WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 3, 688-698. Research Article ISSN 2277– 7105

EFFECT OF PYRACLOSTROBIN (20%WG) ON ENZYMATIC ACTIVITIES OF SDH AND LDH IN FRESHWATER FISH LABEO ROHITA (HAMILTON)

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Article Received on
10 December 2017,

Revised on 31 Dec. 2017,
Accepted on 21 Jan. 2018

DOI: 10.20959/wjpr20183-10825

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ABSTRACT

Water is essential to life and to human existence. The ability to predict the effects of pollutants or chemicals on organisms and to extrapolate toxicant effects from laboratory to population and community levels has become a very important factor. The Indian major carp *Labeo rohita* was exposed to lethal (1.4µg/l) and sub-lethal ($1/10^{th}$ of lethal) concentrations for 24hrs, 5th and 10^{th} days of Pyraclostrobin (20%WG) to examine the SDH and LDH enzymatic activity, in different tissues like gill, liver, kidney, brain and muscle and control group were also maintained. The SDH enzyme activity levels increased and LDH enzyme activity levels decreased when compare with controls.

KEYWORDS: Labeo rohita, pyraclostrobin, Lethal, Sublethal, LDH, SDH.

INTRODUCTION

Aquatic organisms are sensitive to pesticide chemicals, and toxic concentrations may rise not only from excessive spillage of agriculture practices but also from several other sources. Apart from causing death either directly or due to starvation by destruction of food organisms, many pesticides with the evidence of tissue damage. Ideally pesticides should be highly selective, destroying target organisms while leaving non- target organisms unharmed.[1,2] The improper management of pesticides in agriculture crops could result in contamination of water bodies.[3,4] The pesticide stress was known to induce significant change in protein metabolism; it is likely that the amino transferases were also considerably affected. Increased activities of AAT and ALAT in different tissues of fish either increased

operation of transamination or increased synthesis of amino acids from other sources like glucose of fatty acids during Acephate.

MATERIAL AND METHODS

Healthy freshwater fish, *Labeo rohita* (Hamilton) size [6±7 cm total length (TL) and 6.5±7.5 g body weight] were collected from the fish farm, Kuchipudi, Guntur District of A.P, India. Then the fish was acclimatized to the laboratory conditions with sufficient dechlorinated water for 10 to15 days at room temperature 28±2^{0}C. The stock solution of the toxicant was prepared in one liter of 100% pure acetone. Concentration of Pyraclostrobin was found in 96hr 1.4µg/l as a lethal and 1/10th was taken as a sublethal concentration along with control group was maintained for each experiment. The Lactate Dehydrogenase activity (LDH) was estimated by the method of[5] with slight modifications. Succinate dehydrogenase (SDH) activity was estimated by the method of[6] were followed. The fish were exposed to lethal and sublethal concentrations in different tissues like gill, liver, kidney, brain and muscle for 24hrs, 5th and 10th days to determine the enzyme activity levels.

RESULT

The calculated value of lactate dehydrogenase (LDH) activity and the percent change over control along with standard deviation are given in the Table.1&2. and Figure.V.1&2. The activity levels of dehydrogenase in *L.rohita* exposed to pyraclostrobin were expressed as micro moles of formazan/mg /protein/hr. The LDH level of muscle, brain, liver, gill and kidney of control fish were almost stable. The control values of LDH in different tissues of the fish *L.rohita* were in the order of: Kidney >Muscle > Gill> Liver> Brain. Under lethal and sublethal exposure to pyraclostrobin for 24hr, the activity levels of LDH were found to increase in all the tissues of the fish *L.rohita* with percent change.

Under lethal exposure of for 24hr, maximum percentage of depletion was in brain (-31.75) and minimum percentage was observed in gill (-24.35). Under pyraclostrobin sublethal exposure 24hr, maximum percentage of depletion was observed in kidney (-23.80) and minimum percentage was observed in gill (-14.10). Under pyraclostrobin sublethal exposure for 5 and 10 days, maximum percentage of depletion was (29.41) and (33.78) in kidney, minimum depletion was (20.58) and (18.05) in muscle.

