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BIOCHEMICAL VARIATIONS AS IMPEDIMENTS OF A BIOMARKER STUDY IN THE FISH *CATLA CATLA* (HAMILTON) EXPOSED TO BOTH TECHNICAL GRADE CYFLUTHRIN AS WELL AS 10% WP IN ACUTE AND CHRONIC CONCENTRATIONS

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Key words: *Catla catla, Cyfluthrin, 10% WP, Muscle, Gill, Liver, Kidney and Brain, Acute and Chronic levels impediments, toxicants.*

Abstract– The synthetic pyrethroid, Cyfluthrin technical grade (TG) as well as 10% WP is selected for a biomarker study in the fish *Catla catla* that induced biochemical changes of variations which are virtually the impediments of the poikilothermic blood. The study is *in vivo* exposure of both acute and chronic levels in the laboratory for four days and 10 days (l/10th of 96 hours LC_{50} value) respectively, for the two chemical toxicants TG & 10% WP. The selected organs of study include Gill, Liver, Kidney, Brain and Muscle and parameters for assay include as total proteins and glycogen the energy sources and also the enzymes lactate dehydrogenase (LDH), Aspartrate Amino transferases (AAT) and Alanine Amino transferase (ALAT). The prime sources of energy due to proteolysis and glycolysis enhancement that decreased them whereas due to alternative energy pathway, LDH, AAT and ALAT enzymes have increment activity due to shifting of the anaerobic pathway. All the vital organs/tissues/cells had interference of the concentrations of the toxicants to have quantitative and the percent changes in them which varied, all culminated the imbalance in the metabolism and paved the way of the effect of the toxic action in the respective concentrations.

INTRODUCTION

We know that the chemical evolution started with protons, electrons and neutrons and finally figure into simple elements, C, H, N, O, P which made into simple chemical substances proteins, carbohydrates, lipids and finally into nucleic acids.

CHO - Carbohydrates
CHON - Proteins
CHONP – Nucleic acids (life)

The ancient chemical evolution led to the biological evolution. All these are primary constituents of the living cells, prokaryotes and eukaryotes that differentiated into five kingdoms of living organism (Monera, Protista, Fungi, Plantae and Animalia). All of them nutritionally varied into Autotrophs, Heterotrophs, Saprotrophs and Decomponsers. Internally in the cells, there are many biochemical reactions that take place and homeostasis is maintained. The sum of all physiological reactions are non-other than the interconversions of them only, of the biochemical constituents, proteins, carbohydrates, lipids and also the Nucleic acids. Everything is under the control of the neurotransmitter enzyme, AChE.

The other end of the situation, the biosphere is polluted, and one such pollutants are non other than the pesticides. Apart from this, the mother earth is facing the problem of population poverty and pollution (P^3) and of course, one can also add anthropogenic activities and political power all added together (P^4). Due to this several chemicals have come into existence to increase the food production or any produce that are introduced of green, blue and white revolutions. This is the top to bottom approach, and ultimately the biota got effected in the molecules/cells/tissues/organs as bottom to top approach.

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The act of global defilement was visualized and cautioned by Tang *et al.* (2021), Shefali *et al.* (2021), Kelly Macnamara (2021), Rajmohan *et al.* (2020), Pradip Kumar Maurya *et al.* (2019), Anamika Srivastava *et al.* (2018), Akshe *et al* (2018), Indira devi *et al* (2017).

Sana Ullah *et al.* (2019a) reported the studies of reports as biomarkers to understand the effects of the synthetic pyrethroids, Prusty *et al.* (2015) and Ahrar Khan *et al.* (2014) all reiterated that the synthetic pyrethroids do cause the effects in the fish, than other vertebrates.

Even, Anilava Kaviraj and Abhik Gupta (2014) opined that biochemical studies like hyperglycemia, enzymes of energy, enzymes of Nitrogen (AAT & ALAT) and acetyl Cholinesterase (AChE) all as some of the biomarker studies. The same aspects were also mentioned in the review articles of Ullah and Zorrizahara (2015) and Krishna Murthy *et al* (2013).

