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BIOCHEMICAL ALTERATIONS OF TOTAL PROTEINS, GLYCOGEN LACTATE DIHYDROGENASE (LDH) AND TRANSFERASE ENZYMES AMINO TRANSFREASES (AAT) AND ALANINE AMINOTRANSFERASES (AALT) INDUCED BY BIFENTHRIN (A STYNTHETIC PYRETHROID) IN THE FISH *LABEO ROHITA* (HAMILTON)

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Abstract

Bifenthrin type 1 synthetic pyrethroid technical grade and 10% EC induced alterations in the biochemical parameters of total proteins (TP), Glycogen and Lactate dehydrogenase (LDH), Aspartrate Amino Transferase (AAT) and Alanine Amino transferase (ALAT) in the fish *Labeo rohita*, in the laboratory after exposing them in both lethal and sublethal concentrations for four days and 10 days respectively taking into consideration of 96 hour LC_{50} values of the respective toxicants.

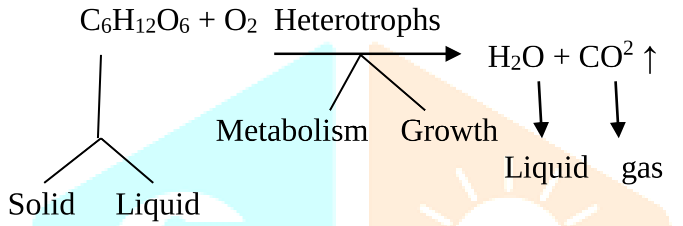
The fish organs, Gill, Liver, Kidney, Brain and Muscle are studied and found an appreciable quantity of percentage as decrement of TP, glycogen and increment in LDH, AAT and ALAT enzymes in this present study. Proteolysis and an hormonal imbalance due to the toxic stress as an effect resulted in the protein break down and gluconeogenesis might be also be reasons of the decrement followed by increment of the three enzymes studied in the biochemical parameters of the fish. Shifting of aerobic pathway to anaerobic resulted LDH activity that got enhanced.

Keywords: Total proteins (TP), glycogen, Aspartate Aminotransferase (AAT) and Alanine Aminotransferase (AALT), Lactate dehydrogenase (LDH)

INTRODUCTION

We know that biology is the science of life and living process only, and the various living organisms that showed diversity had a fundamental similarity made of fundamental units, cells, organized into tissues, which in turn make the organs of the organism organisms as species make a community of the populations and several of such constitute an ecosystem. The biosphere the global ecosystem has energy transactions ATP as dollar, which are governed by laws of thermodynamics. Solar energy and chemical energy both involve the complex reactions and pass it to the other through food chains in the ecosystem and for all that the physiological process, metabolism to occur in all living biota. The Nutritional aspects the living organisms are categorized into Autotrophs, heterotrophs, saprotrophs and few are decomposers. The metabolism starts with CO_2 (gas) mixing with water (H_2O) trapping solar (sun) energy and covert into glucose (carbohydrate) to derive chemical energy. This will be the characteristic feature of all Autotrophs (gas + liquid → solid) and release only (O_2) which is the starting process of metabolism in heterotrophs and convert carbohydrate into chemical energy and release CO_2.

$$\underset{\text{(gas + liquid)}}{CO_2 + H_2O} \xrightarrow[\text{Chlorophyll}]{\text{Solar energy}} \underset{\text{(Solid + gas)}}{C_6H_{12}O_6 + O_2 \uparrow}$$



The cells, the fundamental units of living during the course of evolution from inorganic things only started with chemical evolution only which lead to form elements, simple and compound complexes (started from inorganic to organic) as proteins, LCHON) sugars (CHO) fatty acids, purines and pynmidines organized into nucleosides and nucleotides and finally as polysaccharides, proteins, fats and finally the nucleic acids (CHONP). All together five elements (CHONP) four chemical bonds (Hydrogen, covalent, coordinate and ionic bonds) formed into three substances of chemical forms (sugar, protein, nitrogen bases), which form into two, the nucleoside (sugar + nitrobase) and when combine with phosphate form nucleotides (Nucleoside + Phosphates) and finally one as monomere. The repetition of such monomers is the polymer, DNA (life molecule in living organisms). Hence, monomers of every cell had these chemical substances only and the whole biochemical reactions takes place and the process is non-other than metabolism (The sum of all chemical reactions).

At one end, biosphere is suffering with population dynamics poverty and pollution apart from political pollution and (top to bottom approach) and the biota, in the cells./tissues/organs/organism (bottom to top approach) got affected by such act of defilement.

Hence, the global contaminations lead to the effect of the organisms, and end with all biochemical aspects of reactions impaired and finally the metabolism will be curtailed.

All the chemical reactions are influenced by the enzymes and majority them, if they function normally as a result, homeostasis exists for all physiological actions and are dependent on biochemical process that are involved within them.

But due to the global pollution with reference to pesticides, Mac Namura (2019) shouted the very nature of the agricultural land was at high risk of pesticides pollution only. Tang *et al.* (2021), also cautioned about the regions of high risk due to pollution of pesticides of about 168 countries which also includes India. Ananmika Srivastava *et al.* (2018) in their report of the Indian scenario of the pesticides contamination of water and Indira devi *et al.* (2017), projected the view of pesticides usage more in Indian continent and also the state-wise consumption too is alarming hence the waters are contaminated.

