IMPACT OF HERBICIDE PARAQUAT ON THE INDIAN MAJOR CARP, *Labeo rohita*.

Dr. J. Manju Bashini* and Dr. P. Senthilkumaar
*Department of Zoology, L.N. Government College, Ponneri.
P.G. and Research Department of Zoology, Sir Theagaraya College, Chennai.

ABSTRACT

The fingerlings of the Indian major carp, *Labeo rohita* were exposed to the herbicide, Paraquat for 24 hrs LC_{50} and were analyzed for the variations in their nutritional and enzymatic parameters. After exposure, tissues like brain, muscle, liver and kidney were dissected and subjected to experimentation. Total protein content and carbohydrate content of fingerlings of *Labeo rohita* showed that Paraquat had more effect in all tissues. Total lipid content of *Labeo* showed more effect in brain. The brain and muscle showed very high SDH activity and the brain and kidney showed very less LDH activity. When compared to control, ACP activity increased in all tissues and ALP activity decreased in all tissues.

Key words: Paraquat, *Labeo rohita*, toxicity impact.

INTRODUCTION

Fishes constitute the major food source among aquatic organisms. The Indian major carps capture the main source of income from fishery sources. These pesticides, even when applied in restricted areas are washed and carried away by rains and floods to large water bodies like ponds and rivers and alter the physicochemical properties of water (Bhalchandra *et al*., 2001). Unfortunately, many non-target fresh water organisms like fish, molluscs, prawn, crabs, etc are adversely affected (Yaji *et al*., 2011). Such effect may be at cellular or even at molecular level but ultimately it leads to behavioural, physiological, pathological and biochemical disorders that may prove fatal (Patole *et al*., 2008). When any aquatic animal is exposed to polluted medium, a sudden stress is developed. The animals should meet more energy demand to overcome this toxic stress (Sreenivasan *et al*., 2011). The number of changes in the biochemistry of fish was reported as result of exposure to pesticides. The biochemical studies are good parameters which help to see the effect of toxicants on metabolism of fish (Ghosh, 1986; Kajare *et al*., 2000). These pesticides are proving to be highly toxic, not only to fishes but also to other organisms, which constitute the food for the fishes (Madhab Prasad *et al*., 2002). These in turn alter the physio-chemical characters of water; interfere and interact with various physiological activities of organisms. The present study was to investigate the toxicity effect of pesticide, Paraquat on the fish (*Labeo rohita*).

MATERIALS AND METHODS

Labeo rohita fingerlings were collected from the Thiruthani Taluk, Thiruvallur District, Tamil Nadu, India. The fingerlings were brought to the laboratory in the oxygenated plastic bags and acclimatized for laboratory conditions. The fishes were fed with formulated feed throughout the experiment. The fishes were exposed to pesticide, Paraquat for 24 hrs. After the exposure, the tissues, viz., brain, liver, muscle and kidney were dissected and biochemical parameters were analyzed. The experiment was carried out to determine the toxic effect of the pesticide. Mortality was recorded at 24 hrs of exposure. The LC_{50} values were estimated by following Abbott (1925). The estimation of nutritional and enzymatic parameters were observed following the standard methods; total protein (Bradford, 1976); total carbohydrate (Roe, 1955); total lipid (Folch *et al*., 1957); Succinate dehydrogenase (Nachalas *et al*., 1960); Lactate dehydrogenase (King, 1965) and acid and alkaline phosphatase (Tenniswood *et al*., 1976).

RESULTS

Total protein content and carbohydrate content of fingerlings of *Labeo rohita* showed that Paraquat had more effect in all tissues. Total lipid content of *Labeo* showed more effect in brain. The brain and muscle showed very high SDH activity and the brain and kidney showed very less LDH activity. When

compared to control, ACP activity increased in all tissues and ALP activity decreased in all tissues are listed in the Tables: 1-7.

