International Journal of Advanced Research in Biological Sciences
www.ijarbs.com

Research Article

Acute exposure of Pyrazosulfuron Ethyl induced Haematological and Blood Biochemical changes in the Freshwater Teleost fish *Oreochromis mossambicus*

Ankur Upadhyay[1], Parth Pandya[2] and Pragna Parikh[3]

[1,2]Research scholar, Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara -390002. Gujarat, India.
[3]Associate Professor, Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara – 390002. Gujarat, India.

*Corresponding author e-mail: *php59@yahoo.co.in*

Abstract

Buildup of agriculture pesticides in the aquatic habitat through natural run-off is a major problem faced by developing and developed countries. The present study was designed to evaluate the 96 hr LC_{50} of herbicide, Pyrazosulfuron-Ethyl which belongs to Sulfonylurea group. Using static bioassay with continuous aeration under laboratory conditions, acute toxicity of the herbicide was determined for fresh water fish *Oreochromis mossambicus*. Hematological and biochemical parameters are used as health indicators to detect the functional status of fish under acute exposure. Hence, biochemical parameters like plasma glucose, protein, albumin and globulin were also studied to evaluate the toxic potential of the herbicide. The herbicide led to significant changes in the hematological parameters such as RBC count, Hb, PCV, MCH, MCHC, MCV and WBC count and biochemical parameters. These alterations can be used as non specific biomarkers in herbicide contaminated aquatic ecosystem.

Keywords: Pyrazonsulfuron-ethyl (PE), *Oreochromis mossambicus*, Haemetology, Blood biochemistry.

Introduction

Pesticides are essential for stable and proficient agricultural crop production; however, pesticides used on arable lands can be transported to waterways. Pesticide which are applied on agricultural land, upto 90% of this never reach the intended targets (Sparling *et al*., 2001) as a result, many other non-target organisms sharing the same environment are in disguise unintentionally poisoned. Water bodies became illegally the end point of the discharge of pesticides. Public concern about the adverse effects of pesticides on aquatic organisms, and bioaccumulation in fish and other aquatic invertebrate is increasing; therefore, there have been many monitoring surveys and research on pesticides in freshwater system (Iwafune *et al*., 2011). One of the non-target biological groups mostly affected by pesticides is fishes (Velmurugan, 2007; Majumdar and Gupta, 2009). Contamination of water by pesticides either directly or indirectly can lead to fish kills, reduced fish productivity or elevated concentrations of undesirable toxicant in fresh water edible fish tissue which can affect the

health of humans consuming these fishes (Adedeji *et al*., 2009.).

Sulfonylurea herbicides are an important class of herbicides used worldwide for controlling weeds in all major agronomic crops. Among sulfonylurea products, PE herbicide is widely used for selective post-emergence control of annual, perennial grasses and broad-leaved weeds in cereals, and is currently recommended for use on some relevant crops in over 30 countries (Singh *et al.,* 2012; Giovanni *et al*., 2011). Due its widespread use, it has become a potential water pollutant and presents environmental risk, especially for aquatic organisms, owing to its fairly high water solubility which result in its high mobility. It has been detected in surface and groundwater (Battaglin *et al*., 2000). Phytotoxicity of chlorsulfuron, sulfometuron-methyl and metsulfuron-methyl has been reported for higher plants (Sabater and Carrasco, 1997). Toxicity of triasulfuron on aquatic organisms has been reported earlier (Baghfalaki *et al.,* 2012). However, the toxic potential of PE on fresh water teleost *O.mossambicus* is lacking.

Acute toxicity test usually provide estimates at the exposure concentration causing 50% mortality (LC_{50}) to test organisms during a specified period of the time. The application at LC_{50} has gained acceptance among toxicologists and is generally the most highly rated test for assessing potential adverse effects of chemical contaminats to aquatic life. The exposure of fish to chemical agents induce changes in several hematological variables (Heath, 1995), and are recurrently used to evaluate fish health (Martinez and Souza, 2002). The study of blood parameters in fishes has been extensively used for the detection of physiological alterations in different conditions of stress (Pathak *et al*., 2013 and Parikh *et al*., 2014) Hematological parameters such as hematocrit, hemoglobin, number of erythrocytes and white blood cells are indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in aquatic animals (Sancho *et al*., 1997; Adedeji *et al*., 2000). Moreover, haematological and biochemical parameters are used as health indicators to detect the structural and functional status of fish under stress condition (Ramesh and Saravanan, 2010). In recent years, biochemical variables are used more to determine the effects of external stressors and toxic substances. Therefore, the biochemical evaluations are gradually becoming a routine practice for determining the health status in fish (Padma *et al*., 2012). Hence in the present study an attempt is made to have an insight to the toxicity deviations on the hematological as well as biochemical alteration on *O.mossambicus* on acute exposure of the herbicide.

Materials and Methods

Experimental design

The freshwater fish, *O. mossambiccus* of similar size in length (12 ± 2 cm) and weight (25 ± 1.9 g) were brought from a local pond of Baroda district and were acclimatized at laboratory conditions for 10 days in well aerated test aquaria containing de-chlorinated water. They were fed with commercial fish pallets. 30% water was renewed every day to provide freshwater, rich in oxygen. To evaluate the acute toxicity of the PE herbicide 96-hour LC_{50} values were determined. The day before and during the tests the fish were not fed. For each concentration, 10 fish were tested and the experiment was repeated thrice. Probit analysis (Finney, 1971) was followed to calculate the LC_{50} values.

Experimental Procedure

The experiments were conducted in a series of glass aquariums filled with 40 liter de-chlorinated tap water. Healthy fishes *O. mossambicus* was selected for the test (n=10) to determine the LC_{50} value of each fish. Based on the pilot experiments, the experiment was conducted to determine the toxicity in different concentrations. The concentrations used included 50 mg/l, 100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l, 500 mg/l, 600 mg/l, 700 mg/l, 800 mg/l and 900 mg/l and 1000 mg/l with three replicates each. The stock solutions were prepared and the required quantity of PE was drawn from the stock solution to find out the LC_{50} values for 96 h. Group 1 served as control, while Group 2, 3, 4 and 5 were

treated with PE with concentration 50 mg/l, 100 mg/l, 200 mg/l and 400 mg/l respectively. Ten acclimatized fishes of uniform size were exposed to each concentration. The control and the exposed fish were aerated frequently to prevent hypoxic condition of the medium. Feeding to fishes was stopped during the experiment. Mortality of the fish was recorded from time to time till 96 hours.

Haematological estimation of fish

After the completion of 96 hr acute toxicity Test, fishes were collected from each aquarium for blood analysis. About 3 - 4ml of blood was collected from the caudal peduncle using separate heparinized disposable syringes. The blood was stored in -4°C prior to estimation of hematology. Haemoglobin estimation (HB), Pack Cell Volume (PCV), blood glucose level and total serum protein were analyzed by NIHON KOHDEN Automated Hematology Analyzer (Celtac alpha, Japan). Red blood cell count (RBC), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) was determined using the formulas given below.

MCHC = HB/PCV*1000 g/dL

MCV =PCV*1000/ RBCs fL

MCH = HB/RBCs pg

Statistical analysis

LC_{50} value was determined by Probit analysis using StatPlus 2009 Professional, 5.8.4.version software. Statistical analysis was performed using Graph pad prism 6 software. The data was analyzed using one-way ANOVA test. Results were presented as mean ± SD. The significance was set as $P<0.05$, $P<0.01$ and $P<0.001$.

Results

Table I shows the relation between concentration of PE and mortality rate of fish. The LC_{50} values according to Probit regression curves was found to be 501.65 mg/l, however the Lower Confidence Limit (LCL) value and Upper confidence limit (ULC) were 407.83 mg/l and 595.47mg/l respectively (Fig. 1 & 2). While the LC_{10}, LC_{16}, LC_{84} and LC_{90} were found to be 95.68mg/l, 184.91 mg/l, 818.38mg/l and 90-907.62mg/l respectively.

A significant increase ($p<0.01$) with 50 and 100mg/L dosage in the values of RBC count, HB, and PCV was obtained in the exposed group compared to control (Table – 2). On the other hand MCV showed an insignificant increase while MCHC showed a significant decrease with insignificant alteration in MCH. There were no significant changes in the parameters at higher doses (Table 2, Fig. 3). WBC count and blood glucose showed a significant ($p<0.05$, $p<0.01$) increase compared to control groups (Table-2, Fig-3 & 4). Serum protein level showed a dose dependant significant increase ($p<0.05$) in the experimental groups compared to the control. There was a significant increase ($p< 0.05$) in the globulin and decrease ($p<0.05$) in albumin values (Table 2, Fig. 4).

Discussion

PE, a new rice herbicide belonging to the sulfonyl urea group has recently been registered in India for weed control in rice crops (Singh *et al*., 2012).Several studies indicate that these group of herbicides on leaching enters ground water and tend to persist (Battaglin *et al*., 2000). However once it enters surface waters it may affect other organisms such as fish as a non-target organism in natural conditions (Aktar *et al*., 2009).From the LC_{50} value detected in the present study PE can be categorized into least toxic compound.

Blood is a pathophysiological reflector of the whole body and therefore, blood parameters are important in diagnosing altered physiological status of fish exposed to toxicants (Adhikari *et al*., 2004). PE exposure resulted into a significant increase in the hematological parameters: RBC count, haematocrit (PCV), and HB compared to control. Maximum alteration was noticed at 50 mg/l, followed by a decrease. The initial increase could be due to increased hypoxia (Rifkind *et al*., 1980 and wepener *et al*., 1992). Liver through activating erythopoiesis is probably preventing the physiological hypoxic

Table 1. The relation between concentration of PE and mortality rate of *O. mossambicus*

Concentration	Actualpercent%	Log10 conc.	Total no.	No. dead	Probit
Control	-----		10	0	-
50	0.025	1.699	10	0	3.0396
100	0.1	2.0	10	1	3.3183
200	0.2	2.301	10	2	4.1585
300	0.3	2.4771	10	3	4.476
400	0.4	2.6021	10	4	4.7471
500	0.5	2.699	10	5	5.0
600	0.6	2.7782	10	6	5.2529
700	0.7	2.8451	10	7	5.524
800	0.8	2.9031	10	8	5.8415
900	0.9	2.9542	10	9	6.2817
1000	0.975	3.0	10	10	6.9604

Table 2. Haemetological Parameter, Blood Glucose and Total Protein in *O.mossambicus* affected by acute exposure of PE

Parameters	Concentration mg/l of PY (Mean ± SD)				
	Control	50mg/l	100mg/l	200mg/l	400mg/l
RBCs 10^6/µL	1.04± 0.025	1.90±0.027	1.69 ±0.023	1.40±0.028	1.28±0.022
HB g/dL	5.3±0.154	9.7±0.159	8.6±0.152	6±0.157	5.80±0.162
PCV(Htc)%	14.6±0.555	29.6±0.551	32.6±0.558	23.4±0.557	19±0.554
MCV fL	140.38±3.52	155.79±3.56	192.9± 3.54	144.44±3.57	142.42±3.51
MCHC g/dL	36.3±1.03	32.77±1.03	26.38±1.03	34.62±1.03	31.3±1.03
MCH pg	50.96± 1.66	51.96± 1.68	50.89± 1.64	50± 1.65	53.54± 1.69
TotalWBC10^3/µL	45,000± 655	151,600±653	109,500±653.64	79,600±657.89	76,800±652.23
Glucose	138±3.162	215± 3.113	322±3.15	133±3.19	222±3.14
Protein	10.3± 0.462	11.9± 0.471	12.7± 0.469	14.3± 0.465	16.5±0.473
Albumin	5.64± 0.48	3.4± 0.49	2.40±0.48	4.80±0.47	5.70±0.48
Globulin	6.3±0.354	6.9±0.355	5.50±0.357	8.00±0.360	10.8±0.359

Figure 1. Plot of adjusted probits and predicted regression line of PE to *O.mossambicus*

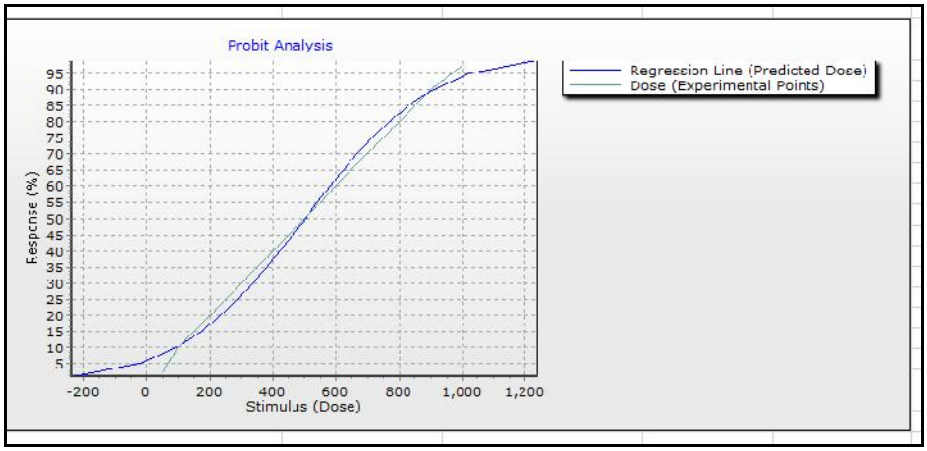


Figure 2. Plot of adjusted probits and predicted regression line of PE to *O.mossambicus* in log10base

