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ETHION (50%EC) EFFECT ON LDH AND SDH ENZYMATIC ACTIVITY IN FRESH WATER FISH *CATLA CATLA*

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ABSTRACT:

Organophosphates are one of the most preferred pesticides due to their highly effective nature in the environment. Ethion (50% EC) is an organophosphate pesticide widely used in agriculture absorb rapidly via different routes and slowly accumulate into various vital organs of freshwater organisms ultimately causes adverse effects. In the Present study, *Catla catla* fish were exposed to Lethal(96hr LC_{50}) 2.8µg/l and Sublethal ($1/10^{th}$ of 96hr LC_{50}) 0.28µg/l concentrations of Ethion (50% EC) for a period of 1, 4 and 8 days to evaluate LDH and SDH enzymatic levels in different organs such as liver, gill, brain, kidney and muscle tissues. Results of the present study indicates that the maximum percentage of LDH activity levels increased in muscle (+28.36) and minimum in gill(+10.41)under exposure to lethal concentrations of Ethion(50%EC) for 1 day whereas, the maximum percentage depletion of SDH levels was in liver (-40.25) and minimum depletion was in gill (-19.44.) SDH activity levels was decreased in all the tissues of the experimental animal when compared with the controls.

Keywords: *Catla catla*, Ethion (50% EC) sub-lethal and lethal concentrations, LDH and SDH enzyme activity.

I.INTRODUCTION:

Pesticides are well known for their high levels of persistence and ubiquity in the environment, and because of their capacity to bio accumulate and disrupt the food chain, they pose a risk to animals and humans. Fish represent an essential component of freshwater and marine ecosystems and play an important role in maintaining their balance (Okuwosa*etal*.,2019) While acute pesticide exposure might

result in abrupt fish die-off, low-dose but long-term exposures can cause cellular and tissue damage, which happens to end up impairing health status and making them vulnerable to additional stressors (Vignet*etal.*,2019).Therefore, they are important to be focused while studying the toxic effects of various pesticides and pollutants on fish organisms are considered as most sensitive and earliest events of any pollutant damage. The changes in enzymatic system may alter the metabolic processes. More recently changes in enzymes concentrations are being employed in the evaluation of toxicological responses (Raja Saha and Sangita Maiti Dutta.,2024). Toxicologists have developed interest in studying the responses of individual enzymes or groups of enzymes to toxic insult. Some enzymes show an increased activity while others are show a decreased one with progressive days of pesticide exposure.

Lactate dehydrogenase (LDH) is an enzyme that facilitates the transfer of hydrogen and catalyses the conversion of L-lactate to pyruvate by utilising NAD^+ as a hydrogen acceptor (Inyang *et al.,* 2016). LDH, a marker of anaerobic metabolism, was anticipated to exhibit higher activity under conditions of low oxygen levels. It facilitates the final stage of anaerobic glycolysis, the reversible transformation of lactate into pyruvate, which occurs in the cytoplasm of all cells in the body. It is commonly used as a measure to identify intact cells and provides information about the cellular glycolic pathway. Consequently, LDH plays a significant role in fulfilling the energy needs of fish within a restricted period of time.

Succinate dehydrogenase (SDH) is one of the important enzymes in the Krebs's cycle. It plays an important role in mitochondria, which are structures inside cells that convert the energy from food into a form that cells can use. Within mitochondria, the SDH enzyme links two important cellular pathways in energy conversion: the citric acid cycle and oxidative phosphorylation. This catalyzes the oxidation of succinate to fumarate (Huang *et al*.,2013). The teleost fish *Catla catla* was selected for the present study due to its wide availability and suitability as model for toxicity testing and also due to sustainability in laboratory conditions. Hence, the present study is undertaken to comprehend the LDH and SDH alterations induced by Ethion (50%EC) in different tissues of the fish, *Catla catla*.