Table: 1 Impact of Paraquat on total proteins (mg/g)

Tissues	Mean	Std. Deviation	Std. Error	P -VALUE
Brain - Control	5.58	0.24	0.017	0.002
Paraquat	4.31	0.24	0.017	0.002
Muscle - Control	8.51	0.34	0.023	0.002
Paraquat	6.28	0.25	0.018	0.002
Liver - Control	25.31	0.29	0.021	0.005
Paraquat	19.26	0.33	0.022	0.005
Kidney - Control	22.28	0.33	0.023	0.004
Paraquat	18.45	0.32	0.022	0.005

Table: 2 Impact of Paraquat on total carbohydrates (mg/g)

Tissues	Mean	Std. Deviation	Std. Error	P-VALUE
Brain - Control	1.36	0.56	0.035	0.001
Paraquat	1.12	0.38	0.021	0.001
Muscle - Control	3.21	0.42	0.032	0.001
Paraquat	2.25	0.43	0.032	0.005
Liver - Control	20.82	0.44	0.034	0.001
Paraquat	19.62	0.43	0.032	0.001
Kidney - Control	3.85	0.36	0.027	0.004
Paraquat	2.92	0.43	0.032	0.005

Table: 3 Impact of Paraquat on total lipids (mg/g)

Tissues	Mean	Std. Deviation	Std. Error	P –VALUE
Brain - Control	12.21	0.35	0.027	0.002
Paraquat	11.23	0.43	0.031	0.001
Muscle - Control	4.36	0.44	0.032	0.003
Paraquat	3.82	0.17	0.077	0.001
Liver - Control	10.46	0.28	0.021	0.002
Paraquat	9.23	0.37	0.025	0.001
Kidney - Control	8.92	0.87	0.027	0.001
Paraquat	7.43	0.33	0.024	0.001

Table: 4 Impact of Paraquat on the succinate dehydrogenase (MIU/min/mg)

Tissues	Mean	Std. Deviation	Std. Error	P-VALUE
Brain - Control	5.46	0.36	0.026	0.002
Paraquat	4.85	0.37	0.024	0.002
Muscle - Control	9.73	0.29	0.023	0.001
Paraquat	8.24	0.27	0.023	0.001
Liver - Control	5.25	0.43	0.031	0.003
Paraquat	4.91	0.36	0.027	0.003
Kidney - Control	5.29	0.42	0.031	0.004
Paraquat	4.54	0.44	0.032	0.002

Table: 5 Impact of Paraquat on the lactate dehydrogenase (µg/100mg)

Tissues	Mean	Std.Deviation	Std. Error	P-VALUE
Brain - Control	3.64	0.43	0.031	0.004
Paraquat	2.82	0.44	0.033	0.005
Muscle - Control	7.91	0.45	0.032	0.001
Paraquat	8.36	0.48	0.034	0.002
Liver - Control	5.67	0.38	0.026	0.003

Paraquat	6.32	0.51	0.031	0.003
Kidney - Control	5.28	0.47	0.033	0.003
Paraquat	4.68	0.45	0.034	0.005

Table: 6 Impact of Paraquat on the acid phosphatase (µg/ PNPP to PNP/100 mg)

Tissues	Mean	Std. Deviation	Std. Error	P-VALUE
Brain - Control	4.77	0.36	0.027	0.002
Paraquat	5.26	0.36	0.025	0.002
Muscle - Control	3.95	0.37	0.027	0.003
Paraquat	4.69	0.34	0.026	0.002
Liver - Control	5.56	0.35	0.025	0.002
Paraquat	5.64	0.28	0.023	0.002
Kidney - Control	5.48	0.27	0.025	0.002
Paraquat	5.29	0.29	0.026	0.002

Table: 7 Impact of Paraquat on the alkaline phosphatase (µg/ PNPP to PNP/100 mg)

Tissues	Mean	Std. Deviation	Std. Error	P-VALUE
Brain - Control	7.26	0.36	0.026	0.001
Paraquat	6.29	0.34	0.025	0.001
Muscle - Control	4.38	0.43	0.032	0.003
Paraquat	3.72	0.48	0.036	0.005
Liver - Control	7.36	0.34	0.026	0.001
Paraquat	6.38	0.37	0.027	0.001
Kidney - Control	6.57	0.38	0.026	0.002
Paraquat	5.44	0.36	0.027	0.002

DISCUSSION

Each body cell is composed mainly of protein. Protein makes up the membrane surrounding the cell and also occurs within the cell. Protein plays a vital role in the formation of enzymes, antibodies and hormones and other substances that regulate the body process (Sujatha, 2011). Sherif-El *et al*., (2009) observed slight reduction or decrease in the intensity of proteins in diazinon treated fish Nile Tilapia, which indicates that these proteins were highly affected by the stress caused by the pesticides. The fishes tend to resist the sudden stress for shorter duration, later with increases of time the decrease of protein content was observed. However increase of consumer concentration of pesticide decreases the protein in tissues of the fish *Labeo rohita*. The present study coincides with the reported data that the protein content was decreased in muscle and liver (Sastry and Siddiqui, 1984; Durairaj and Selvarajan, 1992; Anusha Amali *et al*., 1996; Yeragi *et al*., 2000; Tilak *et al*., 2005); Rajini *et al*., 2015; Bawa *et al*., 2017).

