Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 22 (2) : 2020 : 338-345

ISSN-0972-3005

CHANGES IN GLYCOGEN AND LACTATE DEHYDROGENASE ENZYME ACTIVITY INDUCED BY THE PERMETHRIN (A SYNTHETIC PYRETHROID) TECHNICAL GRADE (TG) AND 25% EC IN THE FISH *CTENOPHARYNGODON IDELLA* (GRASS CARP)

S. SATYANARAYANA[1]*, G. SRINIVASA RAO[2] AND N. GOPALA RAO[3]

[1] Govt. Junior College, Chagallur 534 342, West Godavari (Dt.), A.P., India
[2] Department of Zoology, S.N. Govt. Junior College,
Chebrolu 522 212, Guntur Dist., A.P., India
[3]Department of Zoology and Aquaculture,
Acharya Nagarjuna University, Nagarjunanagar 522 510, A.P., India

(Received 3 November, 2019; accepted 4 February, 2020)

***Key words**: Permethrin, Type I Synthetic Pyrethroid, Ctenopharyngodon idella, Glycogen and Lactate dehydrogenase (LDH).*

Abstract – Permethrin, type I synthetic pyrethroid technical grade (TG) and 25% EC induced alterations in the biochemical aspects of the glycogen and Lactate dehydrogenase enzyme (LDH) activity in the fish *Ctenopharyngodon idella* in the laboratory after exposing them to both the lethal and sub lethal concentrations for 4 days and 10 days respectively taking into consideration of 96h, LC_{50} values of both the toxicants. The fish vital organs, Gill, Liver, Kidney, Brain and Muscle are studied after the exposure and found that glycogen a chief and rich energy source of the fish is utilized to cope the demand of the toxic stress. It is also observed that the lactate, (lactic acid) substrate supposed to be acted on by the enzyme Lactate dehydrogenase showed changes in the activity levels. Glycolysis, to release the sugar to have the metabolism by aerobic pathway and accumulation of the lactic acid due to stress prevailing the anaerobic pathway are also observed in the present study.

INTRODUCTION

Advantages and disadvantages are there while using the pesticides and is a must to protect the agricultural crops and also in the disease management of the pond while in aquaculture practices. In the former, venture definitely they provide the advantages but when transported into the aquatic environment as in the later venture, the disadvantages prevail. When transported by various means these chemicals, into the aquatic environment the ambient living organisms as non-target ones particularly the fish are affected. This is not only the global but also the local problem as opined by the environmental, toxicological reports in the field of ecotoxicology by Kaushik Mondal *et al*. (2014); Velisek *et al*. (2011) and Jarslava *et al.* (2019).

According to Liliana *et al*. (2019), who emphasized that the non-target organisms, Fish and Frogs are affected by many chemical types of pesticides. It is not the quality but the quantity, when used sometimes indiscriminately resulting changes as biochemical, haematological and histopathological in the fish and will be the indices of the toxic stress and are also designated as biomarkers. This contamination of the toxic chemicals is noticed even in developing countries like, India as mentioned by Agarwal *et al.* (2010) and also by Sreeya *et al.* (2017).

Ahrar Khan *et al.* (2012) reported the biochemical changes that are induced by pyrethroid pesticides the one aspect described above which also include the present studied chemical, permethrin the synthetic pyrethroid of type I. Such studies serve as indicators in the field of toxicological and same is

dwelled by Anilava Kaviraj and Abhik Gupta (2014). Velisek *et al*., (2011, 2009a, b, c, 2008, 2007, 2006a, b) too worked and reported as individual work of research, finally even in the reviewed article by them, stressed the need to know the biochemical changes in the fish that caused were as alterations of the physiological disturbance while in the toxic stress.

The interactions between the pollutants as toxicants, introduced into the environment as a defilement act, gave rise to biochemical disturbances in the fish (Banace, *et al*. (2011) and also mentioned in the review articles by Sana and Zorriezehra (2015); Prusty *et al*. (2015). By various researchers any notable restyling effect of the toxic stress lead to physiological susceptibility of the organisms like fish which were well documented by various authors.

Hence, the aim of the study is to examine, the biochemical alterations in glycogen and the enzyme activity of LDH in the fish exposed to the permethrin technical grade as well as 25% EC to evaluate them as biomarkers in the field of ecotoxicology.

MATERIALS AND METHODS

The fresh water grass carp, *Ctenopharyngodon idella* is an edible and economically important fish was selected with a range of size about 3 to 5 cm and 3 to 4.5 grams of weight, irrespective of their sex, have been chosen as the test organisms for the 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, 2012; 2005; 1998) 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 Sudharshan Chemical Industries, Pune and 25% EC was purchased from 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 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±2°C. A batch of fish, 50 numbers were exposed for 4 days in lethal concentrations of technical grade of 0.21 µg/L and another batch of 50 numbers for 25% EC as of 0.122 µg/L and similarly another batches of the same numbers in sublethal concentrations for 10 days 0.021 µg/L for technical grade and 0.122 µg/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 were taken for the further biochemical analysis of glycogen and LDH enzyme activity from their respective tissues/cells. The glycogen was estimated by the method of Kemp *et al*. (1954) whereas the Lactate dehydrogenase activity (LDH) was estimated by the method of Srikantham and Krishna Murthy (1955).

