Effect of Thimet 10% (CG) on biochemical and enzymatic alterations in *Cyprinus carpio* (Hamilton)

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Abstract: Thimet is an organophosphate insecticide that is extensively used throughout the world and in India and Andhra Pradesh. It is used as a broad-spectrum insecticide on numerous commercial crops including paddy and groundnut. It is a soil and systemic insecticide and miticide, commonly used for the control of chewing and sucking insects, mites and soil dwelling pests. Due to enormous use, it is accumulating in the environment and washed away due to heavy rains and floods causing alarming environmental pollution. Hence, the present study was contemplated to evaluate the biochemical alterations in osmoregulatory organs such as gill (GL) and kidney (KY) and non-osmoregulatory organs such as liver, muscle and brain of the fish *Cyprinus carpio* on exposure to sublethal toxicity of Thimet for 5-day and 10-days. The sublethal concentrations of Thimet altered the levels of blood glucose, and total carbohydrates significantly ($P<0.05$) in the all target organs of the experimental fish when compared with the control group. The other parameters evaluated includes the estimation of enzyme activity of different enzymes like Acid Phosphatase, Lactate Dehydrogenase, Succinate Dehydrogenase, Maltase Dehydrogenase, Aspartate aminotransferase, and Alanine aminotransferase. All the enzymes showed alterations in their presence and activity.

Keywords: Cyprinus carpio Thimet 10%CG pesticide Biochemical analysis

INTRODUCTION

Environmental pollution caused by pesticides, particularly in aquatic ecosystems, has become a severe problem. These pesticides even when applied in small quantities are washed and carried away by rains and floods to large water bodies like ponds and rivers and thereby altering the physicochemical properties of water, this proved to be highly toxic, not only to the fishes but also to aquatic life forms and their environment. Contamination of surface waters has been well documented worldwide and constitutes a major issue at local, regional, national, and global levels (Nwani CD et al., 2010).

Organophosphates are one of the most preferred pesticides due to their effectiveness and low persistence in the environment. Organophosphate pesticides directly inhibit acetylcholinesterase enzyme activity in fishes and invertebrates. Phorate {O, O-diethyl-S- [(ethylthio) methyl] phosphorodithioate}, is one of the widest used organophosphorus insecticides used in agriculture. Commercial names of phorate are Thimet, Rampart, etc., and its molecular formula is $C_7H_{17}O_2PS_3$ (Lakshmaiah G 2014).

Toxicity studies have long played an important role in modifying the effects of his activities on the biota. The toxicity studies are especially useful for sensitive species of an ecosystem that can be used as a role in man's efforts to monitor and determine the indicator species for a particular type of pollution. The results of toxicity are generally reported in terms of median lethal concentration LC50 and or median tolerance. Fishes come into contact with multiple contaminants in the aquatic environment as determined pollutants. These pollutants build up in the food chain and are responsible for adverse effects and death in aquatic organisms. Fish are largely being used for the assessment of the quality of the aquatic environment and as such can serve as bio-indicators of environmental pollution (Martin-Reina J *et al*., 2017). The changes in the enzymatic system may alter the metabolic processes. More recently changes in enzyme concentrations are being employed in the evaluation of toxicological responses. Toxicologists have developed an interest in studying the responses.

MATERIALS & METHODS

Collection and Maintenance:

The freshwater fishes were collected from the Bapatla region of Andhra Pradesh, India and brought to the laboratory for acclimation. The length and weight of the fish used for the experimentation were measured and were having an average length of 6 to 9 cm and a weight of about 10-14 gms each. The fishes were disinfected with 0.1% potassium permanganate solution. Fishes were cultured in a rectangular aquarium containing tap water. During the acclimation period, water was renewed daily and fish were fed once a day with artificial food pellets available from the local market. The fishes were acclimated for 15 days and the fishes were divided into 10 groups, each containing 10 fishes. These groups of fishes were placed separately in the plastic tubs containing 10 litres of well-aerated water. Feeding was stopped 24h before the exposure of fishes to the pesticides for acute toxicity tests.

