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RESEARCH ARTICLE

BIOCHEMICAL ALTERATIONS AS TOTAL PROTEINS (TP) ASPARTATE AMINO TRANSFERASE (AAT) AND ALANINE AMINOTRANSFERASE (ALAT) INDUCED BY PERMETHRIN AND 25% EC IN THE FISH CYPRINUS CARPIO (L.)

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Abstract

Permethrin, a synthetic pyrethroid, technical grade and 25% EC when exposed to both lethal and sublethal concentrations, in the fish Cyprinus carpio in vivo, in the laboratory induced alterations in the vital organs. When had an exposure of four days and 10 days respectively for both the toxicants.The tissues Gill, Liver, Kidney, Brain and Muscle are only tested. The biochemical alterations that are assayed includes total proteins (TP), Aminotransferases Aspartate and Alanine (AAT & ALAT). The fish, in the vital organs that are studied had appreciable quantity of percentage as decrement of TP and increment of AAT and ALAT enzymes. Proteolysis and inhibition of protein synthesis resulted by which decrement and gluconeogenesis as a consequence increment of the amino transferases. An hormonal imbalance that resulted due to toxic stress that might had an impact on the effect of the toxicants. They also served as indices of the pollutants as toxicants.

Introduction:-

The environment is continuously loaded with organic chemicals (xenobiotics) released by urban communities and industries.The rapidadvancement in industrial, agricultural sectors, due to alarming rise in human population is resulting tremendous environmental contamination. The aquatic environment is affected by different types of chemical toxicants to the organisms that are originated from both natural and anthropogenic source. The pesticide has been one of the major sources of aquatic pollution not only globally and alsoin India. The effluents from pesticides are so often disposed into water reservoirs such as lakes, ponds, rivers and oceans. Pesticides that leach into aquatic habitats may influence various physiological processes of fishes. Pesticides has credited with economic potential to enhance production of food and fibers and ameliorated in vector-borne diseases, the long-term use has caused effects on human health and the environment including aquatic ecosystem that evolved new branch of aquatic toxicology (Kausiket al., 2015 and Aktaret al, 2009). The presence of a xenobiotic compound in a segment of an aquatic ecosystem does not, by itself, indicate injurious effects, connections must be established between external and internal levels of exposure, of the tissuesdue to contamination and early adverse effects. The natural physiological functioning of an organism gets disturbed on exposure to toxicant stress. It induces its effect first at cellular or even at molecular level, but ultimately causes physiological, pathological and biochemical alterations. It is, necessary to focus attention on changes in biochemical composition of organisms, which are constantly under

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pollutant threat. When the pesticides come in contact with the internal organs, irreversible changes in metabolic activities take place that eventually cause biochemical changes.

Due to the interactions between the pollutants introduced in the environment and the biotic system give rise to biochemical disturbances in the fish. Prominently, these biochemical variations are usually the first detectable and quantifiable responses to environmental changes of animal health and internal environment of the organism (Zeeshan and Parveen, 2022 and Monterio et al., 2006). Hence, the biochemical parameters of the different important tissues in the aquatic organisms are used as possible biomarkers to evaluate the toxicity of chemicals.

The use of biochemical measurements in non-target organisms as indicators of pollution, give information about the adaptive or deleterious responses in organism exposed to a certain amount of chemicals. Such analysis provides early warning signals before other toxicological points, including death are evident (Taufik Mohammadet al., 2022).

Biochemical changes are good indices of damage caused by pollution than other pathological changes. Measurement of blood biochemical parameters are used as important diagnostic tool for the detection of abnormalities in the liver and other tissues (Khalid 2014). Liver serves to protect the body from potentially harmful ingredients absorbed from the intestinal tract, gills, and skin as well as toxic by-products of metabolism by various mechanisms, such changes in biochemical parameters are important to indicate the susceptibility of altering physiological function by pollutants with reference to the toxicant stress; the toxicologists for the chronic effects have been documented by various researchers, as referred by Suchianget al., (2021); AyselGuven (2021) SanaUllah et al., (2019a & b); Ullah and Zorriezahra (2015):Prustyet al., (2015); AyselGuven (2021) and Khalid (2014).

The biochemical studies by using different fish reported earlier by Ullah et al., (2019a and b); Satyanarayana et al., (2019), Martinez (2018), Classen et al., (2018), Neelima et al., (2017& 2016), Ullah et al., (2014), Prustyet al., (2011) for the synthetic pyrethroids.