RESULTS AND DISCUSSION

The amount of glycogen (mg g^{-1}) in the different tissues over control, in the fish *Cyprinus carpio* exposed to Permethrin Technical (TG) & 25% EC was graphically represented in Fig. 1. The decrement was observed as the effect of the toxicants acting as a stress agent of the studied target organs, the gill, liver, kidney, brain and muscle over control (fish organs not exposed to the toxicants) in the present study.

In the present work, the depletion of glycogen in the tissues is in the order of Kidney > Gill > Muscle > Brain > Liver, for technical grade in sub lethal concentration and Kidney > Gill > Brain > Liver > Muscle for 25% EC.

In lethal concentration the decrement is in the order of Kidney > Gill > Brain > Liver > Muscle for the technical grade in sub lethal concentration and Kidney > Gill > Brain> Muscle > Liver for 25% EC.

During the stress conditions, the carbohydrate is utilized much in different tissues and is maximum in kidney for elimination of toxic metabolites. Liver and muscle are least changed due to high content and less utilization in liver and moderate content in

muscle.

Exposure to the Permethrin caused significant changes in the glycogen contents in liver and muscle of the fish *Channa punctata* after exposure to 0.25 ppm of in 30 days, whereas maximum reduction was 43.53% in liver and 36.86%, also in the muscle of *Heteroneusteus fossils* after exposure to 0.25 ppm in 30 days that was reported by Siroshi (2007) which also agrees with the present work of decrement.

Prusty *et al*. (2015), in their review article of the synthetic pyrethroids mentioned that some of the reported studies exposing to different toxicants of type II, and overall the depletion is the result of the glycolysis, either increase in glucose value for energy synthesis in toxic stress or decrease for spending much of the energy precursor, as in the present study.

Hasibur Rehman *et al*. (2014) in their review article of toxicity of Deltamethrin type II synthetic pyrethroid mentioned also, that due to higher toxic action than permethrin of the present study, had an impact on the biochemical alterations in the fish. Similarly, the review articles by Sana and Zorriehzahra (2015) and Murthy *et al* (2013) too mentioned about the biochemical alterations in the glycogen as a parameter when the fish are exposed even to the different pesticides apart from the synthetic pyrethroids.

Velisek *et al*. (2011, 2007 & 2006) while exposing the toxicants cypermethrin and bifenthrin both of them are type II synthetic pyrethroids in the fish *Oncorhynchus mykiss,* the rainbow trout. The glycolysis resulted releasing more glucose, which is required for the toxic stress on energy demand, to combat the effect, only the possible mechanism as in the present study. They used only 10% EC, by name Talstar as in the present experiment 25% EC of permethrin which caused alterations and both are different types. They also expressed in their study the of depletion of the energy source and also in the enzymes of energy metabolism as a biomarker as opined by Anilava and Gupta (2014). As of an increase of glucose levels due to depletion of glycogen might be due to a failure in carbohydrate metabolism due to the condition of physical and chemical stress being exposed to the toxicant (Ogueji and Auta 2007 and Ibraham *et al*. (2013) by whom the same was reiterated. Srivastava *et al*. (2016) recently referring the pesticide toxicity to fish opined that a depletion of glycogen, aerobic pathway of energy synthesis due to toxic stress, when in hypoxic condition fish derives energy by anaerobic pathway and both might be the reasons for the decrement. Anita *et al*. (2010 & 2012) while using fenvalerate as toxicant in the fish *Catla catla, Cirrhinus mrigala* and *Labeo rohita* and in the fish *Ctenopharyngodon idella* by using the same toxicant (Tilak *et al*., 2002) and in the fish *Cyprinus carpio* by using the cypermethrin (Neelima *et al*., 2015) all synthetic pyrethroids of type II reported the same result as in the present study.

Adeyemi Olalekan (2014) reported a study of a biochemical response of *Clarias gariepinus* fish to Cypermethrin as the toxicant and mentioned about the decrement of carbohydrates, of 0, 5, 10, 15 and 20 µg/L over a period of five days and also the glycogen level as glycolysis that was significantly decreased in the tested tissues.

The biochemical changes of different tissues are reported as mentioned in their review article of Sana and Zorriehzalra (2015) and Murthy *et al*., (2013) where the chemical constituents of the carbohydrates are found to result in the decrement and are detrimental to culminate toxic action under stress. The decrement of the glycogen which is the chief energy source was due to the pesticide stress that demanded more utilization.