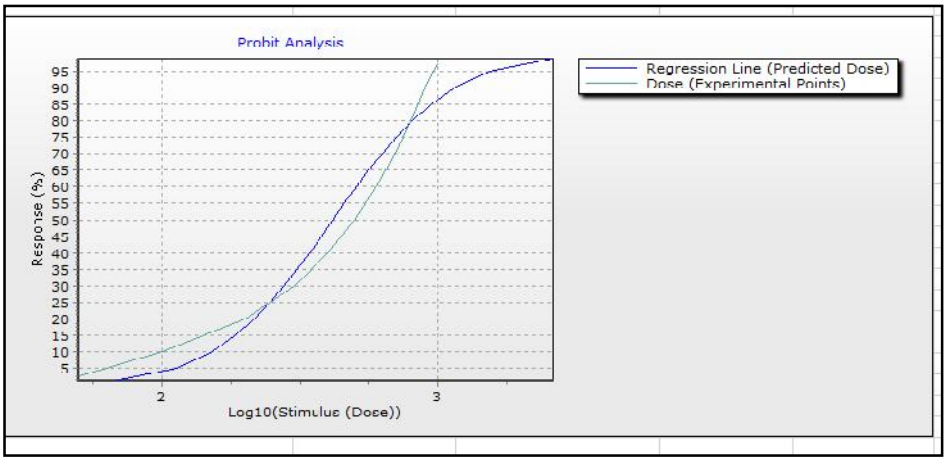
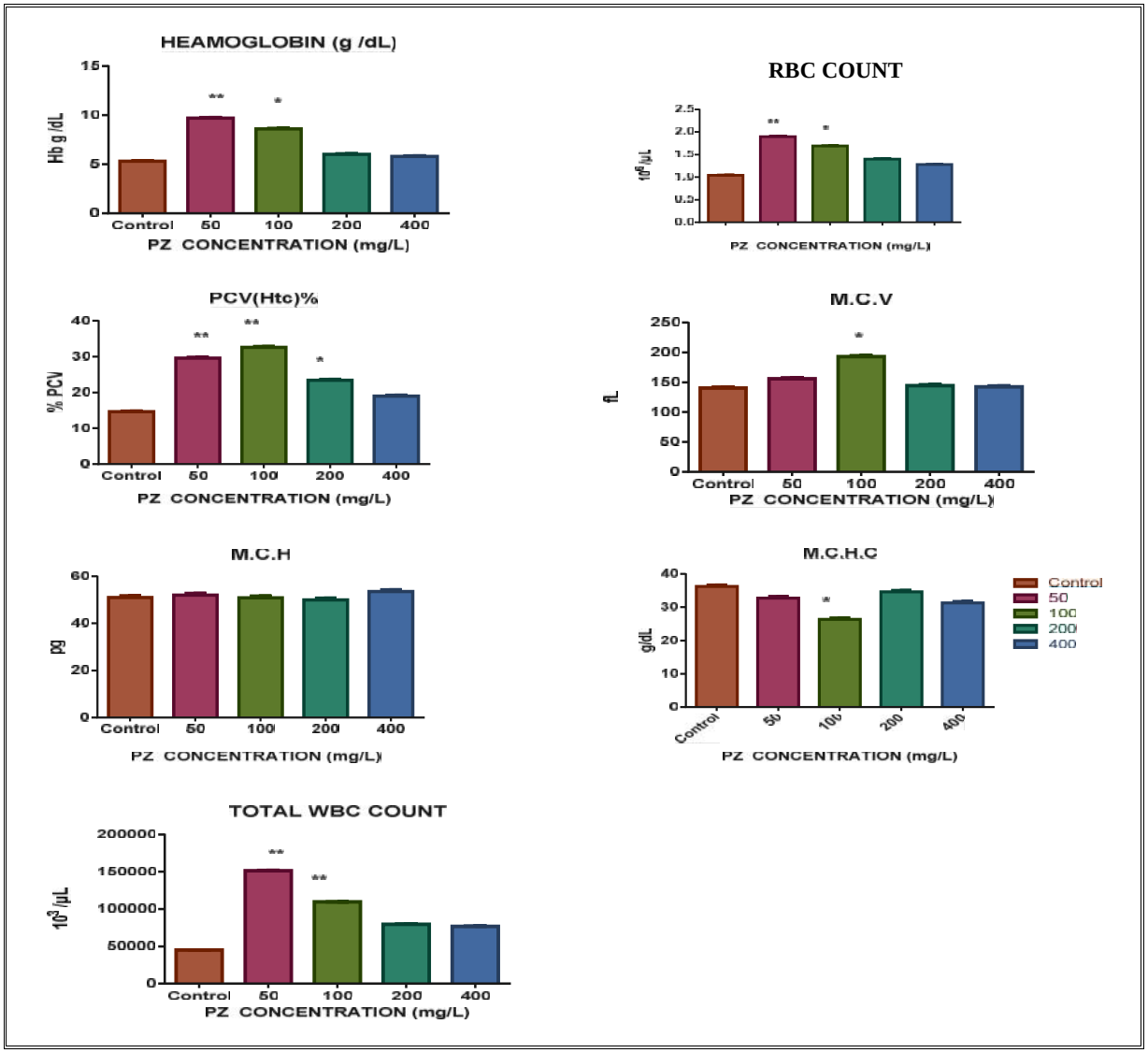
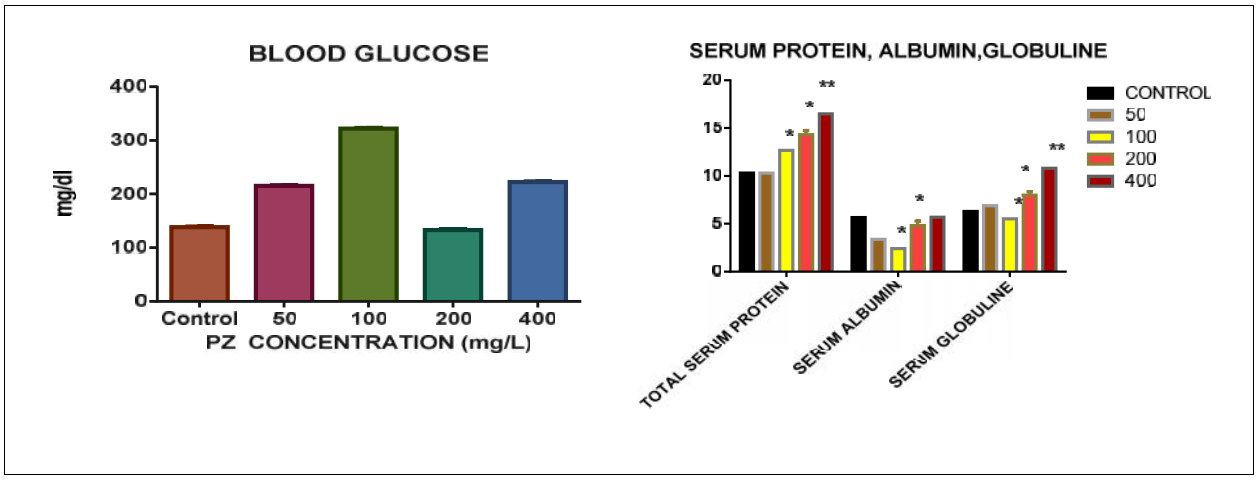


Figure 3. Changes in Haemetological Parameter of PE treated fish *O.mossambicus*.



Values are mean ± SD of five individual observations, Values are significant at (*) $p<0.05$, (**) $p<0.01$

Figure 4. Changes in Blood Glucose and Total Serum Protein of PE treated fish *O.mossambicus*



Values are mean ± SD of five individual observations, Values are significant at (*) $p<0.05$, (**) $p<0.01$

condition as this mechanism work well for the short-term variation in oxygen concentration in blood (Nespolo and Rosenmanm, 2002). Gills are the first organ to come in contact with the toxicant, damaging the gills and impairing the oxygen transport. The increase in PCV is likely to be due to either gill damage or due to increased metabolic demand or both (Varadarajan, 2010). It has been shown that the erythrocyte number and haemoglobin level may vary with oxygen requirements (Hubrec *et al*., 2000; Tavares *et al*., 2004). Similar results were found in reports of acute intoxication by dichlorvos in *Clarias batrachus* (Benarji, 1990), by quinalphos in *O. mossambicus* (Sampath, 1993). Parallel with an increase in the RBCs, WBC also showed a significant increase. Joshi and his co worker (2002) are of the views that increase in WBC count is suggestive of an increase in antibody production for survival and recovery of the fish exposed to pesticides, lindane and Malathion. Thus, the increased WBC counts indicate hypersensitivity of immune cell resulting into immunological reactions to produce antibodies to cope up with the stress induced by PE (Ramesh and Saravanan, 2008).

Biochemical analysis provides valuable information for monitoring the health condition of fishes. Biochemical variations depend on the fish species, age, sexual maturity and health condition. Analysis of glucose concentration in blood is widely used as indication of stress response. Studies have also reported blood glucose to be a sensitive indicator of environmental stress in fish. By and large glucose is continuously required as an energy source by all body cells and therefore must be maintained at adequate levels in the plasma. In the present study the significant increase in glucose may be the manifestation of stress induced by herbicide was seen. The increase of glucose can be interpreted as a consequence of glycogenolytic activity of catecholamines and gluconeogenetic effect of glucocorticoids as in response of an organism to the stress induced by PE. Our results are agreement with the earlier work of Ramesh and Sarvanan (2008).

Proteins are mainly involved in the structural architecture of the cell. During stress conditions fish need more energy to detoxify the toxicant to overcome pesticide trauma. PE exposure resulted into a dose dependent increase in the proteins. Stress increases the physiological activity which in turn will demand mobilization of proteins to meet the energy required. To overcome the stress there is an increase in the protein synthesis (Martinez *et al.*, 2004;Sweety *et al*., 2008). Furthermore, serum protein mainly contains albumin and globulin. Albumin is thought to have three basic functions in fish: osmotic regulation of blood volume, source of protein reserve and is also involved in transport functions of exogenous chemicals and endogenous metabolites (Andreeva, 1999; Baker, 2002). Hence the significant decrease in the albumin is probably

equipping the fish for the removal of the PE; being an exogenous chemical. However there is an overall increase in the proteins which may be due to concomitant increase in the globulin.

Hence, from the present study the mild toxic nature of the herbicide PE is apparent by the significant changes in the hematological and biochemical changes in the blood, and that the fresh water fish *O. mossambicus* are sensitive to herbicide. The alterations of the parameters may provide the early sign for the determination of acute toxic level of herbicide and their effects on aquatic medium. The findings of present study also provide a better understanding of toxicological endpoint of aquatic pollutants and safer level of these herbicides in the aquatic environment and protection of aquatic habitats.

References

Adedeji, O. B., Adeyemo, O. K. and Agbede, S. A. 2009. Effects of diazinon on blood parameters in the African catfish (*Clarias gariepinus*). African. J. Biotechnol. 8:3940-3946.

Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C. T. and Ayyappan, S. 2004.Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). Ecotoxicol. Environ. Safety.58(2):220-226.

Aktar, W., Sengupta, D. and Chowdhury, A. 2009.Impact of pesticides use in agriculture: their benefits and hazards. Interdisci. Toxicol. 2 (1):1-12.

Andreeva, A.M. 1999.Structural and Functional Organization of the Blood Albumin System in Fish.Vopr. Ikhtiol. 39:825-832.

Ayotunde, E.O., Fagbenro, O.A and Offem, B.O.2009. Haematological characteristics of African bony tongue, *Heterotis niloticus* (Teleostei: Arapaimidae), in south-western Nigeria. African. J. Aquatic Sci. 34(1): 97–101.

Baghfalaki, M., Shaluei, F., Hedayati, A., Jahanbakhshi, A. and Khalili, M. 2012.Acute Toxicity Assessment of Tribenuron-Methyl Herbicide in Silver Carp (*Hypophthalmicthys molitrix*), CommonCarp(*Cyprinus carpio*) and Caspian Roach (*Rutilus rutilus caspicus).* Global Veterinaria. 8(3): 280-284.

Baker,M.E. 2002 Albumin, steroid hormones and the origin of vertebrates. J. Endocrinol.175:121–127.

