BIOCHEMICAL ALTERATIONS AS TOTAL PROTEINS (TP), ASPARTATE, AMINOTRANSFERASES (AAT) AND ALANINE AMINO TRANSFERASES (ALAT) INDUCED BY CHLORPYRIFOS (AN ORGANOPHOSPHATE) IN THE FISH CHANNA PUNCTATA (BLOC

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Abstract

Chlorpyrifos, an organophosphate, technical grade and 20% EC induced alterations in the biochemical parameters as total proteins (TP), Aspartate, Amino Transferase (AAT) and Alanine Amino Transferases (ALAT) in the fish *Channa Punctata* (Bloch), in the laboratory after exposing them in both lethal and sub-lethal concentrations for four days and 10 days respectively taking into consideration of 96 hour LC_{50} values of the respective toxicants.

The fish vital organs, Gill, Liver, Kidney, Brain and Muscle are studied and found an appreciable quantity of percentage as decrement of TP and increment in 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 breakdown and gluconeogenesis might be also be the reason of the decrement followed by increment of the activity of the two enzymes that are studied in the biochemical parameters of the fish, which ultimately resulted increase of free Amino acids.

***Keywords*:**

Chlorpyrifos, Organophosphate, *Channa punctata*, Total Proteins (TP), Aspartate Aminotranferase (AAT) and Alanine Amino Transferases (ALAT).

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INTRODUCTION

First, there was a chemical evolution which lead to biological evolution, Saprotrophs, Autotrophs and Heterotrophs having three different lines of nutrition saphotrophic, Autotrophic and finally heterotrophic respectively. The evidence for such is attributed to electrons, protons, neutrons assembled as simple and complex substances, gaseous, liquid and finally solid state, was as ions of anion and cations organized into proteins, elements, then to carbohydrates and fats chemical substances, simple and combinations glycoproteins, glycolipid finally the nucleic acids made by hydrogen, nitrogen, oxygen, carbon and inorganic phosphorous the five very few with sulphur (HCNOP&S) with hydrogen, covalen coordinate and ionic bonds (four bonds) as three substances for proteins, carbohydrates, fats and finally the nucleic acid which contain Nucleosides and Nucleotides and all are the biochemical substances present in the cells/tissues/organs and all such made the organism. These chemical substances are formed within them, the ultimate fundamental units, cells have the metabolism, the concept of life activities. It includes, glycolysis, glycocogenesis, glyconeogenesis, protein synthesis, proteolysis, nucleic acid, synthesis and few as cori cori cycles, urea synthesis and also Acetyl cholinesterase function – all are the biochemical actions that normal take place in the cells/tissues/ organs.

All these reactions are influenced by enzymes and majority of the enzymes if they function normally, homeostasis exists for all physiological activities and are dependent on biochemical process that are involved within them.

But due to the processes of severe aspects, Mac Namara (2021) shouted the very nature of the global farm land at high pesticide risk of pesticides 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, Anamika Srivastava *et al*. (2019) 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 statewise 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 respectively.

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 also as a biomarker study which Sana Ullah *et al*. (2019) 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.

Such reports studies of biochemical alterations serving as biomarkers for synthetic pyrethroids by Sana Ullah *et al*. (2019a) and even for other pesticides by Ullah and Zorrizahara (2015), Krishna Murthy *et al*. (2013) and for Chlorpyrifos (Styanova *et al*. 2020), Sunanda *et al*. (2016), Pallavi Srivastava *et al*. (2016) and Deb and Das (2013) apart from Pallavi Srivastava *et al*. (2016) for organophosphates in general.

Such reports of study promoted to know the effects of chlorpyrifos technical grade and 20% EC exposure in the laboratory conditions in both lethal and sublethal concentrations in the fish *Channa punctata*. The biochemical parameters selected are total proteins (TP), Alanine aminotransferase (AAT), Amino alanine transferase (ALAT).

MATERIALS AND METHODS

The fresh water snake head fish *Channa punctata* an edible and economically important fish was selected with a range of size about 10 to 12 cm and 10 to 12 grams of weight, irrespective of their sex, as the test organisms for present investigation. Healthy and active fish were obtained from local market of 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 ± 1ºC. 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) 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.