II. MATERIALS AND METHODS:

Fish *Catla catla* of size 7 ± 8 cm and 7.5± 8.5 g weight were brought from a local fish farm located in Kuchipudi, Guntur District, Andhra Pradesh, India. They were then acclimatized at 28 to 2ºC in the laboratory for a duration of 15 days. Such acclimatized fish were exposed to sublethal and lethal concentrations of Ethion (50% EC) commercial grade for 1 day, 4 and 8 days. The vital tissues like muscle, brain, liver, gill and kidney of the fish were taken for the estimation of LDH and SDH enzyme activity along with controls. LDH was estimated by the method Srikanth and Krishnamurthy (1955) with slight modifications and SDH activity was estimated by the method of Nachlas *et al*.,(1960) were performed spectrophotometrically.

III. RESULTS AND DISCUSSION:

LDH is an anaerobic enzyme. The calculated values of LDH content along with percent change over the controls and standard deviations in the muscle, brain, gill, liver and kidney of the treated fish *Catla catla* at sublethal and lethal concentrations of Ethion(50%EC) for 1day, 4days and 8days were given in Tables 1&2 and Figures 1&2. Values are presented as micromoles of formazan formed/mg protein/h. Since $P<0.05$ in all the tissues, there is a significant variation between the various exposure days was found.

In the present study, under lethal and sub lethal exposure the LDH activity was showed significant increase in all the tissues of fish *Catla catla* exposed to Ethion (50%EC) indicating LDH is an enzyme that participates in the anaerobic process of carbohydrate metabolism. The increase in LDH activity is a frequently used diagnostic marker for identifying increases in anaerobic metabolism caused by energy depletion under anaerobic and environmental stress conditions. The increased LDH activity can be related to the conversion of stored pyruvate into lactate, which is transferred through muscle to the hepatopancreas, as well as the regeneration of glucose and glycogen to provide energy to fish exposed to insecticides. In other words, increased LDH activity in the muscle and liver indicates a potential improvement in tissue glycolytic capacity. The present investigation is in agreement with the findings of Bantu *et al*., (2017).

Table: 1. Changes in the LDH content (µ moles of formazan/mg protein/hr) and % change over the control, in different tissues of the freshwater fish, *Catla catla* exposed to sub-lethal and lethal concentrations of Ethion (50%EC) for 1 day.

LDH Tissues	Control	Sublethal (mg/gm)	% Change	Lethal (mg/gm)	% Change
Gill	2.40 ±0.07	2.65 ±0.20	+10.41	2.70 ±0.28	+12.50
Liver	3.16 ±0.09	3.21 ±0.12	+15.82	3.24 ±0.01	+25.31
Muscle	2.82 ±0.01	3.62 ±0.16	+28.36	3.79 ±0.23	+34.39
Brain	1.56 ±0.12	1.75 ±0.05	+12.17	1.86 ±0.14	+19.23
Kidney	1.28 ±0.02	1.54 ±0.01	+20.31	1.58 ±0.03	+23.43

Values are the mean of five observations; (±) indicates the standard deviation:

Values are significantly at $p < 0.05$

Fig: 1. Changes in the LDH content (µ moles of formazan/mg protein/hr) and % change over the control, in different tissues of the freshwater fish, *Catla catla* exposed to sub-lethal and lethal concentrations of Ethion (50%EC) for 1 day.