Battaglin, W. A., Furlong,E.T., Burkhardt, M. R. and Peter, C. J. 2000. Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the Midwestern United States. The Science of the total environment.248: 123-133.

Benarji, G. and Rajendranath, T.1990. Haematological changes induced by an organophosphorus insecticide in a freshwater fish *Clarias batrachus*(Linnaeus).Tropical freshwater biology. Benin City. 2(2):197-202.

Biron, M. and Benfey, T.J.1994 Cortisol, glucose and hematocrit changes during acute stress, cohort sampling, and the diel cycle in diploid and triploid brook trout(Saluelinus fontinalis Mitchill).Fish Physiol. Biochem.13:153–160.

Ceron, J.J., Sancho, E., Ferrando, M.D., Gutierrez, C. and Andreu, E. 1997. Changes in carbohydrate metabolism in the eel *Anguilla anguilla*, during short-term exposure to diazinon. Toxicol. Environ. Chem. 60:201–210.

Finney, D.J.1971. Probit analysis.Cambridge Univ. Press,Cambridge: 333

Giovanni,L. M, Hilton, A. J., Garcia1, S. and Luis V. 2011.Acute toxicity of PEand permethrin to juvenile *Litopenaeus vannamei***,** Acta Scientiarum. Biol. Sci.Maringá.33: 1-6.

Heath, A. G.1995.Water Pollution and Fish Physiology.CRC. Press, Inc. Boca Raton, Florida, 359pp.

Hubrec, T.C. and Smith, S.A.2000. Haematology of fish. In: Feldman, B.F., Zinkl, J.G. and Jain, N.C.(eds.), Schalm's Veterinary Hematology. Williams & Wilkins, Philadelphia, p. 1120–1125.

Iwafune, T.,Yokoyama, A., Nagai, T., and Horio, T.2011.Evaluation of the risk of mixtures of paddy insecticides and their transformation products to aquatic organisms in the sakura river, japan. Environ. Toxicol. Chem. 30: 1834–1842.

Joshi, P., Harish, D. and Bose, M. 2002.Effect of lindane and Malathion exposure to certain Blood Parameters in a freshwater teleost fish Clarias batrachus. Poll. Res.21: 55-57.

MacQueen, H.A., Wassif, W.S., Walker, I., Sadler, D.A. and Evans, K. 2011. Age-related biomarkers can be modulated by diet in the rat. Food. Nutri. Sci. 2: 884–890.

Majumdar, T.N. and Gupta, A.2009 Acute toxicity of endosulfan and malathion on *Chironomus ramosus* (Insecta : Diptera : Chironomidae) from north Cachar hills, Assam, India. J. Environ. Biol. 30: 469-470.

Martinez, C.B.R., Nagae, M.Y., Zaia, C.T.B.V. and Zaia, D.A.M.2004. Morphological and physiological acute effects of lead in the Neotropical fish *Prochilodus lineatus*. Braz. J. Biol. 64: 797–807.

Nespolo, R.F. and Rosenmann, M. 2002. Intraspecific allometry of haematological parameters in Basilichthys australis. J. Fish Biol.60:1358–1362.

Padma,P.B., Rachel, V. and Maruthi, Y.A. 2012.Acute toxicity effect of imidacloprid insecticide on serum biochemical parameters of fresh water teleost *channa punctatus*. Int. J. Int. sci. Inn. Tech.1(2):18-22.

Parikh, P.H. and Upadhyay, A.A.2014.Pyrazosulfuron-Ethyl Induced Alteration. In: Haematology And Blood Biochemistry of *Oreochromis Mossambicus*: Sub-Acute Study. TheExperiment.18 (3):1245-125.

Pathak, L., Saxena, R.S. and Sharma, H.N.2013. Haematological changes in major Indian carps under stress of malathion and parathion. Ind. J. Biol. Stud. Res. 2(2):112-120

Ramesh, M. and Saravana, M.2010.Haematological and biochemical responses in a fresh water fish. *Cyprinus carpio* exposed to chloropyrifos. International Journal of Integrative Biology. 3(1): 80-83.

Ramesh, M. and Sarvanan, M. 2008.Haematological and biochemical responses in a fresh water fish *Cyprinus carpio* exposed to chlorpyrifos. Inter. J. Integra. Biol. 3:80-83.

Rifkind,R.A., Bank, A., Marks, P.A., Nossell, H.L. Ellison, R.R. and Lindenbaum, J. 1980. *Fundamentals of Hematology*, second ed., Yearbook Medical, Inc.,Chicago.

Sabater, C. and Carrasco, J. M.1997.Effecs of Chlorsulfuron on Growth of Three Freshwater Species of Phytoplankton. Bull. Environ. Contam. Toxicol.58: 807-813.

Samprath, K., S. Velamnial, I.J. Kennedy and R. James, 1993. Haematological changes and their recovery in Oreochromis mossambicus as a function of exposure period and sublethal levels of Ekalus. Acta Hydrobiol., 35: 73-83

Sepaskhah, A.R. and Ghasemi, M.M. 2008.Every-other-furrow irrigation with different irrigation intervals for grain sorghum.Pak. J. Biol. Sci.11:1234-1239.

Singh, S.B., Sharmab, R., and Singh, N. 2012.Persistence of pyrazosulfuron in rice-field and laboratory soil under Indian tropical conditions.Pest. Manag. Sci.68:828–833.

Sparling, D.W., Fellers, G.M., and McConnell, L.S. 2001.Pesticides and amphibian population declines in California USA. Environ.Toxicol. Chem.20: 1581-1595.

Sweety, R. Remyla, Ramesh, M., Kenneth S. S. and Kurunthachalam, S. K. 2008. Influence of zinc on cadmium induced haematological and biochemical responses in a freshwater teleost fish *Catla catla*. Fish. Physiol. Biochem.34:169-174.

Tavares-Dias, M., Ono, E. A., Pilarski, F. and E Moraes, F.R. 2004. Can thrombocytesparticipate in the removal of cellular debris in the blood circulation of teleost fish? Acytochemical study and ltrastructural analysis. J. Appl. Ichthyol.23:709-712.

Varadarajan, R. 2010. Biochemical Effects of Different Phenolic Compounds On *Oreochromis Mossambicus* (Peters) (Doctoral Dissertation, Cochin University Of Science And Technology).

Velmurugan, B., Selvanayagam M., Cengiz, E. I. and Unlu, E.2007. The Effects of Fenvalerate on different tissues of fresh water fish *Cirrhinus mrigala*. J. Environ. Sci. Hlth. 42: 157-163.

Wepener, W., Vuren, Van., Du, J.H.J and Preez, H.H.1992. Bull. Environ. Contamin. Toxicol. 49:613- 619.

ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/278683356

PYRAZOSULFURON-ETHYL INDUCED ALTERATION IN HAEMATOLOGY AND BLOOD BIOCHEMISTRY OF OREOCHROMIS MOSSAMBICUS: SUB-ACUTE STUDY

Article · January 2014

CITATIONS
4

READS
73

1 author:

P.H. Parikh
The Maharaja Sayajirao University of Baroda
41 PUBLICATIONS **107** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Establishment of Insect cell line View project

All content following this page was uploaded by P.H. Parikh on 19 June 2015.

The user has requested enhancement of the downloaded file.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

Parikh P.H *et al*, The *Experiment, 2014., Vol. 18(3), 1245-1253*

PYRAZOSULFURON-ETHYL INDUCED ALTERATION IN HAEMATOLOGY AND BLOOD BIOCHEMISTRY OF *OREOCHROMIS MOSSAMBICUS:* SUB-ACUTE STUDY

ABSTRACT

Aquatic ecosystems are the ultimate descend of natural and anthropogenic inputs of contaminants into the environment. Many of the environmental pollutant especially pesticides enter into aquatic ecosystem through agricultural run off and ultimately affects the different non target aquatic animals like bivalves, crustaceans, molluscs, prawn and fish, which are of great economics important to humans. Herbicides are a known class of chemicals used to treat weeds, one of the known chemical Pyrazosulfuron-ethyl (PE) is a group of sulfonylurea which is widely used in today's world. Among the sulfonylurea herbicides, pyrazosulfuron ethyl received the most attention because there is limited information available on the fate of pyrazosulfuron ethyl in the environment. Blood hematological and serum biochemistry parameters are often used to assess the health status and as stress indicator in fish. The present study was carried out the impact of sublethal toxicity of herbicide pyrazosulfuron ethyl on haematological and biochemical parameter on freshwater fish *Oreochromis mossambicus.* The fishes were treated with PE During the sublethal treatment (7d,14d) study estimation of hemoglobin(Hb),hematocrit(Hct),erythrocyte count(RBC), mean cellular volume(MCV),mean cellular hemoglobin(MCH), mean cellular hemoglobin concentration (MCHC),and total leucocytes count(WBC) Where as the biochemical profile plasma glucose, total serum protein, albumin, globulin, urea, creatinine, blood urea nitrogen measured. In the present study, the herbicide PE caused the alterations on haematological as well as biochemical parameter of *O.mossambicus* and these alterations can be used as non specific biomarkers in pesticide contaminated aquatic ecosystem.

KEYWORDS

Pyrazosulfuron ethyl, Haemetology, Blood Biochemistry, Sub acute

INTRODUCTION

Injudicious and indiscriminate use of agrochemicals such as fertilizers, pesticides, insecticides and fungicides to boost crop production with the sole aim of getting more yield, water bodies like ponds, lakes, river and low lying water areas are continuously getting polluted [1]. Normally these pesticides reach the aquatic environment through surface runoff, sediment transport from treated soil and direct application as a spray to water bodies to control the inhabiting pests [2]. Thus, Aquatic ecosystems are the ultimate descend of natural and anthropogenic inputs of contaminants into the environment. The agrochemicals used in agriculture are posing a great threat to aquatic fauna especially to fishes, which constitute one of the major sources of protein rich food for mankind.

Herbicides are the most commonly used pesticides, and are the most often detected in surface waters [3]. Numerous commercial formulations containing different herbicides (glyphosate, paraquat, sulfonolurea etc) have become popular around the world due to their effective action and low toxicity to mammals [4, 5, 6]. however; they have proved to be harmful to the environment. Sulfonylurea herbicides are an important class of herbicides used worldwide for controlling weeds in all major agronomic crops. Among sulfonylurea products, pyrazosulfuron-ethyl herbicide is widely used for selective post-emergence control of annual and perennial grasses and broad-leaved weeds in cereals. PE is widely used in rice crops in India [7]. There is limited information available on the fate of PE in the environment.

Blood is a liquid vital fluid and important index for health, environmental effect and growth and reproduction cycle. Haematological analyses has been routinely used in determining the physiological state of animals and known to be affected by different environmental factors, it is used as a guide in the diagnosis of many diseases and in evaluating the responses to therapy in both animals and human [8]. Hematology is used as an index of fish health status in a number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia etc. [9, 10] Hence it use is growing and becoming very important for

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

toxicological research. Shah and Altindag (2004) noted that studies on fish blood gives the possibility of knowing physiological conditions within the fish long before there is an outward manifestation of pathological/disease condition because under stressful condition as well as environmental imbalances some parameters in the fish blood changes in response to reflect the change, the present study therefore examine changes in hematological parameters on sub acute exposure of PE after 7th and 14th day. Nutritional value of fish depends on their biochemical composition, which is affected by the agrochemicals [12]. Alterations in biochemical components as response to environmental stress are authenticated by many authors. Biochemical analysis can provide the valuable information for monitoring the health conditions of fishes. There is vast amount of scientific information available on different herbicides' toxicity on different fish's global level for paraquat [13]on Benny fish *Mesopotamichthy* and by [14]on the haematological parameters of the African catfish, *Clarias gariepinus.* Effect of Glyphosate herbicide has been also been reported on the serum growth hormone (GH) levels and muscle proteins in Nile Tilapia (*Oreochromis Niloticus*) by [15] Behavioral alteration of the common carp (*cyprinus carpio*) has been documented by [16]. However the effects of PE on fresh water Tilapia fish *O. mossambicus* are not well understood. There is a paucity of information about the effects of PE on the hematological and biochemical parameters of *O. mossambicus.* The objective of the present study was to determine the sub-acute toxicity of PE and its effects on haematogical and biochemical parameters of *O. mossambicus* in order to enrich the present knowledge on herbicide toxicity and to show the toxic effects of the herbicide.

MATERIALS AND METHODS

Experimental design:

The specimens of freshwater fish, *O. mossambicus* of similar size in length (12 ± 2 cm) and weight (25 ± 1.9 g) were brought to the laboratory from a local pond of Baroda district and were stocked in well aerated tanks containing chlorine free water for 10 days. Temperature, pH, and dissolved oxygen of the water were maintained at 27 ± 2° C, 7.1 ± 0.5, and 3.9 ± 0.02 mg/L, respectively. If in any batch, mortality exceeded 5% during acclimatization, that entire batch of fish was discarded. They were fed with commercial fish pallets. 30% Water was renewed every day to provide freshwater, rich in oxygen. Ten well-acclimatized fish were transferred from the stock to each experimental tank containing 40 L of water exposed to different concentrations of PE. A control group was also maintained in the same condition for the basic test. The LC_{50} values in the respective time intervals were calculated using software by transforming mortalities (percentage values) into a probit scale [17].