These chemicals, are to combat the target pests which can be called targeted organisms but when present in natural waters, the organism inhabitating there, are the non-target organisms also got effected, where concentration is the factor whether lethal or sublethal.

The non-target organism, Fishes, because of their sensitivity, an important source of food, the food chain connections of links and webs naturally more in number got effected. The biochemical studies in them serve as indices of the toxic action and serve as a biomarker study which Sana Ullah (2019a) recognized them and also Anilava Kaviraja and Gupta (2014) too mentioned in their review articles any such study of the biochemical nature of the actions, the impediments caused by the pesticides serve as an index of the pollution load of the environment.

The biochemical studies, by using different toxicant pesticides to different fish while in lethal and sublethal concentrations were carried by individual researches. Such reports are by Ullah *et al.* (2019b), Satyanarayana *et al.* (2019), Martinez (2018), Classen *et al.* (2018), Neelima *et al.* (2017& 2016), Ullah (2015), Ullah *et al.* (2014), Prusty *et al.* (2011) for synthetic pyrethroids. Edwin *et al.*, (2021), Akter *et al.* (2020), Sarmila and Kavitha (2019), Bheema Rao *et al.* (2018), Revathy and Krishna Murthy (2018), Somayya *et al.* (2017), Anitha Bhatnagar *et al.* (2017), Tripathi *et al.* (2015), Imtiyaz *et al.* (2014), Veeraiah *et al.* (2013a&b), Tilak *et al.* (2005) and Tripathi *et al.* (2003) for organic pesticides.

Such reports of study promoted to know the effects of chemicals of pesticides technical grade and commercial formulation EC exposure in the laboratory conditions in both lethal and sublethal concentrations in the different fish. The biochemical parameters selected are total proteins, glycogen, lactate dehydrogenase (LDH), Alanine aminotransferase (AAT), Amino alanine transferase (ALAT).

Considering the role of above biomarkers in the field of eco-toxicology, the present study has been undertaken to understand the biochemical alterations induced by Bifenthrin technical grade and 10% EC on exposure to sublethal and lethal concentrations to fish *Labeo rohita* in the different tissues exposed. The bioassays include Glycogen, Total Proteins, Lactate Dehydrogenase (LDH), Aspartate Aminotransferase (AAT), Alanine Aminotransferase (ALAT).

MATERIALS AND METHODS

The fresh water carp, *Labeo rohita* an edible and economically important fish was selected with a range of size about 3 to 5 cm and 3 to 5 grams of weight, irrespective of their sex, as the test organisms for present investigation. Healthy and active fish were obtained from Ratna Singh Hatcheries, Kuchipudi, Guntur (A.P.) India. The fish were acclimatized to the laboratory conditions in large plastic water tanks for three weeks at a room temperature of 28±1°C. Water was renewed every day with 12-12 h dark and light cycle. During the period of acclimatization, the fish were fed (ad libitum) with groundnut oil cake and rice bran. Feeding was stopped one day prior to the actual toxicity test. All the precautions laid by committee on toxicity tests to aquatic organisms (APHA 1998, 2005 & 2012, OECD 2019) were followed and such acclimatized fish only were used for experimentation. If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish were discarded. Permethrin technical grade (TG) was supplied by 10% EC was purchased from the local market Guntur, A.P., India.

Physical & Chemical properties of water used for the present experiments are (in mg/L): Turbidity–8 silica units. Electrical conductivity at 28°C-8.16 Micro ohms./cm, pH at 28°C-8.2. Alkalinity**:** Phenolphthalein-Nil, Methyl orange as $CaCO_3$-472, Total hardness-320, Calcium Handness-80, Magnesium Hardness-40, Nitrite nitrogen (as N)- Nil, Sulphate (As SO_4) – Trace, Chloride (as Cl^-)-40, Fluoride (as F^-) – I.S. Iron (as Fe)- Nil, Dissolved Oxygen – 8-10 ppm, Temperature - 28±2°C.

A batch of fish, 50 numbers were exposed for 4 days in lethal concentrations of technical grade of 0.22 µg/L and another batch of 50 numbers for 10% EC as of 0.11 µg/L and similarly another batches of the same numbers in sublethal concentrations for 10 days 0.022 µg/L for technical grade and 0.011 µg/L for 10% EC respectively. A control fish of 50 numbers are also maintained during the period of experimentation. From each exposed fish as well as control group the vital organs, gill, liver, kidney, brain and muscle were taken for the further biochemical analysis of the total proteins, glycogen, LDH, AAT and ALAT enzymes from their respective tissues/cells.

The total proteins are estimated as per the method by the Lowry *et al*. (1951), AAT and ALAT enzymes by the Rietman S. Frankel (1957) methods, Glycogen by Kemp *et al*. (1954) and LDH by Srikantham and Krishna Murthy (1955).

RESULTS

Total Proteins: The results obtained for total proteins as data exposed to both technical grade bifenthrin and 10% EC in sublethal and lethal concentrations to the freshwater fish *Labeo rohita* are graphically presented as Fig. 1 for the quantity of total proteins and as Fig.2 also for the percentage changes as alterations compound to the control.

The decremented lyotropic series of the total proteins are Muscle > Brain > Liver > Kidney > Gill for technical grade and for 10% EC Muscle > Brain > Liver > Kidney > Gill in sublethal whereas for lethal, technical grade Brain > Muscle > Liver > Kidney > Gill and for EC Muscle > Brain > Liver > Kidney > Gill.