When these chemicals as pesticides in acute concentrations in the environment, the living organisms, the fish die, but when the concentrations are at chronic level (sublethal) cause effects in the process of glycolysis, glyconeogenesis, glycogenesis and protein synthesis. All are dependent on the enzyme, AChE only when it got inhibited the whole process of biochemical reactions are not going to be normal and finally feeding, locomotion, metabolism etc., all got impaired (Sana Ullah *et al.*, 2019(b).

The biochemical studies, by using different toxicant pesticides to different fish while in lethal and sublethal concentrations were carried by the individual researcher as reports also [Sana Ullah *et al* 2019b, Satyanarayana *et al* 2018, Martinez 2018, Classen *et al.* 2018, Neelima *et al.* 2017& 2016, Ullah 2015, Ullah *et al.* 2014, Prusty *et al.* 2011 for synthetic pyrethroids.

Such reports of study promoted to know the effects of the chemicals of pesticides of technical grade and commercial formulations exposure, in chronic and acute concentrations in different fish. Hence an attempt is for the fish *Catla catla* in the present study. Biochemical parameters selected are the quantitatively as of total proteins. The shunting enzyme, lactate dehydrogenase (LDH), and transferase enzymes Alanine aminotransferase (AAT), Amino alanine transferase (ALAT).

MATERIALS AND METHODS

One of the major carp *Catla catla,* size range of about 3 to 5 cm and 3 to 5 grams of weight, irrespective of their sex, are procured for the present investigation. The healthy and active fish only are obtained from the local fish farms. The fish, then are acclimatized before experimentation to the laboratory conditions in large plastic water tanks as per the guidelines of APHA (1998, 2005 & 2012 and OECD 2019) for three weeks at a room temperature of 28 ± 1°C. The physiochemical parameters that are for water wherein the present experiment is conducted includes:

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) - LS. Iron (as Fe)- Nil, Dissolved Oxygen - 8-10 ppm, Temperature - 28±2°C.

Periodically the water that renewed every day with 12-12 h cycle. During the period of acclimatization, the fish were fed *(ad libitum)* with groundnut oil cake and rice bran. But the feeding was stopped one day prior to the actual toxicity test. All the precautions laid by the committee on toxicity tests to aquatic organisms (OECD, 2019 and APHA, 2012, 2005, 1998) are followed and such acclimatized fish are only used for experimentation. In that process, if the mortality by 5%, occurs in any batch of fish, the entire batch was discarded. The fish were exposed for 4 days in lethal and 10 days in sublethal concentrations of both the technical grade as well as 10% WP by considering the 96 hrs LC_{50} values only of the toxicity experiments.

Similar such exposure of batches of same number for both acute and chronic concentrations apart from control. The exposed concentrations are acute 2.2 μg/L, chronic (1/10th of 96h LC_{50}) 0.22 μg/L for technical grade and for also 10% WP 1.4 μg/L, and 10% WP chronic (l/10th of LC_{50} value) 0.14 μg/L as per recommendations of (OECD, 2019 and APHA 2012, 2005, 1998). From such exposed fish only the survived ones after 4 days (96 hrs) in lethal concentrations and after 10 days in sublethal concentrations of both technical grade and 10% WP are studied for the quantitative estimations of Total proteins, Glycogen, LDH, AAT and ALAT in gill, liver, brain, kidney and muscle tissues of the fish. The technical grade of Cyfluthrin was from Bayer India (Pvt.) Ltd., (Central Avenue Hiranandini estate, Mumbai-400 067) and formulation 10% WP from same company is purchased from the local market. The total proteins are estimated by the method of Lowry *et al.* (1951), Glycogen, Kemp *et al.* (1954), LDH by Srikantham and Krishna Murthy (1955); AAT and ALAT by Rietman and Frankel (1957).

RESULTS

Total Proteins

The results obtained for the total proteins and the percent changes also, exposed to both technical grade Cyfluthrin and 10% WP in sublethal and lethal concentrations to the fresh water fish *Catla catla* are graphically represented as Figure 1 and 2 respectively.