Succinate dehydrogenase (SDH) is a vital enzyme of citric acid cycle, catalyses the reversible oxidation of succinate to fumarate. In this present investigation it can be visualized that there

is a rapid reflection of SDH activity in all tissues of fish *L.rohita* treated with lethal and sublethal concentrations of pyraclostrobin. When compared with respective controls. The calculated values of Succinate dehydrogenase activity and the present change over control along with standard deviation are given in TableV.15.-V.16. Figure. V.15. -V.16. The calculated value of SDH and standard deviation along with percent change over the controls is tissue specific viz., brain, liver, muscle, gill and kidney of fish *L.rohita* exposed to lethal and sublethal concentrations of pyraclostrobin for 24hr, 5 and 10 days. In the tissue of 24hrs control fish, activity of SDH was in the order of: Muscle>Liver>Kidney>Gill>Brain.

Under lethal exposure to pyraclostrobin for 24hr, the activity was found to increase in all the tissues of test fish, maximum increase was observed in Kidney (22.45) and minimum increase was in gill (10.46). Under sublethal exposure to pyraclostrobin for 24hr the activity of SDH was found to increased in all the tissues of test fish and, maximum increase was observed in kidney(14.28) and minimum increase was observed (5.04) in muscle. Under sub lethal exposure to pyraclostrobin for 5 days the activity of SDH was found to increase in all the tissues of test fish and, maximum increase was observed in kidney (25.33) and minimum increase was observed in gill (9.30). Under sublethal exposure to Pyraclostrobin for 10days the activity of SDH was found to decrease in all the tissues of test fish, maximum depletion was observed in gill (21.87) and minimum depletion was observed in kidney (11.95).

Table: 1. Changes in the specific activity levels of Lactate dehydrogenase (LDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Labeo rohita* exposed to sub-lethal and lethal concentrations of Pyraclostrobin (20%WG) for 24hr.

Tissues	Control (mg/g)	Sub-lethal (mg/g)	% Change	Lethal (mg/g)	% Change
Liver	0.76 ±0.02	0.55 ±0.01	-19.11	0.53 ±0.07	-30.26
Brain	0.68 ±0.04	0.60 ±0.04	-21.05	0.52 ±0.04	-31.75
Muscle	0.89 ±0.05	0.73 ±0.09	-17.97	0.64 ±0.01	28.09
Gill	0.78 ±0.09	0.67 ±0.05	-14.10	0.59 ±0.03	-24.35
Kidney	1.05 ±0.05	0.80 ±0.03	-23.80	0.74 ±0.05	-29.52

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

Values are significantly at $P< 0.05$

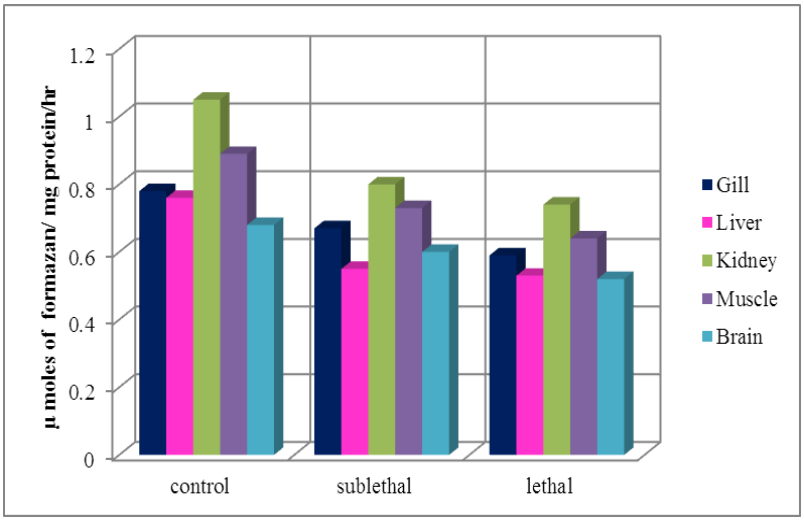

Fig. 1: Changes in the specific activity levels of Lactate dehydrogenase(LDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Labeo rohita* exposed to sub-lethal and lethal concentrations of Pyraclostrobin (20%WG) for 24hr.