BIOCHEMICAL STUDIES

After 5-day and 10-day exposure to the pesticide Thimet 10% (CG), by using LC50 and 1/10th and 1/20th values obtained through experimentation, the alive fishes, 5 from each group were used for dissection. Different organs such as gill, liver, kidney, brain, and muscle were removed, pooled, weighed (100 mg) and used for biochemical estimations.

Estimation of Carbohydrates:

Carbohydrates were estimated according to the method of Trevelyan WE and Harrison JS (1952). The freshly prepared anthrone reagent (5 mL) was pipetted into thick-walled pyrex tubes (150 x 25 mm) and chilled in ice water. The solution under test (1 mL) was layered on the acid, cooled for a further 5 min, and then thoroughly mixed while still immersed in ice water. The tubes were loosely fitted with corks, and heated by vigorously boiling. Then it was made up to 10 mL with water and optical density was determined in a spectrophotometer. The standard graph was plotted with D-glucose as a control. The values were expressed as mg/g wet weight of the tissue.

Estimation of Glycogen:

The total glycogen from fish tissues was estimated by the method of Kemp A and Van Heijingen AJ (1954). 5% homogenate of gill, liver, kidney, brain, and muscle tissues were prepared in 80% methanol and centrifuged at 3000 rpm for 10 minutes. The tissue residue was suspended in 5 ml of trichloroacetic acid (TCA), boiled for 15 minutes at 100°C, and then cooled in running water. The solution was made up to 5 mL with TCA to compensate for the evaporation and then centrifuged. From this, 2 mL of supernatant was taken into the test tube and 6 mL of concentrated H_2SO_4 was added and the mixture was boiled for 10 minutes. The mixture was cooled and the optical density was measured at 520 nm. The glucose was used as a standard. The glucose was converted to glycogen by the multiplication factor of 0.98 and is expressed as mg of glycogen/g wet weight of the tissue.

Estimation of total proteins:

The total protein content of pesticide-exposed fish and control fish was estimated using the Lowry OH *et al*., (1951). Follin phenol reagent method. For this purpose, 5% of the homogenate of tissue was isolated and precipitated with 5% trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 10 minutes. The precipitate was dissolved in 1 mL of 1 N NaOH solution and 0.2 mL of extract was taken into the test tube and mixed with 5 mL of alkaline copper solution (mixture of 2% sodium carbonate and 0.5% copper sulphate in a 50:1 ratio) was added. Then samples were allowed to stand for 10 min. then 0.5 mL of folin phenol reagent (diluted with double distilled water in a 1:1 ratio before use) was added. After 30 minutes, the optical density was measured at 540 nm using a spectrophotometer against the blank. The standard graph was plotted by the aforesaid method with bovine serum albumin (BSA) as standard. The values were expressed as mg/g wet weight of the tissue.

Estimation of free amino acids:

The free amino acid level was estimated by the Ninhydrin method as described by Stein WH, & Moore S (1954). To 1 mL of supernatant, 2.0 mL of ninhydrin reagent was added and the contents were boiled for 5 minutes using a water bath. They were cooled under tap water and the volume was made up to 10 mL with double distilled water.The optical density of the colour developed was measured using a

spectrophotometer at a wavelength of 570 nm against a reagent blank. The standard graph was plotted with Amino Acids standard using the aforesaid method. The values were expressed as mg/g wet weight of the tissue.

Estimation of total lipids:

The total lipid content was estimated by using Barnes H & Blackstock J (1973) method using the vanillin reagent. 50 mg of tissue was homogenized with 10 mL water in a warring blender in a chloroform: methanol (2:1) mixture. The homogenates were filtered through Whatman No. 1 filter paper and the residue was re-homogenized as before and then filtered. The non-lipid matter from the pooled filtrate was removed by shaking vigorously with 0.88% KCl. 1 mL of filtrate was taken in a test tube and evaporated under nitrogen and 1 mL of concentrated H2SO4 was added and boiled for 10 min. For estimation of total lipids, 0.2 mL of solution was taken and 2 mL of vanillin reagent was added. The developed colour was read in a spectrophotometer at 520 nm against a reagent blank. The standard graph was plotted by the above method with cholesterol powder. The experiments were repeated 3 times each for acute and chronic exposure and mean values were expressed as mg/100 mg of wet tissue. Per cent change in tissue glycogen, protein and lipid content over control has been calculated for the presentation of the data using the ANOVA statistical tool.