Glycogen: The results that are obtained for the glycogen content in the fish exposed to both sublethal and lethal concentrations of technical grade and 10% EC are graphically represented as Fig. 3 and percent variation in the Fig. 4.

The glycogen series decreased in the series as follows:

Sublethal
- → Technical grade Kidney > Gill > Muscle > Brain > Liver
- → 10% EC Kidney > Gill > Brain > Liver > Muscle

Lethal
- → Technical grade Kidney > Gill > Brain > Liver > Muscle
- → 10% EC Kidney > Gill > Brain > Muscle > Liver

Lactate Dehydrogenase (LDH): The results obtained as of the increment of the enzyme is graphically represented as Fig. 5 and the percent variants of the result in Fig. 6.

The lyotropical series of the increment for the targeted enzyme are:

Sublethal
- → Technical grade Muscle > Gill > Brain > Kidney > Liver
- → 10% EC Muscle > Liver > Kidney > Gill > Brain

Lethal
- → Technical grade Liver > Muscle > Gill > Brain > Kidney
- → 10% EC Liver > Muscle > Gill > Kidney > Brain

Aspartrate Aminotransferase (AAT): The results as increment of the transferase enzyme AAT of the quantities are represented as graph, Fig. 7 and percent variation of the change over the control as Fig. 8.

The increment series are as follows:

Sublethal
- Technical grade Gill > Muscle > Liver > Kidney > Brain
- 10% EC Gill > Liver > Muscle > Brain > Kidney

Lethal
- Technical grade Gill > Muscle > kidney > Liver > Brain
- 10% EC Gill > Kidney > Muscle > Liver > Brain

Alanine Aminotransferase (ALAT): Alanine Aminotransferase (ALAT) micromoles of pyruvate formed in mg / of protein in the tissues of *Labeo rohita* exposed to bifenthrin technical grade (TG) and 10% EC in sublethal concentration are graphically represented as Fig. 9 and percent changes in Fig. 10.

The increment series for the transferase enzyme is as follows:

Sublethal
- Technical grade (TG) Muscle > Gill > Brain > Liver > Kidney
- 10% EC Muscle > Brain > Gill > Liver > Kidney

Lethal
- Technical grade (TG) Muscle > Brain > Gill > Liver > Kidney
- 10% EC Gill > Liver > Brain > Liver > Kidney

DISCUSSION

Sana Ullah *et al.* (2022) reported on the bifenthrin toxicity in the fish *Ctenopharyngodon idella* and had a biomarkers study in which biochemical aspects of total proteins, antioxidant enzymes including superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD), glutathione peroxidase (GSR-Px), glutathione reductase (GR), DNA, RNA and Acetyl Cholinesterase, etc.

In the tissues of brain, liver, gills and muscle, the protein got depleted due to the stress that was caused by bifenthrin. They mentioned that due to the degradation of protein to fulfill the increased demand of energy to synthesise. They also opined that proteins decrement might also be due to the lesions of the respective tissues./organs that finally damaged by the toxic effect or it might be also due to inhibition of protein synthesis where in the entire machinery got weakened (no transcription).

Yanhan Wang *et al.* (2022) in the fish, *Danio rerio* (zebra fish) due to bifenthrin toxic action, while testing the acute toxicity of different life stages, the stress induced biochemical variation via the genes in action (six-genes) that made mRNA transcription who got inhibited and ultimately resulted changes biochemically, in protein synthesis. They opined that bifenthrin was also endocrine disruption that made all the changes, gluco-neo-genesis paved the way and as fish the selected. Aminoacids as the alternative sources of energy by deamination hence proteins got depleted which might be the same reason even in the present study.

Suchiang *et al.* (2021) in their review article on cat fishes of the toxicity caused by pesticides in biochemical aspects, oxidative stress and histopathological variations resulted as impediments of the biochemical reactions that are to take place normally got altered, hence the quantitative changes of all the biomolecules.

Aysel Güven (2021) in his chapter of the effects of the pesticides biochemically to fish of different species, the alterations are varied by the nature of the species. The organs/tissues/cells fundamental chemical composition as of protein etc., were appreciably affected even though the concentrations are in sublethal level.

Saha and Saha (2021) using bifenthrin as a toxicant on the fish *Clarias batracus*, Saha *et al.* (2021) in the fish *Heteropneustes fossils*, Saha *et al.* (2020) using the same toxicant on the fish *Oreochromis mossambicus* visualized the toxic action and that caused the death of the fish that were in their respective studies cautioned due to the presence of concentration of the toxicant can cause effects of biochemical nature, also apart from others.

Satyanarayana *et al.* (2020) in the fish *Ctenopharyngodon idella* reported on the Glycogen content and the enzyme lactate dehydrogenase (LDH) after exposure to permethrin, a synthetic pyrethroid of type I the toxicant, bifenthrin which is tested also belong to the same group. As in the present study, result of decrease of glycogen and an increase of the enzyme activity of LDH, was reported, by them.

Siroshi (2007) while working on the permethrin toxicity to fish *Heteropneustes fossils* reported the decrement of the glycogen in muscle and liver tissues. Velisek *et al.* (2011, 2007 and 2006), in the fish *Oncorhynchus mykiss, Cyprinus carpio* and *Prussian carp* reported using the synthetic pyrethroid of type II (Deltamethrin and cypermethrin), glycolysis was promoted releasing more energy glucose for the energy synthesis. As long as the Aerobic pathway prevails that is going to be normal but if it is anaerobic pathway lactate production increases that resulted of the activity of the enzyme LDH which also increased.