Table 2: Changes in the specific activity levels of Lactate Dehydrogenage (LDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Labeo rohita* exposed to sub-lethal concentrations of Pyraclostrobin (20%WG) for 5 and 10 days.

Tissue 24hr		5days			10days	
	Control(mg/g)	Sub-lethal (mg/g)	% Change	Control (mg/g)	Sub-lethal (mg/g)	% Change
Gill	0.49 ±0.01	0.37 ±0.05	-24.48	0.42	0.30 ±0.04	-28.57
Liver	1.05 ±0.02	0.75 ±0.01	-28.75	0.93	0.64 ±0.06	-31.18
Kidney	0.85 ±0.05	0.60 ±0.07	-29.41	0.74	0.49 ±0.03	-33.78
Brain	0.67 ±0.09	0.51 ±0.15	-23.88	0.56	0.40 ±0.09	-28.57
Muscle	0.72 ±0.03	0.59 ±0.03	-18.05	0.68	0.54 ±0.05	-20.58

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

Values are significantly at $P< 0.05$.

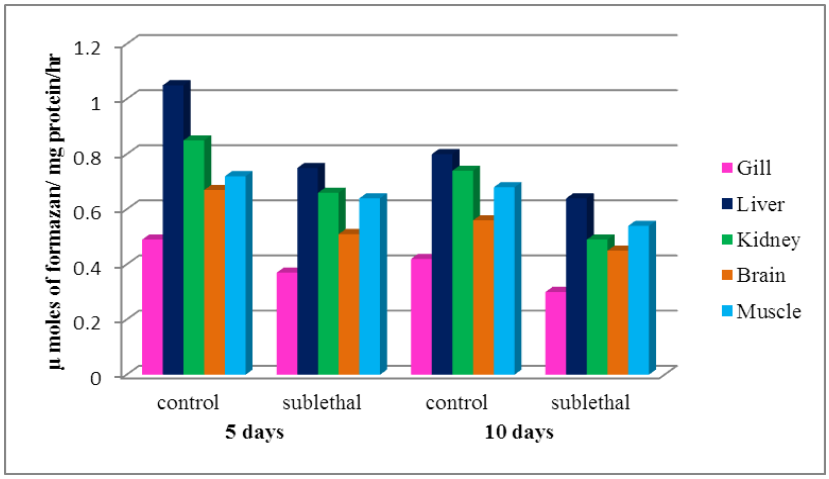

Fig 2: Changes in the specific activity levels of Lactate Dehydrogenage (LDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Labeo rohita* exposed to sub-lethal concentrations of Pyraclostrobin (20%WG) for 5 and 10 days.

Table.3. Changes in the specific activity levels of Succinate dehydrogenase (SDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Labeo rohita* exposed to sub-lethal and lethal concentrations of Pyraclostrobin (20%WG) for 24hr.

Tissues	Control (mg/g)	Sub-lethal (mg/g)	% Change	Lethal (mg/g)	% Change
Gill	0.91 ±0.06	0.96 ±0.05	+6.97	1.02 ±0.09	+10.46
Liver	1.15 ±0.08	1.28 ±0.06	+11.30	1.34 ±0.07	+16.52
Kidney	0.98 ±0.03	1.12 ±0.09	+14.28	1.20 ±0.10	+22.45
Brain	0.81 ±0.04	0.90 ±0.08	+11.10	0.98 ±0.07	+20.98
Muscle	1.19 ±0.10	1.25 ±0.03	+5.04	1.32 ±0.09	+10.92