Naik *et al*., 2016, reported for the cypermethrin as a toxicant in the glycogen content in the tissues of *Labeo rohita*. The reduction was maximum 45% in liver and minimum in the Brain as 13.08%. The reductions were dependent on concentration as well as the exposure period. The report concluded that test fish *Labeo rohita* is more sensitive to the synthetic pyrethroid and demands more energy as a consequence the reserve is decreased.

Rathanamma *et al*., (2007) reported the effect of deltamethrin in the fish *Labeo rohita* and opined that the control of the glycogen metabolism under the control by the enzymes phosphorylase and glycogen synthetase, normally, but because of the toxic stress they were affected. The result revealed that as a consequence of increase in the levels of the phosphorylase activity results the breakdown of the tissue glycogen to enhance the energy output to meet the augmented energy demand under pesticide stress. Deltamethrin affects the carbohydrate metabolism through inhibitory effect of an enzyme system. The study report might be considered and can be the same mechanism in operation for the glycogen decrease in all the tissues tested of the present experiment.

Venkata Ramudu *et al*. (2007) too reported the carbohydrate metabolism in the freshwater fish

Channa punctatus (Bloch) during the sub lethal toxicity of deltamethrin in relation to the sexual dimorphism. They reported on the liver and muscle tissues only not in all as in the present study after exposure to the sub lethal concentration and for different periods of exposure. The study revealed that fish was modulating their physiological and metabolic responses for proper utilization of the energy reserves. The present study can be correlated with the decrement as the exposure period is only 10 days and not as the case of experimentation schedule by as similar report. The hypoxia condition of the toxic stress that prevailed lead to the maximum utilization of the glucose and the possible reason for decreased glycogen.

Veeraiah *et al.* (2013) reported, for cypermethrin 10% EC also induced a change in the food reserve. When exposed to the sub lethal concentration to the freshwater fish *Cirrhinus mrigala* (Hamilton). A decrease of glycogen is reported in the fish exposed to the toxicant. The process of glycolysis leads to be the causative factor for decrease in the glycogen content. In fact when carbohydrate, the glucose is decreased there is no process of glycogenesis due to the toxic stress.

The Pyrethroids also disturb molecular mechanisms in vertebrates with a focus on the fishes as endocrine disruptors as reported by Sussan *et al* (2016). The kidney the excretory organ showed drastic changes in the exposed fish particularly in the sub lethal concentrations that disturbed the adrenal gland functions there by glycolysis is inhibited. A Significant decrease in the glycogen was also reported by Patil and Patole (2012) in *Lepidocephalichthys moltrix* exposed to the sub lethal concentrations of 1/4th and 3/4th of LC_{50} values of 24h of Cypermethrin. Tiwari *et al.* (2012) revealed also that in the sub lethal doses of Cypermethrin (0.129µg/L, 0.258 µg/L for 24 h exposure and 0.082 µg/L; 0.164 µg/L for 96 h exposure of the fish *Labeo rohita* caused significant reduction in liver and muscle respectively.

Saha and Kaviraj (2009) observed a decline in the level of glycogen in *Heteroneustes fossils* due to Cypermethrin exposure of 0.3-0.5 µg/L. The depletion of glycogen in the liver was also observed in the fish *Clarias batracus* exposed to Cypermethrin (Begum, 2005).

It is not the question of the pesticide type, due to stressful condition, the metabolism of the largest gland the liver, by any of the above process are disturbed as mentioned by many researchers. The

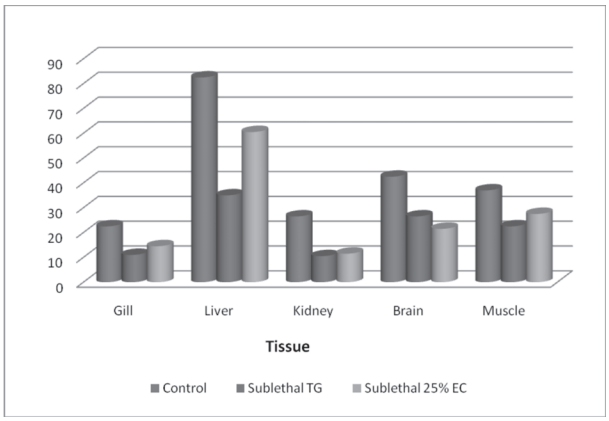

Fig. 1(A). The amount of Glycogen content (mg/g^{-1}) in the tissues of the fish, *Ctenopharyngodon idella* exposed to sublethal concentration of Permethrin Technical Grade (TG) & 25% EC