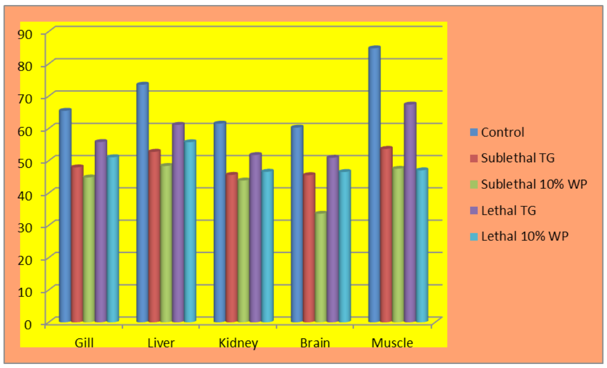

Fig. 1. The amount of total protein content (mg/g wet weight of the tissue) and % change over control in the different tissues of the fish, *Catla catla* exposed to sublethal (SL) and lethal (L) concentrations of Cyfluthrin sublethal technical grade (TG) and 10% wettable powder (WP).

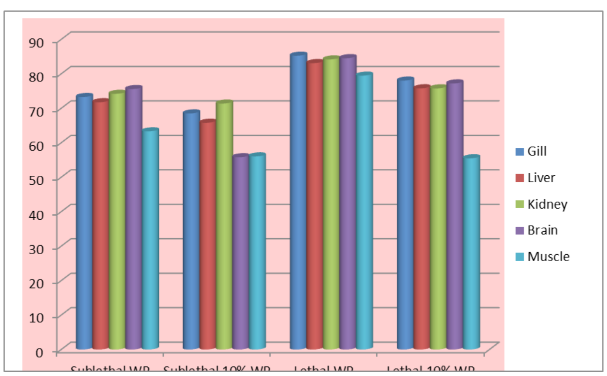

Fig. 2. Percent changes of total proteins mg/g^{-1} in the tissues of the fish *Labeo rohita* exposed to Bifenthrin Technical Grade (TG) & 10% EC in sublethal (SL) and lethal (L) concentrations

The decrement series of the proteins in sublethal concentrations of the technical grade is Brain > Kidney > Gill > Liver > Muscle and for 10% WP is Kidney > Gill > Liver > Muscle > Brain. In lethal concentrations for the technical grade series is Brain > Gill > Kidney > Liver > Muscle and for 10% WP Gill > Brain > Liver > Kidney > Muscle.

Glycogen

The results that are obtained for the glycogen are graphically depicted as Fig. 3 and for the percent changes in Fig. 4. The glycogen series decreased in the series as follows:

Chronic
- Technical grade Kidney > Gill > Liver > Brain > Muscle
- 10% WP Kidney > Gill > Brain > Liver > Muscle

Acute
- Technical grade as Gill > Brain > Kidney > Muscle > Liver
- 10% WP Gill > Kidney > Brain > Muscle > Liver

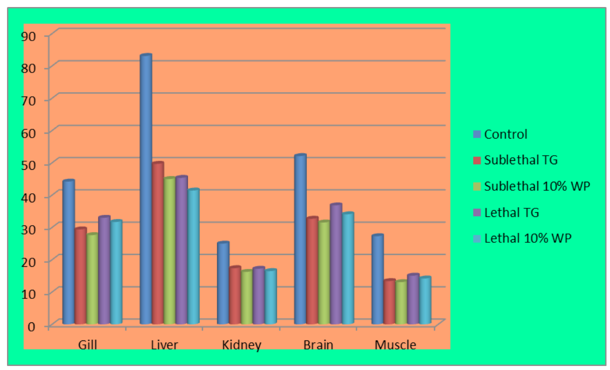

Fig. 3. The amount of Glycogen content (mg/g wet weight of the tissue and % changes in over control in different tissues of the fish *Catla catla* exposed to sublethal (SL) and lethal (L) concentrations of Cyfluthrin, technical grade(TG) and 10% Wettable powder (WP)