Hence, in the present, using permethrin and its formulation of 25% EC as the toxicants, in the fish Cyprinuscarpio, the common carp is tested for the biochemical changes both in lethal and sublethal concentrations in the laboratory as effects.

Materials and Methods:-

The fresh water fish Cyprinus Carpio an edible and economically important fish was selected with a range of size about to 3 to 5 cm and 3 to 6 grams of weight, irrespective of their sex, as the test organisms for present investigation. Healthy and active fish were obtained from local hatchery ofGuntur (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, 2012, 2005 &1998) are followed and such acclimatized fish only are used for the experimentation. If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish were discarded.

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 $CsCO_3$-472, Total Hardness-320, Calcium Hardness-80, Magnesium Hardness-40, Nitrite nitrogen (as N) Nil. Sulphate (As SO.) - 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 are exposed for 4 days in lethal concentrations of technical grade of 2.4 mg/l and another batch of 50 numbers for 25% EC as of 2.7 mg/L and similarly another batches of the same numbers in sublethal concentrations for 10 days 0.24 mg/L for technical grade and 0.27 mg/L for 25% 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 are 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:-

The calculated values graphically represented (Fig. 1) for the amount of total protein (mg g^{-1}) in the different tissues over control, in the fish Cyprinus carpio exposed to Permethrin Technical (TG) & 25% EC. The decrement was observed to be in gill, liver, kidney, brain and muscle over control viz., 59.04±0.37, 62.08±0.19, 53.19±0.81, 56.17±0.16 > 70.31±0.12; 58.32±0.65, 59.41±0.11, 49.32±0.04, 52.78±0.34 > 85.46±0.20; 59.12±0.42, 60.13±0.16, 52.16±0.86, 54.11±0.06 >74.56±0.03; 60.13±0.15, 53.98±0.05, 41.13±0.05, 39.65±0.04 > 78.35±0.96; 55.83±1.10, 57.21±0.02, 43.12±0.11, 41.58±0.08 > 91.52±0.71 respectively.

The trend in the five tissues tested i.e., gill, liver, kidney, brain and muscle, the changes in total proteins are in the decrement order as muscle> brain> liver> kidney>gill, both in technical and commercial formulations exposures (Fig. 1). Protein decrement has a profound effect on the growth of the fish. Protein breakdown (proteolysis) and synthesis inhibited simultaneously in all the tissues when synthesis is more than only growth results. But during pesticide stress breakdown of protein occurs which used as alternative source of energy. The fish exposed to toxic stress that stimulated protein metabolism, rather than synthesis due to decline in nucleic acids,Anithaet al (2012).

Anita et al (2010) studied on the biochemical changes in the tissue of Labeorohitaand Cirrhinusmirigalaexposed to Fenvalerate, a synthetic Pyrethroid. The decreased protein content may be due to metabolic pathway for the synthesis of glucose or due to metabolic utilization of the ketones to gluconeogenesis pathway for the synthesis of glucose or due to the directing free amino acids for the synthesis of necessary proteins or for the maintenance of osmotic and ionic regulation (Prustyet al, 2015). In sublethal concentrations the decrease was more significant which may be due to the detoxification of enzymes or mechanisms apparently slow. Further the insecticide probably acts continuously on the animal system for the longer periods, thereby rendering detoxification of mechanisms less efficient and thus making recovery slow at sublethal concentrations. Changes in varying protein profiles in different animals probably depend on the structural configuration of various Pyrethroids and other chemicals also on differential tissue responses as reported. All, were ably summarized in Gharninet al., (2020) and Sana Ullah et al., (2019a&b);Anithaet al., (2012 & 2010).

Neelima et al. (2016) reported cypermethrin as a biomarker of toxicity and the biochemical alterations in Cyprinus carpio a freshwater edible fish. The fish underwent physiological stress which is correlated with functional changes of cellular proteins. The study is also in the agreement with the present study.

Infact, the report of Sussane M Brander (2016), Pyrethroid pesticides as endocrine disruptors and molecular mechanism in fishes, too emphasize less endocrinal activating finally leading to protein breakdown of other ways of energy synthesis The transferage enzymes incorporated the ketoacids into the TCA cycle via the regeneration of glutamate (Transamination process followed by the conversion of α kutogluterate through oxidative deamination in favour of glucoleogenesis for energy synthesis. Mitochondrial damage and release of cytochromes were responsible for such thing due to tissue disruption which might be the same even in the present study (Sana Ullah et al., 2019a & b).