Carbohydrate is an important biochemical constituent of animal tissues. They not only act as building blocks of the cells but also serve as a reservoir of chemical energy to be increased or decreased according to organismal need (Jagannath Bose, 2004). The results of the present findings showed a significant decrease in carbohydrate content in all the tissues studied. The decrease in carbohydrates content may result in impairment of carbohydrate metabolism due to toxic effect (Thenmozhi *et al*., 2010). The carbohydrate reduction suggests the possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress condition. Many workers have reported a similar trend of decrease in carbohydrate (Venkatramana Sandhya *et al*., 2006; Thenmozhi *et al*., 2011). However, Remia *et al*., (2008) reported that the elevation of carbohydrates might be due to the stress induced by the insecticides as physiology of organisms with the help of corticosteroids.

Abirami *et al*., (2012) reported that when pesticide is used, the lipid content of the tissue decreased with increase in the concentration of pesticides like that of protein. Similar observations were reported by Manohar Patil and Kulkarni (1995) in the fresh water fish, *Channa punctatus* when the fish was exposed to the pesticide, Summach. Tazeen *et al*., (1996) too observed decline in the total lipid content when the catfish *Mystus vittatus* was exposed to the pesticide, Nuvan.

Succinate dehydrogenase or succinate-coenzyme Q reductase (EC 1.3.5.1) is the only enzyme that participates in both the citric acid cycle which catalyses the reversible oxidation of succinate to fumarate and the electron transport chain. SDH is very sensitive to environment pollutants and the activity of SDH may be taken as an indication of the level of operation of the TCA cycle (Bhagyalakshmi *et al*., 1984). The general decrease in SDH activity during pesticides stress was associated with the inhibition of mitochondrial respiratory mechanism or rearrangement in ultra structure, architectural integrity and permeability of mitochondria (Tripathi and Priyanka, 2004). This prevents the transfer of electrons to molecular oxygen, resulting in the inhibition of SDH activity and shifting the aerobic metabolism to anaerobiosis (Shailendra Kumar Singh *et al*., 2010); Pallavi Srivastava and Ajay Singh, 2013; 2014).

Lactate dehydrogenase (EC 1.1.1.27) catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+. It converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply and it performs the reverse reaction during the Cori cycle in the liver. At high concentrations of lactate, the enzyme exhibits feedback inhibition, and the rate of conversion of pyruvate to lactate is decreased. It is also very sensitive to environmental toxicants like pesticides. LDH is generally associated with cellular metabolic activity. It acts as a pivotal enzyme between the glycolytic pathway and the TCA cycle. LDH forms the centre for a delicately balanced equilibrium between catabolism and anabolism of carbohydrates (Everse and Kaplan, 1973) and also associated with cellular metabolic activity (Abston and Yarbrough, 1976). The non-availability of oxygen, inhibition of SDH and simultaneous elevation of LDH may suggest a bias towards the anaerobic glycolytic pathway. The increase in LDH activity may reflect an increased dependence on anaerobic carbohydrate metabolism by the muscle and liver of the fishes and further this increased LDH activity in fish might also result to lactate conversion into pyruvate at the expense of NAD (Al-Ghanim and Mahboob, 2012). The decrease in the pyruvate levels and increase in lactate levels can be attributed to the toxic stress resulting in the inhibition of pyruvate oxidation under hypoxic conditions which indicates the shifting of aerobic to anaerobic respiration. LDH levels increased in the tissues due to stress when exposed to the toxicants (Tilak *et al*., 2003; Kamal Attia and Ashraf El-Badawi (2015); Bhaskara Tataji and Vijaya Kumar, 2016).