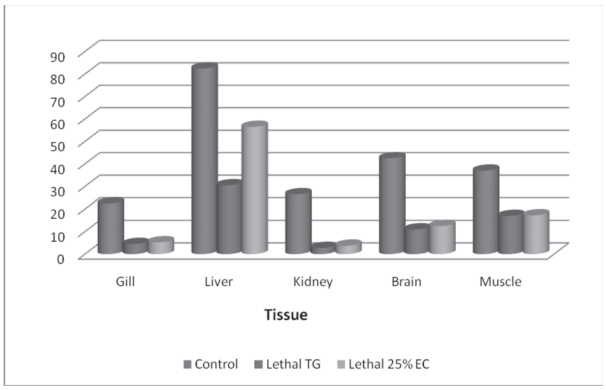

Fig. 1(B). The amount of Glycogen content (mg/g^{-1}) in the tissues of the fish *Ctenopharyngodon idella* exposed to lethal concentration of Permethrin Technical Grade (TG) & 25% EC

biochemical reactions for growth and reproduction are inadequate that lead to a profitable loss in the cultivable species as in the present studies. If, it had the similar situation not suitable for culture and sustenance of in a long run, threatening the pollutant effects in nature even in the sub lethal concentrations and that sort of information can be the outcome of the present result.

Lactate dehydrogenase (LDH)

LDH is one of the most sensitive enzyme and alteration of its functioning has a profound effect as is located at the key point of glycolysis and TCA cycle. Its strategic location to the cori-cori cycle also, any fluctuations in the cellular environment alters the activity of metabolic path way. Fish, a good swimmer needs a control of this enzyme since its catalyses, the reversible oxidation-reduction

reaction involving the lactate and pyruvate conversions to have the continuation of electron transport system of FAD and NADH of ATP the energy currency.

The amount of Lactate dehydrogenase (LDH) (mg g^{-1}) in the different tissues over the control (without the toxicant), in the fish *Ctenopharyngodon idella* exposed to Permethrin, Technical (TG) & 25% EC was graphically represented in Figure 2A and 2B. The deviations that were observed in gill, liver, kidney, brain and muscle over control are significant.

In sub lethal concentrations exposed to technical

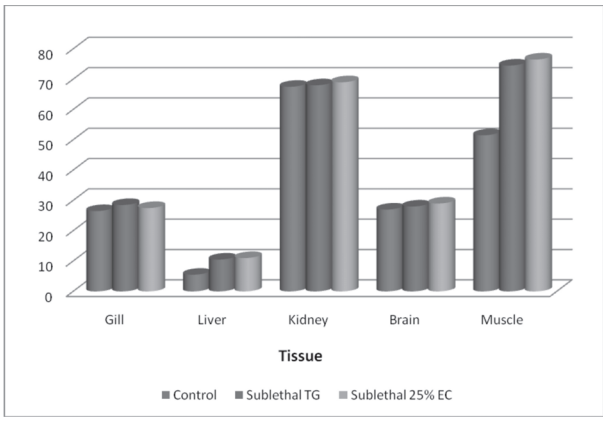

Fig. 2A. Lactate dehydrogenase (LDH) (mg of lactic acid g^{-1} wet weight of tissue) in the different tissues of *Ctenopharyngodon idella* exposed to sublethal concentrations Permethrin Technical Grade (TG) & 25% EC

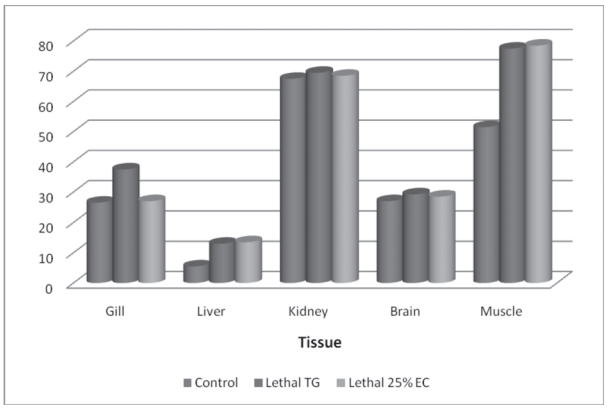

Fig. 2B. Lactate dehydrogenase (LDH) (mg of lactic acid g^{-1} wet weight of tissue) in the different tissues of *Ctenopharyngodon idella* exposed to lethal concentrations Permethrin Technical Grade (TG) & 25% EC

grade toxicant the gradation of alteration is liver > muscle > brain > gill > kidney; and for 25% EC it is liver > muscle > brain > gill >