The decrement of the glycogen and increase of glucose as a result of the failure of carbohydrate metabolism as a result of the physical and chemical stress of the toxicant. Ogueji and Auta (2007) and Ibrahim *et al.* (2013) who reiterate this line of defense. Srivastava *et al.* (2016) too reported that due to the toxic stress the glycogen was depleted (aerobic pathway). Due to hypoxic condition, anaerobic pathway prevailed and that was the valid reason of the decrement. Anitha *et al.* (2012 and 2010) also in the fish *Catla catla*, *Cirrhinus mrigala* and *Labeo rohita* due to Fenvalerate toxicity (synthetic pyrethroid type II) and Tilak *et al.* (2002) in the fish *Ctenopharyngodon idella* using the same toxicant.

Neelima *et al.* (2015) using cypermethrin as the toxicant in the fish *Cyprinus carpio*, Adeyemi olaekam (2014) in the fish *Clarias gariepinus* using the same toxicant as above, Naik *et al.* (2016) in the fish *Labeo rohita* using the same toxicant, Rathnamma *et al.* (2007) using deltamethrin as the toxicant reported the same result of decrement (due to the enzymes phosphorylase and glycogen synthase failure). Even Venkataramudu *et al.* (2007) in the fish, *Channa punctatus* using the Deltamethrin as the toxicant reported that the fish is modulating the physiological and metabolic responses for proper utilization of the energy sources). Veeraiah *et al.* (2013) in the fish *Cirrhinus mrigala* (Hamilton) using cypermethrin as the toxicant due to promotion of glycolysis decrement of glycogen was mentioned as the reason.

Akbar *et al.* (2012) in the fish *Helicoverpa armigera* due to permethrin the LDH enzyme activity increased. Ibrahim *et al.* (2013) reported in the fish *Solea sengalensis* by using cypermethrin and permethrin (Type II & I, respectively), because of the demand of more energy of toxic stress utilization of available way of substrate for energy synthesis LDH activity increased. The decrement of glycogen and increase of anaerobic pathway of the energy synthesis, LDH activity increased which energy was also the reiterated by Neelima *et al.* (2015) in the fish *Cyprinus carpio* using Cypermethrin as the toxicant and, Mozhdeganloo *et al.*(2016) in the fish *Oncorhynchus mykiss* using permethrin as the toxicant (lipid peroxidation and damage to liver were the reasons that explain the enhanced activity of the enzyme, LDH. The same was reiterated by Velisek (2011, 2009, 2007, 2006) in different fish where LDH, CK and transaminases all had of increased activity. Due to the stress shift of glucose (six carbon atom) to lactate (3 carbon atom) by which the LDH enzyme activity increased.

Shehzad Ghayyur *et al.* (2022) in the fish *Cirrhinus mrigala* due to pesticide toxicity of mixtures (Chlorfenapyr Dimetheoate and Acetamiprid). LDH, ALT and AALT activity got increased as in the present study of Bifenthrin, with *Labeo rohita*. Histopathological lesions of damage of liver paved the way of increase of enzyme activity. Mayada R. Farag *et al.* (2021) due to cypermethrin toxicity, biochemical alteration, in LDH was mentioned in the different fish.

Tanseem and Yasmeen (2020) in the fish *Cyprinus carpio* due to sublethal concentration exposure of the toxicant of a biopesticide, Darison. Due to the failure of total metabolism as a result of the damage of the tissues and organs the contradictory result of the present study was reported.

Ghanim *et al.* (2020) using fenvalerate, type II synthetic pyrethroid, selecting zebra fish *Danio rerio* as a model fish studied the biochemical changes of total proteins, AAT and ALAT. The decrement of the protein in the gill tissue was analysed by SDS-PAGE and when control gill and treated gill and muscle showed stress proteins (high intensity). Due to more enzyme activity of transferase enzymes (AAT & ALAT), deamination process was enhanced (Gluconeegenesis) and also Glycolysis to a certain extent was opined.

In the review article of Biomarkers that are for the synthetic pyrethroids showed the scope and aim of them by Sana Ullah *et al.* (2019a). The biochemical aspects of the biomarkers study was reported as mention by the earlier studies. Sana Ullah *et al.* (2019b) for the fish *Hypopthalmichthys molitrix* using deltamethrin as the toxicant reported decrement of total proteins in blood, gills, liver and muscles and brain.

Viera and dos Reis Martnez (2018) in the fish *Prochilodus lineatus* using cyahalothrin as the toxicant, Clasen *et al.* (2018) using the same toxicant in the fish *Cyprinus carpio*, Ullah *et al.* (2015) in the fish *Labeo rohita* using cypermethrin as the toxicant, Suvetha *et al.* (2015) in the same fish using deltamethrin as the toxicant, Ullah *et al.* (2014) in the fish *Tor putitova* using cypermethrin as the toxicant, Prusty *et al.* (2011) in the fish *Labeo rohita*. Using fenvalerate and Nillos *et al.* (2010) in the fish *Oryzias latipes* using permethrin as the toxicant were also the earlier reports for synthetic pyrethroids which had effect on biochemical parameters and the results also support the present studied one.

The review article of Sana Ullah (2019a) mentioned that fish had no hydrolase enzyme because of it the pyrethroids have effect on them and all the effects are due to toxic action whereas the mammals had hydrolase enzyme and are not toxic to them.