Experimental Procedure

On basis of LC_{50} value sub acute study dose $LC_{50}/10$ was chosen for hematological and biochemical studies. The experimental regime was maintained in the laboratory for14 days. A control group was also maintained. The experiment was performed semi statically with a group of 10 fish in two experimental aquaria, one control aquaria. Hematological and biochemical examinations of the experimental as well as the control fish were carried out at 7th and 14th days of exposure. All the groups were kept under continuous observation during the experimental period. Commercially food pallets were given to fish's once in day during the experiment Ad libitum. Behavior of the test fishes was observed during the experiment period. After the completion of the exposure fish were caught very gently using a small dip net, one at a time with least disturbance. They were slowly released in the tough containing 1% clove oil to make it immobile, and then after each fish was held and wrapped with a clean, dry towel and the posterior half of its body were blotted with a clean coarse filter paper. The total body weight was noted.

Haematological and biochemical estimation of fish: The caudal peduncle of the fish was severed with a single stroke from a heavy, sharp seizure. After discarding the first drop of blood, the freely oozing blood was collected using separate heparinized disposable syringe. The blood was then transferred to the ependrof containg anticoagulant, thoroughly mixed using a thin, blunt glass rod, during the process of collection itself. The blood was stored in -4°C prior to hematological and biochemical estimations. An alteration in the hematology and biochemical profile was performed using NIHON KOHDEN Automated Hematology Analyzer (Celtics α, Japan). The difference between the control and the PE exposed fishes was determined by Multivariate Test (MANOVA). If there was significant difference, Tukey's Post Hoc was employed to recognize difference in the alteration. The significant level of the tests was set at 5% ($p<0.05$).

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

Parikh P.H *et al*, The *Experiment, 2014., Vol. 18(3), 1245-1253*

RESULTS AND DISCUSSION

The alterations in the hematology and biochemical parameters are presented in Table 1 and in Fig 1 and 2. The results of this investigation are as presented in table 1 indicating wilk's lambda test. Levene's test of Equality of error variances and dunnett's test respectively. The positive value in the post hoc tests indicates increases in the activity of the parameter studied and vice versa. The exposure of fish to PE caused clear significant decrease ($p<0.05$) in hematocrit and hemoglobin levels (Fig 1) after 7^{th} and 14^{th} days of exposure periods, respectively, in comparison with control. The results also revealed that after PE exposure, other hematological indices including MCV, MCH experienced considerable decrease (Fig 1). Blood forms a unique compartment between external and internal environments and any agent including toxic substances that causes stress and can alter blood composition either directly or indirectly by altering osmotic and ion regulation. Blood parameters are considered good physiological indicators of the whole body conditions and therefore can be used in diagnosing the structural and functional status of fish exposed to toxicants [18, 19]. The exposure of *O.mossambicus* to sub acute concentrations of PE resulted into a significant and progressive decrease in the PCV with time i.e on day 7^{th} and 14^{th} respectively compared to the control. A decrease in the erythrocyte count or in the percent of haematocrit indicates the worsening of an organism state and developing anaemia. In the light of the present study, PE exposure led to anemia, as indicated by the significant decrease in Hb and PCV values leading to anemia and as a response might have led to a fall in the red blood cell count, hemoglobin concentration and haematocrit volume. Hypoxia, anemia, and hyperthermia are related stresses causing an osmotic imbalance and decreased capacity of the RBC to carry sufficient oxygen unless otherwise compensated by erythropoiesis or suitable physiological adjustments. The anemic condition in fish results from an unusually low number of red blood cells or too little hemoglobin in the red blood cells. According to [20]the pesticide induced anemia in fish may be due to the inhibitory effect of the toxic substance on the enzyme system responsible for the synthesis of haemoglobin. It may also be due to impaired intestinal absorption of iron, as suggested by [1] Furthermore, this is an indication of disruptive effects of PE on erythropoietic tissues as well as cells viability [21]. Moreover, there are reports that dilution of blood in an organism is an indication of suppressed osmoregulation [22, 23, 24] Similar kind of actions has previously considered by other researchers for herbicides Paraquat [13] Roundup [25. 26], for Atrazine [27] and some of the pesticides such as Imidachloprid. The MCV, MCH and MCHC also decreased considerably compared to the control. This is in agreement with the work of [28] following a short-term exposure of tench (*Tinca tinca*) to agrochemical metal. These alterations were attributed to direct responses of structural damage to RBC membranes resulting in haemolysis and impairment in haemoglobin synthesis, stress related release of RBCs from the spleen and hypoxia, induced by exposure to agrochemical [28].

Biochemical analysis can provide valuable information for monitoring the health conditions of fishes. Biochemical changes depend on the fish species age, the cycle of sexual maturity and health condition. Moreover, analyses of serum biochemical constituents' levels have shown useful information in detection and diagnosis of metabolic disturbances and diseases in fishes ([29]. Determination of glucose concentration in blood serum is widely used as an indication of stress response. Generally, glucose is continuously required as an energy source by all body cells and must be maintained at adequate levels in the plasma. In many fish species, the blood glucose level has the tendency to increase due to experimental stress. In the present study the significant time dependent increase in glucose may be considered to be manifestation of stress induced by PE herbicide. Blood glucose is caused by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stresses. A variety of stressors stimulate the adrenal tissue resulting in increased level of circulating glucocorticoids and catecholamines. Both of these groups of hormones produce hyperglycemia. It is generally thought that, under conditions of stress, hyperglycemia may provide additional energy during times of high metabolic need such as "fight and flight" response [10].

Concerning the results of total protein, albumin, globulin and A/G ratio proteins are the most important and abundant macromolecules in living beings, which play a vital role in architecture and physiology of the cell and in cellular metabolism. Also proteins play an important role in the metabolism and regulation of water balance [30]. Proteins play a vital role in physiology of living organisms. All biological activities are regulated by enzymes and hormones, which are also proteins. Assessment of protein content can be considered as

a diagnostic tool to determine the physiological phases of the cells [31]. Results revealed total hypoproteinaemia, hypoglobulinaemia, hypoalbuminaemia and increased A/G ratio in exposed fish during sub acute exposures .The survival ability of animals exposed to stress mainly depends on their protein synthesis potential. The decrease in protein content was probably due to reduced/perturbation of microsomal protein synthesis suggested as suggested by many workers. The degradation of protein suggests the increase in proteolytic activity and possible utilization on their products for metabolic purpose and cause damage. The depletion of protein fraction in serum in present study may have been due to their degradation and possible utilization of degraded products for metabolic purposes [32] The quantity of protein is dependent on the rate of protein synthesis or on rate of its degradation. Decreased protein level may be attributed to stress mediated immobilization of these compounds to fulfil an increased element for energy by the toxicant.(Seth and Saxena 2003). Serum albumin measures as considerable diagnostic value in laboratory animals because it relates general nutritional status, the integrity of the vascular system and liver function. Albumins in fish organism participate in plastic metabolism and perform transport functions of substances necessary for life activities. In our study Protein, albumin and globulin were decreased significantly. [24] also observed same results in Sevin exposed to *Clarias battrachus.*

Stress results in an increase in cortisol levels in fish stimulating both glycogenesis and gluconeogenesis, as well as an increase in protein catabolism and ammonia production. Ammonia is toxic to all vertebrates. It can be converted to the less toxic urea, but this is a metabolically expensive process which is found only in terrestrial vertebrates. Teleost fishes are primarily amminotelic but their blood contains significant amount of urea and indeed in some teleosts it may account for 20 % or more of the total nitrogen excreted. Occurrence of uremia was reported by many workers [33, 34] Hence, freshwater fish excrete ammonia along with a small quantity of urea as they use urea as an osmotic filter. [35] Furthermore, renal disorders also are known to elevate serum urea values, which are parallel with an significant increase in the BUN, suggesting renal disturbances. The Lake Magadi tilapia excretes all nitrogenous waste as urea produced via the ornithine urea cycle .Many fish embryos have an active ornithine urea cycle and convert ammonia to urea to avoid ammonia toxicity during the early stages of development .Thus urea formation is used by several fish species during development or under certain environmental conditions, such as air exposure or alkaline water pH, to avoid problems of ammonia accumulation and toxicity [36].

Creatinine is derived mainly from the catabolism of creatine found in muscle tissue and its catabolism to creatinine occurs at a steady rate. Severe kidney damage will lead to increased creatinine levels. In the present study serum Creatinine showed insignificant decrease in experimental group in comparison to control animal suggesting that there occurs an alteration in glomeruli filtration rate. Excretion occurs through a combination of glomerular filtration (70 to 80%) and tubular secretion [37, 38]. The alteration in the levels of serum creatinine may, therefore, be due to a combination of these two factors. [39, 40]. Our results are in agreement with the results of [41] who reported that the level was significantly unaffected in O. niloticus exposed to sublethal concentration of atrazine. However, they, disagree with that of [42, 43] This difference in results may be due to the difference in fish species and the nature of the pesticide used.

BUN levels should be viewed by clinicians as a potential indicator of disrupted nitrogen excretion. Elevated blood urea nitrogen level in teleost may serve as a clinical indication of respiratory and excretory compromise due to respiratory epithelial cell hypertrophy and hyperplasia [44]. Thus the elevated blood urea in the present study can be expalined as a protection of fish against oxyhaemoglobin oxidation in the blood [45].Furthermore, as there is no significan change in the Creatinine level it is possible that the increased level of blood urea nitrogen is trying to overcome the respiratory stress induced by the herbicide.

To conclude, the PE exposure of *O.Mossambicus* at sub-acute concentration caused alterations to hematological and biochemical indices, all of which resulted in stress to the organism. The herbicide therefore can be classified as toxic for fish. It also points to the fact that the haematological parameters are the most sensitive parameters in monitoring the toxicity.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

Parikh P.H *et al*, The *Experiment, 2014., Vol. 18(3), 1245-1253*

Table 1: Haemetological and blood biochemical Parameter in *O.mossambicus* affected by sub acute exposure of PE:

Variables	Control vs Treated (7 d)	Control vs Treated (14 d)
	MD± SE	MD± SE
Haematological parameter		
Hb	-0.413 ± 0.197	-0.866±0.197***
RBC	-0.056±0.022	-0.106±0.022**
PCV	-1.166±0.224**	-2.133±0.224***
MCV	-5.266±1.614*	-8.733±1.614**
MCH	-3.166±1.298	-8.350±1.298**
WBC	-6090±5.140***	-9070.0±5.140***
Biochemical parameter		
Glucose	-73.666±1.981**	-53.333±1.981***
Protein	-5.833±0.538**	-4.600±0.538***
albumin	-3.833±0.243**	-3.366±0.243***
globulin	-3.366±0.372**	-3.200±0.372***
Urea	12.766±0.787**	28.800±0.787***
BUN	6.000±0.41096**	13.000±0.410***
Creatinine	-0.6000±0.176	-0.300±0.176
Ratio	18.566±0.568**	18.833±.568***

