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Endosulfan mediated some physiological and biochemical changes in a fresh water fish

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ABSTRACT

In the present study, an attempt has been made to analyze the toxicity of the endosufan on fresh water fish *Labeo rohita* (Rohu). It has been found that the acid phosphomonoesterase activity was decreased by 23.78% in liver, 17.93% in muscles, 16.97% in kidney and 44.31% in gills of *Labeo rohita* at 0.25 ppm of Endosulfan after an exposure of 15 days. The toxicity of a pesticide could vary from species to species. The alkaline phosphomonoesterase activity in Labeo was reduced by 26.15% in liver, 23.24% in muscles, 2.25% in kidney and 2.18% in gills. Exposure of endosulfan caused significant change by descreasing the lipase activity of Labeo fish. The maximum reduction was 57.74% in muscle and 52.28% in liver. The minimum change in lipase activity was 3.74% and 5.65% in gills and kidney respectively. Choline esterase activity was reduced by 35.66% in liver and 38.60% in muscles and the maximum decrease 52.54% in gills and 41.21% in kidney. Endosulfan caused 30.79%, 55.59%, 41.21% and 52.54% decrease in the activity of Trypsin in liver, muscle, kidney and gills respectively. It caused significant changes in glycogen content in liver, muscle, kidney and gills of the fish. The maximum reduction of glycogen was was noted (43.53%) in liver and minimum (26.13%) in gills. Protein concentration was reduced by 22.89% in liver, 31.81% in muscles, 43.94% in kidney and 26.22% in gills. The maximum decrease in total lipids founds were 3.095% in liver, 2.73% in muscles, 0.21% in kidney and 2.64% in gills. Therefore it can be said that these are the biochemical changes in fish when exposed to the sublethal concentration of the pesticide and it also showed irritation, very fast movements of operculum, violent action of pelvic fins and spreading of the fins.

Key words: Endosufan, p Biochemical changes, *Labeo rohita*

INTRODUCTION

The use of pesticides has increased with the growing awareness about their utility in increasing agricultural production and protecting live stock and animal husbandry. In India, large number of pesticide substances derived from the plant origin have been used during last two centuries for protection

of the crop and their products. However the extensive use of pesticides has caused a lot of pollution and threatened the health of nontarget terrestrial and aquatic animals by disturbing the ecosystem. The mobilization of these pesticides on land and in water brings out lethal and sublethal effects on nontarget animals. Temporal variation due to over contamination with cyacnophyceae, chlorophyceae, bacillariophyceae and euglenophyceae in Madhav sagar dam, Sikar was notified by Brij Mohan Singh, (2020). Physicochemical parameters were not found in the suitable range in Jaisamand Lake, Alwar by Singh *et al.,* (2014).

Many groups of pesticides such as insecticides, rodenticides, herbicides, fungicides and nematicides are being used frequently. The pesticides used for various purposes reach aquatic environment either directly or indirectly through run off from agricultural fields, spray drifts, rain water, sewage and effluents from industries. The persistence of these pesticides in aquatic environment is more dangerous for the survival of the fish because the fish can easily absorb the pesticides from water through gills and skin. Effect of toxicity caused by pesticides is not limited to single perspective of fish but usually diversified entirely to all metabolic, structural and various stages of their life cycle. Therefore, pesticides of plant extracts are more safer and gaining more popularity than synthetic ones.

Synthetic pyrethroids constitute the newest major class of insecticides used in agriculture, domestic and veterinary applications. These include a series of more than 20 compunds like endosulfan, cypermethrin, λ-Cyhalothrin, fenvalerate, Deltamethrin etc are being sold in the market with different trade name such as Ambush, Pounce, Ectibon, Kafil, Torpedo, Bestox, Acquit, Apex, Metador, Charge etc. These compounds are introduced in the market in 1970s and now account for more than 30% of insecticides being used worldwide. The synthetic pyrethroids are based upon pyrethrins, which are derived from the flowers of *chrysanthemum cinerariaefolium, Chrysanthemum cocconeum* and *Chrysanthemum roseum.* Endosulfan ($C_9H_6Cl_6O_3S$) is an off-patent organochlorine pesticide which has two isomers exosulfan and endosulfan are known popularly as I and II in the market.

Exposure of insecticide influences some important physiological functions of fish. It greatly affects fish production and human health too through ecological cycling and biological magnification. Accumulated insecticide acts as a pro-oxidant and enhances oxyradicals generation by reacting with oxygen. Fishery is emerging as a single largest industry in our country employing about 10 million people. Most importantly it is a source of livelihood for a large section of economically backward people of the world. Freshwater fishes are facing a major challenge due to indiscriminate use of different types of pesticides in agriculture. Annual pesticide consumption has increased by 20 to 25 folds during over three decades in India. They are used to control a wide range of insect pest.

