fungicide evaluated and found to be 0.50,0.55,0.60,and 0.66 mg/L for 24, 48, 72 and 96 h respectively as shown in (Table 1). The mortality data was subjected to probit analysis and graphs were plotted between concentrations of thiram fungicide and percent mortality of fish. The percent mortality was gradually increased with the increase in concentration of fungicide. During the experiment, the LC_{50} values were reduced and the exposure time was increased along with Log concentration from 24 to 96 h and no mortality was observed in control group.

Table 1: Effect of Thiram fungicide on survival of *cyprinus carpio* for sub-lethal concentrations

Time(hr)	Conc. of toxicant (mgl-1)	Log conc.	No. of exposed fish	No. of dead fish	Present of Mortality	Probit Mortality
24	0.50	0.30	10	5	50	5.00
48	0.55	0.25	10	5	50	5.00
72	0.60	0.22	10	5	50	5.00
96	0.66	0.18	10	5	50	5.00

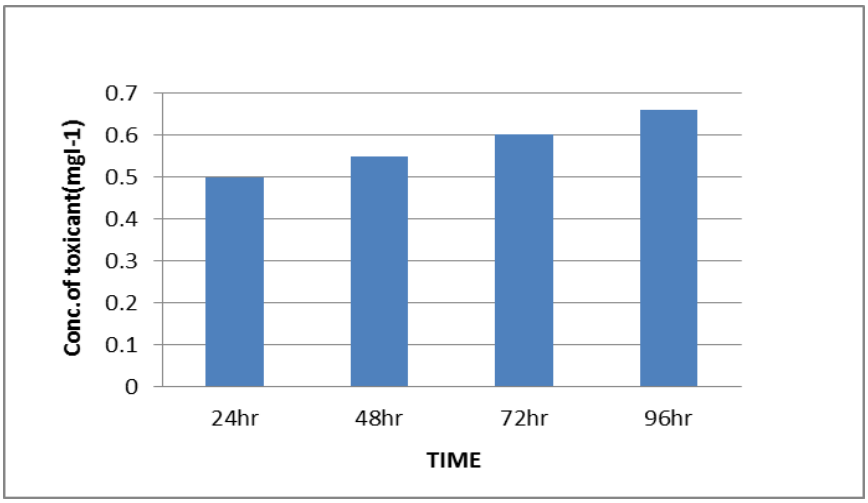

Fig 1: Sub-lethal concentrations of Thiram fungicide on survival of *cyprinus carpio* from 24hr to 96hr

Muscle and liver tissues of *cyprinus carpio* were examined for glycogen levels in the present study and the results are tabulated (tables 2 and 3). Relative to control, a significant decrease in glycogen levels was observed at day1, followed by day 7, 15and 30th day Thiram intoxicated fish. Relative to control, a significant increase in the blood glucose level was observed at day 1 to day 30 in the thiram exposed fish and results are tabulated (table 2).

Table 2: Glycogen contents in the muscle and liver of freshwater fish, *cyprinus carpio* exposed to sub-lethal concentrations of thiram in different exposure periods 30 days.

Organs		Control	24hrs	7th days	15th days	30th days
Muscle	Mean±S.D	6.353±0.233	4.683±0.224	4.373± 0.120	3.970±0.202	4.640±0.362
	% change		-26.3	-31.9	-37.49	-26.93
Liver	Mean±S.D	30.26±1.209	28.69±0.884	23.61± 1.067	16.87±1.037	22.19 ± 1.17
	% change	-	-5.18	-21.98	-44.25	-26.67

SD – Standard Deviation; % – Percent change

Carbohydrates are one of the main energy yielding nutrients of living organisms. If the intake is more, the excess carbohydrate is stored in the form of glycogen and is utilized whenever there is stress. Hence, glycogen is known to be a ready and prime source of energy. The decrease in the levels of glycogen during thiram exposure is an indication of rapid utilization, since glycogen is considered to be one of the first organic constituent to be utized under stress condition. Similar trend in the decrease of glycogen levels were observed in different fish exposed to various toxicants [20, 21]. It could be also due to stimulation of hormones that accelerate glycogen breakdown. The decline of glycogen levels in both the tissue indicates the breakdown of glycogen which releases glucose into circulatory system to meet the increased energy requirements in a stressed condition which indicates the prevalence of anaerobic conditions and also indicates then possibility of active glycogenolysis [22].

Table 3: Exposure of Thiram on Blood glucose level in fish, *cyprinus carpio* exposed to sub-lethal concentrations in different exposure days

Sub-lethal exposure period					
S. No	**Control**	**24hrs**	**7th day**	**15th day**	**30th day**
Blood glucose	87.79	118.8	82.5	131.74	93.94
± S.D	0.36	0.035	0.040	0.035	0.030
% change		+35.6	-5.63	+5.53	+7.38

SD – Standard Deviation;% – Percent change

The difference between control and 1or 7or 15 or 30 are found to be statistically significant ($p<0.05$) value expressed as mg of glucose/100ml of blood glucose level Each value is mean ±SD of 6 individual observations.

The disturbances in the carbohydrate metabolism caused by the action of toxic compound and the complimentary shift appear to be essential in the cell and tissue metabolism the decrease in muscle glycogen could be due to increased glycogenolysis for synthesis of glucose to provide sustained energy to the fish under stress [23, 24, 25]. Blood parameters are considered pathaphysiological indicator of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to various toxicants [26, 27]. In the present investigation, a significant increase in blood glucose was observed. This may be due to stimulated gluconeogenesis and glucogenolysis and also may be due to reduced insulin secretion. The increased blood sugar level that is hyperglycaemic condition may be due to the conversion of stored glycogen into catecholamine' s by toxicants [28, 29]. Similar results were observed in *Oreochromis mossambicus* when exposed to quinolphos [30]. The elevation in the blood glucose levels suggests physiological response of the fish to meet the critical need of energy under stressed condition [31]. Hence, all type of stress in fish results in increase the circulation of catecholamine's and corticosteroids referred to as the primary effects. The endocrine changes induce secondary biochemical responses as pronounced increase in the blood sugar level [32]. In the present investigation the significant decrease levels and increase in blood glucose levels were observed. This can be due to reduced insulin secretion and stimulated gluconeogenesis and glycogenolysis.

The present study was undertaken to analyze the impact of sub lethal concentrations of thiram exposure a fungicide on glycogen and blood glucose levels of freshwater, C*yprinus carpio.*

4. Conclusion

The present study concluded was investigated on Freshwater fish, C*yprinus carpio* exposed with technical grade di-thiocarbamate a fungicide, Thiram were the LC_{50} value gradually decreased with the increase of exposure period and the mortality rate increased with increase in concentration with fungicide exposure and the depletion of glycogen levels in liver, muscle and elevation of blood glucose levels were observed.

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