Akbar *et al*. (2012) reported that in their study of exposure to Permethrin and fenvalerate in the fish *Helicoverpa armigera change in the enzyme LDH.* The two toxicants belong to the two categories of the synthetic pyrethroids and former is a type I of the present study whereas the latter is type II. In both the cases an increase which can be considered as 'leak' and might alter the antioxidant enzymes leading to stress in the cells of oxidative nature. Along with enzyme acetyl cholinesterase (AChE) inhibition, a neurotoxic effect on the growth is curtailed and the present studies one is also cultivable along with the other major carps. We can say that oxidative reactions are stopped due to toxic influence and the further research in the line of lipid per oxidation and enzyme work of glutathione reductase would be good aspect for the better explanation. Ibrahim *et al.* (2013) reported in the fish *Solea sengalensis* by using the cypermethrin and permethrin (Type II and Type I synthetic pyrethroids) induced biochemical changes including the LDH values which were similar as in the present study. A demand for more energy, due to toxic stress might be the reason as explanation for increase of the activity of the enzyme (LDH). The same was also reported for Lamba cyhalothrin (Type II synthetic pyrethroid) to the fish *Clarias gariepinus* and by Ogueji and Auta (2007) and also by Neelima *et al*. (2015) to the fish *Cyprinus carpio* using the toxicant the cypermethrin (type II).

Mozhdeganloo *et al.* (2015) reported that permethrin had an impact in the liver of the fish, Rainbow trout (*Oncorhynchus mykiss*) wherein the LDH activity increased and is attributed to the lipid peroxidation for its enhancement and damage of the liver might be the explanation for increase as in the present study. The liver damage of the present studied fish *Ctenopharyngodon idella* can be the fitting explanation of similar lines when an attempt is made to study histopathologically also. The cell membrane damage, that lead to a change in the permeability levels and leakage of the enzyme and any of the further consequences of the toxicant effect had induced a change in the LDH activity.

The same line of explanations were given in the review articles of Prusty *et al*. (2015) Ahrar Khan *et al*. (2012) for pyrethroids and for other classes pesticides in general by Sana and Zorriezehra (2015) and Murthy *et al*. (2013).

Specifically in the liver LDH enzyme, of the fish *Cyprinus carpio* using Glyphosphate as toxicant by

Stryanova *et al*. (2015) and the work of Banaee *et al*. (2013) also the explanation of the present work is similar in situation demand and the anaerobic pathway of energy synthesis fits good.

Scot and Slomsan (2014), due to pollutants effect in the fish that showed complex behavior and physiological alterations which were due to hormonal imbalance there by various enzymes had impact by the toxicants. Herein the present study the LDH activity as an effect might be the reason of endocrine disruption finally leading to the energy demand and is by anaerobic pathway only.

Velisek *et al*. (2011, 2009, 2007, 2006) while working on the fish *Oncorhynchus mykiss* (Rainbow trout) reported an increase of LDH activity due to deltamethrin and cypermethrin (type II synthetic pyrethroids) intoxification. The enzymes LDH, CK and Transaminases AST and ALT activity levels are increased. As a result of which glycogen catabolism and glucose shift to form lactic acid in a stressed fish is the explanation given that sounds good even in the present study. This was reiterated even in the report of Svoboda (2001) after the exposure to the cypermethrin pesticide in the fish due to stress.

Shailendra Kumar Singh *et al*., (2010) reported the toxicological and biochemical alternations of cypermethrin, synthetic pyrethroid against freshwater teleost fish *Colisa fasciatus,* wherein the LDH activity was also increased. The experiments are conducted in the laboratory temperature of 23±0.7°C whereas the present experiments temperature is slightly higher. Olalekhan (2014) studied on *Clarias gariepinus* exposed to the Cypermethrin and reported the levels of tissue lactate content acts an index of anaerobic pathway, a condition of hypoxia. The lactic acid accumulation in the muscle is an augmentation of glycolytic pathway as consequences of stress. The present study is in the similar lines of the previous reports. This was also confirmed by Muhammad Jamal Haider (2014) in the fish *Cirrhinus mrigala* using Diazinon an organophosphate as toxicant.

Saha and Kaviraj (2009) also found an increase in glucose level coupled with enzyme LDH in gill, kidney, intestine, brain and liver tissues as a result of Cypermethrin exposure and even by the same toxicant reported by Osman *et al* (2013) in different fish.

The fish are affected by different pesticides of different class including the Synthetic Pyrethroids, LDH activity alterations meet the demand of energy, in stress conditions and the present work is no exception. In culture practices, to take the fish feed either primary or secondary supplementary feed, the movement of the fish is a dire necessity and if muscle is involved in such activity the accumulation of lactic acid has bearing on the enzyme LDH. This is going to be indices for other cultivable species also which are involved in the aquaculture practices.

Das and Mukherjee (2003) in *Labeo rohita* observed Cypermethrin induced changes in LDH along with the other biochemical changes. LDH activity in all the tissues of *Channa punctatus* (Bloch) showed a very significant increase over the control. Increasing trend was more significant in lethal concentration than in sublethal concentrations was reported by Tilak *et al.,* (2009).