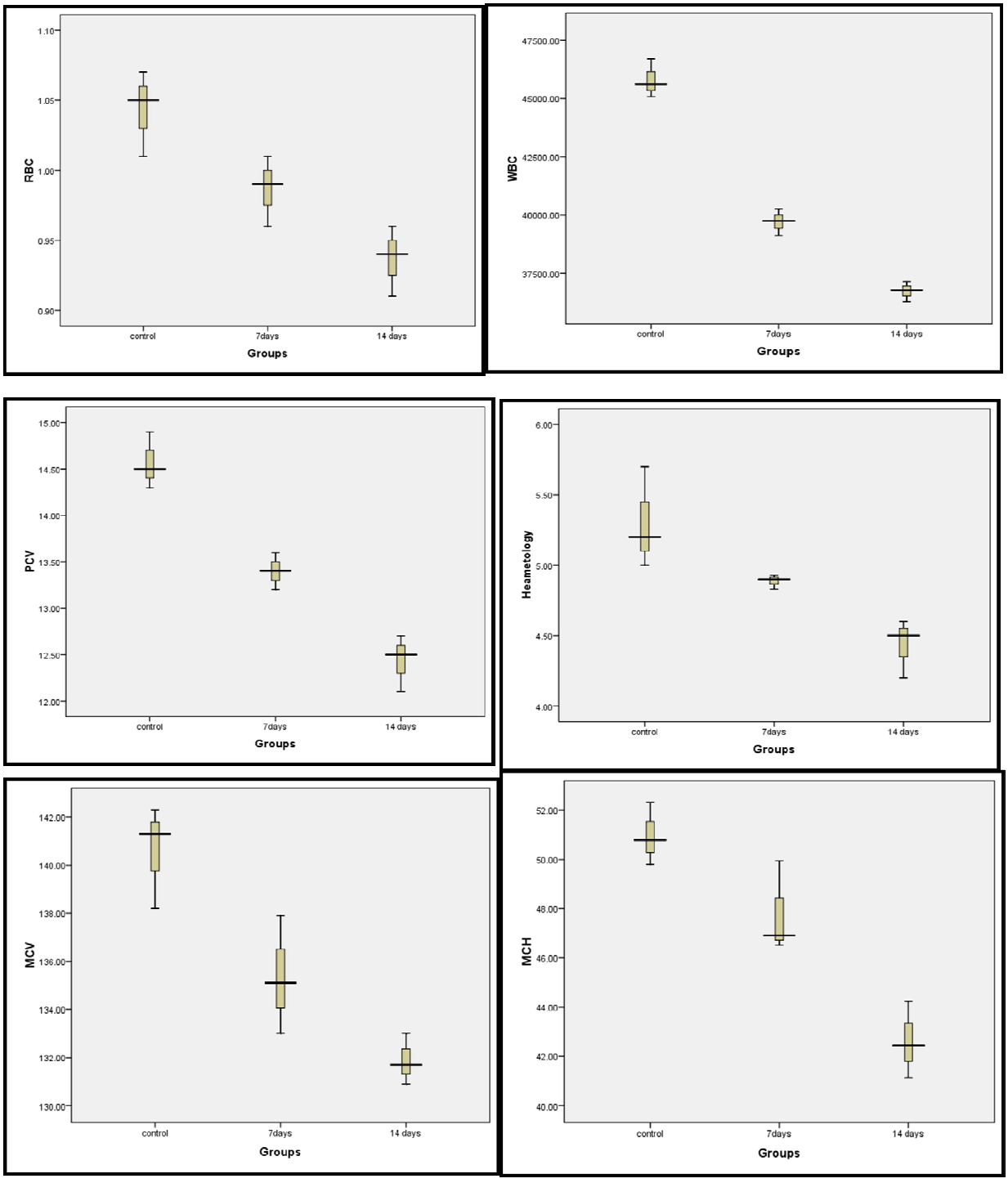
MD± SE Mean difference ± Standard error
The mean difference in significant at 0.05 level
*indicates mean difference is significant at 0.05 level.
**indicates mean difference is significant at 0.001 level.
*** indicates mean difference is significant at 0.0001 level.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

Fig 1: Changes in Haemetological Parameter of PE treated fish *O.mossambicus*. values are mean ± SE of five individual observation



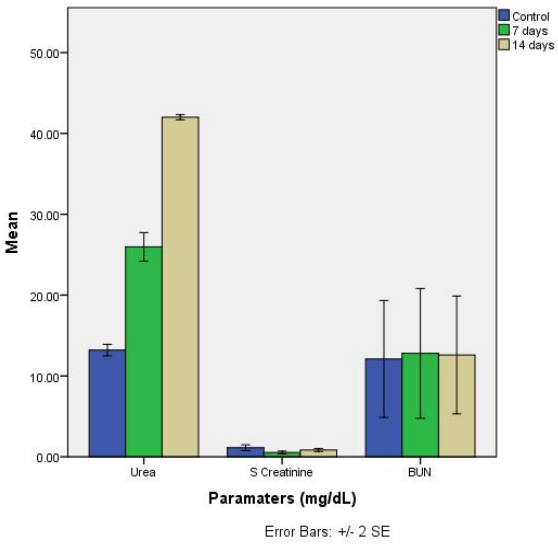
THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

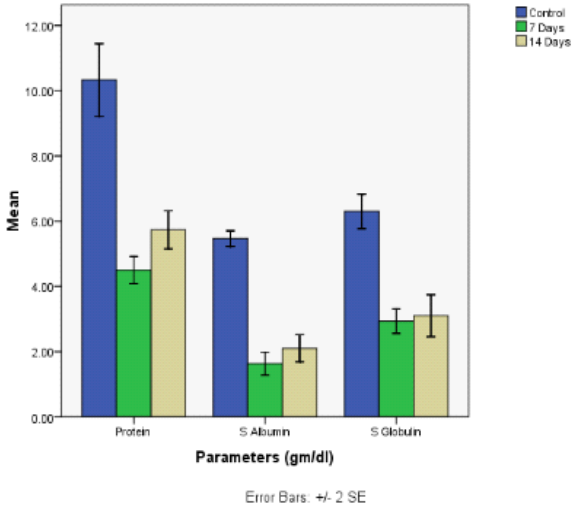
ISSN-2319-2119

RESEARCH ARTICLE

Parikh P.H *et al*, The *Experiment, 2014., Vol. 18(3), 1245-1253*

Fig.2 Changes in Blood Urea, Creatinine, BUN, and Total Serum Protein of PE treated fish *O.mossambicus*





REFFERENCE

1. Joshi PK, Bose M, Harish D.Haematological changes in the blood of Clarias batrachus exposed to mercuric chloride. Ecotoxicol Environ Monit 2002; **12**:119-122.
2. Jayaprakash C, Shett N.Changes in the hematology of the freshwater fish, Channa punctatus (Bloch) exposed to the toxicity of deltamethrin. J Chem Pharm Res 2013; **5**(6):178-183.
3. Frans LM. Pesticides detected in urban streams in King County Washington, 1999-2003:U.S. Geological Survey Scientific Investigations Report 2004-5194.
4. Corberaa M, Hidalgoa M, Salvado V, Wieczorek P. Determination of glyphosate and aminomethylphosphonic acid in natural water using the capillary electrophoresis combined with enrichment step. Analytica Chimica Acta 2005; **540**: 3–7.
5. Zhang C, Xu J, Liu X, Dong F, Kong Z, Sheng Y, Zheng Y. Impact of imazethapyr on the microbial community structure in agricultural soils. Chemosphere 2010; **81**: 800-806.
6. Giovanni LM, Hilton AJ, Silvano G, Vinatea L.. Acute toxicity of pyrazosulfuron-ethyl and permethrin to juvenile Litopenaeus vannamei. Acta Scientiarum. Biological Sciences 2011; **33**:1-6.
7. Singh SB, Sharmab R, Singh N. Persistence of pyrazosulfuron in rice-field and laboratory soil under Indian tropical conditions.Pest Manag Sci 2012; **68**: 828–833.
8. Solomon SG, Okomoda VT. Effect of photoperiod on some biological parameters of Clarias gariepinus juvenile. Journal of Stress Physiology & Biochemistry 2012; **8**(4): 47-54.
9. Satheeshkumar P, Ananthan G, Senthil Kumar D, Jagadeesan L.. Haematology and biochemical parameters of different feeding behaviour of teleost fishes from Vellar estuary, India. Comp Clin Pathol -2011; 1259-7
10. Ramesh M, Saravanan M. Haematological and biochemical responses in a freshwater fish Cyprinus carpio exposed to chlorpyrifos. Intern. J Integr Biol 2008; **3**(1):80-83.

11. Shah S, Altindag A. Hematological parameters of tench (tinca tinca l.) after acute and chronic exposures to lethal and sublethal mercury treatment. Bull. Environ. Contam toxicol. 2004;**73**: 911-918
12. Prado R, Rioboo C, Herrero C, Cid A. The herbicide paraquat induces alterations in the elemental and biochemical composition of nontarget microalgal species. Chemosphere 2009; **76**: 1440-144.
13. Safahieh A, Hedaiati A, Savari A, Movahedinia A. Effect of sublethal dose of mercury toxicity on liver cells and tissue of yellowfinseabream.Toxicology and Industrial Health. 2011; 1-10
14. Kori-Siakpere O, Adamu, KM, Okenabirhie J. Sublethal effects of paraquat on some plasma organic constituents (metabolic parameters) of African catfish: Clarias gariepinus (Osteichthyes: Cariidae). Intl J. of Zool Resv 2007; **3** (4): 213-217.
15. El-Shebly, Abdalla A, Mohamed AH, El-kady. Effects of Glyphosate Herbicide on Serum Growth Hormone (GH) Levels and Muscle Protein Content in Nile Tilapia (Oreochromis Niloticus L.).Research Journal of Fisheries and Hydrobiology 2008; **3**(2): 84-88
16. Sarikaya R,Yılmaz M..Investigation of acute toxicity and the effect of 2,4-D (2,4-dichlorophenoxyacetic acid) herbicide on the behavior of the common carp (Cyprinus carpio L., 1758; Pisces, Cyprinidae). Chemosphere 2003; **52** (1): 195–201
17. Finney. D.J. Probit analysis. Cambridge Univ. Press, Cambridge, 1971; 333
18. Adhikari S, Sarkar B,Chatterjee, A, Mahapatra CT, Ayyappan S.. Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost; *Labeo rohita* (Hamilton). Ecotoxicol Environ Safety 2004; **58**: 220–226
19. Seriani R, Dias DC, Silva MRR, Villares EO, Abessa DMS, Moreira LB, Ranzani-Paiva MJT, Rivero DHRF.Hematological parameters of *Oreochromis niloticus* from a polluted site. Annals of World Aquaculture Society Vera Cruz, México.2009
20. Pamila D, Subbaiyan PA, Ramaswamy M.. Ind. J. Environ. Hlth. 1991; **33**: 218-224.
21. Feldman BF, Zinkl, JG, Jain NC. Schalm's Veterinary Hematology. 5th ed.Lippincott Williams & Wilkins. 2000; 1120-1124.
22. Sancho E, Ceron JJ, Ferrando MD.Cholinesterase Activity and Hematological Parameters as Biomarkers of Sublethal Molinate Exposure in Anguilla Anguilla. Ecotoxicology and Environmental Safety 2000; **46**:81-86.
23. Barcellos LJG, Kreutz LC, Rodrigues LB, Fioreze I, Quevedo RM, Cericato L, Conrad J, Soso AB, Fagundes M, Lacerda LA , Terra S.Haematological and biochemical characteristics of male jundiá (Rhamdia Quelen, Quoy & GaimaRDT, Pimelodidae): changes after acute stress. Aquacul Res 2003; **34**:1465– 1469.
24. Patnaik L, Patra AK.Haematopoietic alterations induced by carbaryl in Clarias batrachus (linn). J Applied Sci Environ Manage 2006; **10**:5-7.
25. Giesy JP, Dobson S, Solomon KR. Ecotoxicological risk assessment for roundup herbicide. Environ Contam Toxicol 2000; **167**: 35–120.
26. Glusczak L, Miron DS, Crestani M, Fonseca MB, Pedron FA, Duarte MF, Vieira VLP.Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (Leporinus obtusidens). Ecotoxicol Environ Saf 2006; **65**: 237–241.
27. Soorena A, Jourdehi YA, Rezvanollah K, Yazdani MA.Effects of Atrazine (Herbicide) on Blood Biochemical Indices ofGrassCarp(Ctenopharhyngoden idella) . Journal of the Persian Gulf (Marine Science) 2011; **2**(5):51-56.
28. Shah SL. Hematological parameters in tench Tinca tinca after short term exposure to lead. J Appl Toxicol 2006; **26**(3): 223-228.
29. Ferrari A, Venturino A, Pechén AM de D'Angelo.. Effects of carbaryl and azinphos methyl on juvenile rainbow trout (Oncorhynchus mykiss) detoxifying enzymes. Pesticide Biochemistry and Physiology 2007; **88**(2) :134–142.
30. Heath A G.Water Pollution and Fish Physiology.CRC. Press. Inc. Boca Raton, Florida. 1995; 359.
31. Kapila M,Raghothaman G. Mercury, copper and cadmium induced changes in the total protein level muscle tissue of an edible estuarine fish Boleophthalmus dessumieri. Cuv J Envi Biol 1999; **20**(3):231-234.
32. Padma PB, Rachel V, Maruthi YA. Acute toxicity effect of imidacloprid insecticide on serum biochemical parameters of fresh water teleost channa punctatus. Int J Int sci Inn Tech Sec A Jun.2012; **1**(2):18-22.
33. Gupta RC, Bhargava S.Practical Biochemistry. CBS Publishers And Distributors, Delhi (India).1985