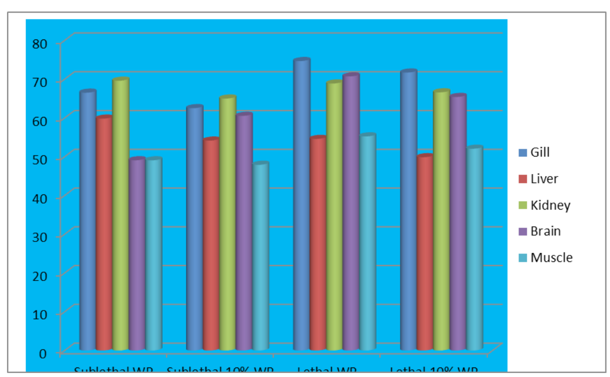

Fig. 4. Percent changes over Glycogen content (mg/g wet weight of the tissue and % changes in over control in different tissues of the fish *Catla catla* exposed to sublethal (SL) and lethal (L) concentrations of Cyfluthrin, technical grade(TG) and 10% Wettable powder (WP)

Lactate Dehydrogenase (LDH)

The results obtained as of the increment of the enzyme Lactate dehydrogenase (LDH) are graphically presented as Fig. 5 and the percent changes in the graph as Fig.6. The lyotropical series of the increment for the targeted enzyme are:

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

Lethal
- Technical grade as Kidney > Liver > Brain > Gill > Muscle
- 10% WP Kidney > Brain > Liver > Gill > Muscle

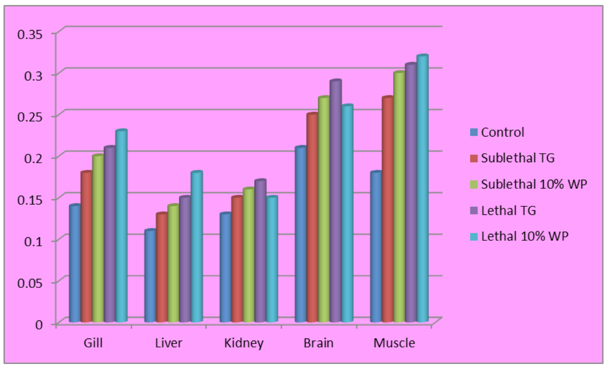

Fig. 5. Lactate dehydrogenase (LDH) μ moles of formazan/mg/protein/hr/ and % change in over the control in different tissues of the fish *Catla catla* exposed to sublethal (SL) and lethal (L) concentrations of Cyfluthrin, technical grade (TG) and 10% wettable power (WP)

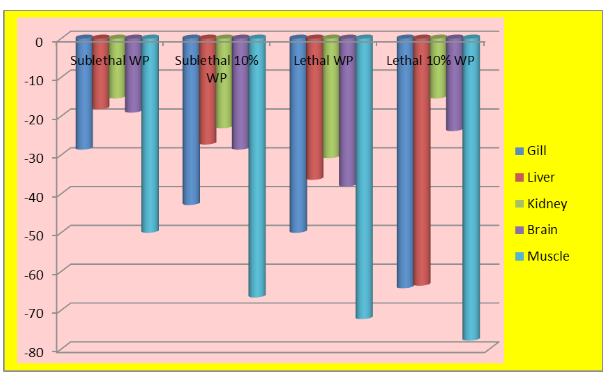

Fig. 6. Percent changes of Lactate dehydrogenase (LDH) μ moles of formazan/mg/protein/hr/ and % change in over the control in different tissues of the fish *Catla catla* exposed to sublethal (SL) and lethal (L) concentrations of Cyfluthrin, technical grade (TG) and 10% wettable power (WP)

Aspartrate Aminotransferase (AAT)

One of the transferase enzyme, Aspertate Amino Transferase (AAT) that resulted as alterations in the fish *Catla catla* exposed to both technical grade Cyfluthrin and 10% WP are graphically presented in Fig. 7 and the percent changes in Fig. 8 as a graph.

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

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

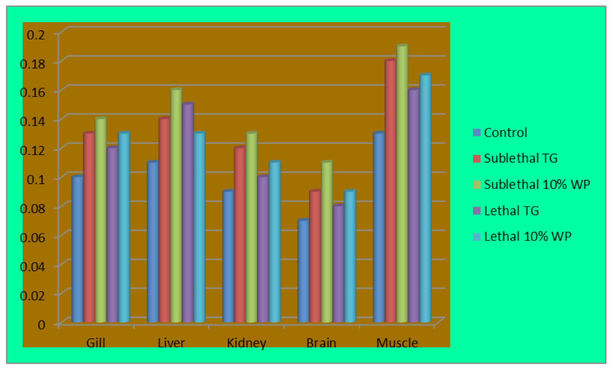

Fig. 7. Aspartrate Aminotransferase (AAT) μ moles of pyruvate formed / mg / protein / hr and % changes over the control in different tissues of fish *Catla catla* exposed to sublethal (SL) and lethal (L) concentrations of Cyfluthrin, technical grade (TG) and 10% wettable powder (WP)