Estimation of Nucleic acids:

After completion of the acute exposure period of 5 day and 10 day, gill, liver, kidney and muscle tissues were quickly removed and weighed. The homogenate of tissue was prepared in 10 ml of ice-cold distilled water. This homogenate is further used to determine ribonucleic acid (RNA) and Deoxyribonucleic acid (DNA) by following the method of Results have been confirmed after repetitive experimentation of acute and chronic exposure at least 3 times. The values obtained were compared with control and per cent change has been calculated for presentations of the data. Values obtained were used for RNA/DNA ratio calculation (Volkin E & Cohn WE (1954).

Estimation of Enzymes:

After chronic 5 days and 10 days of exposure to pesticide Thimet, the live fishes were sacrificed (5 from each group) for enzyme study. Intestine and liver were quickly removed and weighed. The tissue was homogenized and tissue concentration was made to 10 mg/ml with 0.9 % ice-cold NaCl. The homogenate was centrifuged at 3000 rpm for 10 minutes. Aliquots of supernatant were used as the source for enzyme estimation.

Acid Phosphatase (ACP):

The activity of acid phosphatase (ACP) was determined by Bhawane Nikami GP *et* al., (2007). The tissues were homogenized with 10 mg/ml of 0.9 % chilled saline solution, the homogenate was centrifuged towas0 rpm for 10 minutes. For acid phosphatase, a citric acid buffer substrate of pH 4.8 was used; the assay was similar to alkaline phosphatase. In alkaline and acid phosphatases, specific activities of enzymes have been determined and expressed in terms of mg para nitrophenol liberated / 100 mg tissue/hr.

Lactate Dehydrogenase (LDH):

The Lactate Dehydrogenase (LDH) activity was estimated by the method of Srikanthan TN and Krishna MGR (1955). 2% homogenate of the tissue was prepared in 0.25 M ice-cold sucrose solution and centrifuged at 1000 rpm for 15 minutes. The supernatant served as the enzyme source. The reaction mixture of 2 mL comprises of 0.5 mL of lithium lactate, 0.5 mL of phosphate buffer, 0.2 mL of INT or [2-(4-iodophenyl)-3(4- nitrophenyl)-5-phenyl-2H-tetrazolium chloride] and 0.2 mL of NAD (Nicotinamide adenine dinucleotide) and 0.6 mL of supernatant. The reaction mixture was incubated at 37°C for 30 minutes and after the incubation, the reaction was stopped by adding 5 mL of acetic acid. Zero time controls were maintained by adding 5 mL of acetic acid prior to the addition of homogenate. The formazan dye was extracted in 5 mL of cold toluene. The intensity of colour developed was read at 495 nm against a reagent blank in a spectrophotometer. The activity was expressed as μ moles of formazan formed/mg protein/h.

Succinate Dehydrogenase (SDH):

The Succinate Dehydrogenase (SDH) activity was estimated by the method of Nachlas et al. (1960). 5% percent homogenate (w/v) of the tissues were prepared in cold 0.25M sucrose solution and centrifuged at 1000

rpm for 15 min. The supernatant acts as the enzyme source. The reaction mixture of 2 mL contains 0.6 mL of supernatant, 0.5 mL of phosphate buffer (pH 7.2), 0.5mL of sodium succinate, 0.2 mL of INT or [2-(4-iodophenyl)-3(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride] and 0.2 mL of distilled water. The reaction mixture was incubated at 37°C for 30 min and after incubation, the reaction was stopped by adding 5 mL of acetic acid. Zero time controls were maintained by adding 5 mL of acetic acid prior to the addition of homogenate. The formazan dye formed was extracted overnight in 5 mL of cold toluene. The intensity of colour developed was read at 495 nm against a reagent blank in a spectrophotometer. The activity was expressed as µ moles of formazan formed/mg protein/h.