Chlorpyrifos technical grade (TG) was supplied by Hindustan Agro Chemicals (India) and 20% 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 28ºC-8.16 Micro ohms/cm, pH at 28ºC-8.2.

Alkalinity

Phenolphthalein-Nil, Methyl orange as $CsCO_3$-472, Total Hardness-320, Calcium Hardness-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±2ºC.

A batch of fish, 50 numbers were exposed for 4 days in lethal concentrations of technical grade of 2.61 mg/L and another batch of 50 numbers for 20% EC as of 1.41 mg/L and similarly another batches of the same numbers in sublethal concentrations for 10 days 0.26 mg/L for technical grade and 0.14 mg/L for 20% 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, 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.

RESULTS AND DISCUSSION

Changes in Total Proteins

Calculated values for the total proteins along with standard deviation and percent changes in the respective tissues / organs that were tested and the amount of total protein (mg g^{-1}) in the different tissues over control, in the fish *Channa punctata* exposed to chlorpyrifos Technical (TG) & 50% EC are graphically plotted and presented as in Fig. 1 and also percent changes in Fig. 2. The decrement that was observed in the tested tissues gill, liver, kidney, brain and muscle of the fish over the control were found significant.

The trend as the changes as per the table in the five tissues tested, in the total proteins are in the decrement order as muscle > brain > liver > kidney > gill, both in technical grade and commercial formulations exposures (Fig. 1). The Protein decrement that will have a profound effect on the growth of the fish when such toxic action prevail.

As chlorpyrifos might negatively affect the availability of oxygen while during inspiration an hypoxia condition resulted. The lactic acid accumulation, anaerobic path way was the only alternative source of energy, a demand during the stress and the proteins were the only alternate sources. By degeneration of the aminoacids, proteolysis resulted and glyco-neogensis pathway might also be possible which all favoured depletion of the cellular backbones of the biochemical constituent, while working on the fish, *Hypothalmichthys moltrix* (Sana Ullah *et al*. 2019b) in the blood, liver, gill, muscle, brain and also glucose levels, the depletion of proteins in the fish were reported. When muscle got effected it results in energy deficiency which will have bearing the fish movement. Ullah (2015) too, reported in the fish *Labeo rohita*, even using cyhalothrin as the toxicant, the protease activity increased resulting the depletion of the proteins. Ullah *et al*. (2014) reported by using cypermethrin as the toxicant in the fish *Tor tor*, Prusty *et al*. (2011) using fenvalerate as the toxicant and Montana *et al*. (2014) in the fish *Rhamdia quelen*, using cypermethrin as the toxicant reported in the similar lines of the decrease of the proteins and due to the toxic stress to cope the energy demand as they are the only the alternative sources by which means they got depleted.

The permethrin study of the depletion of the proteins by Satyanarayana *et al*. (2019) in the fish *Ctenopharyngodon idella*, Neelima *et al*. (2016), in the fish *Cyprinus carpio* using cypermethrin EC as toxicant, Neelima *et al*. (2017) in the fish *Cirrhinus mrigala* using cypermethrin (25% EC) and Neeraja and Giridhar (2014a) in the fish *Labeo rohita* all, also reported the depletion of the total proteins and all of them mentioned that the cause as the proteolysis to augment the energy crisis due to the toxic stress which also supported by using the triazine and deltamethrin in the fish Prussian carp and common carp a report of Velisek *et al*. (2011). All the above that are mentioned the synthetic pyrethroids, that along are with organophosphates, too are the inhibitors of Acetyl Cholinesterase enzyme (Prusty *et al*. 2015), Hasibur Rahman *et al*. (2014), Ahrar Khan *et al*. 2012). Styanova *et al*. (2020) in the fish liver of *Cyprinus carpio* using chlorpyrifos as the toxicant, explained that, was a study of biomarker where tissue/organ the architectural damage was the main reason of the depletion of the proteins, in lethal concentration and in sublethals apart from the damage as proteolysis is also the causative factor for the decrement of the proteins.