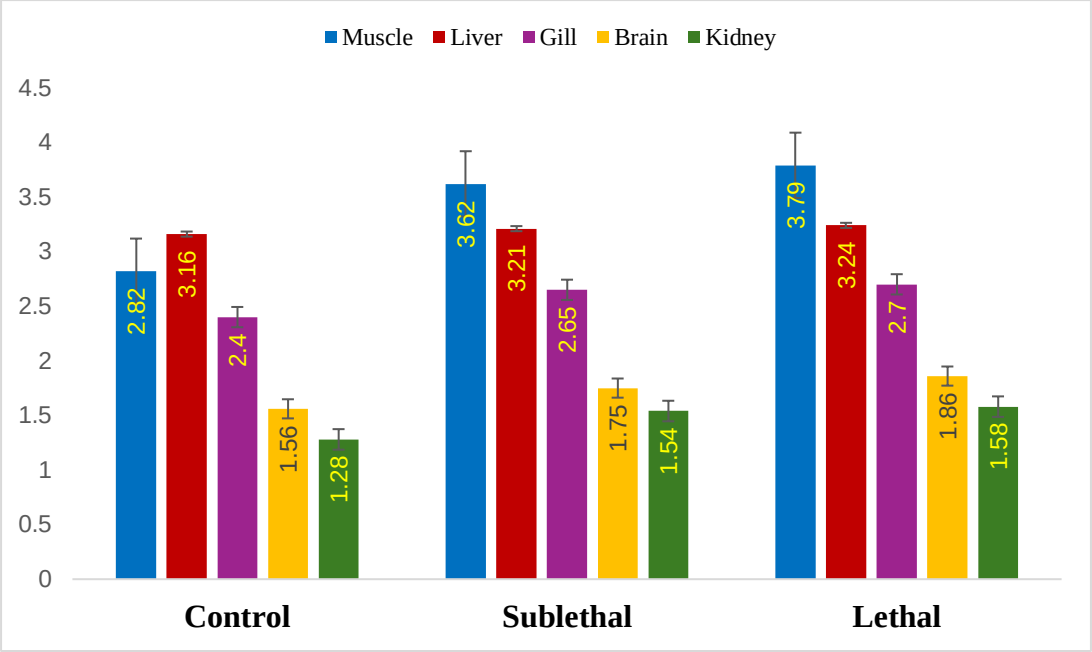


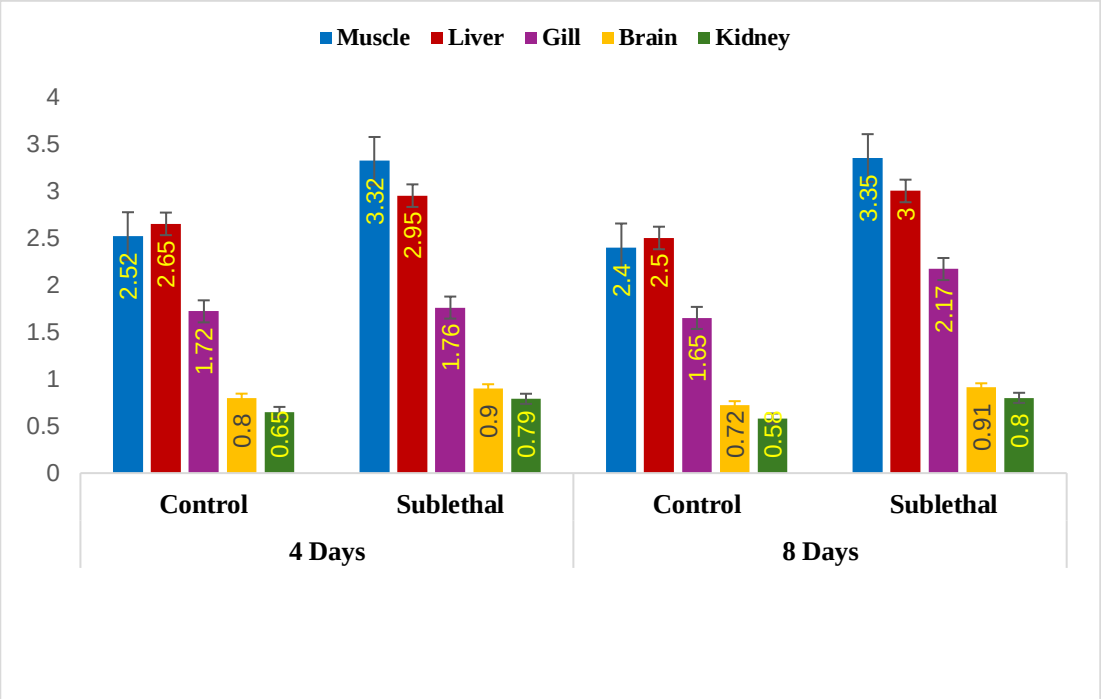
Table:2. Changes in the LDH content (µ moles of formazan/mg protein/hr) and % change over the control, in different tissues of the freshwater fish, *Catla catla* exposed to sublethal concentrations of Ethion (50%EC) for 4 days and 8 days.