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

Values are significantly at $P< 0.05$

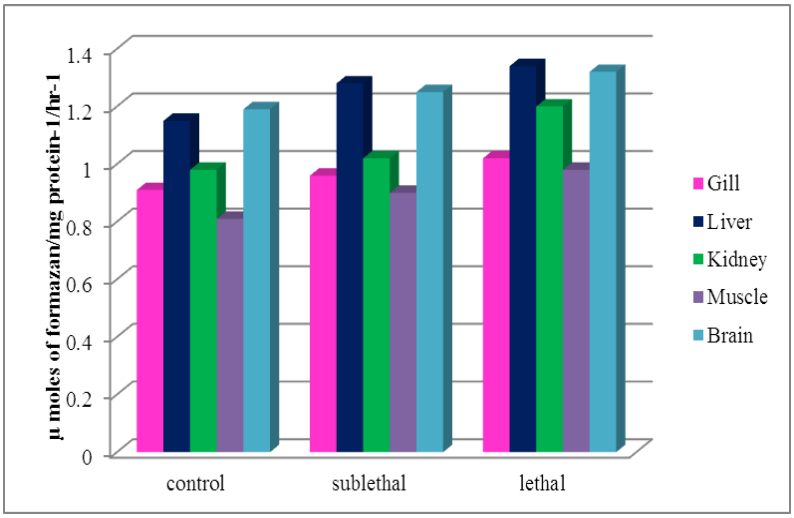

Fig.3. Changes in the specific activity levels of Succinate dehydrogenase(SDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Labeo rohita* exposed to sub-lethal and lethal concentrations of Pyraclostrobin (20%WG) for 24hr.

Table.4. Changes in the specific activity levels of Succinate dehydrogenase(SDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Labeo rohita* exposed to sublethal concentrations of Pyraclostrobin (20%WG) for estimation of SDH content in 5 and 10 days.

Tissues	5days			10days		
	Control (mg/g)	Sub-lethal (mg/g)	% Change	Control (mg/g)	Sub-lethal (mg/g)	% Change
Gill	0.75 ±0.07	0.82 ±0.05	+9.30	0.64 ±0.10	0.78 ±0.05	+21.87
Liver	0.94 ±0.06	1.05 ±0.12	+11.70	0.79 ±0.05	0.96 ±0.02	+21.51
Kidney	0.75 ±0.02	0.94 ±0.07	+25.33	0.92 ±0.32	1.03 ±0.06	+11.95
Brain	0.69 ±0.03	0.80 ±0.15	+15.94	0.61 ±0.07	0.70 ±0.18	+14.75
Muscle	0.46 ±0.02	0.52 ±0.18	+13.04	0.38 ±0.05	0.46 ±0.24	+21.05