Fish provide high calorie diet to a large number of people all over the world but indiscriminate use of these pesticides has reduced the fish growth. Synthetic pyrethroid may decrease nutritive value, as well as these compounds may also reach to human body through fish flesh and will cause toxicity to human being also. Keeping in view the seriousness of this problem, present study is designed to investigate the physiological and biochemical effects of endosulphan on fresh water fish *Labeo rohita* (Rohu).

MATERIAL AND METHODS

In present investigation fishes were identified through morphological characters (Talwar and Jhingran, 1991) and collected from local water body (Koat dam, Sikar) and acclimatized in laboratory conditions for a time period of 15 days. After acclimatization fishes were divided in to control and experimental groups and transferred to different aquaria having 25 litres of water in each aquarium.

Pesticide exposure and experimental design

Endosulfan pesticide of different concentrations were added in to water of aquaria. To find out different desired concentrations lethal, sublethal and acute concentrations of these compounds for experimental fishes LC_0, LC_{50} and LC_{100} were determined by conducting a series of experiments up to a maximum period of 30 days. The sublethal concentration is used to study the effect of synthetic pyrethroids on the fishes. Fish is exposed to sublethal concentration of endosufan insecticide (0.024 ppm) for a defined period (15 days). Fresh concentration of endosufan is prepared by changing water on alternate day in order to avoid toxicity of excretory products. Dead fish were removed periodically from the container, if found. The

control group of fishes was maintained in tap water without pesticides. To study the effect of these pesticides, liver, muscles, kidney and gills were collected from the body of sacrificed and exposed fishe. These body parts were washed, weighed and homogenized in homogenizer using 10 ml distilled water. The homogenate thus obtained were spun at 10,000g at 4˚C for 20 minutes in centrifuge. The supernatant if necessary, were stored at 0˚C up to a maximum 5 days. Fresh homogenate were prepared at 5 days intervals and following methodologies were applied for the related activities.

Methodologies

1. Various physicochemical features of water were estimated as per standard method of APHA (2005) and Trivedy and Goel (1986).
2. Acid phosphomonoesterase activity was determined by the method of Mather and Di Giulio (1991).
3. Alkaline phosphomonoesterase activity by the enzymatic method of Luck, H. (1974).
4. Lipase activity was measured by Malin *et al*.(1990).
5. Choline esterase activity was estimated by the method of Nishimoto *et al.*(1991).
6. Trypsin and lipase enzymes were done according to the method of Somero and Childress (1980) and Aknes and Njaa (1981), respectively.
7. Parihar and Duvey (1995) methods for estimation of the glycogen were used.
8. The protein content of the sample was determined according to the method of Lowry *at el.* (1951) using crystalline bovine serum albumin standard (**Figure**-9).
9. The total lipids were extracted based on the procedure of Di Giulio *et al.* (1989).
10. Statistical Analysis**:** Various statistical analyses like mean deviation, standard error, t-test, variance (ANOVA) and DMRT etc. are performed with help of a computer in order to ascertain the level of significance.

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RESULTS AND DISCUSSION

The effects of pesticide on all four tissues (liver, muscles, kidney and gills) of Labeo fish were compared with untreated control fishes to observe the activities of acid phosphomonoesterase, alkaline phosphomonoesterase, lipase, choline esterase, trypsin enzyme, glycogen, protein and total lipids as shown in the tables 4 to 11 and comparative variations showed in **Figure**s 1 to 8.

As shown in table-4 and **Figure**-1, the acid phosphomonoesterase activity was decreased by 23.78% in liver, 17.93% in muscles, 16.97% in kidney and 44.31% in gills of *Labeo rohita* at 0.25 ppm of endosulfan after an exposure of 15 days. The acute toxicity of cypermethrin and λ Cyhalothrin to *Channa punctatus* has been reported by Kumar *et al*. (2007).

The alkaline phosphomonoesterase activity in Labeo was reduced by 26.15% in liver, 23.24% in muscles, 2.25% in kidney and 2.18% in gills as in table-5, Figure-2. Acute toxicity of Chlorantraniliprole to fresh water fish (*Channa punctatus*) affects physiology and pathological changes and their bioconcentration and bioaccumulation in fish tissue (Nagaraju and Venkata, 2013).

Table 1: Physicochemical analysis of water as per standard methods of APHA (2005) and Trivedy and Goyal (1986)

Physicochemical parameters	Amount figure
Turbidity (NTU)	8
Electrical conductivity (in mho)	816 micro mho/cm
pH	8.1
Temperature ˚C	28 ± 3
Total hardness (mg/l)	320
Calcium hardness (mg/l)	80
Mangnecium hardness (mg/l)	40
Nitrate (mg/l)	1.12
Sulphate (mg/l)	Trace
Chloride (mg/l)	40
Fluoride (mg/l)	0.8
Dissolved oxygen (ppm)	8

Table 2: Fish showed the following behavioural changes after exposure.