34. Kurde S.Effect of textile mill effluents and dyes on the heamatological parameters in albino rats. Ph.D.Thesis.1990.
35. Barad VS Kulkarni RS.Haematological changes induced by short – term exposure to copper in the indian freshwater fish, notopterus notopterus (pallas).The bioscan 2010; **5**(2) :313-316.
36. Saha N, Das L, Dutta S, Goswami UC.. Role of ureogenesis in the mud-dwelled Singhi catfish (Heteropneustes fossilis) under condition of water shortage. Comp BiochemPhysiol 2001; **128**A :137–146.
37. Ravel R. Clinical Application of Laboratory Data, In: Clinical Laboratory Medicine : Mosby-Year Book Inc., St Louis, USA. 6th edition 1995; 309–330.
38. Ahmed SM, Hussein SY,El-Nasser MA. Comparative studies on the effect of herbicide atrazine on freshwater fish Oreochromis niloticus and Chrysichthye auratus at Assuit. Egypt Bull Envion Contam Toxicol 1996; **57**: 503 – 510.
39. El-Bagori HM.Pathological studies on some environmental pollution on some freshwater fish in Sharkia Governorate. M.V.Sc. Thesis, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt. 2001.
40. Abbass HH, Zaghloul KH, Mousa MAA.Effect of some heavy metal pollutants on some biochemical and histopathological changes in blue tilapia; Oreochomis niloticus. Egyptian Journal of Agricultural Research 2002; **80**(3): 1385-1411.
41. Mekkawy IA, Hussein SY, Abdel – Nasser M, Ahmed SM. Comparative studies on the effects of herbicide atrazine on some blood constituents and protein electrophoretic patterns of Oreochromis niloticus and Chrysichthyes auratus. Assuit. J Egypt Ger Soc Zool 1996; **19** (A):283 – 319.
42. Haggag NZ. Physiological studies on the changes induced by some non – traditional pesticides to the Nile catfish. M.V.Sc. thesis. Girls college, Ain shams Univ., Cairo. 2004
43. Radwan OA, El –Said MM.Biochemical studies on residues of two different formulations of profenfos insecticide in O.niloticus. J Biol Chem Environ Sci 2006; **1**(3): 491 – 519.
44. Nelson K, Jennifer Jones SJO, Reimschuesse R.Elevated Blood Urea Nitrogen (BUN) Levels in Goldfish as an Indicator of Gill Dysfunction. Journal of Aquatic Animal Health 1999; **11**:52–60.
45. Nikkhah A, Mirzaei M , Khorvash M, Rahmani HR, Ghorbani GR.Chromium improves production and alters metabolism of early lactation cows in summer. J Anim Physiol Anim Nutr (Berl). 2011; **95**(1):81-9.

Upadhyay A.A. and Parikh. P.H.*
Department of Zoology, Faculty of Science,
The Maharaja Sayajirao University of Baroda,
Vadodara – 390002.
Gujarat,India

View publication stats

ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/303951616

Impact Factor(JCC): 1.8207- HISTOLOGICAL CHANGES IN THE TISSUES OF OREOCHROMIS MOSSAMBICUS AND LABEO ROHITA ON EXPOSURE TO IMIDACLOPRID AND CURZATE

Article · April 2016

CITATIONS
0

READS
152

3 authors, including:

Ankur Upadhyay
The Maharaja Sayajirao University of Baroda
5 PUBLICATIONS **5** CITATIONS
SEE PROFILE

P.H. Parikh
The Maharaja Sayajirao University of Baroda
41 PUBLICATIONS **107** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Establishment of Insect cell line View project

All content following this page was uploaded by P.H. Parikh on 14 June 2016.

The user has requested enhancement of the downloaded file.

IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(E): 2321-8851; ISSN(P): 2347-4580
Vol. 4, Issue 5, May 2016, 149-160
© Impact Journals

HISTOLOGICAL CHANGES IN THE TISSUES OF *OREOCHROMIS MOSSAMBICUS* AND *LABEO ROHITA* ON EXPOSURE TO IMIDACLOPRID AND CURZATE

BHAVIKA PATEL, ANKUR UPADHYAY & PRAGNA PARIKH

Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

ABSTRACT

An attempt is made to evaluate the effect of Imidacloprid (IMI) and Curzate (CZ) on the histopathological alterations in gills and kidney of *O. Mossambicus* and *L. Rohita.* Histological observations envisaged the deleterious anatomical and morphological alterations induced in gill and kidney by sub-lethal toxicity of the IMI and CZ agrochemicals. Each tissue showed specific sterical changes and revealed the incapability of these tissues to withstand the toxic effects induced by IMI and CZ. Histological damages in the tissues were found to intensify with increase in concentration and duration. The histopathological changes observed in the kidney were severe necrosis of tubular epithelial cells, thickening of the Bowman's capsule and shrinkage of the glomeruli along with severe degenerative and necrotic changes in the renal tubules with focal areas of necrosis and hemorrhage, haemolysis. Vacuolar degenerations in the epithelium of renal tubules. The histological changes are more prevalent and more pronounced in the gills of both the fish were curling of secondary lamellae followed by disorganization, rupture in the secondary lamellae. Haemorrhage at primary lamellae and bulging at the tip of primary filament were also noticed. As a conclusion, the findings of the present histological investigations demonstrate that the exposure of adult fresh water teleost fish, *O. Mossambicus* and *L. Rohita* caused moderate to severe damaging to gills and kidney.

KEYWORDS: IMI, CZ, *O. Mossambicus, L. Rohita*, Kidney & Gills

INTRODUCTION

Industrial, agricultural and domestic activities continuously contaminate the aquatic environment by releasing their toxic chemicals. Pesticides are one of the major classes of toxic substances used for management of pest in agricultural lands and control of insect vectors of human disease (1). The runoff from treated areas enters the river and aquaculture ponds that are supplied by rivers. Such rivers and the adjacent aquaculture ponds are likely to be contaminated by pesticides. Fish is a suitable indicator for monitoring such contamination because they concentrate toxins in their tissues directly from water and also through their diet. The tolerance of aquatic organisms to toxicants in domestic effluents may vary among species and their integrative effects may lead to reproductive failure or reduction of fish species number (2). The response to chemical stress can be used as biomarkers of environmental conditions. Biomarkers are early responses or measurable biological event due to exposure to pollutants after acute or chronic exposure and the morphological findings has been largely considered in biomonitoring studies (3, 4). Histopathological events are considered fast and efficient for detection of acute and chronic adverse effects in fish; and may express the health condition of exposed contaminants (5, 6, and 7).

Imidacloprid (IMI) is a systemic insecticide (8) and Curzate (CZ) a fungicide which is a mixture of Cymoxanil

and mancozeb, has got systemic action that enters the target pest via ingestion or direct contact. A review of toxicity data of IMI and CZ toxicity for terrestrial non-target organisms such as Mammals, birds, and amphibians as well as aquatic organisms such as fish, amphibians and various invertebrates suggests that they are mild to moderately toxic (9,10,11,12 and 13).

Gills are the first organs which come in contact with environmental pollutants. Paradoxically, they are highly vulnerable to toxic chemicals because firstly, their large surface area facilitates greater toxicant interaction and absorption and secondly, their detoxification system is not as robust as that of liver. Additionally, absorption of toxic chemicals through gills is rapid and therefore toxic response in gills is also rapid. (14,15). Therefore, lesions in gill tissues can be the start of imbalance of the physiological and metabolic processes, thus any harm in the gills leads to impairment of vital functions revealing respiratory distress, impaired osmoregulation and retention of toxic wastes. Fish, as in higher vertebrates, the kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment. The kidney excretes nitrogen-containing waste products from the metabolism such as ammonia, urea and Creatinine. Kidney of fishes receives much the largest proportion of postprandial blood and therefore renal lesion might be expected to be good indicators of environmental pollution (16).

The exposure to chemical contaminants can induce a number of lesions and injuries to different fish organs (17) but gills and kidney represent important target organs suitable for histopathological examination in searching for damages to tissues and cells (18). Hence, in the present study an attempt is made to evaluate the effect of IMI and CZ on the histopathological alterations in gills and kidney of *O. Mossambicus* and *L .Rohita.*

MATERIALS AND METHODS

Experimental Design

Two freshwater teleosts, *O. Mossambicus* and *L. Rohita* of similar size in length and weight (12 ± 2 cm; 25 ± 1.9 g) and (25 ± 3 cm; 110 ± 5 g) respectively were brought from a local pond of Baroda district. Animals were transported to laboratory in large aerated plastic container and were acclimatized in glass aquaria containing 50 liter of well aerated dechlorinated tap water (with physic-chemical characteristics: pH 6.5- 7.5, temperature 25±3ºC and dissolved oxygen content of 7-8ppm) for ten days. During an acclimation period of 10 days, the fish were kept under natural photoperiod and fed two times a day (10:00 and 16:00h) with commercial pelleted diet. The acclimatized healthy fishes of both sexes were selected randomly for the studies.

Sub-lethal Exposure

Based on the result of the 48 h LC_{50}, 30 tilapia fish were divided in 3 groups, 10 fish for each group: Group 1 served as control without any treatment of Agro-chemicals. Group 2 were treated with low dose of IMI and CZ (LC_{50} / 10). Group 3 were treated with high dose of IMI and CZ (LC_{50} / 20) for a period of 21 days. Each concentration was replicated two times. Constant amount of the test chemical and test media were changed every 24 hours to maintain the toxicant strength and the level of dissolved oxygen as well as to minimize the level of ammonia during experiment. The fishes were fed once in a day throughout the duration of the sub-lethal toxicity tests. At the end of the experiment the fish were carefully netted to minimize stress, and the fish weighed. After this, Fishes were sacrificed by pithing. Then, kidney and gills tissues were removed, weighed and processed for histological observations.

Histological Observation

After measuring length and weight fresh tissues were fixed in 4% paraformaldehyde for 24 hrs, dehydrated, embedded in paraffin wax and sectioned at 10-12µm then stained with heamatoxylin and eosin and examined microscopically and photographed using digital camera (Sony).

RESULTS

Figure 1 A-E, 2A-E, 3A-E and 4A-Edepicts the histological changes observed in the gills and kidney of *O. Mossmbicus* and *L. Rohita* subjected to IMI and CZ. A dose dependent change was observed for IMI as well as CZ Figure 1A and 2A shows normal histological structures of the gills of *O. Mossambicus* and *L. Rohita*. The common histopathological observations in the gills of *O. Mossambicus* and *L. Rohita* includes ploriferation of the epithelium of the gill filaments and secondary lamellae, resulting in fusion of secondary lamellae, degenerative necrotic changes in gill filaments and secondary lamellae, curling of secondary lamellae and mucus cells proliferations. Edematous changes, characterized by epithelial detachment in gill filaments and secondary lamellae, associated with aggregations of inflammatory cells in gill filaments. Comparatively, the degree of pathological changes observed on IMI exposure was more prominent compared to CZ for *O. Mossambicus* (Figure 1B, 1C, 1D and 1E) as well as *L. Rohita* (Figure 2B, 2C, 2D, and 2E). Distinct feature observed was hyperemia and hemorrhages in primary and secondary gill lamellaeon CZ exposure and on IMI exposure in *L. Rohita*.

Figures 3A and 4A show the normal histological structure of kidney. Histological alterations in the kidney of both the fish consist of severe degenerative and necrotic changes in the renal tubules with focal areas of necrosis and hemorrhage, haemolysis. Vacuolar degenerations in the epithelium of renal tubules and dilation in the capillary tubes of renal tubules were observed. Also edema of Bowman's capsule with atrophy in the glomeruli and dilation in the renal blood vessels were observed. Kidney tissue from *O. Mossambicus* (Figure 3B, 3C, 3D and 3E) and *L. Rohita* (Figure 4B, 4C, 4D and 4E) showed mild necrosis and tubular degeneration on CZ exposure whereas on IMI exposure it showed severe necrosis, vacuolation and tubular degeneration.