CONCLUSION

Thus the fish, *Ctenopharyngodon idella* when exposed to the toxicants both technical grade and 25% EC induced in the two important biochemical parameters, glycogen and Lactate dehydrogenase enzyme due to the toxic stress. Aerobic and anaerobic pathways for the synthesis of energy on demand is the study result that is not going to be a good thing for the fish as it is the one among the cultivable, hindering the growth.

Figure 2A: Lactate dehydrogenase (LDH) (mg of lactic acid g^{-1} wet weight of tissue) in the different tissues of *Ctenopharyngodon idella* exposed to sublethal concentrations Permethrin Technical Grade (TG) & 25% EC

Figure 2B: Lactate dehydrogenase (LDH) (mg of lactic acid g^{-1} wet weight of tissue) in the different tissues of *Ctenopharyngodon idella* exposed to lethal concentrations Permethrin Technical Grade (TG) & 25% EC

REFERENCES

Adeyenni Olalekar, 2014. Biochemical Response of *Clarias gariepinus* to cypermethrin. *Environmental & Analytical Toxicology*. 5 (1) : 5 pages doi 10.4172/2161-0525.1000250.

Agarwal Afa Ravi, S. Pandey and Bechan Sarma, 2010. Water pollution with special reference to pesticide contamination in India. *J. Water Resource and Protection.* 2 : 432-448.

Akbar, S.M.D., Sharma, H.C., Jayalakshmi, S.K. and Sreeramulu, K. 2012. Effect of Permethrin and Fenvalerate on the oxidative stress of *Helicoverpa armigera* change in the enzyme LDH. *World Journal of Science and Technology.* 2 (1) : 01-05.

Ahrar Khan, L. Ahmmad and Khan, M.Z. 2012. Haemato biochemical changes induced by pyrethroid insecticides in avian, fish and Mammalian species. *International Journal of Agriculture and Biology.* 14 : 834-842.

Anilava Kaviraj and Abhik Gupta, 2014. Biomarkers of type II Synthetic pyrethroid pesticides in freshwater fish. *Biomedical Research International*. Article ID 928063. 7 pages.

Anitha Susan, T. Sobha and Tilak, K.S. 2012. Toxicity and histopathological changes in three Indian major caps *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton). *International Journal of Plant and Animal and Environmental Sciences*. 2 (1) : 1-15.

Anita Susan, Sobha, K. and Tilak, K.S. 2010. Studies on biochemical changes in the tissue of *Labeo rohita* and *Cirrhinus mrigala* exposed to Fenvalerate, a synthetic Pyrethroid. *J. Toxicol. Environ. Health Sci.* 2(5) : 53-62.

APHA, AWWA and WEF. *Standard Methods For The Examination Of Water And Waste Water,* 22nd Edition, Clesceri L.S. Greenberg A.E. and Eaton A.D. (Eds). American Public Health Association, American Water Works Association Water Environment Federation, Washington, DC, USA. 2012.

APHA, AWWA and WEF. *Standard Methods for The Examination Of Water And Waste Water,* 21st Edition, Clesceri L.S. Greenberg A.E. and Eaton A.D. (Eds). American Public Health Association, American Water Works Association Water Environment Federation, Washington, DC, USA. 2005.

APHA, AWWA and WEF. *Standard Methods for the Examination of Water and Waste Water,* 20th Edition, Clesceri L.S. Greenberg A.E. and Eaton A.D. (Eds). American Public Health Association, American Water Works Association Water Environment Federation, Washington, DC, USA. 1998.

Banaee, M. 2011. Physiological dysfunction in fish after insecticides exposure. In S. Tridan (Ed.) *Insecticides – Development and Safer and more Effective Technologies.* Tech. Croaba, pp. 103-143.

Begum, G. 2005. *In vivo* biochemical changes in liver and gill of *Clarias batrachus* during Cypermethrin exposure and following cessation of exposure. *Pestic Biochem Phys.* 82 (3) : 185196.

Das, B.K. and Mukherjee, 2003. Toxicity of cypermethrin in *Labeo rohita* fingerlings biochemical enzymatic and haematological consequences comparative Biochemical Physiology. *Toxicology and Pharmacology.* 134 : 109-121.

Hasibur Rehman, Al Thbiani, Shalini Saggu, Zahid Khorshid, Abbar Anand Mohan Abid and Ansari, A. 2014. Systematic review on pyrethroid toxicity with special reference to deltamethrin. *Journal of Entomology and Zoology Studies.* 2 (6) : 60-70.

Ibrahim, A. EI-Elaimy, M.F.F. Baryomy, A.G. Kalief, G.M. Radriguez and Mancera, J.M. 2013. Pyrethroid Neurotoxic impacts on medieterranean fish Stress and growth and metabolism. *Egypt Journal Expt. Bio.* 9 (1) : 61-71.

Jaroslea Lidova, Milos Bric Autonin Kouba Josef Velisek, 2019. Acute toxicity of two pyrethroid insecticides for five non-indigenous crab fish species in Europe. *Veterinarni Medicina*. 64 (03) : 125-133.