LDH Tissues	4 days			8 days		
	Control	Sublethal (mg/gm)	% Change	Control	Sublethal (mg/gm)	% Change
Liver	2.65 ±0.20	2.95 ±0.05	+11.32	2.50 ±0.13	3.00 ±0.07	+20.00
Brain	0.80 ±0.01	0.90 ±0.04	+12.50	0.72 ±0.02	0.91 ±0.32	+26.38
Muscle	2.52 ±0.12	3.32 ±0.01	+31.74	2.40 ±0.24	3.35 ±0.05	+39.58
Gill	1.72 ±0.05	1.76 ±0.08	+23.61	1.65 ±0.05	2.17 ±0.12	+31.51
Kidney	0.65 ±0.15	0.79 ±0.32	+21.53	0.58 ±0.11	0.80 ±0.18	+37.93

Values are the mean of five observations; (±) indicates the standard deviation:

Values are significantly at $p < 0.05$

Fig:2. Changes in the LDH content (µ moles of formazan/mg protein/hr) and % change over the control, in different tissues of the freshwater fish, *Catla catla* exposed to sublethal concentrations of Ethion (50%EC) for 4 days and 8 days:



Lactic dehydrogenase (LDH) serves as the focal point for a finely balanced equilibrium of carbohydrate catabolism and anabolism. The stimulation of LDH and the high rate of glycolysis in fish *Catla catla* treated with Ethion (50%EC) seen in the current study indicate that the end product of glycolysis, pyruvate, was routed through the lactic acid cycle rather than Kreb's cycle under hypoxic conditions, resulting in lactic acid buildup. A similar increase in LDH activity was observed in the tissues of fish *Labeo rohita* after exposure to sublethal amounts of Deltamethrin (Mohan *et al.,* 2017).An increase in LDH activity suggested organ-specific toxicity and represented the severity of cellular harm in Ethion-treated fish. Disruption of the respiratory epithelium might have induced tissue hypoxia, resulting in oxidative metabolism, which might be responsible for an increase in LDH activity during toxicant stress (Annamalai malarvizhi *et al.,* 2012; Khan *et al.,* 2020).LDH activity was elevated in all tissues of *Oreochromis mossambicus* and *Channa punctatus* following sublethal exposures to the fungicides azoxystrobin and hexaconazole throughout the experiment (Neelanjana*et al.,* 2017).

In the present study, under lethal and sublethal exposure, LDH activity is increased indicates increased anaerobic respiration to fulfil the energy needs when aerobic oxidation decreased and reduced oxidative metabolism. LDH activity was increased in fish *Cyprinus carpio* when exposed to chlorpyrifos (Kareema A & M Venkateshwarlu,2022).Metabolic enzymes such as citric acid synthesis and lactate dehydrogenase are part of the respiratory enzymatic system. Normal activity of LDH patterns was altered in situation of chemical stress (A Anitha *et al.*,2017). Leakage of LDH is a marker of membrane permeability and cell death. They also suggested that an increase in LDH activity might to stabilization of cytoplasmic membrane (KhazaeiMonfared, Y *et al.*,2022).

The findings indicated that impacted organs or tissues may have been harmed or malfunctioning as a result of Ethion (50% EC) exposure. Furthermore, higher LDH activity could be due to induced oxidative stress, which generates reactive oxygen species (ROS) and causes cellular damage. As a result, measuring LDH activity could aid in determining organ-specific toxicity and the level of cellular harm caused by pesticide exposure (Banaee *et al.*, 2023). The results showed that administering of Ethion (50%EC) could potentially increase the LDH activity to overcome toxic stress.

Succinate dehydrogenase (SDH) activity:

In this present investigation, it can be seen that there is a rapid reflection of SDH activity in all tissues of fish *Catla catla* treated with lethal and sub-lethal concentrations of Ethion (50%EC) when compared to respective controls. The calculated values of succinate dehydrogenase activity along with percent change over controls and standard deviations are given in Tables 3 and 4 &Figures 3 and 4.SDH is a key enzyme in the citric acid cycle, catalyses the reversible oxidation of succinate to fumarate.