LeninSuvethaet al. (2015) reported on Deltamethrin toxicity and also enzymatic changes and a decrement which was observed in the fish Labeorohita which coincides with the present study result.

Jaya and Shettu (2015) reported changes in the biochemical constituents of the fresh water fish Channa punctatus (Bloch) exposed to the toxicity of Cypermethrin, the total proteins which also confirms the gluconeogenesis. The reports of the above similar lines of the present.

Prustyet al. (2015) in their review article mentioned about the nature of another type II synthetic pyrethroid as toxicant to fish, effecting the different enzymes particularly the carbonic anhydrase of the gill as a result of which not only the decrement but also the oxygen carrying capacity in protein content.

AndeyemiOlarekan (2014) reported biochemical responses of Clarias gariepinus to cypermethrin in which the total proteins the muscle and lever decreased due to exposure. There is a shift from aerobic pathway to anaerobic process argumented by the toxicant toxicity. The same conclusion can be drawn from the present study, even though the toxicant belongs to different type of synthetic pyrethroid of class, due to the action of proteolysis.According to Ullah et al., (2014) cypermethrin exposure to the fish resulted also a decrease in total proteins in the tissues/organs of the fish, for putifora using the same toxicant cypermethrin.

Ibraham, A. Elaimyet al (2013) reported that of sublethal exposure to Cypermethrin and Permethrin; significantly increased total proteins in Cypermethrin treated fish, while Permethrin altered oppositely. Their report concluded that Cypermethrin and Permethrin enhanced stress and inhibited the growth axes. One of the cultivable species, the Cyprinus carpio, a decrease in the gene expression of Insulin like growth factors that resulted in retardation of growth, which is not a good sign for the culture practice.

Satyavardhan (2013) reported the effect of fenvalerate and Malathion on the biochemical constituents of the freshwater fish Ctenopharyngodonidella (Valenciennes) and observed that due to toxicant that influenced the proteins and glycogen levels. The result also coincides with the present study and the toxicant is type II synthetic pyrethroid and fish is different.

Shailendra Kumar Singh et al., (2010) reported the toxicological and biochemical alterations of cypermethrin, the synthetic pyrethroid against the teleost fish Colisa fasciatus at different seasons. The toxicant altered the total protein levels. They opined that the depletion of proteins due to their degradation and possible utilization of degraded products for the metabolic purposes. The proteins are mainly the components of the cell and are the chief source of nitrogenous metabolism and during chronic period of the stress, it is the source of energy. The quantity of proteins depends on the rate of its synthesis or degradation. An impairment of incorporation of aminoacids into polypeptide chain a decrement in RNA level which also the same as in the present study.

The toxic stress is exhibited even in sublethal concentration, accumulation, concentration, pathological changes and alterations in the biochemical parameters viz., proteolysis is evident even in the fish with Permethrin as toxicant as according to other synthetic Pyrethroids of either class I and II type.

Aminotransferases (ALAT) & (AAT)

Aminotransferases are important as they convert amino acids into keto acids incorporate them in TCA cycle. Both alanine aminotransferase (ALAT) and aspartate aminotransferase (AAT) level in the present study after exposure to Permethrin for both technical grade and 25% EC is graphically presented as Fig.2 and Fig.3. For technical grade exposure in sublethal concentration the observed deviation as alterations is in the order Gill > muscle > brain > kidney > liver. Whereas in lethal concentration of technical grade Kidney > gill > brain > liver > muscle and for 25% EC gill > kidney > muscle > liver > brain.

The increment of aspartate aminotransferase (AAT) and alanine aminotransferase (ALAT) (mg g^{-1}) in the different tissues over control, The deviation for AAT was observed between gill, liver, kidney, brain and muscle over control were 128.47±0.68, 131.39±0.12, 138.32±0.68, 150.27±0.14 > 74.63±0.14; 286.13±0.72, 224.51±0.34, 336.89±0.81, 342.65±0.38 > 209.24±0.6; 178.21±0.15, 184.31±0.21, 283.59±0.48, 293.17±0.81 > 149.89±0.96; 152.87±0.34, 164.46±0.12, 203.07±0.11, 208.14±0.19 > 129.41±0.37; 87.96±0.16, 89.14±0.34, 116.83±0.42, 113.28±0.13 > 58.26±0.74 respectively.