The Acid phosphatase (EC 3.1.3.4) is a hydrolytic lysosomal enzyme released by lysosomes for the hydrolysis of foreign materials and increase in its activity is probably related to the cellular damage. The acid phosphatase has a role in eliminating certain toxins by the detoxification function. It is difficult however to tolerate the decrease in ACP activity with necrosis. Increase in acid phosphatase and alkaline phosphatase activities can be interpreted as a shift, which emphasize on energy breakdown pathway from normal ATPase system which includes phosphorylation. The phosphatases, ACP and ALP are active at specific pH and are usually called as phosphomonoesterases. The toxicity of pesticide, increases ACP and ALP activity in fishes (Tejendra *et al*., 1990). Increased ACP enzyme activity at all the concentrations of Butachlor might be due to increase in protease activity which caused damage to the lysosomal membrane, thus permitting the leakage of lysosomal enzyme into cytoplasm. Changes in the enzyme activity are due to adverse effect of xenobiotics on the cell and its organelles. In the present study, the mean value of ACP activity in the kidney, liver and gill of *L. rohita* increased during the long time of exposure. This increased phosphatase activity was due to the cellular damage caused by hepatotoxins or a response to overcome toxicity of Butachlor. The significant difference in phosphatases activities between the control and experimental groups of fish species might be considered due to the damage of hepatic tissue with dysfunctions of organs. The elevation in ACP activity proposes an increase in the lysosomal mobilization and cell necrosis due to the pesticide toxicity (Venkateswara Rao, 2006). The enzyme ACP activity elevation in brain tissue was described in stress-exposed *C. punctatus* (Abdul Naveed *et al*., 2011) and *Anabas testudineus* (Santhakumar and Balaji, 2000). The sub-acute exposure pesticide chlorpyrifos revealed increased activity of ACP content in the liver and kidney tissues of *Gambusia affinis*, and ACP activity is a conventional indicator of liver damage in the fish (Sabiha Khan and Neelam Sharma, 2012). Dose dependent and significant increase in the activity of acid phosphatase may be attributed to the hepatic and renal damage (Sreenivasan *et al*., 2010). The increased ACP activity appears as a result from improved enzyme turn over under pesticide stress. It plays an important role in carbohydrate metabolism. This enzyme can be found inside the membrane of lysosomes (Sabiha Khan, 2014). So, any damage to the membrane of lysosomes can cause the release of this enzyme into muscle and increase its levels. The elevation of ACP activity in all the tissues of fish has been noticed in the present experiment. Many researchers have

supported the increase in ACP activity levels by studying various species exposed under different toxicants and pesticides (Rao, 2006; Nchumbeni *et al*. 2007; Nte *et al*., 2011). Thus in the present study, the pesticides intoxication produced elevation in the activity levels of ACP in all the tested tissues of the fish. The results of the present experiment are in correlation with the previous work done on various fish species exposed to different toxicants where ACP levels were increased (Neelam Sharma, 2014; Umamaheswari and Senthilnathan, 2014a). The increase was associated either with the decrease in stability of liver lysosome membrane or with the liver damage (Moraes *et al*., 1998). The enzyme is known to be associated with lysosomal activity. It has been speculated that the ACP elevation reflects proliferation of lysosomes in attempt to sequester the toxic xenobiotic (Gill *et al*., 1992; Karra Somaiah and Kanikaram Sunita, 2015).

Alkaline phosphatase (EC 3.1.3.1) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins and alkaloids. The process of removing the phosphate group is called dephosphorylation. It is a metallo enzyme which catalyses the non-specific hydrolyses of phosphate mono esters (Qing-Xi-Chen *et al*., 2000). ALP is a brush border enzyme that splits various phosphorous esters at an alkaline pH and mediates membrane transport. It is also involved in synthesis of certain enzymes, protein synthesis, glycogen metabolism and secretory activity. A clear reduction in values of ALP is an indication of inhibition of this enzyme and this may indicate inactive transamination and oxidative deamination in the kidney and liver cells (Inyang *et al*., 2016). The decrease in ALP in the various organs may be attributed to such factors as a decline in the rate of synthesis caused by lowered metabolic demands and also due to electrolytic imbalance caused by tissue over hydration (Anderson *et al*., 2002). Alkaline phosphatase is a membrane bound enzyme found at bile pole of hepatocytes and also found in pinocytic vesicle and golgi complex. It is present on all cell membranes where active transport occurs and hydrolase and transphosphorylase in function. It is often employed to access the integrity of plasma membrane (Akanji *et al*., 1993). Inhibition of ALP reflects alteration in protein synthesis and uncoupling of oxidative phosphorylation (Verma *et al*., 1984). This decrease may be due to the damage and dysfunction of the liver. The decrease in ALP by stressors probably indicates an altered transport of phosphate (Engstrom, 1974; Kori-Siakpere *et al*., 2010; Chimela Wala *et al*., (2014); Palas Samanta *et al*., (2014); Ahsan Khan *et al*., (2016); Inyang *et al*., (2016).