Succinate dehydrogenase (SDH) is a key and active regulating enzyme in the Krebs or TCA cycle. It is essential for mitochondria, which are structures within cells that transform food energy into a form that cells can use. Within mitochondria, the SDH enzyme connects two important cellular energy conversion pathways: the citric acid cycle and oxidative phosphorylation. This catalyzes the conversion of succinate into fumarate (Huang and Millar, 2013).Inhibition of NAD-dependent SDH activity resulted in a decreased passage of intermediates into the citric acid cycle (Khare *et al*., 2019). Reduced SDH activity clearly indicates TCA cycle depression, or oxidative metabolism depletion at the mitochondrial level. The decreased SDH activity indicates inhibition of O_2 at mitochondrial level (Srivastava *et al.*, 2016).

In the present study a comparable decrease in SDH activity was reported in freshwater fish *Catla catla* exposed to 50% EC of Ethion. The kidney had the largest concentration of SDH, followed by the liver and muscle, while the gills had the lowest concentration. As the number of days exposed increased, the concentration of SDH decreased significantly in all four organs. Because, succinate dehydrogenase (SDH) is mitochondrial localized, increased SDH activity in the liver and muscle reflects a greater distribution of mitochondria in the tissues. A similar decrement in SDH activity was observed in the tissues of fish *Labeo rohita* on exposure to sublethal concentrations of deltamethrin, (Mohan *et al.*,2017).

Table: 3. Changes in the succinate dehydrogenase (SDH) content (µ moles of formazan/mg protein/hr) and % change over the control, in different tissues of the freshwater fish, *Catla catla* exposed to sub-lethal and lethal concentrations of Ethion (50%EC) for 1 day.

SDH Tissues 24hr	Control	Sublethal (mg/gm)	% Change	Lethal (mg/gm)	% Change
Liver	1.54 ±0.05	1.14 ±0.08	-25.97	0.92 ±0.05	-40.25
Muscle	1.25 ±0.01	0.99 ±0.07	-20.80	0.87 ±0.03	-30.40
Kidney	1.16 ±0.04	0.89 ±0.10	-23.27	0.74 ±0.02	-34.48
Brain	0.98 ±0.03	0.77 ±0.05	-21.42	0.69 ±0.20	-29.59
Gill	0.72 ±0.18	0.63 ±0.01	-12.50	0.58 ±0.11	-19.44

Values are the mean of five observations; (±) indicates the standard deviation

Values are significantly at $p<0.05$

Fig: 3. Changes in the succinate dehydrogenase (SDH) content (µ moles of formazan/mg protein/hr) and % change over the control, in different tissues of the freshwater, *Catla catla* exposed to sub-lethal and lethal concentrations of Ethion (50% EC) for 1 day.

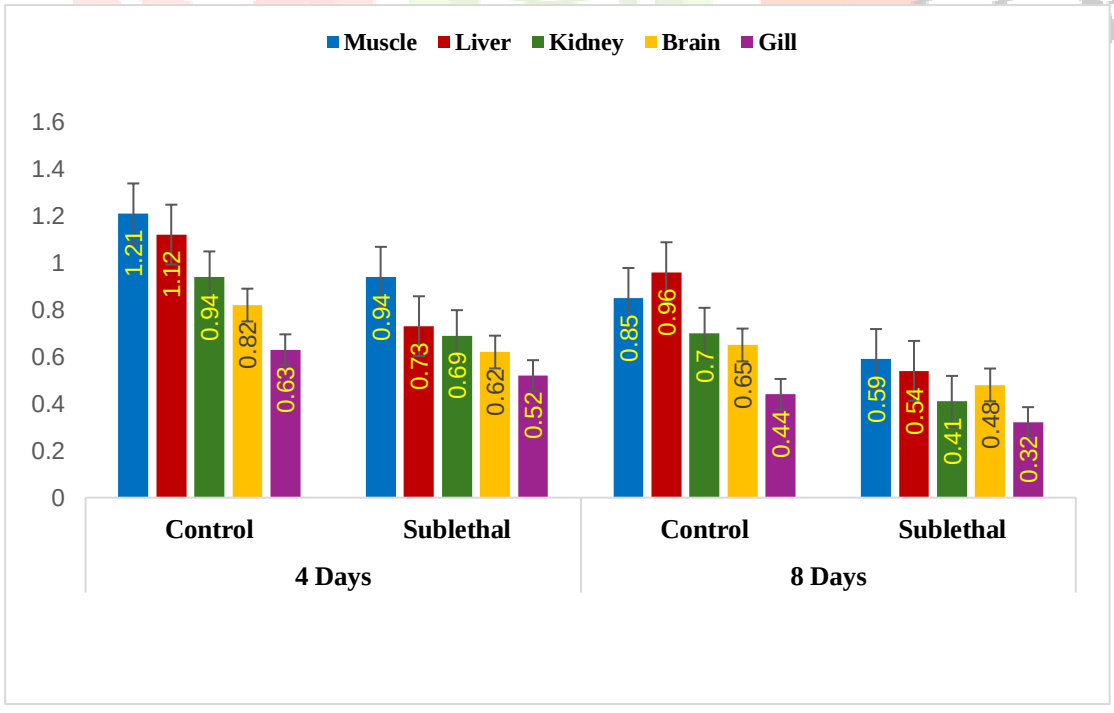
Table: 4. Changes in the SDH content (µ moles of formazan/mg protein/hr) and % change over the control, in different tissues of the freshwater fish, *Catla catla* exposed to sublethal concentrations of Ethion (50%EC) for 4 days and 8 days.