The deviation for ALAT was observed between gill, liver, kidney, brain and muscle tissues are 87.46±0.18, 84.39±0.16, 126.54±0.28, 127.23±0.34 >64.83±0.14; 263.75±0.92, 284.13±0.74, 426.89±0.11, 421.36±0.68 >216.43±0.38; 218.41±0.51, 224.91±0.14, 318.14±0.28, 315.17±0.91 > 196.14±0.18; 102.45±0.24, 109.87±0.17, 152.87±0.41, 154.89±0.18 > 96.46±0.13, 91.17±0.64, 112.03±0.32, 101.02±0.16 > 54.16±0.11 respectively.

The increment for ALAT activity as alterations are in the grade of muscle >gill > brain > liver > kidney in sublethal concentration of technical grade and for 25% EC it is Muscle > brain > gill > liver > kidney.

Similarly, in lethal concentration exposure for technical grade gradation changes from the normal value is muscle > brain > gill > liver > kidney, whereas for 25% EC Gill > brain > liver > muscle > kidney.

The increased trend in both AAT and ALAT activities indicates that there is a more conversion of amino acids into ketoacids than that is utilized as an alternative for energy synthesis.

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 (Naquash Khan et al. 2021).

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 Labeorohitacan be considered 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 (Satyanarayana et al., 2020).

The depletion of proteins under the stress of pesticide toxicity observed indifferent tissues of Labeorohitaindicates 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 (Ghamimet al., 2020).

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 (Ullah et al., 2019a and b). The aspartate amino transferase catalyses the inter-conversions of aspartic acid, and α-ketoglutaric acid to oxaloacetic acid and glutamic acid, while alanine amino transferase catalyses the inter-conversion of alanine and α-ketoglutaric acid to pyruvate and glutamic acid.

Neelima et al., (2016) reported biomarker of cypermethrin synthetic pyrethroid toxicity and biochemical alterations in Cyprinus carpio. The study mentioned about the alteration of the AAT and ALAT which can be used as indices of toxicity as in the present study.

Asma Karim et al., (2016) reported on the fish silver carp Hypophthalmichthys molitrix after acute exposure to pyrethroid and the study revealed the enhancement of amino-transferases AAT & ALAT. Due to cytotoxic nature of the cells which leaked into the plasma, the enzymes increased. This report coincides with the present study, toxicant is one and the same both the fish are exotic fish commonly known as carps.

Fishes are sensitive to the environmental contamination of water. Hence pollutants such as insecticides may affect significantly certain physiological and biochemical processes that different kinds of insecticides can cause serious impairment to health status of fishes (Farid Sohman Sabra and Ei Syed EI DeebMehana, 2015). Due to changes biochemically in different tissues the ultimate result is none other than death.

Parmith Bhattacharjee and Suchismitha Das (2014) in their review article of Deltamethrin mentioned the reports of AAT and ALAT wherein a significant increase is resulted.The elevation of AAT activity provides the oxaloacetate required for the gluconeogenic pathway to meet the additional supply of glucose to produce energy under reduced phase of oxidative metabolism.

Banaeeet al., (2014) too reported alterations in the freshwater fish Alburnusmossulensis exposed to sublethal concentrations of Fenpropathrin. The report also mentioned about the significant raise of AAT and ALAT amino transferases. AST and ALT enzymes are important in cellular nitrogen metabolism, oxidation of amino acids and liver gluconeogenesis. In stress situation increased activity of liver enzymes has stimulatory effects on gluconeogenic mechanism. Thus, increased levels of these aminotransferases activities played a role in energy supply in toxic conditions.

Kaviraj and Gupta (2014) recognized enzymes of nitrogen metabolism as biomarkers that too increased activities of enzymes such as AAT & ALAT in response to cypermethrin.

AhrarKhan et al (2012) reported haemato-biochemical changes induced by Pyrethroid insecticides in Avian, Fish and Mammanlian species and stressed the chronic use and excessive doses of pesticides like Pyrethroids may however become part of food chain leading to a series of haematologicaland biochemical alterations rendered by them.