REFERENCES

1. Abbott, W.S., (1925) A Method of Computing the Effectiveness of an Insecticide. *J. Econ. Entomo.,* **18**: 265-267.
2. Abdul Naveed and C. Janaiah. (2011) Effect of Triazophos on protein metabolism in the fish, *Channa punctatus* (Bloch). *Current Research Journal of Biological Sciences*., **3(2):** 124-128.
3. Abston, P.A. and J.D. Yarbrough. (1976) The *in-vivo* effect of mirex on soluble hepatic enzymes in the rat. *Pest*. *Biochem*. *Physiol*., **6(2):** 192-199.
4. Ahsan Khan., Ali Muhammad Yousafzai., Nazish Shah., Muhammad., Munawar Saleem Ahmad., Muhammad Farooq., Fawad Aziz., Muhammad Adnan., Muhammad Rizwan and Shahibzada Muhammad Jawad. (2016) Enzymatic Profile Aactivity of Grass Carp (*Ctenopharyngodon idella*) After Exposure to the Pollutant Named Atrazine (Herbicide). *Pol. J. Environ. Stud*., **25(5)**: 2003-2008.
5. Akanji, M.A., O.A. Olagoke and O.B. Oloyede. (1993) Effect of chronic consumption of metabisulphate on the integrity of the kidney cellular system. *Toxicol*., **81(3):** 173-179.
6. Al-Ghanim, K.A. and S. Mahboob. (2012) Effect of sodium cyanide on the activities of some oxidative enzymes and metabolites in *Clarias gariepinus*. *African Journal of Biotechnology*., **11(41):** 9849-9854.
7. Anderson, T., L. Forlin, J. Hardig and A.A. Larsson. (2002) Physiological disturbances in fish living in the coastal water polluted with bleached Kraft pulp mill effluents. *Can. J. Fish Aquat. Sci*., **45(9):** 1525-1536.
8. Anusha Amali, A., L. Cyril Arun Kumar, F.X. Elizabeth Jayanthi and M. Selvanayagam. (1996) Quinolphos induced biochemical anomalies in *Cirrhinus mrigala*. *J. Environ. Biol*., **17(2):** 121-124.
9. Bawa, V., J.K. Kondal., S.S. Hundal and Harpinder Kaur. (2017) Biochemical and Histological Effects of Glyphosate on the Liver of *Cyprinus carpio* (Linn.). *American Journal of Life Sciences*., **5(3-1)**: 71-80.