S.N.	Behavioural change
1	Irritation
2	Fast opercular movement
3	Violent actions of pelvic fin and spreading of the fins
4	Loss of equilibrium
5	Mucous covering

Table 3: Summary of water toxicity test result with Endosulfan. (Normal laboratory conditions)

SN	Endosulfan Conc. in ppm in water	% survival and mortality							
		24 Hrs		48 Hrs		72 Hrs		96 Hrs	
		S	M	S	M	S	M	S	M
1	0.0029	100	00	100	00	100	00	100	00
2	0.0031	100	00	100	00	100	00	100	00
3	0.0033	100	00	100	00	60	40	80	20
4	0.0035	80	20	60	40	60	40	40	60
5	0.0052	40	60	40	60	40	60	20	80
6	0.0070	80	20	40	60	40	60	60	40
7	0.0087	60	40	60	40	20	80	20	80
8	0.0100	00	100	00	100	00	100	00	100

LC_{50} 24 Hours = 0.0049ppm S = Survival
LC_{50} 48 Hours = 0.0042ppm M = Mortality
LC_{50} 72 Hours = 0.0038ppm
LC_{50} 96 Hours = 0.0035ppm

Biochemical changes:

Biochemical change…. in table 4 to 11 and figure 1 to 8

Table 4. Acid phosphormonoesterase activity of control group and 0.025ppm of pesticide exposed fish

Tissues	control group 15 days (µg/g)	Sublethal 15 days exposure group	Difference (%)
Liver	45.77 ± 2.42	34.89 ± 2.42	10.88 -(23.78%)
Muscle	39.62 ± 2.56	32.52 ± 2.31	07.10 -(17.93%)
Kidney	6.54 ± 1.79	5.43 ± 2.56	1.11 -(16.97%)
Gills	4.22 ± 0.56	2.35 ± 2.56	1.87 -(44.31%)

Data represents means ± SD of six individual values; significant at p≤ 0.05

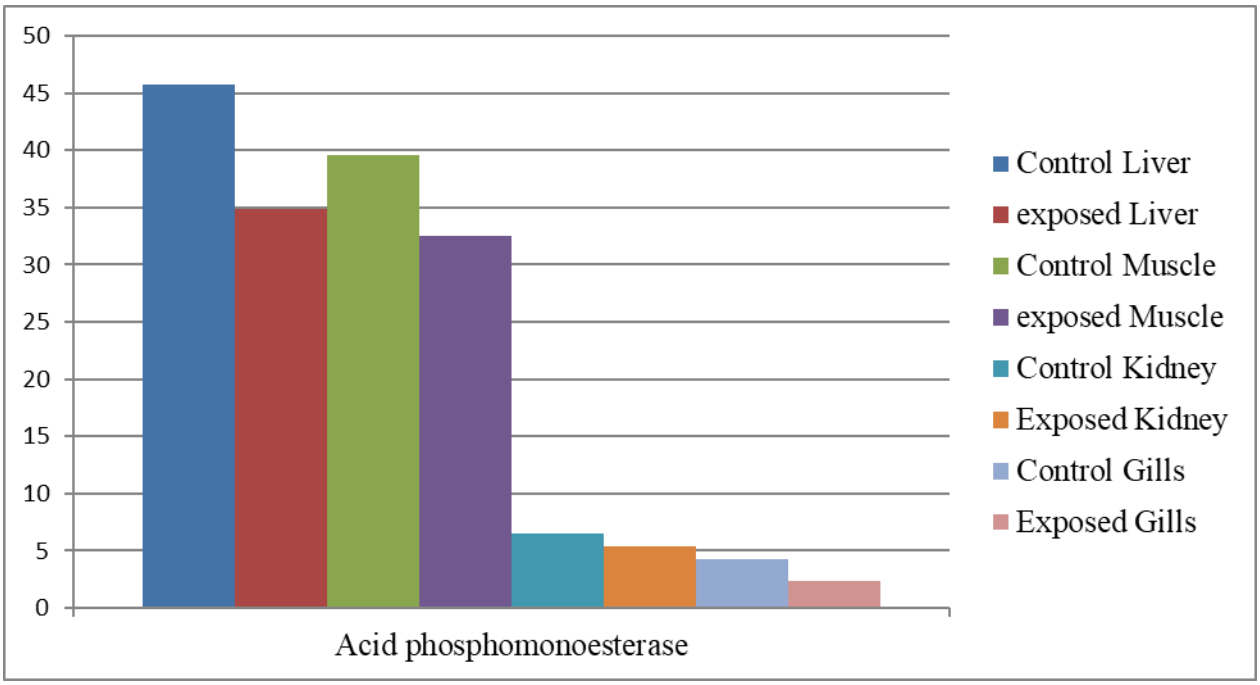

Figure 1: Shows Acid phosphomonoesterase activity of control group and 0.025ppm of pesticide exposed fish