Gabriel et al (2012) observed the effects of sublethal concentration of Cypermethrin on metabolic enzymes i.e., AAT, ALAT and ALP in gill, kidney, liver and muscle of Clarias gariepinus for 10 days exposure and the enzyme activity is significantly affected. The activities of these enzymes in all the organs showed concentration dependent activities which decreased significantly.

The increase of ALAT and AAT activities are increased as per the reports of Al Ghanim (2014); Ojutikuet al (2013); Gabriel et al (2012); Firatet al (2011).Aminotransferases are important as they convert amino acids into keto acids and incorporate them into TCA cycle. Both ALAT and ASAT level that increased in tissues of fish, tested suggesting the conversion of amino acids 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 (Veliseket al., 2011).Hereported in common carp Cyprinus carpio and rainbow trout the present studied one, due to the toxicant tested now, had the same result.

Anita Susan et al (2010), studied on biochemical changes in the tissues of Labeorohita and Cirrhinusmrigala exposed to Fenvalerate technical grade and emphasized decrease in AAT activity was more conspicuous in sublethal concentrations than in lethal concentrations which might in some circumstances be more dangerous than the brief period at a high concentration because the survivors from the latter way may be able to metabolize a excrete the toxicant over the subsequent period if no further exposure occurred. The biochemical changes induced by different pesticides to the fish was reviewed by Ullah and Jallil (2015).

Figure 1:- The amount of total protein (mg g^{-1}) in the tissues of the fish, Cyprinus carpio exposed to Permethrin Technical Grade (TG) & 25% EC.