Maltase Dehydrogenase (MDH):

The Maltase Dehydrogenase (MDH) activity was estimated by the method of Nachlas MM *et al*., (1960). 2% homogenate of different tissues was prepared in ice-cold 0.25M sucrose solution and centrifuged at 1000 rpm for 15 min. The supernatant was used as the enzyme source. The reaction mixture of 2 mL contains 0.6 mL of supernatant, 0.5 mL of phosphate buffer (pH7.2), 0.5 mL of malate, 0.5 mL of INT or [2- (4-iodophenyl)-3(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride] and 0.2 mL of NAD was added. The reaction mixture was incubated at 37°C for 30 min. Zero time controls were maintained by adding 5 mL of acetic acid prior to the addition of homogenate. The formazan dye formed was extracted overnight in 5 mL of cold toluene. The intensity of colour developed was read at 495 nm against a reagent blank in µ moles of formazan formed/ mg protein/h.

Aspartate aminotransferase (AST):

Aspartate aminotransferase activity was estimated by the method of Mohun AF and Cook IJY (1957). 10% homogenate of different tissues was prepared in 0.33M sucrose solution and centrifuged at 1000 rpm for 15 minutes. The supernatant obtained was used as the enzyme source. The reaction mixture of 1.5 mL contains 1 mL of phosphate buffer (pH 7.4), 0.1 mL of L-aspartate (L-Aspartic acid), and 0.1 mL of α-ketoglutaric acid and 0.3 mL of supernatant as enzyme source. The reaction mixture was incubated at 37°C for 30 minutes. After incubation, 0.3 mL of the enzyme was added to the control. Then 1 mL of 2, 4-dinitrophenyl hydrazine (DNPH) reagent was added and kept at room temperature for 20 minutes. The reaction was stopped by addition of 10 mL of 0.4 N NaOH, vortexed and kept at room temperature for 5 minutes. The colour developed was read at 540 nm in a spectrophotometer against a reagent blank. The AST activity was expressed as µmoles of pyruvate formed/mg protein/h.

Alanine aminotransferase (AAT):

Alanine aminotransferase activity was estimated by the method of Mohun AF and Cook IJY (1957). 10% homogenate of different tissues was prepared in 0.33 M sucrose solution and centrifuged at 1000 rpm for 15 minutes. The supernatant obtained was used as the enzyme source. The reaction mixture of 1.5 mL contains 1 mL phosphate buffer (pH 7.4), 0.1 mL of L-alanine, 0.1 mL of α-ketoglutarate and 0.3 mL of the supernatant as enzyme source. The contents were incubated at 37°C for 30 minutes. After incubation, 0.3mL of the enzyme was added to the control. Then 1 mL of 2, 4-DNPH reagent was added and kept at room temperature for 20 minutes. The reaction was stopped by the addition of 10 mL of 0.4 N NaOH, vortexed and kept at room temperature for 5 minutes. The colour developed was read at 540 nm in a spectrophotometer against a reagent blank. The AST activity was expressed as µ moles of pyruvate formed/mg protein/h.

RESULTS & DISCUSSION

In the present study, fish *Cyprinus carpio* was subjected to different concentrations of organo-phosphate pesticide Thimet 10% CG and evaluated different biochemical parameters. The calculated mean values and alterations in the levels of total Carbohydrates, Glycogen, Protein, Free Amino Acids (FAA), Lipids, DNA and RNA and % change over control in different tissues of fish *Cyprinus carpio* were reported in Tables 1 to 7. In most cases the liver is shown the highest depletion of biochemical contents. In the fish reared as a control for 5-days and 10-days, the total biochemical contents were highest in the liver followed by muscle, and brain, moderate values were observed in gill, and low in kidneys respectively. The total biochemical content significantly decreased ($p < 0.05$) during both

sublethal and lethal exposures of Thimet when compared to the control fish group.