Table 5. Alkaline phosphomonoesterase activity of control group and 0.025ppm of pesticide exposed fish

Tissues	Control group 15 days (µg/g)	Sublethal 15 days exposure group	Difference
Liver	78.66 ± 4.03	58.09 ± 4.03	-20.56 (26.15%)
Muscle	74.08 ± 4.21	56.87 ± 4.02	-17.21 (23.24%)
Kidney	23.27 ± 2.22	18.04 ± 2.12	-05.23 (2.25%)
Gills	18.10 ± 1.80	14.16 ± 1.70	-03.94 (2.18%)

Data represents means ± SD of six individual values; significant at p≤ 0.05

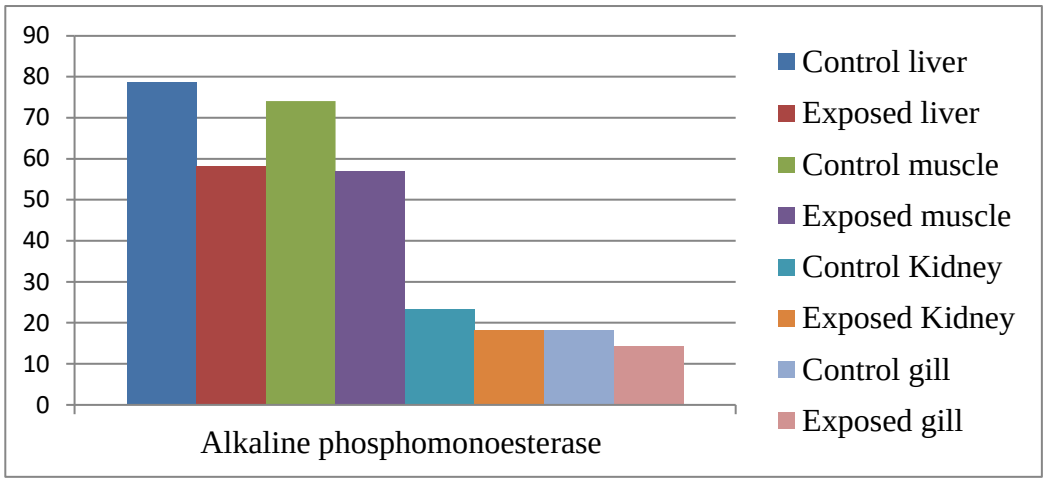

Figure 2: Shows alkaline phosphomonoesterase activity in muscle, liver, kidney and gills of Labeo fish.

Table 6. Lipase activity in liver, muscle, kidney and gills of control and treated fish.

Tissues	control group 15 days (µg/g)	Sublethal 15 days exposure group	Difference (%)
Liver	435.79 ± 35.61	413.01 ± 35.26	-22.78 (52.28%)
Muscle	361.07 ± 32.93	340.23 ± 32.44	-20.84 (57.44%)
Kidney	17.30 ± 2.001	16.40 ± 2.011	-0.90 (5.65%)
Gills	09.77 ± 3.43	9.405 ± 1.30	-0.365 (03.74%)