SDH Tissues	4 days			8days		
	Control	Sublethal (mg/gm)	% Change	Control	Sublethal (mg/gm)	% Change
Muscle	1.21 ± 0.02	0.94 ± 0.03	-22.31	0.85 ±0.05	0.59 ±0.04	-30.58
Liver	1.12 ±0.03	0.73 ± 0.06	-34.82	0.96 ±0.01	0.54 ±0.12	-43.75
Kidney	0.94 ± 0.03	0.69 ± 0.04	-26.59	0.70 ±0.05	0.41 ±0.15	-41.42
Brain	0.82 ± 0.04	0.62 ± 0.04	-24.40	0.65 ±0.08	0.48 ±0.01	-26.15
Gill	0.63 ±0.02	0.52 ±0.05	-17.46	0.44 ±0.01	0.32 ±0.03	-27.27

Values are the mean of five observations; (±) indicates the standard deviation

Values are significantly at $p < 0.05$

Fig: 4. Changes in the SDH content (µ moles of formazan/mg protein/hr) and % change over the control, in different tissues of the freshwater fish, *Catla catla* exposed to sublethal cncentrations of Ethion (50%EC) for 4 days and 8 days.



Decreased SDH activity was observed in *Cyprinus carpio* exposed to chlorpyrifos (Kareema Ambareen and M.Venkateshwarlu, 2022). The results of the present study are also in conformity with those of the earlier observations. (Mounika *et al,* 2023), in *Ctenopharyngodone idella* under phenthoate 50% EC toxicity. (Al-Ghanim&Shahid,2012), in fish *Clarias gariepinus* under sodium cyanide toxicity. Sankar Samipillai and U. Uma (2018), in fish *Catla catla* under copper sulphate toxicity.

Pesticidal stress causes a fast decrease in SDH activity in all tissues of the fish *Labeo rohita* when exposed to sublethal and lethal dosages of profenofos and carbosulfan. The general decrease in SDH activity during pesticide stress was linked to the regulation of the mitochondrial respiratory mechanism of rearrangement on ultra structure, architectural integrity, and mitochondrial permeability (Bantu *et al.*, 2017). Decreased SDH activity might observe due to depletion in the oxidative metabolism at the mitochondrial level (Pradeep Kiran and Bhaskar, 2016). This limits electron transport to molecular oxygen, inhibiting SDH function and switching from aerobic to anaerobic metabolism. The decline activity of respiratory oxidative enzyme SDH in liver and kidney indicates decline in enzyme synthesis, since Ethion (50%EC) disrupt the membrane bound enzyme.