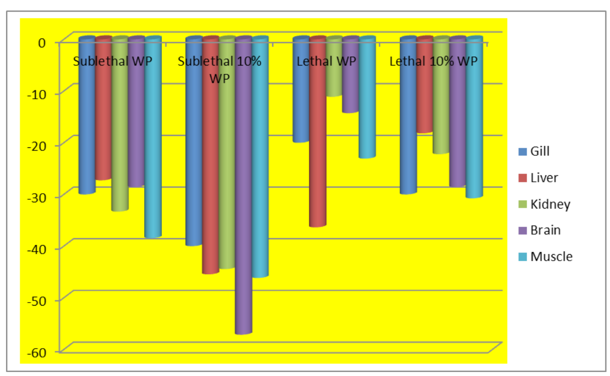

Fig. 8. Percent changes of Aspartrate Aminotransferase (AAT) μ moles of pyruvate formed / mg / protein / hr and % changes over the control in different tissues of fish *Catla catla* exposed to sublethal (SL) and lethal (L) concentrations of Cyfluthrin, technical grade (TG) and 10% wettable powder (WP)

Alanine Aminotransferase (ALAT) Enzyme

The quantitative values of the increase for the transferase enzyme Alanine Amino Transferase (AALT) that are increased are graphically presented as Fig. 9 and its changed values as of percent are also graphically represented as in Fig. 10. The increment series for the transferase enzyme is as follows:

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

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

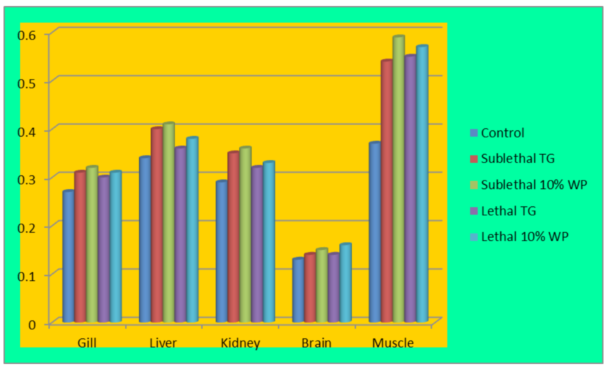

Fig. 9. Alanine Amino Transferase activity (ALAT) μ moles of pyruvate formed / mg/ protein / hr and % change over in control in different tissues of the fish *Catla catla* exposed to sublethal (SL) and lethal (L) concentrations of Cyfluthrin, technical grade (TG) and 10% wettable power (WP).

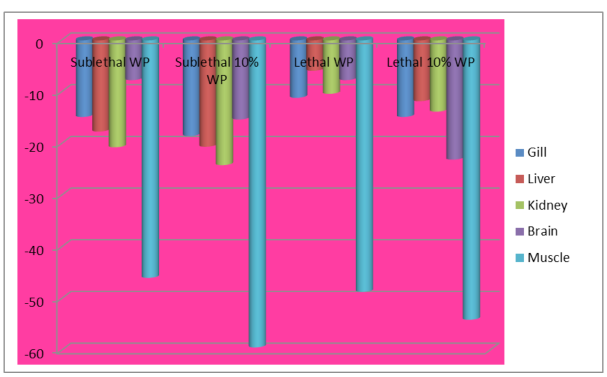

Fig. 10. Percent changes of Alanine Amino Transferase activity (ALAT) μ moles of pyruvate formed / mg/ protein / hr and % change over in control in different tissues of the fish *Catla catla* exposed to sublethal (SL) and lethal (L) concentrations of Cyfluthrin, technical grade (TG) and 10% wettable power (WP).

DISCUSSION

Shefali *et al.* (2021), while mentioning the pesticides impact on the aquatic bodies (where by 2050 year it becomes 10 billion tonnes) opined that growth is the important aspect when metabolism got impaired due to the variations in the biochemical constituents of the fish.