Yanchova *et al*. (2019) in the fish *Cyprinus carpio* due to chlorpyrifos toxic stress the architectural damage of the gill, resulting a respiratory distress that caused not to have

aerobic process of the energy synthesis that paved only the anaerobic process which resulted the alternative source of the energy, hence proteins decreased. Trivany Edvin (2019) too, chlorpyrifos, the present tested toxicant, in the fish *Oreochromis niloticus* and *Cyprinus carpio* reported due to the architectural damage of gills, the lethal action prevailed whereas in sublethals depletion.

Swarnima Kumari (2019) in the report of organophosphate causing biochemical alterations differentiated the changes of the total proteins either decrease/increase or in majority decreased in the blood and tissues/organs. Toxic action induced including chlorpyrifos which is no exception due to toxic stress the metabolism is impaired. Oxidative stress, degeneration of the tissues and the main action of the enzyme AChE that all resulted the causative reason of the depletion.

Tripathi and Rajesh (2015) in the fish *Labeo rohita*, phenthoate as the toxicant reported the depletion of the proteins, exposing to 1/10th of 96 h LC_{50} value, for 1, 4 and 8 days and also reported in the similar lines as above.

Imtiyaz *et al*. (2014) reported in their biochemical toxicity of organophates to fishes mentioned that proteins not only the main constituents of cells which form the backbone of the architecture, during the stress conditions it requires more energy to evert the toxic action mainly not to have it which all require more in terms of quantity of energy, proteins are used that cause the depletion which will be also the support and offer a good explanation for depletion even in the present study.

Illavazhahan, M. *et al*., (2017) in the fish *Labeo rohita* exposed to monocrotophos 36% EC (an organophosphate) and reported at the end of 24, 48, 72 and 96 hrs toxicity determination duration end periods, the results of the total proteins as depleted. Proteolysis the alternative source of energy synthesis resulted due to the toxic action and deamination that resulted to be in the TCA cycle as substrates (Glyco-neo-genesis) were the resulted aspects.

Sunanda *et al*. (2016) and Deb and Das (2013) in their review articles mentioned about the biochemical alterations in different fishes as reported by different researchers.

Majimdar and Anilava (2017) in the fish *Oreochromis niloticus* due to chlorpyrifos toxic stress due to the technical grade and 20% EC exposure reported the biochemical alterations of the proteins due to the toxic stress via oxidative nature resulted anaemic condition that paved the anaerobic pathway metabolism, hence proteins were the alternative source of energy a reason for decrement in the total proteins.

Somayya *et al*. (2017) in their summary report of four decades of research of organophosphorus pesticides of liver due to hepatotoxic action all OP compounds including chlorpyrifos resulted impaired metabolism that caused the depletion of liver proteins which we can interpret for other tissues/organs too, without any exception.

Anitha Bhatnagar *et al*. (2017) reported in the fish *Cirrhinus mrigala* exposed to 1/20th, 1/10th and 1/5th of 96 h for 90 days and tested for the biochemical alterations. They reported either the inhibition of the protein synthesis, denaturation of proteins or even interruption also in Amino acid synthesis the valid reasons of decrement.

Bheem Rao *et al*. (2018) in the fish *Heteroneusteus fossils* exposed to Methyl parathion another organophosphate and reported the depletion of the proteins with EC for the periods of 24, 48, 72 and 96 hrs of sublethal concentrations, only. Metabolic dysfunction not of any specific nature reported as the cause for such decrease even though they studied only EC, the study is not a comparison of both lethal and sublethal concentration and also not with technical grade and the result was at the end of each exposure of duration of 24, 48, 72 and 96 a different one of the present study.

Revathy and Krishna Murthy (2018) in the fish *Channa striatus* due to chlorpyrifos toxic action at 0.18 ppm, concentration which they have taken $1/10^{th}$ and $1/30^{th}$ of value for 5, 10 and 15 days exposure. They reported only in the three tissues gill, liver and muscle and comparison is focused on different days of exposure period but not a comparison of the tissues. The reason that they mentioned in the report as due to degradation as well as possible source of energy in its utilization. The present study is the exposure of 10 days in sublethals and lethal 4 days of both technical grade and 20% EC where EC had also significant decrement.