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

Values are significantly at $P< 0.05$

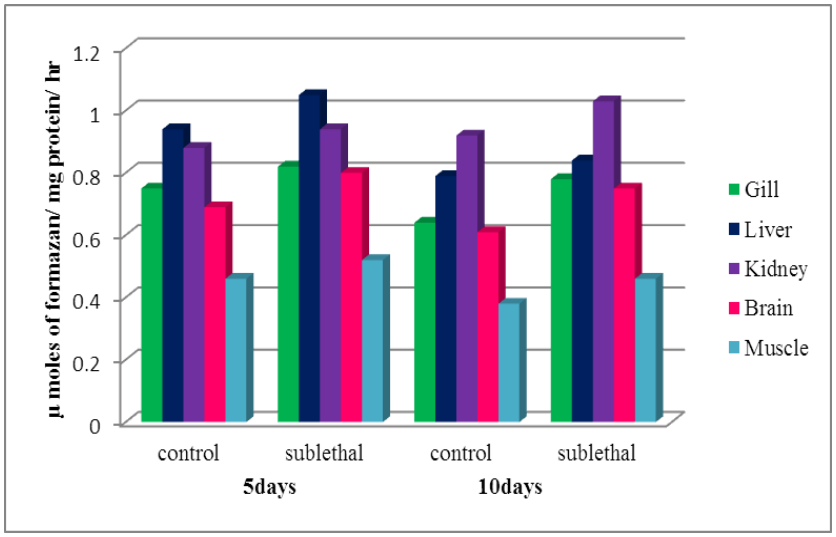

Fig.4. Changes in the specific activity levels of Succinate dehydrogenase(SDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Labeo rohita* exposed to sublethal concentrations of Pyraclostrobin (20%WG) for estimation of SDH content in 5 and 10 days.

DISCUSSION

In the present study, it is observed that the activity of LDH was highly elevated following pyraclostrobin exposure indicating increased anaerobic respiration to meet the energy demands where aerobic oxidation is lowered. Further disruption of respiratory epithelium might have caused tissue hypoxia resulting in LDH activity was more in toxicant stress.[7] Lactate dehydrogenase (LDH) converts the lactate to pyruvate and it plays a very important role in carbohydrate metabolism. LDH involved in carbohydrate metabolism, any change in protein and carbohydrate metabolism may cause change in LDH activity. Elevated LDH activity in pyraclostrobin treated fish *Labeo rohita* suggests that aerobic catabolism of glycogen and glucose has shifted towards the formation of lactate, which may have adverse effects on the organism.

Metabolic enzymes such as citric acids synthesis and lactate dehydrogenase (LDH) are part of the respiratory enzymatic system, which can be effected by from the detoxification enzyme systems under stress conditions in fish.[8] Normal activity of LDH patterns was found to be altered in situations of chemical stress.[9]

Elevation of LDH activity in the mosquito fish, Gambusia holbrooki after acute exposure to Clofibric[10] Disturbances in their catalytic process due to xenobiotics compounds can cause cellular homeostasis affecting different enzymatic systems, which can lead to effects at

higher levels of biological organization such as tissues, organs, or individuals.[11][12] reported that LDH activity in fish Channa punctatus significantly increased in skeletal muscle (2.2) fold followed by liver (1.8) fold, gill (1.6) fold and brain (1.4) in response to treatment with alphamethrion for 14days,due to an increase in anaerobic respiratory activity and production of more lactate for completion of metabolic process.

[13]Reported that with increasing ammonia concentration, there was a progressive increase LDH activity in gill, liver, kidney and brain of the exposed fingerlings Cirhinus mrigala, may have due to stress induced increase in the rate of glycolysis. As the rate if glycolysis increases, the pyruvate is not routed to Krebs's cycle, later catalyses to lactate; thereby shifting the respiratory metabolism from aerobiosis to anaerobiosis.

Increased LDH activity in the liver and muscle of fish Cyprinus carpio exposed to drug carbamazepine (CBZ), due to disruption of respiratory epithelium might have caused tissue hypoxia resulting in oxidative metabolism which may be responsible for in LDH activity in toxicant stress.[14] Similar observations on LDH activity were made under deltamethrin stess in prown by.[15] [16] Disturbances in their catalytic process due to xenobiotic compounds can cause cellular homeostasis affecting different enzymatic systems, which can lead to effects at higher levels of biological organization such as tissues, organs, or individuals.[11]

In the present study, it was observed that the LDH activity in the fish Labeo rohita under exposure to lethal and sublethal concentrations of pyraclostrobin was elevated, indicating that the anaerobic stress and to overcome the toxic stress. LDH is associated with cellular metabolic action, particularly in conditions of chemical exposure and stress when high levels of energy may be required in a period of time. The general decrease in SDH activity during pesticide stress was associated with the inhibition of mitochondrial respiratory mechanism of rearrangements on ultra structure, architectural integrity and permeability of mitochondri.[17] The inhibition in SDH actives were observed in fish *Colisa fasciatus* due to toxicity of ethanolic extract of Nerium indicum milk latex.[18]