Saumya Biswas *et al.* (2019) in his review article of the synthetic pyrethroids too had a mention of some of the studies that were earlier reported in the lines of the work of biochemical alterations.

Tamzin *et al.* (2019), while studying on the toxicity of bifenthrin (nanen capsulated-nanoform) in the fish *Oncorhynchus mykiss*, reported decrement of the total proteins and other enzymes.

Satyanarayana *et al.* (2018) using permethrin as the toxicant reported alterations of total proteins and Aminotransferase in the fish *Ctenopharyngodon idella*. Decrement of the total proteins and increment of activity of the enzymes, AAT and AALT. The mention of the Sussane *et al.* (2016) wherein the pyrethroids are of endocrine disruptors as result gluconeogenesis was promoted and also proteolysis which might be the valid reasons of decrement for proteins and the same can be visualized even in the present study. It was also mentioned that in the opinion of Velisek *et al.* (2011), to meet the necessity of energy on demand, deamination followed by stunting of the respective acids of TCA cycles that resulted the decrement of proteins. More Aminoacids without increase of protein synthesis due to the toxic stress cytotoxicity paved the way and the transferase activity had a role.

Prem Sagar *et al.* (2017) using bifenthrin, in the fish *Channa punctata* exposure reported on the effect of the haemopoietic/excretory organ of Ammonotellic nature, the glycogen content got reduced due to toxic stress, then it requires sufficient energy which was by glycolysis only (Glycogen → Glucose) and the synthesis of strength (Absolutely there was no possibility Glycogenesis (Glucose → Glycogen). There is every possibility of gluconeogenesis (either Fatty acids or protein as a source/non-carbohydrate source for energy synthesis).

Huma Naz *et al.* (2017), in the major carps, *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* due to mixture toxicity (bifenthrin, chlorpyrifos and Endosulphan) studied the effect also wherein the enzyme activity of antioxidant enzyme, SOD (Superoxide dismutase activity). Neelima *et al.* (2017) in the fish *Cirrhinus mrigala* when exposed to cypermethrin resulted a decrement of the total proteins. They opined that proteins are mobilized as source of substrates for energy synthesis (via oxidation) and destruction of the organs/tissues resulting inhibition of either protein synthesis or no protein synthesis. One of thinking line was impaired food intake, increased energy cost of homeostasis, tissues repair and detoxification mechanism while during toxic stress all responsible for decrease of the proteins.

Shailendra Kumar Singh *et al.* (2010) due to the cypermethrin action of the toxic nature when exposed in the fish when exposed enhanced activity of the enzyme LDH in *Colisa fasciatus*. According to them, the enzyme is located at the key point that associated with anabolism and ketabolism and is responsible for inter-conversions of lactic acid and pyruvic acid and during anaerobic conditions. Hence, in their study as well as in the present study the same explanation can be thought off.

Velisek *et al.* (2009b) due to bifenthrin exposure to the fish *Onchorhynchus mykiss* (rainbow trout) (2009a) in the fish *Cyprinus carpio* reported biochemical alterations. The opinion expressed mentioned earlier of that Velisek *et al.* (2011) holds good as explanation for decrement of energy sources and paved the way for alternative sources for the synthesis of energy.

Synthetic pyrethroids and organophosphates and carbamates (both) share common point of Endocrine disruptors and also the inhibitors of Acetyl cholinesterase enzyme (AChE).

Even the following studies of organophosphates and carbamates have biochemical manifestations of total proteins, glycogen, LDH, AAT and AALT alterations.

Juginu and Binukumari (2021) while studying the toxic effect of Malathion that induced biochemical changes in proteins, carbohydrates and lipid metabolism aspects in the fish *Labeo rohita*. The proteins were decreased and the reason mentioned that the stress and its demand for more energy oxidation of Aminoacids (deamination) only. The carbohydrates are the first immediate sources of energy for the teleost where the swimming activity is vigorous in them, as result glycolysis or hexose monophosphate pathway made the decrement of glycogen.

Naquash Khan *et al.* (2021) while using the chlorpyrifos as the toxicant in the fish *Ctenopharyngodon idella* reported on the biochemical alterations mainly due to histopathological architectural damage of the organs/tissues that resulted such changes.

Sandya Rani and Rasool (2021) in the fish *Channa punctatus* due to the exposure of mild dose of Malathion reported the decrements of energy sources both proteins and glycogen. The antioxidant enzymes made all the effected the biochemical biomolecules of proteins and glycogen.

Rajyalakshmi and Raju (2017) in the fish *Gambusia affinis*, when exposed to Malathion LDH, AAT and ALAT were all effected, quantitatively in the brain tissue only. Stress factor, anaerobic pathway of energy synthesis made LDH activity to increase and consequently even shift to transferase enzymes of AAT and ALAT enhancement of its activity.

Mini and Kumar (2019) in the fish *Labeo rohita*, *Catla catla* due to phenthoate intoxification resulted decrement of total proteins and glycogen in the tissues tested apart from the present tested tissues and also in the gut. To augment the energy demand due to the toxic stress the energy sources that were available fully utilized.

Serum glucose study in the fish *Cirrhinus mrigala* by using chlorpyrifos as toxicant by Anitha Bhatnagar *et al.* (2017) and using the same toxicant in the fish *Oreochromis mossambicus* by Shezad Ghayyur *et al.* (2017) too reported decrement and increment of glucose levels. They are the primary source of energy and impropose utilization that enhance glucose in the blood as a result increase.