10. Bhagyalakshmi, A.P., P.S. Reddy and R. Ramamurthy. (1984) Sub-acute stress induced by sumithion on certain bio-chemical parameters in *Oziotelphusa senex*, the fresh water rice field crab. *Toxicol. Lett*., **21(2):** 127-134.
11. Bhalchandra, B. Wayker, V.S. Lomte. (2001) Acute toxicity of pesticides Carbaryl and endosulfan to freshwater bivalve, *Parreysia cyclindrica*. Poll. Res ., 20 (1): 25- 29.
12. Bhaskara Tataji, P. and M. Vijaya Kumar. (2016) Biochemical Changes Induced by Butachlor and Machete 50% EC to the Freshwater Fish *Channa punctata* (Bloch). *International Journal of Science and Research*., **5(3)**: 2048-2052.
13. Bradford, M.M., (1976) A rapid sensitive method for the quantification of microgram quantities of protein utilizing principle of protein dye binding. *Anal. Biochem.*, **72:** 248-254.
14. Chimela Wala., Abdulraheem Bawa-Allah and Azubuike Adams. (2014) Alkaline Phosphatase (ALP) Activity in Selected Tissues & Organs of *Clarias gariepinus* Exposed to Different Levels of Paraquat. *Journal of Biology, Agriculture and Healthcare*., **4(21**): 8-10.
15. Durairaj, S. and V.R. Selvarajan. (1992) Influence of quinolphos, an organophosphorous pesticide, on the biochemical constituents of the tissues of fish *Oreochromis mossambicus*. *J. Environ. Biol*., **13(3):** 181-185.
16. Engstrom, L. (1964) Studies on bovine liver alkaline phosphatase, phosphate incorporation. *Biochem. Biophys. Acta- Specialized Section on Enzymological Subjects*., **92(1)**: 71-78.
17. Everse, T. and N.O. Kaplan. (1973) Lactate dehydrogenase: Structure and function. *Advances in Enzymology and Related Areas of Molecular Biology*., **37:** 61-133.
18. Folch, J., S.M. Lee and G.H. Slone-Stanley (1957) A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**: 497-508.
19. Ghosh, T.K., (1986) Effect of Dimethoate on tissue glycogen content of some freshwater fish. *Env. Eco.*, **4**: 554-557.
20. Gill, T.S., H. Tewari and J. Pande. (1992) Short and long-term effects of copper on the rosy barb, *Puntius conchonius*. *Ecotoxicol. Environ. Saf*., **23(3):** 294-297.
21. Inyang, I.R., N.C. Okon and S.C. Izah. (2016) Effect of glyphosate on some enzymes and electrolytes in *Heterobranchus bidosalis* (a common African catfish). *Biotechnol. Res*., **2(4)**:161-165.
22. Jagannath Bose, M.T. (2004) Effect of endosulfan, an organochlorine insecticide on the edible freshwater field crab, *Spiralothelphusa hydrodroma* (Herbst) of Irulenchery village, Tiruvallur District, Tamilnadu. *Ph.D. Thesis*., University of Madras. *pp*. 152.
23. Kajare, A., S. Singh and K. Shrivastava. (2000) Malathion induced biochemical changes in the kidney of freshwater fish *Clarias batrachus*. *J. Ecotoxicol. Environ. Monit.*, **10**: 11-14.
24. Kamal A. Attia and Ashraf A. El-Badawi. (2015) Effects of short or long-term exposure of dithiopyr on certain blood, growth and tissue biochemical parameters in catfish (*Clarias garipinus*). *Int. J. Appl. Sci. Biotechnol*., **3(2)**: 314-321.
25. Karra Somaiah and Kanikaram Sunita. (2015) Biochemical changes induced by Butachlor, pre-emergent herbicide to sub lethal concentrations in the freshwater fish *Labeo rohita* (Hamilton). *Journal de Afrikana*., **2(4)**: 99-122.
26. King, J., (1965) In:Practical Clinical Enzymology. D.Van Norstrand Co., London.
27. Kori-Siakpere, O., R.B. Ikomi and M.G. Ogbe. (2010) Variations in acid phosphatase and alkaline phosphatase activities in the plasma of the African catfish: *Clarius gariepinus* exposed to sublethal concentration of Potassium permanganate. *Asian J. Exp. Biol. Sci*., **1(1):** 170-174.
28. Madhab Prasad, Bandyopadhaya and AjitKumar Aditya. (2002) Xenobiotic impact on sensitivity in *Anabas testudineus* (Bloch). *J. Ecobiol .*, **14 (2):** 117-124.
29. Moraes, G., L.H. de Aguiar., I.M. Avilez., A.E. Altran and C.F. Correa. (2004) Metabolical effects of Folidol 600 on the neotropical freshwater fish matrinxa, *Brycon cephalus*. *Environ. Res.*, **95(2)**: 224-30.
30. Nachlas, M.M., S.I. Margulius and A.M. Selligman. (1960) A colorimeteric method for the estimation of SDH. *J. Biol, Chem.*, **235:** 499-503.
31. Nchumbeni, H., D.R. Kulkarni and B.G. Bhavita Chavan. (2007) Effect of arsenic on the enzymes of the *rohu carp, Labeo rohita* (Hamilton, 1822). *The Raffles Bulletin of Zoology Supplement*., **14**: 17-19.