Data represents means ± SD of six individual values; significant at p≤ 0.05

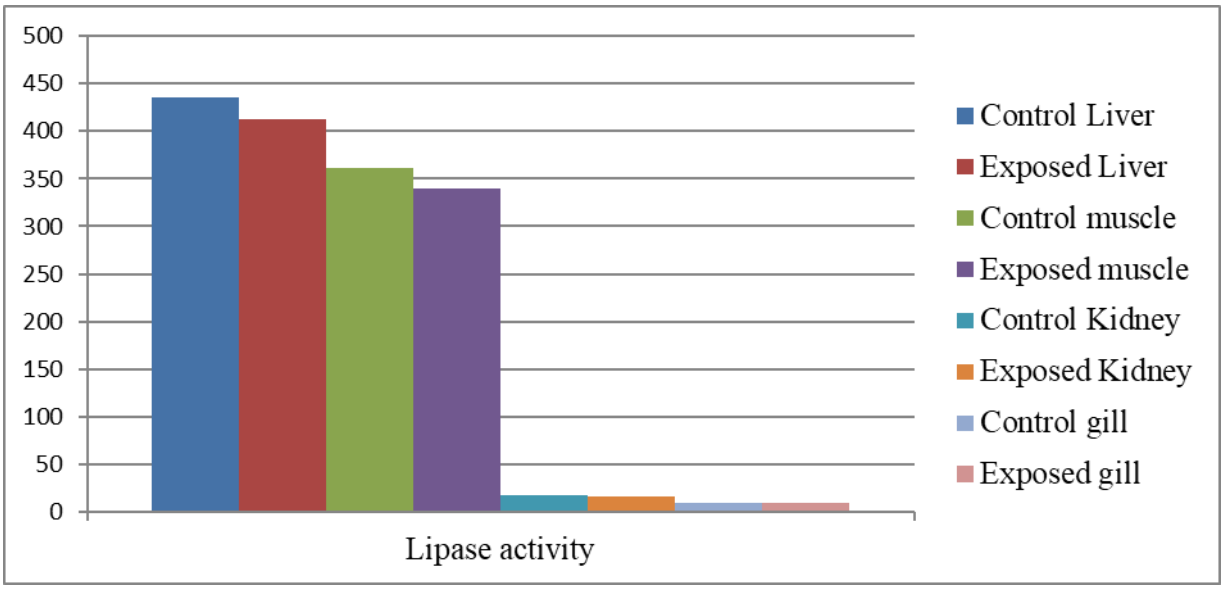

Figure 3: Shows Lipase activity in mucle, liver, kidney and gills of Labeo fish.

Table 7. Choline esterase activity in liver, muscle, kidney and gills of control and treated fish.

Tissues	control group 15 days (µg/g)	Sublethal 15 days exposure group	Difference (%)
Liver	12.35 ± 2.17	7.94 ± 2.77	-4.404 (35.66%)
Muscle	18.72 ± 3.02	13.51 ± 3.00	-7.22 (38.60%)
Kidney	1.34 ± 0.17	0.661 ± 0.12	-0.679 (50.70%)
Gills	1.78 ± 0.13	0.89 ± 0.12	-0.895 (50.3%)

Data represents means ± SD of six individual values; significant at p≤ 0.05

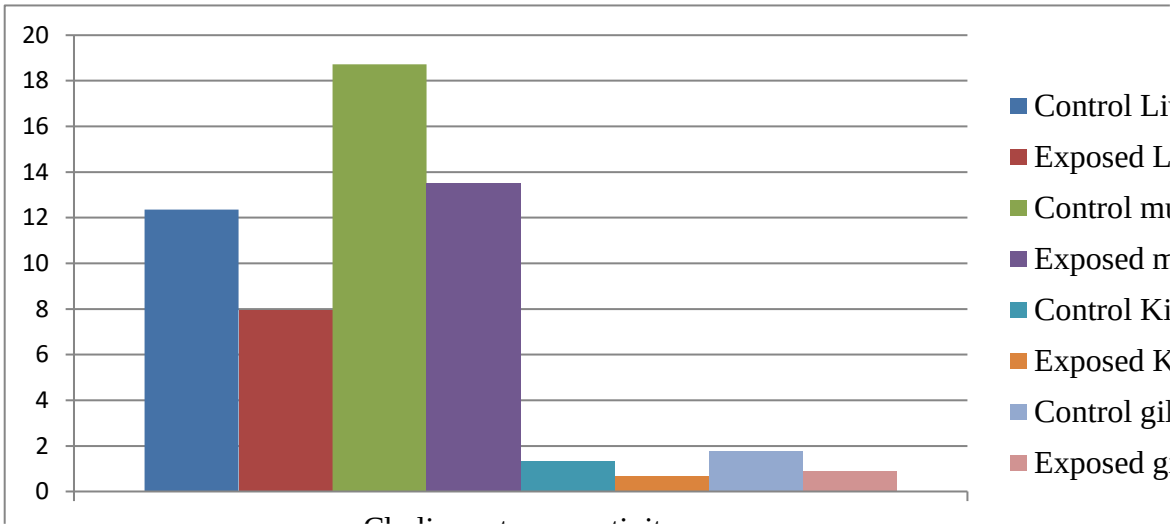

Figure 4: Shows Cholin esterase activity in muscle, liver, kidney and gills of Labeo fish.

Table 8. Trypsin activity in liver, muscle, kidney and gills of control and treated fish.

Tissues	control group 15 days (µg/g)	Sublethal 15 days exposure group	Difference (%)
Liver	2.49 ± 0.79	1.725 ± 0.75	-0.765 (30.79%)
Muscle	2.36 ± 0.81	1.049 ± 0.83	-1.311 (55.59%)
Kidney	0.32 ± 0.03	0.19 ± 0.03	-0.131 (41.21%)
Gills	0.27 ± 0.01	0.129 ± 0.01	-0.141 (52.54%)