Christopher *et al.* (2016) in the fish *Labeo rohita* exposed to phosphomidon reported on the effects of biochemical parameters, total proteins and total free sugars in the blood. The proteins and free sugars decreased as a result of energy synthesis while during the toxic stress due to glycolysis and gluconeogensis to augment the energy demand of the toxic effect.

Karra Somaiah *et al.* (2015), due to phenthoate toxic effect in the fish *Labeo rohita* reported decrement of the total proteins and glycogen whereas Sunitha *et al.* (2015) in the same fish and toxicant reported on the effects of the biochemical changes that are resulted due to the toxic action in total proteins, glycogen, AAT and ALAT. The transferase enzymes incorporated the ketoacids into the TCA cycle via the regeneration of glutamate (Transamination process followed by the conversion of α-kelogluterate through oxidative deamination to favour gluconeogenesis for energy synthesis. Mitochondrial damage and release of cytochromes were responsible for such thing due to tissue disruption, which, the same can be visualized even in the present study.

Ramesh *et al.* (2015) in the fish *Cyprinus carpio* due to furadon (carbofuran EC, a carbamate pesticide) had enzymatical changes in the blood, particularly the glutamate transaminase as a result the total proteins, decreased whereas AAT and ALAT increased.

Shezia Quadir *et al.* (2014) in the fish *Labeo rohita* due to exposure of Imodacloprid, Suneel Kumar (2014) in the fish *Channa punctata* due to Nuvan exposure, Chitra *et al.* (2013) due to Quinolphos exposure, Veeraiah *et al.* (2013b) in the fish *Labeo rohita* due to indoxacarb. Thenmozhi *et al.* (2011) in the fish *Labeo rohita*, Lakshmaiah (2016) in the fish *Cyprinus carpio* (only LDH) earlier studies all reported the biochemical alterations.

CONCLUSION

Thus, for different fishes for different chemicals of pesticide. The present studied and other parameters proteins, glycogen the chief energy sources got decreased, whereas the enzymes LDH, AAT and ALAT activity enhanced. The targeted enzymes is not for target species of pests and also for the non-target organisms like fish even in sublethal concentration, the fish especially the culturable one is not comfortable. The Bifenthrin is type I class of pesticide whereas Cypermethrin, Deltamethrin and Fenvalerate is of type II. The behaviour is same in both the types as the case of the present and other parameters study and result is the death of the fish. Both organophosphates and synthetic pyrethroids inhibit the enzyme AChE and variation in others and fish got so effected.

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Fig. 1. The amount of total protein mg g^{-1} in the tissues of the fish, *Labeo rohita* exposed to Bifenthrin Technical Grade (TG) & 10% EC in sublethal and lethal concentrations

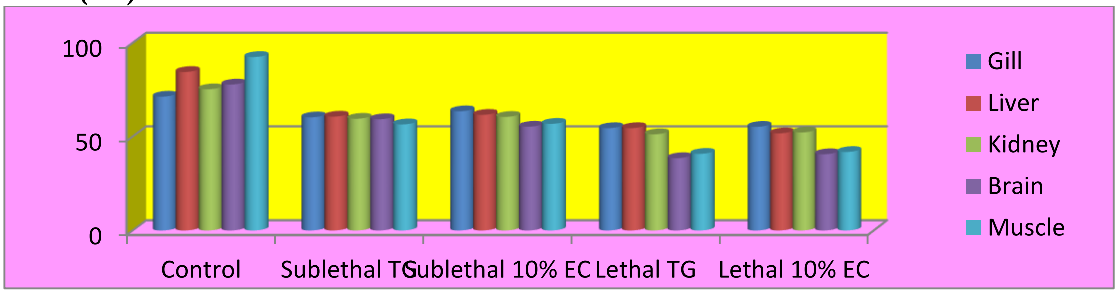

Fig. 2. The amount of Glycogen content mg g^{-1} in the tissues of the fish, *Labeo rohita* exposed to Bifenthrin Technical Grade (TG) & 10% EC in sublethal and lethal concentrations

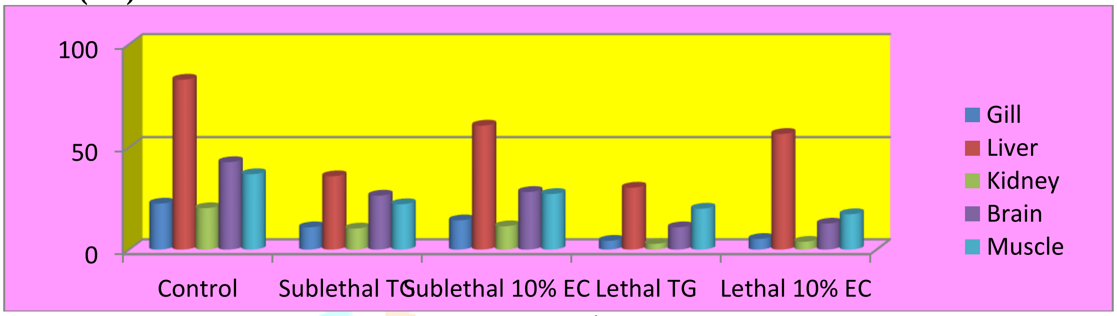

Fig. 3. Lactate dehydrogenase (LDH) mg/of lactic acid g^{-1} wet weight of tissue in the different tissues of *Labeo rohita* exposed to Bifenthrin Technical Grade (TG) & 10% EC in sublethal and lethal concentrations.