DISCUSSIONS

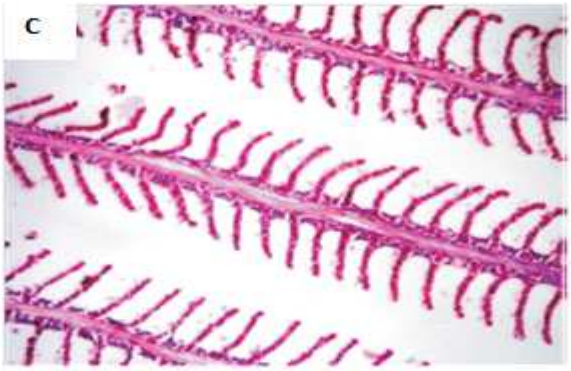
Results of the study revealed that *O. Mossambicus* and *L. Rohita* on exposure of IMI and CZ manifest histopathological changes in gills and kidney. It is possible that the pathological alterations in the tissues of both studied fish with both IMI and CZ could be a direct result of the pesticides induced stress. Gill tissue from *O. Mossambicus* on low dose exposure of CZ (Figure 1B) showed depicting proliferation of the epithelium of the primary lamellae, curling of secondary lamellae and enlargement of primary lamellae. However, at high dose of CZ (Figure 1C) exposure, there were loss of epithelial lining and degeneration of primary lamellae. At low dose of IMI (Figure 1D) exposure there were loss of secondary lamellae and degeneration of primary lamellae along with the distortion of epithelial lining of primary lamellae. At high dose (Figure 1E) of IMI exposure led to severe curling and clubbing of secondary lamellae accompanied by proliferation of epithelial cells. Whereas the gill tissue from *L. Rohita* on low dose exposure of CZ (Figure 2B) exposure showed loss of epithelial lining as well as distortion of primary lamellae and curling of secondary lamellae. At high dose (Figure 2C) of CZ exposure showed branchial filament with hyperplasia and fusion of secondary lamellae. While, At low dose of IMI (Figure 2D) exposure gill showed uplifting epithelial lining and degeneration of secondary lamellae and

brachial hemorrhage and at high dose (Figure 2E) of IMI exposure led to complete severe distortion of secondary lamellae and enlargement of primary lamellae. These pathological changes may be a reaction to toxicant intake or an adaptive response to prevent the entry of the toxicant through the gill surface. Besides, alterations like proliferation of epithelial cells, partial and total fusion of secondary lamellae as well as lifting of epithelium are defense mechanisms as this would result in the increase of the distance between the external environment and the blood thereby serving as a barrier to the entrance of the pesticides (16, 19). The cellular damage observed in the gills in terms of epithelial proliferation, separation of epithelial layer from supported tissue and necrosis can adversely affect the gas exchange and ionic regulation (20, 21). The observed edematous changes in the gill filaments and secondary lamellae are probably due to increased capillary permeability. More prevalent and more pronounced changes in the gills of both the fish on IMI exposure were curling of secondary lamellae followed by disorganization, rupture in the secondary lamellae. Hemorrhage at primary lamellae and bulging at the tip of primary filament were also noticed. Our results are parallel with earlier findings on the histopathological changes in the gills of teleost fish exposed to different pesticides (22, 23, and 24).

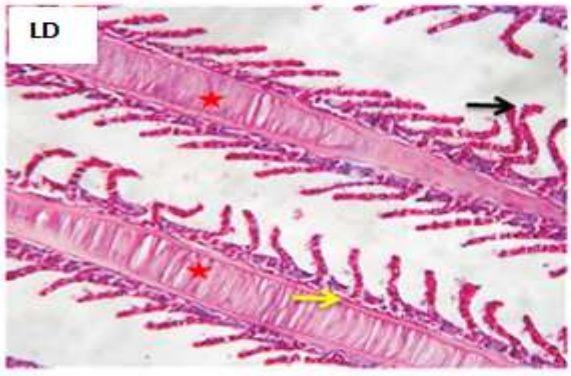
Morphologically, the nephron of the control fish consists of intact structures of glomerulus, tubules and collecting ducts. The glomeruli, a cluster of capillaries surrounded by the Bowman's capsule were very clearly seen. The structure of the proximal and distal convoluted tubules was undamaged. The teleostean kidney is one of the first organs to be affected by contaminants of the water (25). The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only involved in removal of wastes from blood but it is also responsible for selective reabsorption which helps in maintaining volume and pH of blood and body fluids as well as erythropoesis (26). Kidney tissue from *O. mossambicus* on low dose exposure of CZ (Figure 3B) showed mild necrosis and shrunken glomeruli, at high doses of CZ (Figure 3C), the changes were more severe and the normal histoarchitecture of the kidney was lost. At low dose of IMI (Figure 3D) exposure led to complete degeneration of blood vessels in the glomeruli. The interstices of the tubules were seen to be enriched with hematopoietic tissue. At high dose of IMI (Figure 3E) there was complete degeneration of tubular epithelial cells and complete disorganized Bowman's capsules. Kidney tissue from *L. Rohita* on low dose exposure of CZ (Figure 4B) showed mild swollen proximal tubular epithelial cells with dilated nuclei and at high dose (Figure 4C) it showed severe swelling of tubules with necrosis. At low dose of IMI (Figure 4D) kidney showed expansion of space inside the Bowman's capsule and glomerular atrophy and at high dose of IMI (Figure 4E), severe degeneration of tubules, cloudy swelling and severe necrosis in nephritic tissue was observed. The degenerative necrosis of the renal tubules affects the metabolic activities and may promote metabolic abnormalities in the fish (28). The present results are in agreement with those observed in *C. Mrigala* exposed to lambda-cyhalothrn and fenvalerate (29); in *O. Niloticus* exposed to alchlor (Peebua *et al*., 2008)(30) and in *O. Mossambicus* exposed to Dimethoate (23). It is believed that kidney tissues are a sensitive indicator of environmental pollution as they act as primary osmoregulatory organs and function in cellular immunity (31). As an important organ of the immunity response) the observed mild to severe changes in the histoachitecture of the kidney may induce defense system changes damaging the animal's homeostasis and health. Adaptive immune system of several teleost has been explored by immunotoxicological analysis by various scientists (32-35). However, in the present study the main focus was to have an insight in to the histological aspects. Hence, at this juncture it is difficult to propose the immunotoxic effect of the agro-chemicals and these aspects demands more detailed analysis for understanding the immunotoxicological effects and mechanisms as well as risks that may have on human consumers as consequence of the bioaccumulation.

CONCLUSIONS

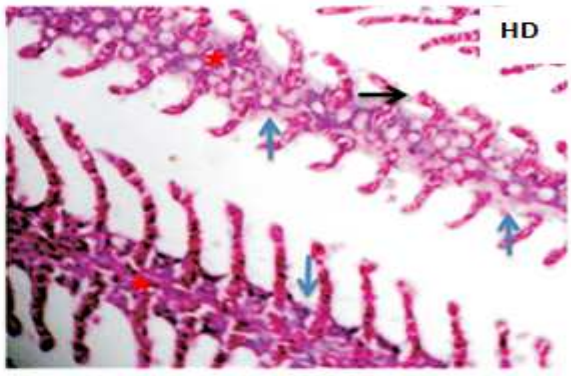
As a conclusion, the findings of the present histological investigations demonstrate that the exposure of IMI and CZ on adult fresh water teleost fish, *O. Mossambicus* and *L. Rohita* caused moderate to severe damaging to gills and kidney.



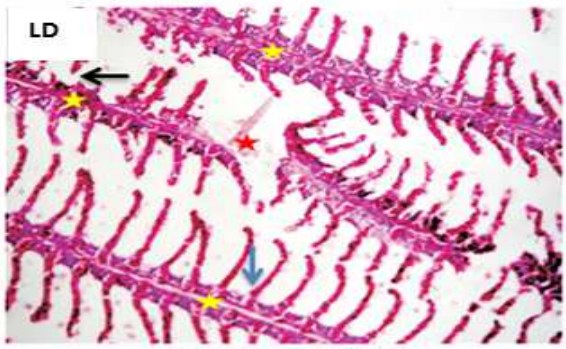
Photomicrograph 1A, 200X



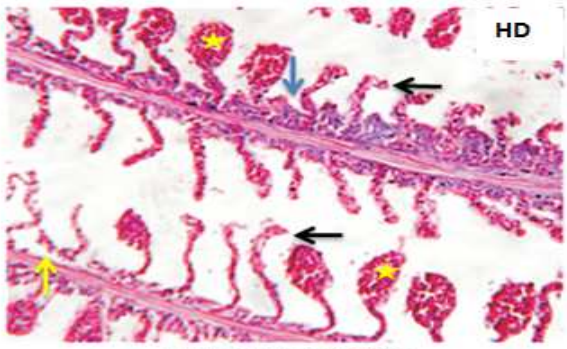
Photomicrograph 1B, 200X



Photomicrograph 1C, 200X



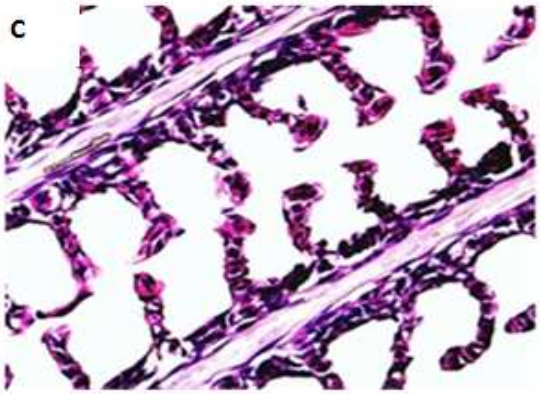
Photomicrograph 1D, 200X



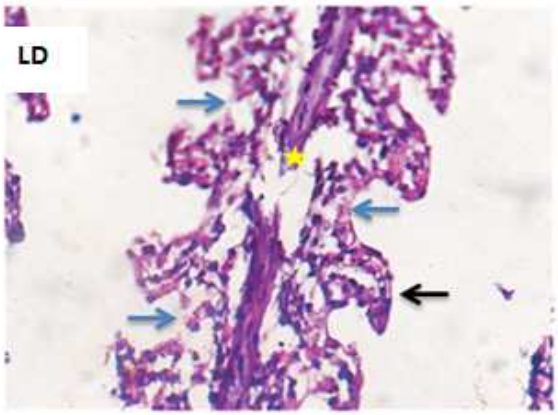
Photomicrograph 1E, 200X

Photomicrograph 1A shows a normal structure of gill tissue of *O. mossambicus* with well defined primary and secondary lamellae. 1B depicting proliferation of the epithelium of the primary lamellae (yellow arrow) curling of secondary lamellae (black arrow) and enlargement of primary lamellae (red star). 1C shows degeneration of primary lamellae (red star), loss of epithelial lining (blue arrow) and curling of secondary lamellae. 1D shows loss of secondary lamellae (black arrow) and primary lamellae (red star), degeneration of primary lamellae (yellow star) along with distortion of epithelial lining of primary. 1E showing curling (black arrow) and clubbing (yellow star) of secondary lamellae along with proliferation of epithelial cells (blue arrow) at high dose of IMI exposure. IMI- Imidacloprid, CZ- Curzate, HD- High Dose, LD- Low dose