All the tested tissues of the fish organs i.e., muscle, brain, gill, kidney and muscle have shown a rapid decline in glycogen content during 10-day sublethal exposure of Thimet when compared to 5-day and 10-day sublethal exposure. Thus, a fall in glycogen levels shows its rapid consumption to meet the enhanced energy demand in pesticide-exposed animals through glycolysis or hexose monophosphate pathway (Aziz H & Mohiuddin SS 2022). These reductions in glycogen content might also be due to the prevalence of hypoxic or inhibition of the enzyme glycogen synthases. Pesticides are known to act on the endocrine system, which contributes to glycogen synthesis. Reduced glycogen synthesis is also attributed to the inhibition of the enzyme glycogen synthases, which mediates glycogen synthesis (Anita S *et al*., 2010). Though brain tissue is metabolically active, lower glycogen content was observed, since it lacks the inherent potential to store glycogen and is dependent on blood glucose for all its metabolic activities. The results of glycogen depletion are reported in table-1.

Table-1: Alterations in the levels of total Glycogen, (mg/g wet weight of the tissue) and % change over control in different tissues of fish *Cyprinus carpio* treated with 5 Day and 10 Day sublethal doses of Thimet.

Tissues	5 Days exposure			10 Days exposure		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% Change
Gill	34.1±0.03	28.1±0.03	-17.5	31.2±0.03	25.4±0.02	18.5
Liver	51.5±0.03	42.1±0.03	-18.25	56.2±0.03	40.19±0.03	28.48
Kidney	38.1±0.03	32.2±0.03	15.48	36.5±0.03	29.30±0.03	19.72
Brain	42.1±0.02	33.3±0.03	20.90	39.7±0.03	27.85±0.03	29.77
Muscle	45.8±0.03	34.7±0.03	28.89	42.3±0.02	28.5±0.03	32.6

The carbohydrates are the major source of energy in the cells and play an essential role in the cellular metabolism by acting as fuel and providing energy to the body cells. In the present study, maximum carbohydrate depletion was observed in liver and muscle during lethal and sublethal concentrations. The depletion of carbohydrate content suggests possible utilization of carbohydrates to meet the energy demands during pesticide stress (Tilak KS and Swarna Kumari R 2009). In vertebrates, generally from fishes to mammals; blood glucose level corresponds to the standard metabolic rate (Umminger BL 1977). The change in carbohydrate content supports that the carbohydrate metabolism is affected by the toxicant. The results of carbohydrates estimation were represented in table-2.

Table: 2. Alterations in the levels of total Carbohydrates (mg/g wet weight of the tissue) and % change over control in different tissues of fish *Cyprinus carpio* treated with 5 Day and 10 Day sublethal doses of Thimet.

Tissues	5 Days exposure			10 Days exposure		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% change
Gill	16.23±0.01	14.26±0.01	12.13	15.14±0.03	3.43±0.02	77.34
Liver	15.46±0.01	13.56±0.01	12.28	16.54±0.02	14.15±0.02	14.44
Kidney	14.54±0.02	12.25±0.02	15.74	14.44±0.02	12.54±0.02	13.15
Brain	14.75±0.01	13.15±0.02	10.847	12.45±0.02	10.44±0.02	16.144
Muscle	16.85±0.02	13.85±0.02	17.80	11.17±0.02	19.84±0.01	-77.61

In the present investigation, protein content decrement was more apparent in sublethal concentrations than in lethal concentrations. It may be due to the detoxification of enzymes or mechanism, which is apparently slow. Several other investigations also revealed a gradual decrease in protein profiles was observed with organophosphate compounds (Magar RS and Afsar Shaikh 2012). The results of total protein estimation were represented as table-3.

Table: 3. Alterations in the levels of total Protein (mg/g wet weight of the tissue) and % change over control in different tissues of fish *Cyprinus carpio* treated with 5 Day and 10 Day sublethal doses of Thimet.