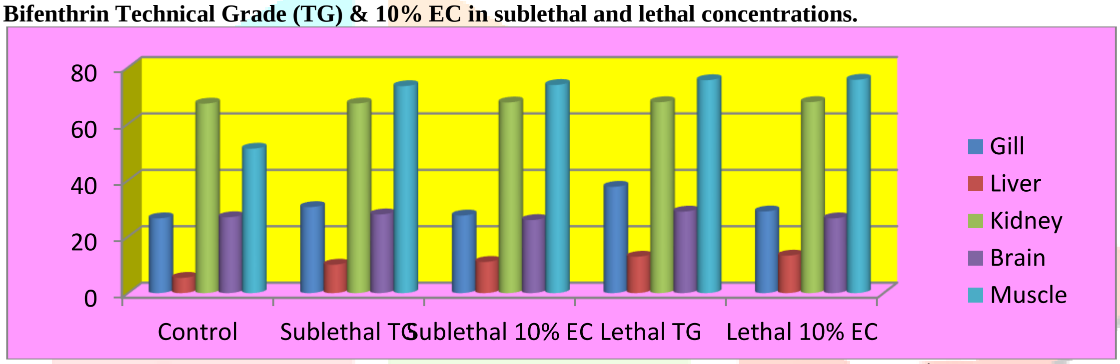

Fig. 4. Aspartrate Aminotransferase (AAT) µ moles of pyruvate formed mg of protein^{-1} in the tissues of the fish *Labeo rohita* exposed to Bifenthrin Technical Grade (TG) & 10% EC in sublethal and lethal concentrations.

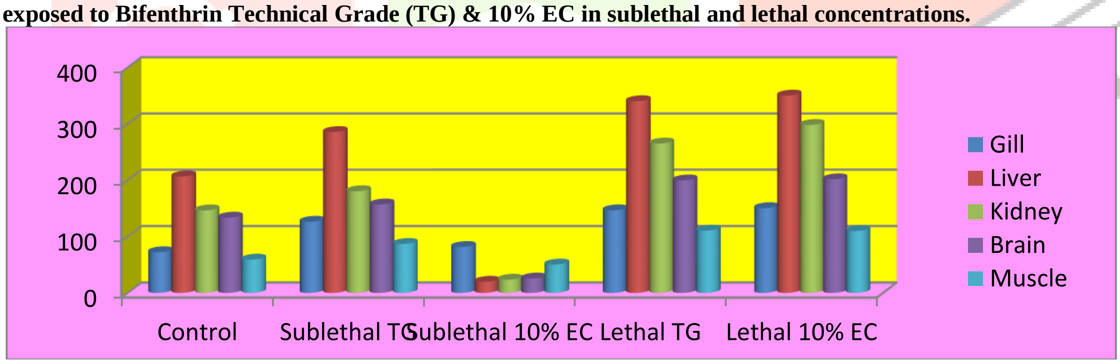

Fig. 5. Alanine Aminotransferase (ALAT) micromoles of pyruvate formed in mg/of protein in the tissues of *Labeo rohita* exposed to Bifenthrin Technical Grade (TG) and 10% EC in sublethal and lethal concentrations.

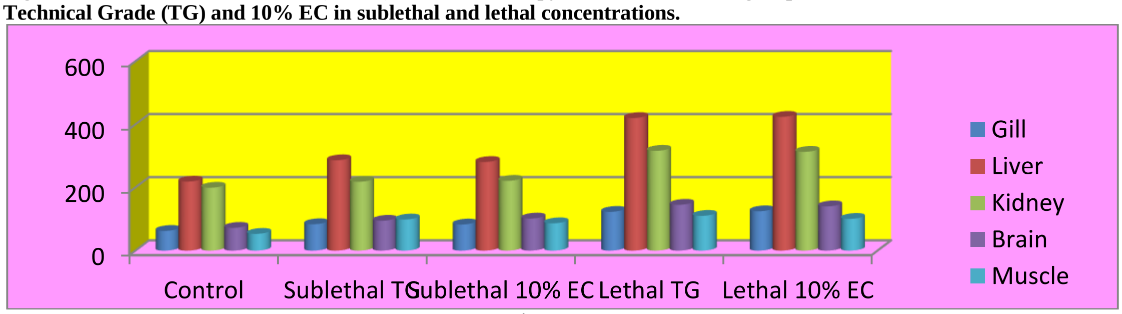

Fig. 6. Percent changes of total protein mg g^{-1} in the tissues of the fish, *Labeo rohita* exposed to Bifenthrin Technical Grade (TG) & 10% EC in sublethal and lethal concentrations