As per the reports of earlier researchers, for the different fish the total proteins had a decrement in the blood component as well as in tissues/organs for both the synthetic pyrethroid as well as organophosphates. They have one point in common both are the inhibitors of the enzyme Acetyl cholinesterase which is important for all the vital organs gill, liver, muscle, kidney and brain (the site of origin) at the synaptominal junction point and them flow to the target organs for their functional aspects. As a consequence, the metabolism goes in not in an orderly manner, if any changes, the biochemical actions get impaired and as a consequence it is reflected on the quantities of the total proteins which are enzymes for all the metabolic reactions. The present studied fish *Channa punctata* is a palatable flood, costlier than major carps in the market, if the proteins are depleted the fish meat had a protein depletion.

Aminotransferases (AAT & ALAT)

They play an important role as they convert aminoacids into keto acids and incorporate them in tricarboxilic acid (TCA) cycle.

Both transferases that showed alterations, Aspartrate amino transferase (AAT) and Alanine aminotransferase (AALT) are graphically presented as Fig. 3 and Fig.4 respectively apart from depicting the percent changes as Fig. 5 and Fig. 6 respectively.

In both sublethal and lethal concentrations of technical grade and 20% EC of chlorpyrifos it showed accumulation of the aminoacids as AAT and ALAT due to the toxic action in all the tissues/organs tested.

For AAT, in sublethal concentration of technical grade chlorpyrifos the gradation series of increment that is observed as Gill > Muscle > Liver > Kidney > brain whereas for 20% EC, it is Gill > Muscle > Kidney > Brain > Liver. For AAT, in lethal concentrations of technical grade, it is Gill > Muscle > Kidney > Liver > Brain, whereas for 20% EC it is Gill > Kidney > Muscle > Liver > Brain.

For AALT in sublethal concentration of technical grade chlorpyrifos the gradation series of increment it is Muscle > Gill > Liver > Brain > Kidney and for 20% EC it is Muscle > Brain > Gill > Liver > Kidney.

AALT in lethal concentrations for technical grade chlorpyrifos, the increment series is in the order of Muscle > Gill > Liver > Brain > Kidney whereas for 20% EC Gill > Brain > Liver > Muscle > Kidney.

For AAT, the most accumulated organ of both lethal and sublethal concentrations of the two toxicants the most effected organ is gill and least effected for one brain and kidney.

For AALT, the most accumulated organs of both lethal and sublethal concentration of the two toxicants, the most effected one muscle and least effected one kidney.

Fishes are sensitive to the environmental contamination of water. Hence pollutants (Scot and Sloman, 2004) and insecticides (Sana Ullah *et al*., 2009b) effect the physiological and biochemical processes that caused serious impairment in enzymes, which had an impact on the health status of the fish. The result of such changes in lethal concentrations result in death while in sub-lethal the impairment of metabolic pathways.

The increased activities of the two vital enzymes AAT & ALAT in different tissues of fish suggest either increased action as gluconeogenis which is mainly by increased transamination partially also true may be due to increased synthesis of Aminoacids. The cytolysis action of the lethal concentration resulting more free enzymes and enhancement of enzymes AAT and ALAT. For synthetic pyrethroids this was reported by Sana Ullah *et al*. 2019b), Lenin Suvetha *et al*. (2015), Banaee *et al*. (2014); Vani *et al* (2011); Velisek *et al*. (2011) and Anitha Susan *et al*. (2012).

Shehzad *et al*. (2021) made a comparative study of three toxicants, of organophosphates, chlorofenapyryl, Dimethoate and Acetamiprid and the toxic action was ranked, respectively also where due to the exposure of 10, 20 and 30 days the result as enhanced level of AAT and ALAT enzymes was reported. Mentioning the increase of the cortisol level, metabolism is enhanced, which was also mentioned by Lenin Srivetha *et al*. (2015), the same reason as the causative action by the hormone, cortisol a steroid hormone which involves in the energy metabolism. Due to the toxic stress, cell membrane was distructed as a result there was a outflow of the enzyme (Rahman *et al*. 2019). Similar reports of Dawood *et al*. (2020) and also by Hussain *et al*. (2019) while working with organochlorines/synthetic pyrethroids. The work on the AAT/ALAT, enzymes in different fish by using chlorpyrifos as well as other organophosphates by different researches reiterate the same fact, that was mentioned in the review articles of organophosphates, general, Swarnima Kumari (2019); Pallavi Srivastava *et al*. (2016), and Deb and Das (2013) for chlorpyrifos. The individual reports of Bheem Rao *et al*. (2018) using methyl parathion in the fish, *Heteroneusteus fossils*, in the fish *Aphanius dispar* using the same toxicant Nafisa Saoaib and Siddiqui (2016), Banaee *et al*. (2013), in the fish *Cyprinus carpio* using curecron (an OP compound), Baby Joseph and Raj (2011) and Tilak *et al*. (2005) in the major carps using chlorpyrifos as toxicant also reiterated the same.