Tissues	5 Days exposure			10 Days exposure		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% Change
Gill	78.543±0.02	66.43±0.01	15.422	69.26±0.02	53.24±0.03	23.13
Liver	92.54±0.01	78.45±0.01	15.22	83.64±0.02	65.7±0.45	21.3
Kidney	74.66±0.01	62.34±0.02	16.50	72.14±0.02	56.43±0.02	22.10
Brain	65.25±0.01	57.32±0.01	12.1	63.25±0.02	51.26±0.02	18.95
Muscle	62.24±0.03	53.19±3.00	14.54	55.62±0.01	44.08±0.05	20.74

In the present study, the depletion in FAA in all tissues of *Cyprinus carpio* at sublethal and lethal concentrations may be due to their utilization for the production of new proteins or for the production of energy to survive with the prevailing toxic conditions due to intoxicant induced stress. The results were represented in table-4.

Table: 4. Alterations in the levels of total Free Amino Acids (FAA) (mg/g wet weight of the tissue) and % change over control in different tissues of fish *Cyprinus carpio* treated with 5 Day and 10 Day sublethal doses of Thimet.

Tissues	5 Days exposure			10 Days exposure		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% Change
Gill	10.50±0.08	9.11±0.02	10.47	7.85±0.009	5.7±0.04	27.3
Liver	14.20±0.08	12.13±0.02	14.57	9.19±0.04	8.07±0.03	12.18
Kidney	12.26±0.03	10.19±0.01	16.93	10.15±0.03	7.36±0.04	27.48
Brain	12.36±0.04	10.34±0.01	12.29	7.22±0.04	5.21±0.04	27.83
Muscle	11.37±0.02	9.09±0.04	19.47	9.50±0.13	7.91±0.04	16.73

The total lipids values of *Cyprinus carpio* for the sublethal 5 days and sublethal 10 days exposure periods are given in Table-5. Total lipids decreased in the blood of *Cyprinus carpio* under the toxicity of Thimet.

Table: 5. Alterations in the levels of total Lipids (mg/g wet weight of the tissue) and % change over control in different tissues of fish *Cyprinus carpio* treated with 5 Day and 10 Day sublethal doses of Thimet.

Tissues	5 Days exposure			10 Days exposure		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% change
Gill	69.5±0.04	38.1±0.04	45.1	58.1±0.06	30.1±0.06	48.1
Liver	87.1±0.05	49.6±0.05	43.0	77.5±0.04	32.4±0.11	58.1
Kidney	62.2±0.05	41.1±0.05	33.9	49.6±0.06	38.1±0.06	23.1
Brain	55.2±0.04	40.2±0.06	27.1	46.2±0.06	25.7±0.05	44.3
Muscle	38.3±0.03	25.4±0.06	33.6	32.1±0.05	18.49±0.04	42.3

DNA and RNA % change over control in different tissues of fish *Cyprinus carpio* treated with 5 days and 10 days sublethal doses of Thimet along with standard deviations values and percent changes over control are represented in Table-6 and 7. The decrease in DNA and RNA content when compared to control groups may be due to inhibitory action of Thimet on DNA synthesis machinery or increased degradation.

Table: 6. Alterations in the levels of total DNA (mg/g wet weight of the tissue) and % change over control in different tissues of fish *Cyprinus carpio* treated with 5 Day and 10 Day sublethal doses of Thimet.

Tissues	5 Days exposure			10 Days exposure		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% change
Gill	10.1±0.05	8.4±0.07	16.8	9.1±0.04	5.5±0.06	28.5
Liver	13.1±0.04	11.8±0.06	9.9	11.8±338.7	8.1±0.05	31.3
Kidney	16.4±0.06	13.2±0.06	19.5	12.5±0.46	10.29±0.06	17.68
Brain	11.5±0.06	9.2±0.05	20	11.1±0.05	8.8±0.06	20.7
Muscle	9.1±0.05	8.2±236.7	9.8	8.1±0.06	6.5±0.06	19.7

Table: 7. Alterations in the levels of total RNA (mg/g wet weight of the tissue) and % change over control in different tissues of fish *Cyprinus carpio* treated with 5-Day and 10-Day sublethal doses of Thimet.