REFERENCES:

1. Al-Ghanim K A and S Mahboob. Effect of sodium cyanide on the activities of some oxidative enzymes and metabolites in *Clariasgariepinus*. *African Journal of Biotechnology* Vol. 11(41), pp.9849-9854 (2012).
2. Banaee, M., Sureda, A., Mirvagefei, A.R. and Ahmadi, K. 2013. Histopathological alterations induced by diazinon in rainbow trout (*Oncorhynchus mykiss*). *International Journal of Environmental Research*, 7(3): 735-744.
3. Bantu, N, Hagos, Z, Krishna, C., Krihnan, G., Rathnamma, V., Suneetha K., Nirmala, BK., 2017. Impact of Profenofos and Carbosulfan on Dehydrogenase activity of Freshwater fish, *Labeorohita* (Hamilton), *J. Biol. Today's World*. 6(1): 9-20.
4. Dr.S.SankarSamipillai and U. Uma (2018).Effect of copper sulphate on succinate dehydrogenase and lactate dehydrogenase in the selected tissues of freshwater fish *Catlacatla*.IJSRST. Vol.4 (11), 18401157.
5. Huang S, Millar AH. Succinate dehydrogenase: the complex roles of a simple enzyme. *Current opinion in plant biology* 2013;16(3):344-9.
6. Inyang.I.Akio.K, ChibuezeIzah.S.(2016). Effect of dimethoate on latate dehydrogenase, creatine kinase and amylase in Clariaslazera. Biotechnological Research,2(4):155-160.
7. KareemaAmbareen and MididoddiVenkateshwarlu (2022). Impact of chlorpyrifos on biochemical constituents of the freshwater cyprinid fish *Cyprinuscarpio* (L).*IndianJ.Applied&Pure Bio.Vol*.37(3),925-937.
8. Khan, A. A., Allemailem, K. S., Alhumaydhi, F. A., Gowder, S. J., &Rahmani, A. H. (2020). The biochemical and clinical perspectives of lactate dehydrogenase: an enzyme of active metabolism. Endocrine, Metabolic & Immune Disorders- Drug Targets (Formerly Current Drug Targets-Immune, Endocrine &Metabolic Disorders), 20(6), 855-868.
9. Khare, A., Chhawani, N., & Kumari, K. (2019). Glutathione reductase and catalase as potential biomarkers for synergistic intoxication of pesticides in fish. Biomarkers, 24(7), 666-676.
10. Mohan, SM., Siva Prasad, BV, Srinu, A., Vijayalakshmi, D., Harold Phillip, G. 2017. Commercial Deltamethrin: Its (Sublethal) Impact on Carbohydrate Metabolism of *Labeorohita*. *Ind. J. Adv. in Chem Sci*,5(4): 245-254.
11. Mounika.G, Abubakkar.V, Aswartha Narayana.A and Venkata Rathnamma V.(2023). LDH and SDH activity in freshwater Fish *Ctenopharyngodone idella*exposed phenthoate 50%. EC (an organophosphate) IJCRT Voll-II, ISSN:2320-2882.

12. Nachlas,M.M., Margulius, S.P., and Seligman,M.,(1960). A colorimetric method for the determination of succinic dehydrogenase activity. *J.Biol.Chem*,235:499-504.
13. Neelanjana Ch., Jayanta T, Ashis Kumar P. 2017. Assessment of Antioxidant Biomarkers and Protein Levels in Tissues of *Oreochromis mossambicus* and *Channa punctatus* Exposed to Toxicity by Fungicides.*Turkish J. Fisheries & Aquatic Sci,* 17: 487-498.
14. Okwuosa O.B., Eyo J.E., Omovwohwovie E.E. Role of Fish as Bioindicators: A Review. *Iconic Res. Eng. J*. 2019;2:354–368.
15. Okwuosa,O.B.;Eyo,J.E;Omovwhohwovie, E.E.Role of fish as Bioindicators: A Review.Iconic Res.Eng.J.2019,2,354-368.
16. Raja Saha and Sangita Maiti Dutta (2024). Pesticides mode of action on aquatic life. *Toxicologyreports,Volume* 13, 101780.
17. Srikanth.T.N and Krishnamurthy.C.R.(1955). Tetrazodium test for dehydrogenases. *J.Sci.Ind.Res*.14:206.
18. Vignet C, Cappello T, Fu Q, Lajoie K, De Marco G, Clerandeau C, Mottaz H, Maisano M, Hollender J, Schirmer K, Cachot J. 2019. Imidacloprid induces adverse effects on fish early life stages that are more severe in Japanese medaka (Oryziaslatipes) than in zebrafish (*Daniorerio*). Chemosphere 225:470–478.