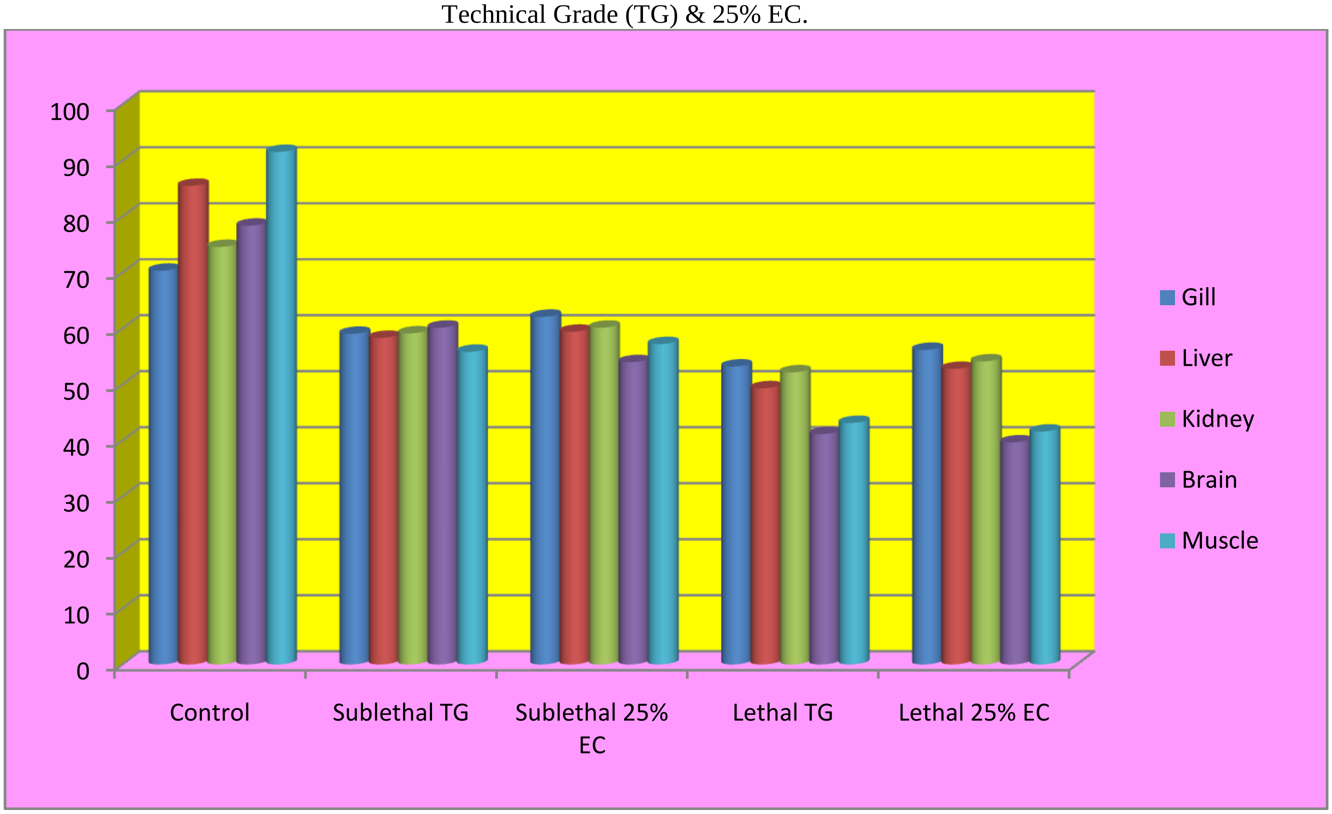


Figure 2:- Aspartate aminotransferase (AAT) µmoles of pyruvate formed mg of protein^{-1} in the tissues of Cyprinus carpio exposed to Permethrin Technical Grade (TG) & 25% EC.