A similar decrement in the SDH activity was observed by[19] in fish exposed to malathion and fenitrothion pesticides. The result of the present study are in agreement with those of [20] on *Labeo rohita*. The inhibition of NAD dependent, SDH activity indicated a decreased pass of intermediates into the citric acid cycle. This might be responsible for suppression of oxidative phase of tissue metabolism under pesticidal stress.[19]

Decrease in Succinate dehydrogenase (SDH) activity was observed in both pesticide treated fish tissues compared to control. Similar decrement in the SDH activity was also observed by the various workers in different species of the fish exposed to different pesticides.[21] reported decrease in the activities of LDH and SDH in fish Colisa faciatus after exposure to cypermethrin. The inhibition in LDH and SDH activities were observed in fish due to toxicity of ethanolic extract of *Nerium indicum* mill latex.[18] Similar decrement in the SDH activity was also observed by various workers in different species of fish exposed to different peptides.

[21]Reported decrease in activities of LDH and SDH on fish *Colisa fasciatus* and *Labeo rohita* after exposure to cypermethrin.[15] reported that decreased SDH activity in fish *Tilapia mossambica* and *Clarias gariepinus* exposed to different type of chemicals, due to depletion in the oxidative metabolism at level of mitochondria leading to depression of TCA cycle.

CONCLUSION

In the present study, it is observed that the activity of LDH was highly elevated following Pyraclostrobin exposure indicating increased anaerobic respiration to meet the energy demands where aerobic oxidation is lowered. Further disruption of respiratory epithelium might have caused tissue hypoxia resulting in LDH activity was less in toxicant stress. The increasing SDH activity during pesticide stress was associated with the exhibition of mitochondrial respiratory mechanism of rearrangements on ultra structure, architectural integrity and permeability of mitochondria.

REFERENCES

1. Ernest Hodgson A Textbook of Modern Toxicology, Third Edition, edited by ISBN 0-471-26508-X, John Wiley & Sons, Inc. Gregory cope, W., 2004. Exposure Classes, Toxicants in air, Water, Soil, Domestic and Occupational settings A Textbook of modern Toxicology, third Edition, edited by Ernest /hodgson ISBN 0-471-26508-X,John Wiley & Sons, Inc., 2004; 4: 40-44.
2. David Hernandez-Moreno, Marcos Perez-Lopez, Francisco Soler, Carlos Gravato, Lu'cia Guilhermino Effect of carbofuran on the sea bass (Dicentrarchus labrax L.); Study of biomarkers and behavior alterations. Ecotoxicology and Environmental Safety, 2011; 74: 1905-1912.
3. Candida Toni, vania Lucia loro., Adriana santi, Charlene cavalheiro de Menezes, Roberta cattaneo, Barbara estevo clasen, Renato zanella Exposure to tebuconazol in rice field and

laboratory conditions induces oxidative stress in carp(*Cyprinus carpio)*. Comparative Biochemistry and physiology, Part C, 2011; 153: 128-132.