According to Tilak *et al*., (2005) the elevation of AAT activity provides the oxaloacetate required for the gluconeogenic pathway to meet the additional supply of glucose for the production of energy under reduced phase of oxidative metabolism. Elevation in the levels of AAT and ALAT in different tissues of brain, liver, muscle, gill and kidney of the fishes *Catla*

catla, Labeo rohita and *Cirrhinus mrigala*, can be considered as a response to the stress induced by chlorpyrifos to generate ketoacids like α-ketoglutarate and oxaloacetate for contributing to gluconeogenesis and as energy production necessary to meet the excess energy demand under the toxic manifestations. Liver ALAT deceased to 59.4% and in plasma ALAT increased to 94.2%. This response, associated with glucose dehydrogenase (GDH) reduction using the recovery period, was attributed to impairment of amino acid metabolism and to the liver damage. The increase of heart and plasma AAT was suggested tissue injury. The tissue damage or hemorrhages in the fish exposed to the various pesticides were reported by Anita Susan (2010 & 2012).

GDH catalyses the reversible deamination of glutamate to *a*-Ketoglutarateammonia.AAT catalysis reverse transamination of glutamate and oxalo-acetate to α-ketoglutarate and Aspartate, while ALAT catalysis the reverse transamination of glutamate and Pyruvate to α-ketoglutarete and alanine. Thus, the aminotransferases with GDH contribute some strategic such as *a*-ketoglutarete, Pyruvate, oxaloacetate, glutamate etc., to oxidative metabolism (Neelima *et al*. 2016).

The elevation of AAT activity provides the oxaloacetate required for the gluconeogenic pathway, to meet the additional supply of glucose for the production of energy under reduced phase of oxidative metabolism. Elevation in the levels of GDH, AAT and ALAT in different tissues of brain, gill, kidney, liver and muscle in the fish studied as a response to stress induced by the pesticides to generate ketoacids like α-ketoglutarate, and oxaloacetate for contributing to gluconeogenesis and or energy production necessary to meet the excess energy demand under the toxic manifestation (Sana Ullah *et al.*, 2019a).

The depletion of proteins under the stress of pesticide toxicity observed indifferent tissues of fish indicates the proteolysis, prompting the suggestion that the proteins were utilized to meet the excess energy demands imposed by the toxic stress. The alterations in the levels of activity of aminotransferases induced by the organochlorine, organophosphate and pyrethroids, pesticides clearly indicate that the stress brings about the metabolic reorientation in the tissues by raising energy resources through Transaminase systems. The ratio of three pesticides as mixed toxicity shows greater impact than individual pesticides, indicating the higher toxicity.

Activities of aspartrate and alanine amino transferases ASAT and ALAT may serve as a strategic links on the behavior of protein and carbohydrate metabolism providing source of keto acids for Kreb's cycle and gluconeogenesis. They have been proposed for monitoring aquatic pollution and are considered as useful biomarkers to determine pollution levels in various aquatic ecosystems (Anitha Susan *et al*., 2010). The aspartate amino transferase catalyses the inter-conversions of aspartic acid, and α-ketoglutaric acid to oxaloacetic acid and glutamic acid, while alanineamino transferase catalyses the inter-conversion of alanine and α-ketoglutaric acid to pyruvate and glutamic acid.

Aminotransferases are important as they convert amino acids into keto acids and incorporate them into TCA cycle. Both ALAT and ASAT level increased in tissues of fish, tested suggesting the conversion of aminoacids released by the proteolysis into keto acids for energy production. The increase in ALAT and ASAT activities in our study supports earlier findings and serves as indicator of tissue damage.