Data represents means ± SD of six individual values; significant at p≤ 0.05

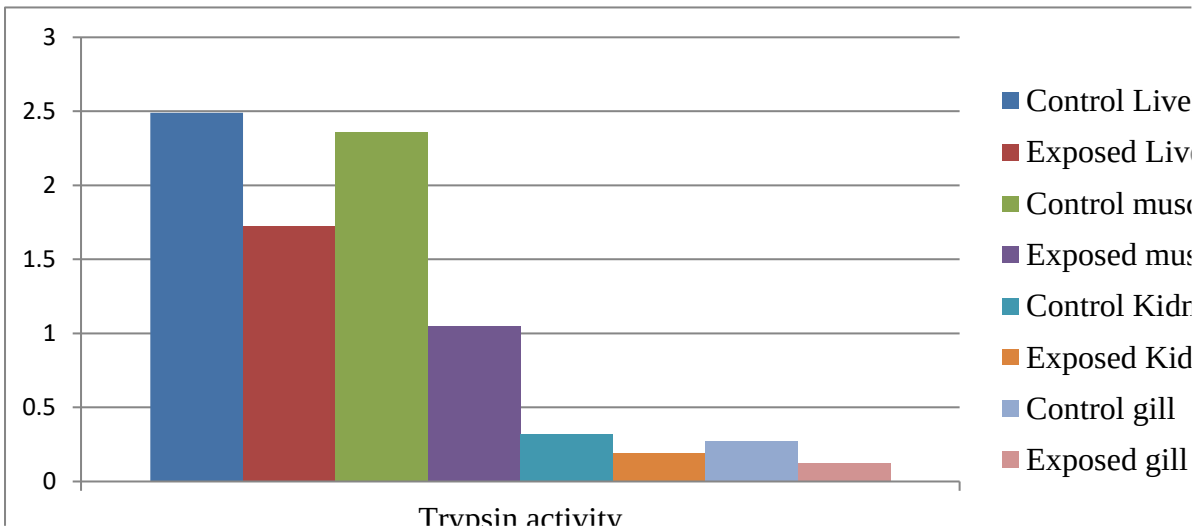

Figure 5: Shows Trypsin activity in muscle, liver, kidney and gills of Labeo fish.

Table 9. Glycogen activity in liver, muscle, kidney and gills of control and treated fish.

Tissues	control group 15 days (µg/g)	Sublethal 15 days exposure group	Difference (%)
Liver	1800 ± 150	1016.46 ± 153	-783.54 (43.53%)
Muscle	1670 ± 120	1054.44 ± 122	-615.56 (36.86%)
Kidney	370 ± 80	208.94 ± 78	-161.06 (43.53%)
Gills	450 ± 82	333.0 ± 81	-117.00 (26.13%)

Data represents means ± SD of six individual values; significant at p≤ 0.05

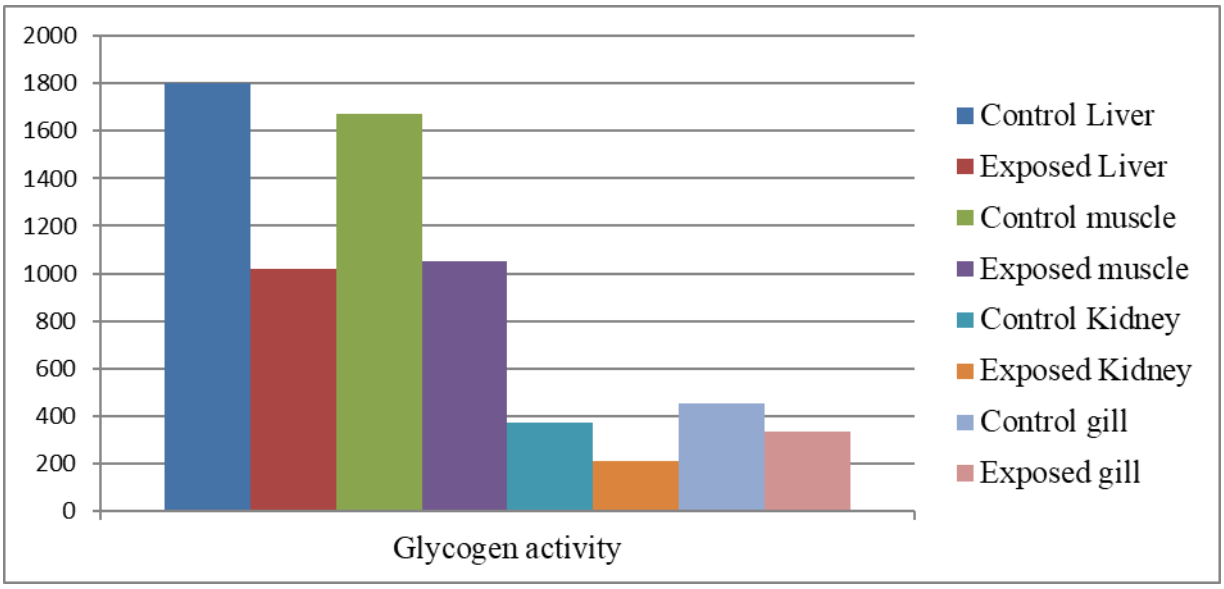

Figure 6: Shows Glycogen activity in muscle, liver, kidney and gills of Labeo fish.