4. Capkin E, Altinok I Effects of chronic carbosulfan exposure on liver antioxidant enzyme activities in rainbow trout. Environ Toxicol Pharmacol, 2013; 36(1): 80-87.
5. Srikanthan, T.N., and Krishnamurthy, C.R Tetrazodium test for dehydrogenases J. Sci.Ind. Res., 1955; 14: 206.
6. Nachlas, M.M., Margulius, S.P.,and Seligman,M., A colorimetric method for the determination of succinic dehydrogenase activity. J. Biol. Chem, 1960; 235: 499-504.
7. Gill, Jaishree Pande and Hema Tiwari Sublethal effect of an organophosphorus insecticide on certain metabolic levels in a freshwater fish Puntius conchonius(Ham). Pestic. Biochem.Physiol., 1990; 36: 290-299.
8. Rodrigo Orrego, Zacharias Pandelides, JohnGuchardi, Douglas Holdway Effects of pulp and paper mill effluents extracts on liver anaerobic and aerobic metabolic enzymes in tainbow trout.*Ecotoxicol.environment. Safe*, 2011; 74: 761-768.
9. Diamantino, T.C., Almeida, E., Soares, A.M.V.M., Guilhermino, L. Lactate dehydrogenage activity as an effect criterion in toxicity tests with *Daphania magna stratus*. Chemosphere, 2001; 45: 553-560.
10. Nunes, B., Carvalho, F.,Guilhermino, L. Acute and chronic effects of clofibrate and clofibric acid on the enzymes acetylcholinesterase, lactate dehydrogenase and catalase of the mosquitofish, *Gambusia holbrooki,* Chemospere, 2004; 57: 1581-1589.
11. Orrego, Rodrigo.,Zacharias Pandelides, John Guchardi, Douglas Holdways Effects of pulp and paper mill effluent extracts on liver anaerobic and aerobic metabolic enzymes in rainbow throut. *Ecotoxicology and environmental Safety*, 2011; 74: 761-768.
12. Thripathi, G and Singh,H Impact of alphmethrin on biochemical parameters of *Channa punctatus* , journal of environmental biology., 2013; 34: 227-230.
13. Pratap Chandra Das,S.Ayyappan,J.K Jena,B.KDas Aqute toxicity of ammonia and its sublethal effects on selected haemological and enzymatic parameters of mrigal *Cirrhinus mrigala*(Hamilton). Aquaculture Research, 2004; 35(2): 134-143.
14. Annamalai MalarviZhi, Chokkalingam. Kavitha, Manoharan Saravanan, Mathan Ramesh. Carbamazepine (CBZ) induced enzymatic stress in gill, liver and muscle of a common carp, *Cyprinus carpio.Journal of Kig Saud University-science*, 2012; 24: 179-186.
15. Al-Ghanim, K, and Mahboob, S Effect of sodium cyanide on the activities of some oxidative enzymes and metabolites in *clarias gariepinus*. African Journal *of* Agricultural *research*, 2012; 7(4): 9849-9854.

16. Cristiana Oliveira., Joana Almeida, Lucia Guihermino, Amadeu M.V.M.Soares, Carlos Gravato Acute effects of deltramethrin on swimming velocity and biomarkers of the common prawn *Palaemon serratus*. *Aquatic Toxicology*, 2012; 124-125: 209-216.
17. Shwatha Alavandi and Basaling B.Hosetti Influence of cyanide on some antioxidant enzymes of fresh water fish, *Cirrihinus mrigala* (Hamilton). *Journal of Agricultural Sciences*, 2011; 58(3): 177-184.
18. Sudhanshu Tiwari and Ajay Singh Changes in some biochemical parameters in the liver and muscle of *Colisa fasciatus* due to toxicity of ethanolic extract of *Nerium indicum* Mill.(Lal Kaner) latex.*Natural producters radiance*, 2009; 8(1): 48-54.
19. Abdel-Salam Mohamed Ibraik Ohaida, Nuha Mahmoud and A. Akrawee The effects of exposure to organophosphorus compounds on some aspects of carbohydrate metabolism in carbohydrate metabolism in *Tilapia zillii*. *The Egyptia Journal of Hospital Medicine*, 2010; 39: 223-228.
20. Suneetha,K Effect of edosulfan and fenvalerate on carbohydrate metabolism of the freshwater fish, *Labeo rohita* (Hamilton). *International Journal of pharmacy and pharmaceutical Science*, 2012; 4(1): 262-268.
21. Shailendra kumar singh, Sunil Kumar Singh and Ram P.Yadav Toxicological and biochemical alterations of cypermethrin (Synthetic pyrethroid) against freshwater fish *Colisa fasciatus* at different Seasons. *World* journal *of* Zoology., 2010; 5(1): 25-32.