Kaushik Mondal, B., Karmkar and Shakir, A. 2014. A Review on effects of pyrethroid pesticides on the fresh water fish behavior and fish reproduction. *Journal of Global Biosciences.* 4 (6) : 2594-2598.

Kemp, A. and J.M. Akits, V. Hejinenja, J.M.A. Kits and V. Hejneuja, 1954. A colorimetric method for the determination of glycogen in tissues. *J. Bio. Chem*. 56: 646-648.

Liliana Christina Soare, Alina Paunesm and Ponepal Christiana Maria, 2019. The morphological, physiological, histopathological and biochemical response of some non-target organisms to the stress induced by the pesticides in the environment. In : *Pesticides, Anthropogenic activities and Health of our Environment*. Doi-10.5772 intechopen 84332. 28 pages.

Luskova, V., Svoboda, M., Kolarova, J. 2001. The effect of Diazinon on blood Plasma Biochemistry in carp *(Cyprinus carpio*). *Acta Vet. Brno.* 71 : 117-123.

Mohammad Jamal Haider, 2014. Sublethal effects of Diazinon on Haematological indices and Blood biochemical parameters in Indian carp *Cirrhinus mrigala* (Hamilton). *Brazilian. Arch. Biol. Technology*. 57 (6) : 1-8.

Mozhdegauloo 2015. Permethrin induced oxidative damage in the liver of rainbow trout (*Concorynchus mykiss*) and its alteration by Vitamin-C. *Indian Journal of Veterinary Research*. 17 (1) : 31-35.

Naik Balakrishna, R. Gopala Rao, N. and Neelima, P. 2016. Sublethal toxicity of cyperkill. A synthetic pyrethroid on glycogen content in the tissues of *Labeo rohita*. *International Journal of Current Research and Academic Review*. 4 (12) : 127-134.

Neelima, Cyrill, C. Arun Kumar, J. Chandra Sekhara Rao and N. Gopala Rao, 2015. Histopathological alterations in gill, liver and kidney of *Cyprinus carpio* (Linn) exposed to cypermethrin 25% EC. *J. Adv. Research Biological Sciences.* 2 (2) : 34-40.

Ogueji Okechuk Wu and Aula, J. 2007. The effects of sublethal Doses of Lambda cyhalothrin on some Biochemical characteristics of the African cat fish *Clarias gariepinus. Journal of Biological Sciences*. 7 (8) : 1473-1477.

Olalekan, A. 2014. Biochemical Responses of *Clarias gariepinus* to cypermethrin. *J. Environ. Analytical Toxicology*. 5 (1) : 250-254.

Patil, V. and Patole, S.S. 2012. Effects of malathion and cypermethrin on biochemical constituents of freshwater fish. *Internationally Indexes and Referred Research*. 4 (39) : 33-35.

Prusty, A.K., Meena, D.K., Mohapatra, S., Panikar, P. Das, P. Gupta, S.K. and Behara, B.K. 2015. Synthetic Pyrethroids type II and fresh water fish culture perils and mitigation: A Review, *International Aquatic Research*. 7 : 163-191.

Rathanamma, V., M. Venkata Kumar, M. Vijayakumar and Phillip, G.H. 2007. Efect of Deltamethrin on glycogen phosphorylase and glucose 6 phosphatase activity in the freshwater fish *Labeo rohita*. *Bulletin of Pure and Applied Science Zoology*. 26 (2) : 1-8.

Saha, S. and Kaviraj, A. 2008. Acute toxicity of synthetic pyrethroid cypermethrin to some freshwater organisms. *Bull. Environ. Contam. Toxicol.* 80 : 49-52.

Sana Ullah, Mohammad Jallil Zorriehzahra, 2015. Ecotoxicology: A Review of Pesticide Induced Toxicity in Fish. *Advances in Animal and Veterinary Sciences*. 3 (1) : 41-57.

Scott, G.R. and Sloman, K.A. 2004. The effect of environmental pollutants on complex fish behaviour integrating behavioural and physiological indicators of toxicity. *Aquatic Toxicology*. 68 : 369-392.

Shailendra Kumar Singh, Sunil Kumar Singh and Yadav, R.P. 2010. Toxicological and biochemical alterations of the cypermethrin (synthetic pyrethroids against fresh water teleost fish *Colisa faseatus* at different seasons. *World Journal of Zoology*. 5 (1) : 25-32.

Sirohi, V. 2007. Effect of synthetic pyrethroids on Biochemical activities of some freshwater fishes. Thesis submitted to MJP Rohilkhand University, Bareilly, U.P. p.358

Sri Kanthan, T.N. and Krishna Murthy, 1955. Tetrazollium test for dehydrogenases. *J. Sci. and Research.* 14 : 206.