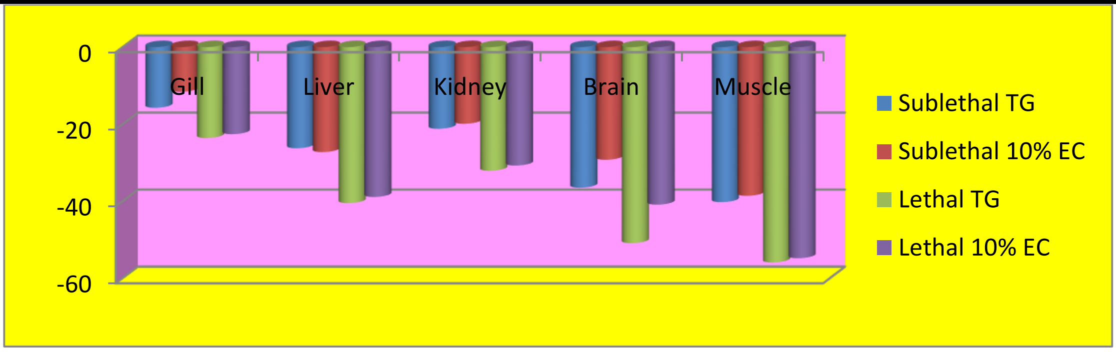

Fig. 7. Percent changes of Glycogen content mg g^{-1} in the tissues of the fish, *Labeo rohita* exposed to Bifenthrin Technical Grade (TG) & 10% EC in sublethal and lethal concentrations

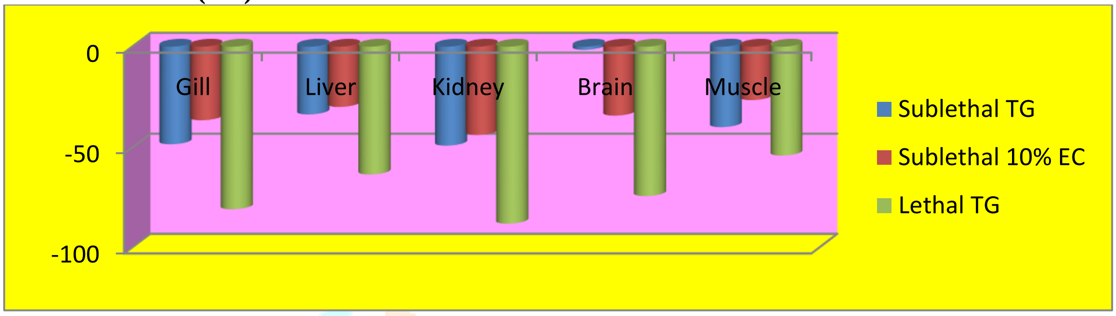

Fig.8. Percent changes of Lactate dehydrogenase (LDH) mg/of lactic acid g^{-1} wet weight of tissue in the different tissues of *Labeo rohita* exposed to Bifenthrin Technical Grade (TG) & 10% EC in sublethal and lethal concentrations.

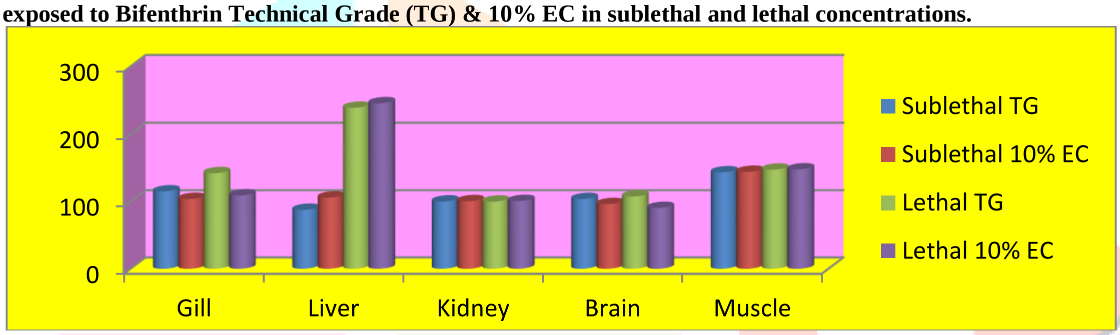

Fig. 9. Percent changes of Aspartrate Aminotransferase (AAT) µ moles of pyruvate formed mg of protein^{-1} in the tissues of the fish *Labeo rohita* exposed to Bifenthrin Technical Grade (TG) & 10% EC in sublethal and lethal concentrations.

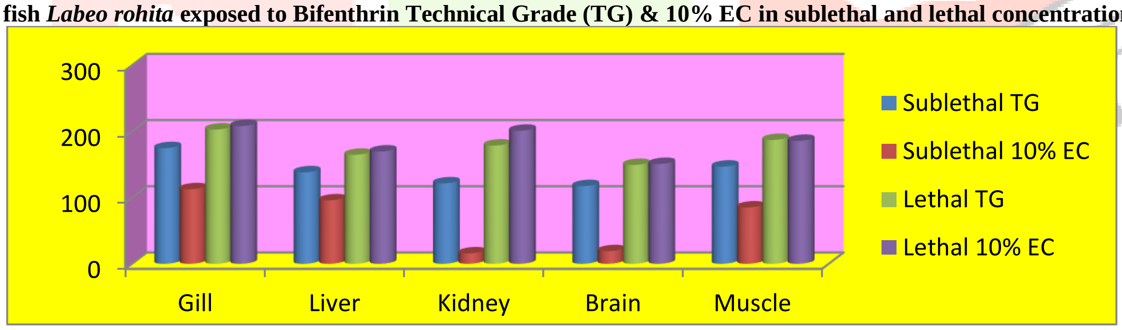

Fig. 5.10. Percent changes of Alanine Aminotransferase (ALAT) micromoles of pyruvate formed in mg/of protein in the tissues of *Labeo rohita* exposed to Bifenthrin Technical Grade (TG) and 10% EC in sublethal and lethal concentrations.