32. Neelam Sharma. (2014) Assessment of the changes in some diagnostic enzyme in fish *Gumbusia affinis* treated with the pesticide with chlopyripos. *International Journal of Research in Pharmacology and Phormcotherapeutics*., **l3(4)**: 205-201.
33. Nte, M.E., A.I. Hart., O.M. Edun and O.A. Akinrotimi. (2011) Alterations in enzymes activities as a biomarker in Black Jaw tilapia (*Sarotherodon melanotheron*) exposed to industrial effluents. *Continental Journal of Biological Sciences*., **4(2)**: 37-44.
34. Palas Samanta, Sandipan Pal, Aloke Kumar Mukherjee, Tarakeshwar Senapati and Apurba Ratan Ghosh. (2014) Effects of Almix Herbicide on Metabolic Enzymes in Different Tissues of Three Teleostean Fishes *Anabas testudineus, Heteropneustes fossilis* and *Oreochromis niloticus. International Journal of Scientific Research in Environmental Sciences*., **2(5**): 156-163.
35. Pallavi Srivastava and Ajay Singh. (2013) Study of in-vivo Effects Caused by Metabolites (1,2,4-Trizole Alanine) of Steroid-Inhibitor Fungicide on Aquatic Life (Fish). *J. Aquac. Res. Development*., **4(4)**: 1-5.
36. Pallavi Srivastava and Ajay Singh. (2014) Potential Effects of Agricultural Fungicide (Mancozeb) on Fish *Clarias batrachus*. *Research journal of Biological sciences*., **9(4)**: 129-134.
37. Patole, S.S., M. Patil and R.T. Mahajan. (2008) Studies on metabolic changes during intoxication by phytotoxicants in fish, *Nemacheilus evizardy* (Day) *Int. J. Pharmacol. Biol. Sci.*, **2**: 63-68.
38. Qing-Xi Chen, Wen-Zhu Zheng, Jing-Yu Lin, Y. Shi, Wen-Zhang Xie and Hai-meng Zhou. (2000) Effect of metal ions on the activity of green crab, *Scylla serrata* alkaline phosphatase. The *International Journal of Biochemistry and Cell Biology*., **32(8):** 879-885.
39. Rajini, A., R.A. Gopi and V. Bhuvana. (2015) Effect of pre-emergence and post-emergence herbicide on new biochemical parameters to freshwater fish *Labeo rohita*. *International Journal of Recent Scientific Research Research*., **6(9)**: 6422-6425.
40. Rao, J.V. (2006) Biochemical alterations in euryhaline fish, *Oreochromis mossambicus* exposed to sublethal concentrations of an organophosphorus insecticide, monocrotophos. *Chemosphere*., **65(10)**: 1814-1820.
41. Remia, K.M., S. Logaswamy, K. Logankumar and D. Rajmohan. (2008) Effect of an insecticide (Monocrotophos) on some biochemical constituents of the fish *Tilapia mossambica*. *Poll. Res*., **27(3):** 523-526.
42. Roe, J.R., (1955) The determination of sugar in blood and spinal fluid with anthrone reagent. *J. Biol. Chem.*, **20**: 335-343.
43. Sabiha Khan and Neelam Sharma. (2012) Study on enzymes acid phosphatase and alkaline phosphatase in the liver and kidney of fish, *Gambusia affinis* exposed to the chlorpyrifos, an organophosphate. *International Journal of Pharmaceutical Sciences Review and Research*., **13(1)**: 88-90.
44. Sabiha Khan. (2014) Endosulphan concentration alters biomarkers in albino rat. *Indian Journal of Pharmaceutical and Biological Research*., **2(2)**: 14-1.
45. Santhakumar, M. and M. Balaji. (2000) Acute toxicity of an organophosphours insecticide monocrotophas and its effects on behaviour of an air-breathing fish, *Anabas testudineus* (Bloch). *Journal of Environmental Biology*., **21(2)**: 121-123.
46. Sastry, K.V. and A. Siddiqui. (1984) Some haemotological, biochemical and enzymological parameters of a fresh water teleost fish *Channa punctatus*, exposed to sublethal concentrations of quinolphos. *Pestic. Biochem. Physiol*., **22:** 8-13.
47. Shailendra Kumar Singh, Sunil Kumar Singh and R.P. Yadav. (2010) Toxicological and Biochemical alterations of cypermethrin (synthetic pyrethroids) against freshwater teleost fish *Colisa fasciatus* at different season. *World Journal of Zoology*., **5(1):** 25-32.
48. Sherif-El, M.S., M.T. Ahmed, El-Danasoury, H.K. Nagwa and E.I. Nwishy. (2009) Evaluation of Diazinon toxicity on Nile Tilapia fish. *J. Fish Aquatic Sci*., **4(4):** 169-177.
49. Sreenivasan, R.S., P. Krishna Moorthy and M. Deecaraman. (2011) Effect of phosphatases activity in the Hepatopancreas and Muscle of the fresh water female field crab, *Spiralothelphusa hydrodroma* (Herbst) treated with Cypermethrin. *IJPSDR.*,**3(2):** 123-126.
50. Sujatha, K. (2011) Nutritional aspects in common edible fishes from two fish landing centres in Coromandal coast with special reference to human health. *Ph.D. Thesis*., University of Madras.