Srivastava, P., Singh, A. and Pandey, A.K. 2016. Pesticides toxicity in fishes Biochemical, Physiological and genitoxic aspects. *Biochemical Cell Arch.* 16 (2) : 199-210.

Sreeya, G. Nair, R. Radha and C. Radha Krishna Nair, 2017. Studies on acute toxicity to pesticides stress in a freshwater fish *Cirrhinus mrigala*. *International Journal of Fisheries and Aquatic Studies*. 5 (4) : 355-358.

Stryanova, S.V. Yancheva, I. ILIEW T. Vasileva, V. Bivolarski. Velcheva and Georgieva, E. 2015. Glyphosphate induces morphological and enzymatic changes in common carp (*Cyprinus carpio*), Liver. *Bulgarian Journal of Agricultural Science*. 21 (2) : 409-412.

Susane, M. Brander Molly K. Gabler Nicholas L. Flower, Richard E. Canon and Daniel S Chlenk, 2016. Pyrethroid pesticides as Endocrine disruptors molecular mechanism in vertebrates with a focus on fishes. *Environmental Science Technol.* 50 (17) : 8977-8992.

Svoboda, M. Luskova, V., Drashchova, M.J., V. Zlabek 2001. The effect of diazonin on haematological induces of common carp (*Cyprinus carpio* L.). *Acta. Vet. Br no.* 70: 457-465.

Tilak, K.S., Satyavardhan, K. and Thathaji, P.B. 2003. Biochemical changes induced by fenvalerate in the freshwater fish *Channa punctata* (Bloch.). *Journal of Ecotoxicology and Environmental Science.* 13(2) : 82-86.

Tilak, K.S. and Yacob, K. 2002. Toxicity and effect of fenvalerate on fish *Ctenopharyngodon idellus. J. Ecotoxicol. Environ. Monit***.** 121: 09-15.

Tiwari S., Tiwari R. and Singh A. 2012. Impact of Cypermethrin on fingerlings of common edible carp (*Labeo rohita*). *The Scientific World J.* 1:1-7.

Veeraiah, 2013. Biochemical changes induced by Cypermethrin 10% EC Pyrethroid compound in sublethal and lethal concentrations to the fresh water fish *Cirrhinus mrigala* (Hamilton). *J. Atoms and Molecules.* 3(6) : 625–634.

Velisek Joseph, Alzbeta Stara and Zdenka Svobodova, 2011. The effects of pyrethroid and Triazine pesticides on fish physiology. Chapter 17 Pesticides in the modern world pests control and pesticides exposure and Toxicity Assessment. pp 377-402.

Velisek, J., Stastra, K., Sudova, E., Turek, J. and Svobodova, Z. 2009a. Effects of subchronic simazine exposure in some biometric, biochemical, hematological and histopathological parameters of the common carp (*Cyprinus carpio* L.,). *Neuroendochrinology Letters*. 30 (1) : 236-240.

Velisek, J., Sobodova, Z. and Diackova, 2009b. Effects of acute exposure to bifunthrin on some haematological and histopathological parameters of rainbow trout *Onchorynchus mykiss*. *Veterinary Medicine.* 54 (3) : 131-137.

Velisek, J., Svobdova, Z. and Machova, J. 2009c. Effects of bifeuthrin on some haematological biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.). *Fish Physiology and Biochemistry.* 35 (4) : 583-590.

Velisek, Svobdova, Z., Piackova, V., Novotny, L., Blalova, J. Sudora, E. and Maly, V. (2008). Effects of metribuzin on rainbow trout *Oncorynchus mykiss. Vetevarnarni Medicina*. 53 (6) : 324-332.

Velisek, J., Jurickova, obsikova, R., Svobodova, Z., Pickova, V., Madhora, J. and Novothy, L. 2007. Effects of deltamethrin on rainbow trout. *Onchorynchm mykiss. Environmental toxicology and Pharmacology*. 23: 297-301

Velisek, J., Dobsikova, R., Svobodava, Z., Modra, H. and Luskova, V. 2006a. Effect of deltamethrin on the biochemical profile of common carp (*Cyprinus carpio* L.). *Bulletin of Environmental Contamination and Toxicology*. 76 : 992-998.

Velisek, J., T. Wlason, P. Gomulka Z. Svobodova, R. Dobsikova, L. Norothy and M. Dudzik 2006b. Effects of cypermethrin on rainbow trout *Oncorynchus mykiss*. *Veterinary Medicine*. 51 (10) : 469-476.

Venkata Ramudu, M., Nagabhushana Reddy, Chennaiah, K. and Indira, P. 2007. Protein changes in freshwater fish *Channa punctatus* (Bloch) during sublethal toxicity of deltamethrin in relation to sex. *Bulletin of Pure and Applied Sciences*. 26A (2) : 7-16.