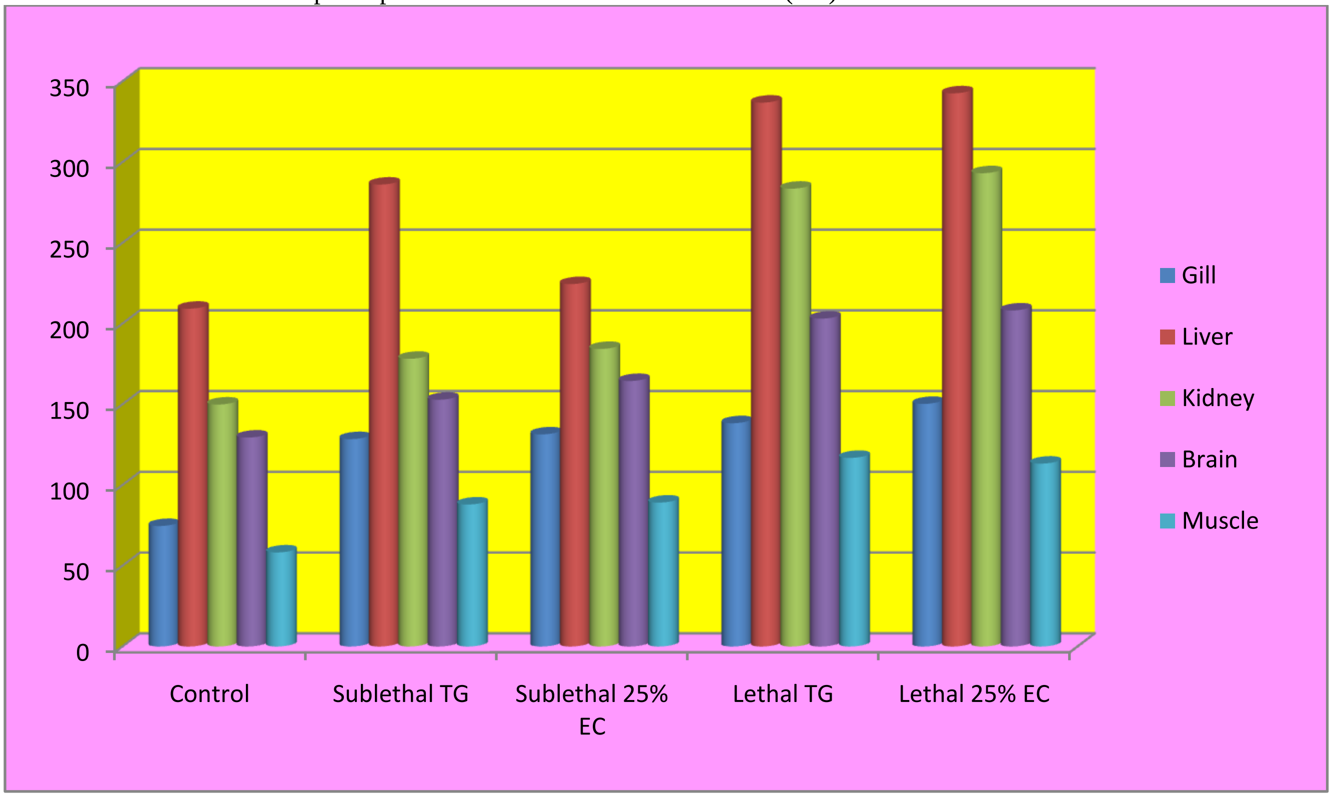
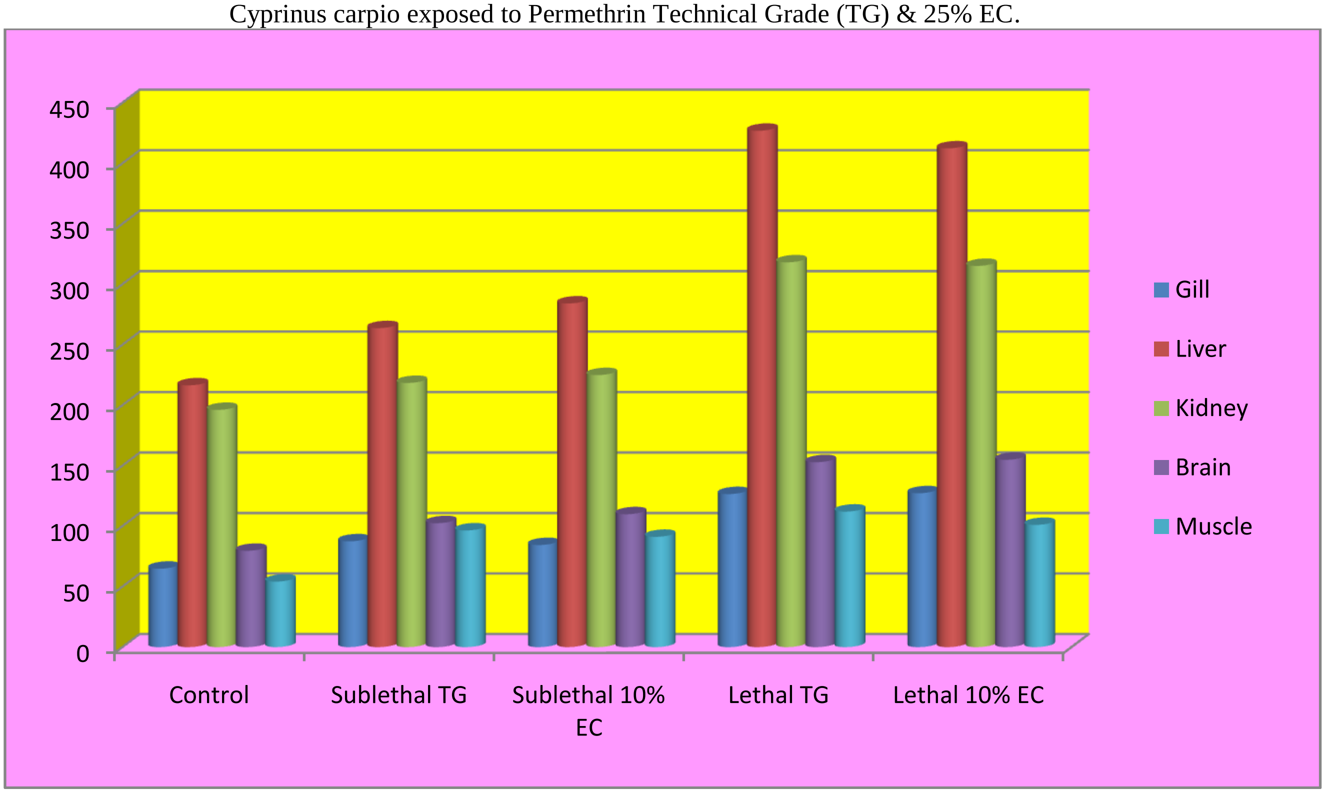


Figure 3:- Alanine aminotransferase (ALAT) µmoles of pyruvate formed in mg of protein $^{-1}$ in the tissues of Cyprinus carpio exposed to Permethrin Technical Grade (TG) & 25% EC.



Conclusion:-

Due to toxic stress, the fish had an effect of the energy source due to lack of prime source, had an determined size depletion which had tremendous impact on the cultivable source. Aerobic oxidation resulted decrement and promoted anaerobic pathway due to which accumulation of free aminoacids that resulted increase of transferase enzymes. Monitoring of the pesticides in the water is a necessity for the control of pollution and its monitoring especially that receive the water for aquaculture practices. These parameters serve as indicators of pollution also.

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