Tissues	5 Days exposure			10 Days exposure		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% change
Gill	13.1±0.05	11.84±0.05	9.6	10.4±0.05	9.1±0.03	12.5
Liver	20.2±0.03	18.4±0.05	8.9	16.1±0.04	14.2±0.05	11.8
Kidney	15.5±0.05	13.8±0.05	10.9	14.5±0.06	12.1±0.05	16.55
Brain	16.1±0.05	15.1±0.06	6.2	15.1±0.05	13.2±0.04	12.5
Muscle	11.1±0.05	9.7±0.05	12.6	8.1±0.08	6.7±0.34	17.28

The calculated mean values of the enzymes ACP, LDH, SDH, MDH, AST, and AAT along with standard deviations values and percent changes over control are represented in Tables. 8 to 13. In this study, the fish was reared as control and exposed for 5-days, and 10-days for enzyme alternations. In most cases, the liver is shown the highest depletion of enzyme content in non-neurological enzymes. The LDH, SDH, MDH, AST, and AAT contents significantly increase or decrease ($p<0.05$) sub-lethal and lethal exposures of Thimet 10% GC when compare to the control fish groups.

On exposure to Thimet, liver, kidney, brain, and gill showed highly elevated LDH activity levels during 10-day sublethal followed by 5-day sublethal periods. Increased LDH activity suggests a significant increase in the conversion of pyruvate to lactic acid, thereby leading to the accumulation of lactic acid. There was a remarkable increase in LDH activity in the liver and kidney during 10-day sublethal exposure to Thimet. The increase in LDH level indicated metabolic changes i.e., the glycogen catabolism and glucose shift towards the formation of lactate occurred in stressed fish (Suneetha, 2012). The results of LDH alterations after exposure were represented in table-8.

Table: 8. Alterations in the specific activity of LDH (µ moles of formazan/ mg protein/hr) and % change over control in different tissues of fish *Cyprinus carpio* treated with 5-day and 10-day sublethal doses of Thimet.

Tissues	5 Days exposure			10 Days exposure		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% Change
Gill	0.70±0.02	0.81±0.01	15.71	0.72±0.02	0.63±0.02	12.5
Liver	0.85±0.02	0.94±0.02	10.5	0.70±0.01	0.83±0.01	18.5
Kidney	0.81±0.01	1.03±0.01	27.01	0.68±0.01	0.79±0.01	16.1
Brain	0.59±0.10	0.74±0.01	25.4	0.45±0.02	0.54±0.01	20.0

Muscle	0.50±0.01	0.63±0.02	26.0	0.32±0.01	0.41±0.01	28.1

Table: 9. Alterations in the specific activity of SDH (µ moles of formazan/ mg protein/hr) and % change over control in different tissues of fish *Cyprinus carpio* treated with 5-day and 10-day sublethal doses of Thimet.

Tissues	5 Days exposure			10 Days exposure		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% change
Gill	0.48±0.01	0.40±0.01	-16.6	0.42±0.02	0.26±0.02	38.0
Liver	1.16±0.02	0.71±0.01	38.7	0.82±0.01	0.52±0.01	36.5
Kidney	1.10±0.01	0.80±0.01	27.2	0.75±0.03	0.59±0.01	29.1
Brain	0.51±0.01	0.43±0.01	15.6	0.48±0.01	0.34±0.02	21.3
Muscle	0.71±0.02	0.52±0.09	26.7	0.61±0.02	0.50±0.01	18.0

Table: 10. Alterations in the specific activity of MDH (µ moles of formazan/ mg protein/hr) and % change over control in different tissues of fish *Cyprinus carpio* treated with 5-day and 10-day sub-lethal doses of Thimet.