According to Rajamohan *et al.* (2020) some of the factors, that the aquatic organism got effected includes the concentration duration and persistence primarily and secondarily the stress factor, antigen mode of immune response, and the toxic action. Biochemically changes due to continuous exposure in chronic levels bring, such variations in the biochemical constituents of the cells/tissues/organs.

Pradip Kumar Maurya *et al.* (2019) too opined that if any exotic toxic chemical when become an integral part of the ecosystem disturbs the ambient organism life that effect biochemically, the food chain relations all, are being altered that pave the way for habitat change.

Sana Ullah *et al.* (2022) reported on the bifenthrin toxicity. In *Ctenopharyngodon idella* that was a biomarker study in which biochemical aspects of total proteins, enzymes of antioxidant nature, which includes superoxide dismutase (SOD), and others such as Catalase (CAT), Peroxidase (POD), glutathione peroxidase (GSR-Px), glutathione reductase (GR), DNA, RNA apart from Acetyl Cholinesterase, etc. all showed alterations.

In the tissues of brain, liver, gills and muscle tissues, 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 for synthesis they were used [Gluconeogenesis]. They also opined that proteins decrement might also be due to the lesions of the respective tissues/organs that finally damaged them by the toxic effect or it might be also be due to inhibition of the protein synthesis where in the entire machinery got weakened (no transcription).

Arsian *et al.* (2022) reported in the bivalve (*Unio delicates*) some of the biochemical variations in the blood also, due to the present tested toxicant.

Zeeshan and Parveen (2022) reported that due to contamination of the pesticides in the river ganga biochemical alterations were resulted in fish, *Rita rita* and *Cyprinus carpio*.

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

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

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

Satyanarayana *et al.* (2020) while studying on *Ctenopharyngodon idella* fish studied and reported in Glycogen content as well as the enzyme lactate dehydrogenase (LDH) exposed to permethrin and bifenthrin which is tested now also belong to the same group of the pyrethroids (Type-I). Even in the present study, result of decrease of glycogen and an increase of the enzyme activity of LDH, the same also that was reported, by them.

Carlos *et al.* (2018) reported on the biochemical changes in the fish *Mochilodus lineatus* due to exposure of lambda cyhalothrin and the same results of the present study.

Siroshi (2007), on the permethrin toxicity in *Heteropneustes fossils* fish even reported that 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* respectively reported that using the synthetic pyrethroid of type II (Deltamethrin and cypermethrin), glycolysis was promoted releasing more energy from glucose for the 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 was due to the result of the physical and chemical stress of the toxicant. Ogueji and Auta (2007) and Ibrahim *et al.* (2013) also reiterate this line of same defense. It is reported that due to the toxic stress the glycogen was depleted (aerobic pathway) and 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 in the fish *Ctenopharyngodon idella,* Tilak *et al*. (2002) reported in the similar lines too.

Neelima *et al.* (2015) cypermethrin as the toxicant in *Cyprinus carpio* fish, Adeyemi olaekan (2014) in the fish *Clarias gariepinus* using the same toxicant as above, Naik *et al.* (2016) in *Labeo rohita* fish using the same chemical reported earlier all. reiterated the same aspect as reason of the decrement.

Rathnamma *et al.* (2007) using deltamethrin exposure that resulted as the same result of now, that was reported of the decrement (due to the enzymes phosphorylase and glycogen synthase failure). In *Channa punctatus* treating with the toxicant also, reported that the fish is modulating the physiological and metabolic responses for the proper utilization of the energy sources) (Venkata Ramudu *et al.* 2007). Veeraiah *et al.* (2013) in *Cirrhinus mrigala* (Hamilton) fish due cypermethrin pesticide due to promotion of the glycolysis decrement of glycogen was mentioned as the reason.