51. Tejendra, S.G., P. Jaishree and H. Tiwari. (1990) Enzyme modulation by sublethal concentrations of aldicarb, phosphamidon and endosulfan in fish tissues. *Pesticide Biochemistry and Physiology*., **38**: 231-244.
52. Tenniswood, M., C.E. Bind and A.F. Clark (1976) Acid phoshpatases androgen dependent markers of rat prostate. *Can. J. Biochem.*, **54(4):** 350-357.
53. Thenmozhi, C., V. Vignesh, R. Thirumurugan and S. Arun. (2010) Impacts of Malathion on mortality and biochemical changes of freshwater fish *Labeo rohita*. *Iran. J. Environ. Health. Sci. Eng*., **8(4)**:189-198.
54. Thenmozhi, C., V. Vignesh, R. Thirumurugan and S. Arun. (2011) Impacts of malathion on mortality and biochemical changes of freshwater fish *Labeo rohita*. *Iran J. Environ. Health Sci. Eng*., **8(4):** 325-332.
55. Tilak, K.S., K. Satyavardhan and P.B. Thathaji. (2003) Biochemical changes induced by fenvalerate in the fresh water fish, *Channa punctatus*. *J. Ecotoxicol. Environ. Monit*., **13**: 261-270.
56. Tilak, K.S., K. Veeraiah and D. Koteswara Rao. (2005) Biochemical changes induced by chlorpyrifos an organophosphate compound in sublethal concentrations to the fresh water fish *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. *J. Environ. Biol*., **26(2):** 341-347.
57. Tripathi, G. and V. Priyanka. (2004) Fenvalerate induced changes in a catfish, *Clarias batrachus*: metabolic enzymes, RNA and protein. *Comparative Biochemistry and Physiology part C*., **138(1):** 75-79.
58. Umamaheswari, S. and S. Senthilnathan. (2014a) Enzymatic changes induced by dibutylphthalate in tilapia *Oreochromis mossambicus. International Journal of Fisheries and Aquatic Studies*., **1(6)**: 243-246.
59. Venkateswara Rao, J. (2006) Sublethal effects of an organophosphorus insecticide (RPR-II) on biochemical parameters of tilapia, *Oreochromis mossambicus*. *Comparative Biochemistry and Physiology*, Part C., **143**: 492-498.
60. Venkatramana Sandhya, G.V., P.N. Rani and P.S. Moorthy. (2006) Impact of malathion on the biochemical parameters of gobid fish, *Glosogobius giuris* (Ham). *Journal of Environmental Biology*., **27(1):** 119-122.
61. Verma, S., M. Saxena and I. Tonk. (1984) The influence of Ider 20 on the biochemical and enzymes in the liver of *Clarias batrachus*. *Environ. Pollu*., **33:** 245-255.
62. Yaji, A.G., J. Auja, S.J. Oniye, J.A. Adakole and J.I. Usman. (2011) Effect of Cypermethrin on behaviour and biochemical indices of freshwater fish, *Oreochromis niloticus*. *Elec. J. Environ, Agric and Food Chem.*, **10:** 1927-1934.
63. Yeragi, S.G., V. Koli and S. Yeragi. (2000) Effect of pesticide malathion on protein metabolism of the marine crab, *Uca merionis*. *J. Ecotoxicol. Monit*., **10(1):** 59-62.