Table 10. Protein concentration in liver, muscle, kidney and gills of control and treated fish.

Tissues	control group 15 days (µg/g)	Sublethal 15 days exposure group	Difference (%)
Liver	1500 ± 156	1156.65 ± 145	-343.35 (22.89%)
Muscle	1800 ± 132	1227.42 ± 127	-572.58 (31.81%)
Kidney	100 ± 5	56.06 ± 4.3	-43.94 (43.94%)
Gills	360 ± 13	265.61 ± 12	-94.39 (26.22%)

Data represents means ± SD of six individual values; significant at p≤ 0.05

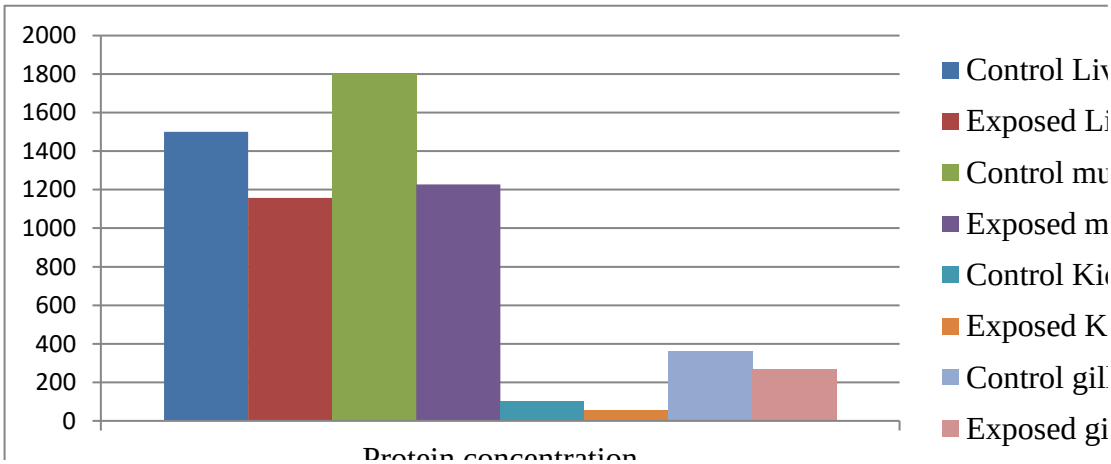

Figure 7: Shows protein concentration in muscle, liver, kidney and gills of Labeo fish.

Table 11. Total lipids in liver, muscle, kidney and gills of control and treated fish.

Tissues	control group 15 days (µg/g)	Sublethal 15 days exposure group	Difference (%)
Liver	735.79 ± 36	713.01 ± 35	-22.78 (3.095%)
Muscle	761.07 ± 39	740.23 ± 32	-20.84 (2.73%)
Kidney	417.30 ± 23	416.40 ± 21	-0.90 (0.21%)
Gills	209.77 ± 12	204.23 ± 13	-5.54 (2.64%)

Data represents means ± SD of six individual values; significant at p≤ 0.05

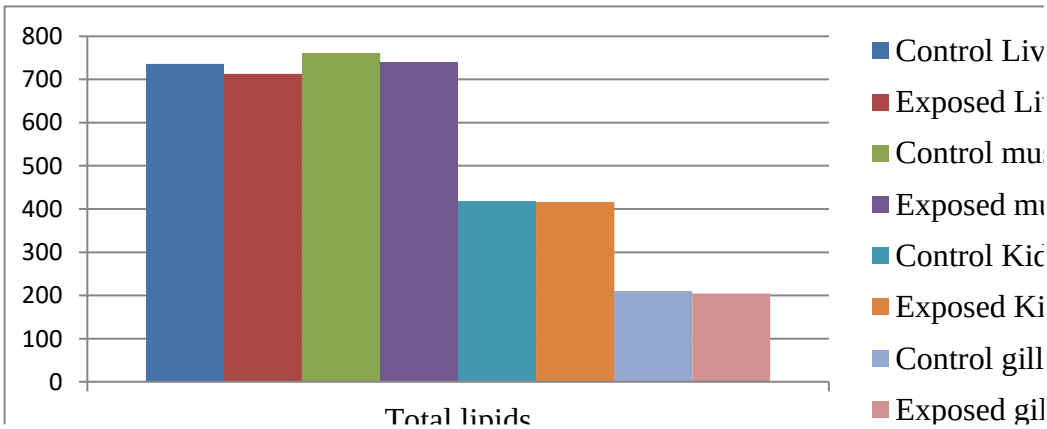

Figure 8: Shows total lipids in muscle, liver, kidney and gills of Labeo fish.