Akbar *et al.* (2012) reported in the fish *Helicoverpa armigera* due to permethrin the LDH enzyme activity increased. Ibrahim *et al.* (2013) in *Solea sengalensis* fish cypermethrin and permethrin as well (Type II & I, respectively), exposure, because of the demand of more energy of the toxic stress the utilization of the available way of the substrate for the energy synthesis hence LDH activity increased that is what they reported. The decrement of the glycogen and increase of anaerobic pathway of the energy synthesis, LDH activity increased by which energy can be synthesized hence, that the report was also the reiterated in *Cyprinus carpio* fish due to by Neelima *et al.* (2015) Cypermethrin, Mozhdeganloo *et al* (2016) in *Oncorhynchus mykiss* with permethrin as the chemical lipid peroxidation as well as 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 the increased activity. Due to the stress shift of glucose (six carbon atom) to lactate (3 carbon atom) only the LDH enzyme activity that got increased.

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

Tanseem and Yasmeen (2020) in *Cyprinus carpio* reported that as due to the sublethal concentration exposure of the toxicant of a biopesticide, Darison in which the same aspect as of the report as outcome. Due to the failure of total metabolism which due to damage of the tissues and organs contradictory result was also reported of the present case.

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

In the review article by Sana Ullah *et al*. (2019a) of biomarkers that are for the synthetic pyrethroids showed the scope and aim of them. All biochemical aspects made as a biomarkers of study in which they mentioned some of earlier studies. In *Hypopthalmichthys molitrix* due to deltamethrin exposure as the toxicant Sana Ullah (2019b) reported a decrement of total proteins in blood, gills, liver and muscles and brain.

In the same article works that were carried by Viera and Martinez (2018); Classen *et al.* (2018) using Cypermethrin in *Prochilodus lineatus* and in *Cyprinus carpio* respectively both were mentioned. Ullah *et al* (2015) *Labeo rohita* testing with cypermethrin, Suvetha *et al.* (2015) in the same fish of above but deltamethrin as the toxicant, Ullah *et al.* (2014) in *Tor putitora* fish of same cypermethrin, Prusty *et al.* (2011) *Labeo rohita* fish due to fenvalerate and Nillos *et al* (2010) *Oryzias latipes* fish with permethrin chemical earlier works of study for synthetic pyrethroids which had effect on biochemical parameters and the results also support the present studied one all were mentioned.

The review article of Sana Ullah (2019a) also 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 hence not toxic to them.

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

Tamzan *et al.* (2019), while studying on the toxicity of bifenthrin (non en-capsulated-nanoform) in the fish *Oncorhynchus mykiss*, reported a 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 was the result they got. The mention of the Sussane *et al* (2016) wherein the pyrethroids are of endocrine disruptors as result the gluconeogenesis was promoted and also proteolysis which might be the valid reasons of the decrement for proteins and the same can be visualized even of the present. It was also reported by Velisek *et al.* (2011), to meet the necessary of energy on demand, wherein deamination followed by shunting of the respective acids of TCA cycles that resulted the decrement of proteins. More Aminoacids without increase of the protein synthesis due to the toxic stress the cytotoxicity only 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 the 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 to Glycogen). There is every possibility of gluconeogenesis (either Fatty acids or protein as a source/non-carbohydrate source for energy synthesis).

In major carps study of mixture toxicity (bifenthrin, chlorpyrifos and Endosulphan) by Huma Naz *et al*. (2017), reported the effect also wherein the enzyme activity of antioxidant enzyme, SOD (Superoxide dismutase activity) showed variations. In *Cirrhinus mrigala* also when due to cypermethrin exposure 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 the thinking line was also due to lack of proper food intake energy waste to attain homeostasis repair of the tissues/organs and finally detoxification too failed due to the stress of the toxicity.

Khalid reported alterations in AAT and ALAT due to exposure of the Cypermethrin in the fish *Cyprinus carpio*.

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

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

CONCLUSION

The fish had the effects in acute concentrations as well as in chronic. The delayed or prolonged effects are slow poisons even in the sublethal concentrations the vital organs got affected in the parameters that are studied. Metabolism the sum of the chemical reactions when got affected physiological aspects, which got delayed or impaired. Generally, the acute death even though not recommended but the chronic concentrations as sublethals are really lethal in the long run. If the fish is not having normal metabolism, the growth factor will be effected and the present studied fish *Catla catla* is a cultivable fish. Practically, the work on biochemical aspects is very meager and this is for the first time all biochemical aspects are studied and reported for the toxicants studied.

Conflict of Interest - None

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