CONCLUSION

Due to toxic stress, the biochemical aspects of energy sources are depleted and proteolysis resulted protein synthesis is not there and accumulation of free aminoacids due to the increase of activity of the Amino transferase enzymes. The cells, tissues and organs and finally the death of the organism resulted.

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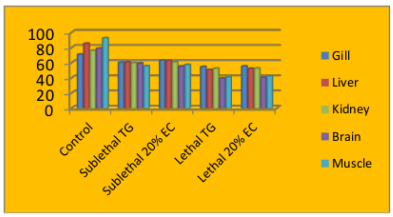
Fig. 1: The amount of total protein (mg g^{-1}) in the tissues of the fish, *Channa punctatus* exposed to Chlorpyrifos Technical Grade (TG) & 20% EC in lethal and sublethal concentrations.

Fig. 2. Percent changes of amount of total protein (mg g^{-1}) in the tissues of the fish, *Channa punctata* exposed to Chlorpyrifos Technical Grade (TG) & 20% EC in lethal and sublethal concentrations.



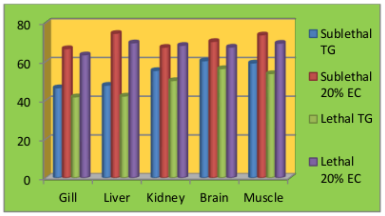
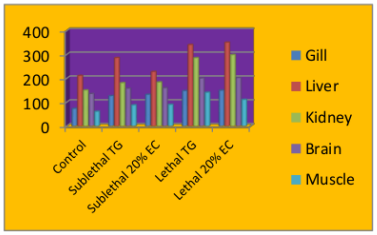


Fig. 3: Aspartrate Aminotranferase activity (AAT) μ moles of pyruvate formed mg $protein^{-1}$, hr^{-1} in the tissues of *Channa punctata* exposed to sublethal and lethal concentrations of chlorpyrifos technical grade and 20% EC.

Fig. 4: Percent changes of Aspartrate Aminotranferase activity (AAT) μ moles of pyruvate formed mg $protein^{-1}$, hr^{-1} in the tissues of *Channa punctata* exposed to sublethal and lethal concentrations of chlorpyrifos technical grade and 20% EC.



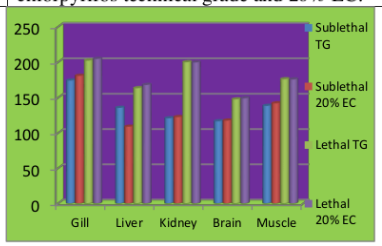
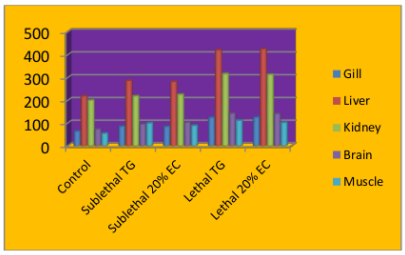
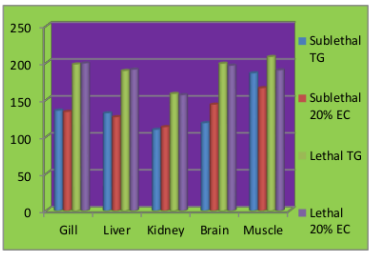


Fig. 5. Alanine Aminotransferase (ALAT) activity μ moles of pyruvate formed $protein^{-1}$, hr^{-1} in the tissues of *Channa punctata* exposed to sublethal and lethal concentrations of chlorpyrifos technical grade and 20% EC.

Fig. 6. Percent changes of Alanine Aminotransferase (ALAT) activity μ moles of pyruvate formed $protein^{-1}$, hr^{-1} in the tissues of *Channa punctata* exposed to sublethal and lethal concentrations of chlorpyrifos technical grade and 20% EC.





BIOCHEMICAL ALTERATIONS AS TOTAL PROTEINS (TP), ASPARTATE, AMINOTRANSFERASES (AAT) AND ALANINE AMINO TRANSFERASES (ALAT) INDUCED BY CHLORPYRIFOS (AN ORGANOPHOSPHATE) IN THE FISH CHANNA PUNCTATA (BLOC

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