Tissues	5 Days exposure			10 Days exposure		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% change
Gill	0.73±0.02	0.59±0.01	19.1	0.62±0.01	0.53±0.01	14.5
Liver	1.11±0.01	0.80±0.01	6.27	0.84±0.02	0.73±0.02	13.0
Kidney	0.81±0.02	0.70±0.01	13.5	0.69±0.01	0.53 0.02	23.1
Brain	0.69±0.01	0.61±0.02	11.5	0.61±0.01	0.43±0.02	29.5
Muscle	0.78±0.01	0.64±0.03	17.9	0.70±0.01	0.61±0.01	12.8

The ACP is a hydrolytic lysosomal enzyme released by lysosomes for the hydrolysis of foreign materials and increases in its activity are probably related to cellular damage. Increased ACP enzyme activity at all the concentrations of Thimet might be due to an increase in protease activity which caused damage to the lysosomal membrane, thus permitting the leakage of lysosomal enzyme into the cytoplasm. Changes in the enzyme activity are due to the adverse effect of xenobiotics on the cell and its organelles. In the present study, the mean value of ACP activity in the brain, muscle and gill of *Cyprinus carpio* increased during the long time of exposure. This increased phosphatase activity was due to the cellular damage caused by hepatotoxins or a response to overcome the toxicity of Thimet.

Table: 11. Alterations in the specific activity of ACP (µ moles of formazan/ mg protein/hr) and % change over control in different tissues of fish *Cyprinus carpio* treated with 5-day and 10-day sublethal doses of Thimet.

Tissues	5 Days exposure			10 Days exposure		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% change
Gill	3.8±0.05	4.05±0.02	-6.578	3.67±0.03	3.53±0.03	-3.79
Liver	4.06±0.03	4.51±0.02	-11.083	4.8±0.13	4.45±0.08	7.29
Kidney	5.38±0.05	5.8±0.02	-7.8	5.08±0.02	5.37±0.04	-5.7
Brain	4.07±0.02	4.64±0.03	-0.58	3.86±0.03	4.16±0.02	-7.7
Muscle	3.07±0.03	3.7±0.03	-20.52	2.84±0.03	3.18±0.05	-11.97

In the present study, the assessment of AST and ALAT enzymes are the best indicators of organophosphate pollution. Variations in AST and ALAT enzyme activity in fish have been used frequently as potential stress biomarker in the contamination of aquatic ecosystems. The results of AST and ALAT were represented tables 12 and 13 respectively.

Table: 12. Alterations in the specific activity of ALAT (µ moles of formazan/ mg protein/hr) and % change over control in different tissues of fish *Cyprinus carpio* treated with 5-day and 10-day sub-lethal doses of Thimet.

Tissues	5 Days exposure			10 Days exposure		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% change
Gill	3.8±0.03	4.0±0.03	5.2	3.7±0.01	3.8±0.03	2.7
Liver	4.0±0.02	4.5±0.02	12.5	4.0±0.02	4.5±0.02	12.5
Kidney	5.4±0.02	5.8±0.03	7.4	5.0±0.02	5.4±0.02	8.0
Brain	4.0±0.02	4.6±0.03	15.0	4.0±0.02	4.1±0.03	2.5
Muscle	3.0±0.03	3.7±0.02	23.3	2.8±0.03	3.2±0.02	14.2

Table: 13. Alterations in the specific activity of AST (µ moles of formazan/ mg protein/hr) and % change over control in different tissues of fish *Cyprinus carpio* treated with 5 days and 10-day sub-lethal doses of Thimet.

Tissues	5 Days exposure			10 Days exposure		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% change
Gill	1.9±0.02	1.4±0.03	25.7	1.71±0.02	1.31±0.01	23.3
Liver	2.6±0.02	2.0±0.03	23.0	2.5±0.02	1.64±0.02	34.4
Kidney	1.9±0.04	1.4±0.03	26.3	1.7±0.03	1.41±0.01	17.0
Brain	2.4±0.03	1.9±0.03	20.8	2.2±0.02	1.83±0.02	16.8
Muscle	2.5±0.03	1.7±0.02	32.0	2.3±0.02	1.68±0.02	26.9

CONCLUSIONS

In the present study, we have contemplated to evaluate the biochemical and enzymatic alteration in fresh water fish *Cyprinus carpio* which is more sensitive to the toxicity of Thimet when compared to control group fish. Accordingly, there is an increasing need to minimize the adverse impacts of these pesticides on the environmental quality by the controlled application of such hazardous chemicals.

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