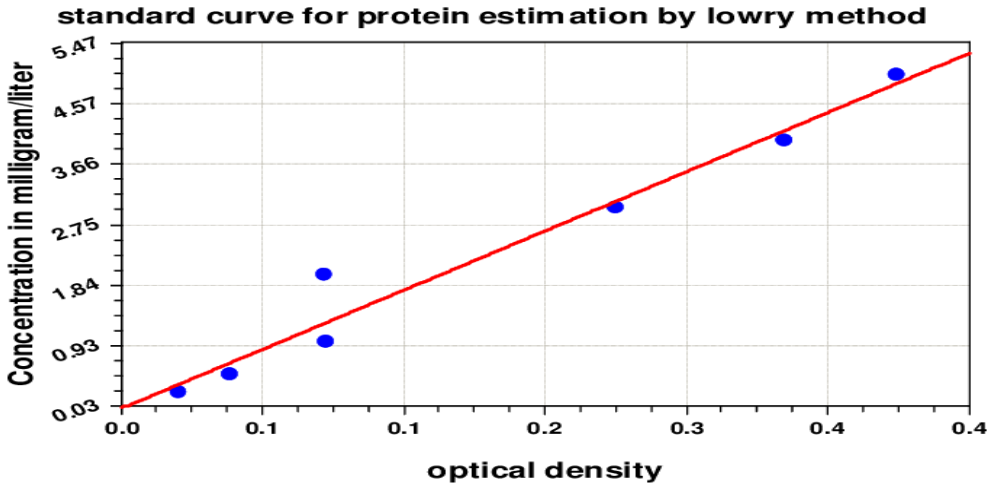

Figure 9: Standard curve for protein estimation by Lowry method

Exposure of endosulfan caused significant change by decreasing the lipase activity of Labeo fish. The maximum reduction was 57.74% in muscle and 52.28% in liver at the concentration of 0.25 ppm. The minimum change in lipase activity was 3.74% and 5.65% in gills and kidney respectively as shown in table-6 and **Figure**-3. The toxicity of a pesticide could vary from species to species. The variation is due to differential tolerance capacities of animals to pesticide exposure. Ferencz and Balog (2010) and Farenhorst (2006) reported the toxic effects of the organophosphate pesticide phosphamidon in thiourea medium, on the freshwater fish, *Sarotherodon mossambica*.

Choline esterase activity was reduced by 35.66% in liver and 38.60% in muscles and the maximum decrease 52.54% in gills and 41.21% in kidney (Table-7 and **Figure**-4).

Table-8 and **Figure**-5 showed 30.79%, 55.59%, 41.21% and 52.54% decrease in the activity of Trypsin in liver, muscle, kidney and gills respectively. In most teleosts, Trypsin is synthesized in the cells of pyloric

ceacum as an inactive precursor trypsinogen which is secreted in to intestinal lumen and activated by enteroprotease (Marcuschi *et al*, 2010).

Exposure of endosulfan also caused significant changes in glycogen content in liver, muscle, kidney and gills of Labeo fish as shown in table-9 and **Figure**-6. The maximum reduction was noted 43.53% in liver and minimum 26.13% in gills. Cypermethrin treated fish showed inhibition in GPT activity in liver and kidney of *Notopterus notopterus* (Gupta *et al*., 1998).

Protein concentration (as in table-10 and **Figure**-7) was reduced by 22.89% in liver, 31.81% in muscles, 43.94% in kidney and 26.22% in gills of exposed fish. *Tilapia mossambica* exposed to sublethal concentration of fenvalerate showed remarkable changes in the level of carbohydrate, protein and amino acid in liver (Radaiah *et al.,* 1989) and similar results were observed by Nagaraju Bantu *et al*, 2013.

Table-11 and **Figure**-8 showed the maximum decrease in total lipids were found 3.095% in liver, 2.73% in muscles, 0.21% in kidney and 2.64% in gills of Labeo fish. Table 3 showed increase mortality rate as the concentration of endosulfan and duration of exposure increases due to acute toxicity of water.

Some other physiological changes:

Pesticide pollution brings about some physiological changes too. It increased thyroxin level in serum and pharyngeal thyroid follicles concurrent with induction of peroxidase activity. It reduces reproductive performance of fish. Ovulation time defers, ova size reduces, and increase mortality rate. High packed cell volume or blood hematocrit level usually indicates hemoconcentration due to gill damage. It has been hypothesized that impairment of iron metabolism and distribution due to deficiency of vitamin C might decrease RBC production. Oxidative toxicity increases in turn enhance oxidative stress ultimately oxidative damages (Zhang *et al.,* 2003).

CONCLUSION

From the above study it can be concluded that endosulfan pesticide is a synthetic pyrethroid which can cause serious damage in liver, muscle, kidney and gills and other parts of the fish and also disturb metabolic and physiological activities when exposed to sublethal concentration of this pesticide.

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Conflict of Interest

The author declares that there is no conflict of interest.

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