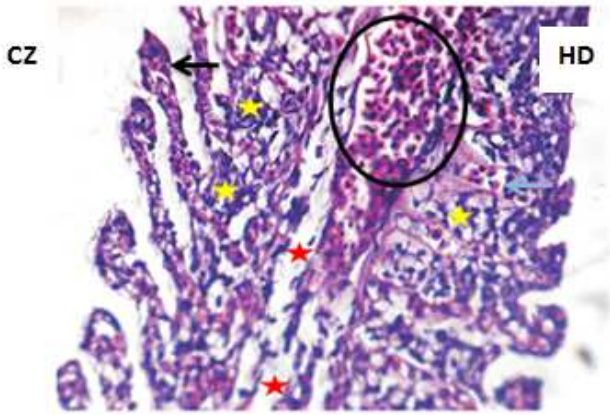
Figure 1



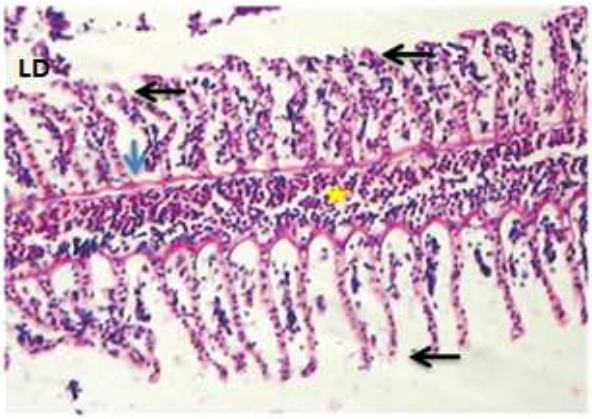
Photomicrograph 2A, 400X



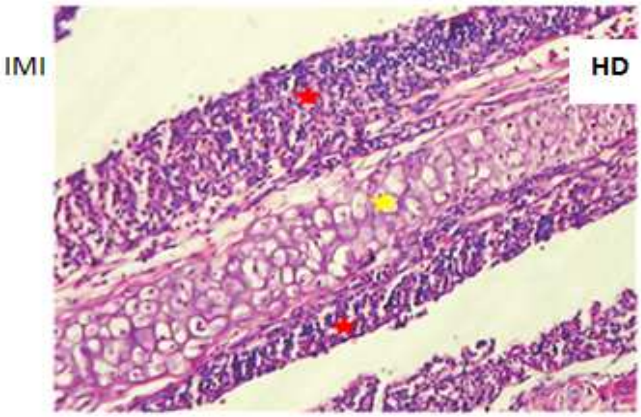
Photomicrograph 2B, 400X



Photomicrograph 2C, 400X



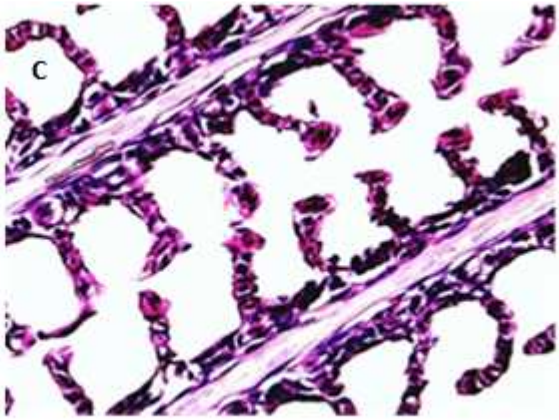
Photomicrograph 2D, 200X



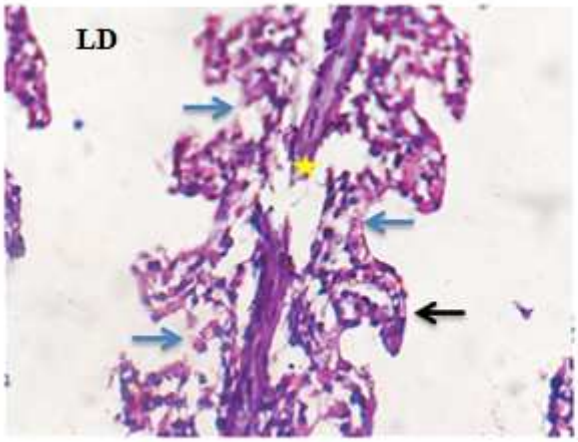
Photomicrograph 2E, 200X

Photomicrograph 2A shows a normal structure of gill of *L. rohita*. 2B shows distortion of primary lamellae (star), curling of secondary lamellae (black arrow) and loss of epitheial lining of primary lamellae (blue arrow). 2C shows branchialfilament with hyperplasia (circle) and fusion of secondary lamellae (yellow star), hemorrhage (blue arrow) and hyperplasia at the tip of secondary lamellae. 2D shows severe degeneration of secondary lamellae (black arrow) and branchialhemorrhage(yellow star), uplifting epithelial lining of secondary lamellae (blue arrow) and degeneration as well as fusion of secondary lamellae (black arrow). 2E complete severe distortion of secondary lamellae (red star) and enlargement of primary lamellae (yellow star). IMI- Imidacloprid, CZ- Curzate, HD- High Dose, LD- Low dose

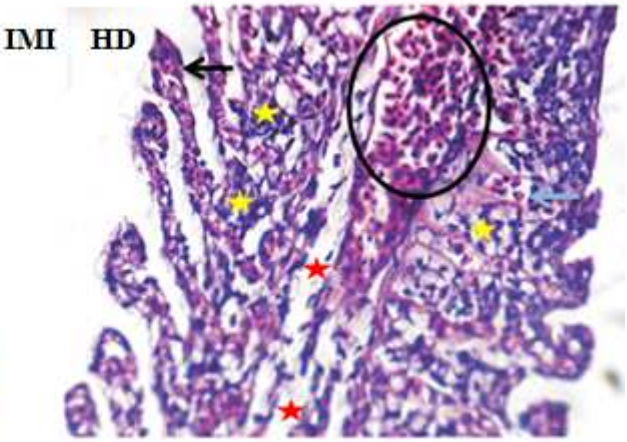
Figure 2



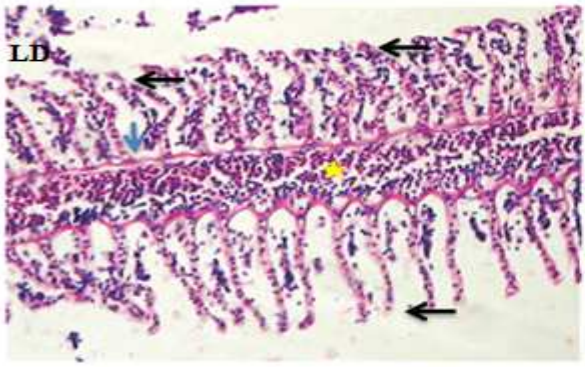
Photomicrograph 2A, 400X



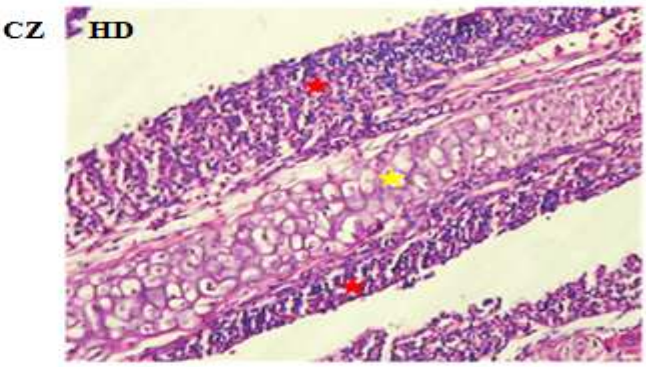
Photomicrograph 2B, 400X



Photomicrograph 2C, 400X



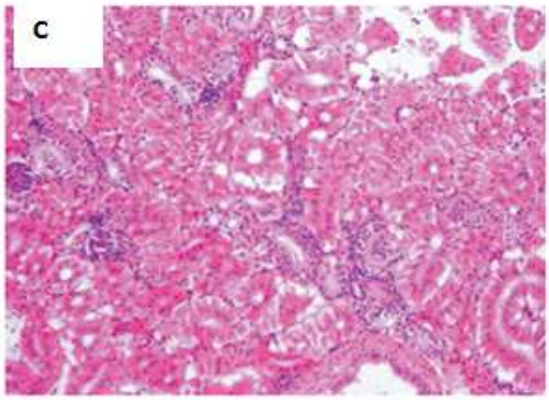
Photomicrograph 2D, 200X



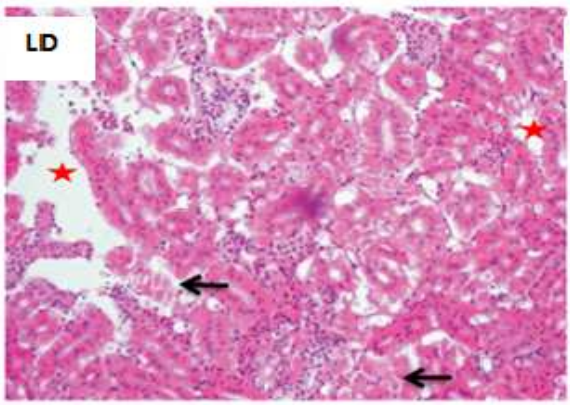
Photomicrograph 2E, 200X

Photomicrograph 2A shows a normal structure of gill of *L. rohita*. 2B shows distortion of primary lamellae (star), curling of secondary lamellae (black arrow) and loss of epithelial lining of primary lamellae (blue arrow). 2C shows branchialfilament with hyperplasia (circle) and fusion of secondary lamellae (yellow star), hemorrhage (blue arrow) and hyperplasia at the tip of secondary lamellae. 2D shows severe degeneration of secondary lamellae (black arrow) and branchialhemorrhage(yellow star), uplifting epithelial lining of secondary lamellae (blue arrow) and degeneration as well as fusion of secondary lamellae (black arrow). 2E complete severe distortion of secondary lamellae (red star) and enlargement of primary lamellae (yellow star). IMI-Imidacloprid, CZ- Curzate, HD- High Dose, LD- Low dose

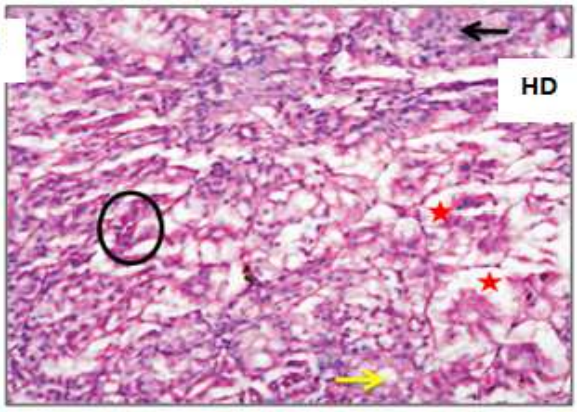
Figure 3



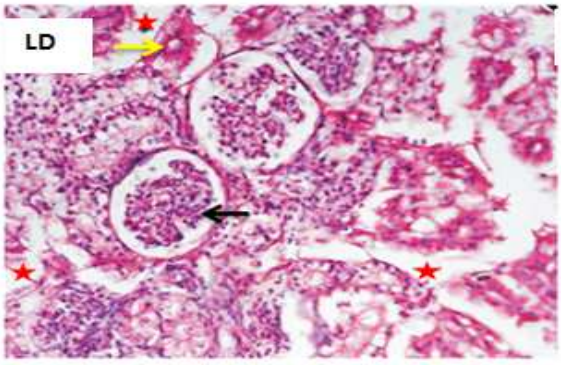
Photomicrograph 3A, 200 X



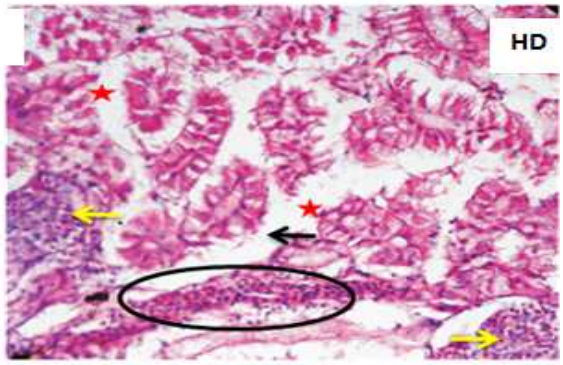
Photomicrograph 3B, 200X



Photomicrograph 3C, 200X



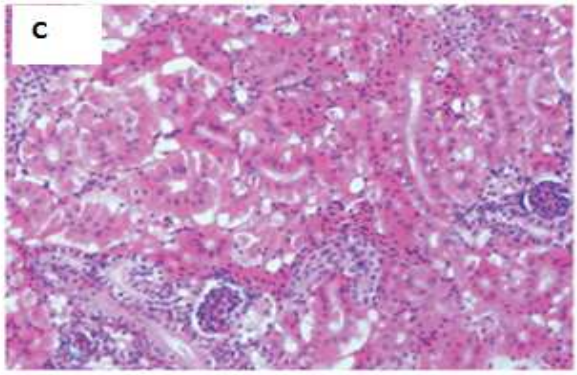
Photomicrograph 3D, 200X



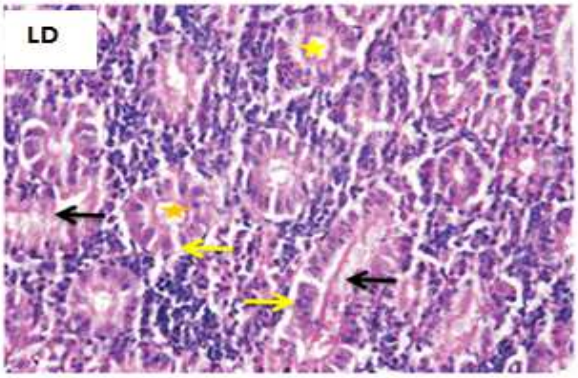
Photomicrograph 3E, 200X

Photomicrograph 3A shows a normal structure of kidney of *O. mossambicus*. 3B showing intracellular vacuolization (red star) and vacuolardegeneration of tubular epithelial cells (black arrow). 3C shows intra cytoplasmic vacuoles in epithelial cells of renal tubules with hypertrophied cells and lumen tubules diminished (red star), renal tubule degeneration (yellow arrow), haemorrhage (circle) and swelling in the epithelial cells of renal tubules (black arrow). 3D shows shrinkage of glomeruli and expansion of space inside the Bowman's capsule (black arrow), degeneration of epithelial cells of renal tubules (yellow arrow) and increase intra cellular space (red star). 3E showing increase intracellular space (red star), haemorrhage and dilation of renal blood vessels (circle), severe degenerative and necrotic changes in renal tubules and glomeruli with focal area of necrosis (yellow arrow), vascular degeneration of tubular epithelial cells (black arrow) and severe necrosis in the epithelium of renal tubules (blue arrow). IMI- Imidacloprid, CZ- Curzate, HD- High Dose, LD- Low dose

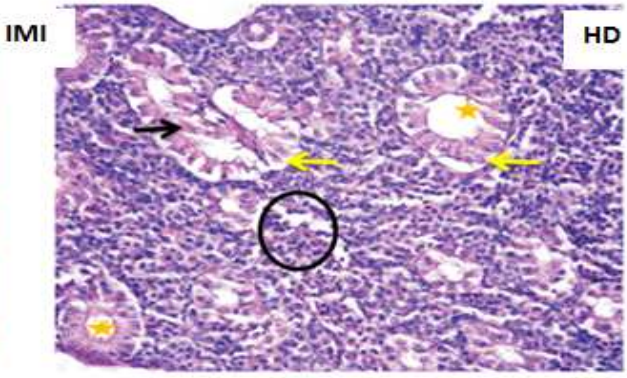
Figure 4



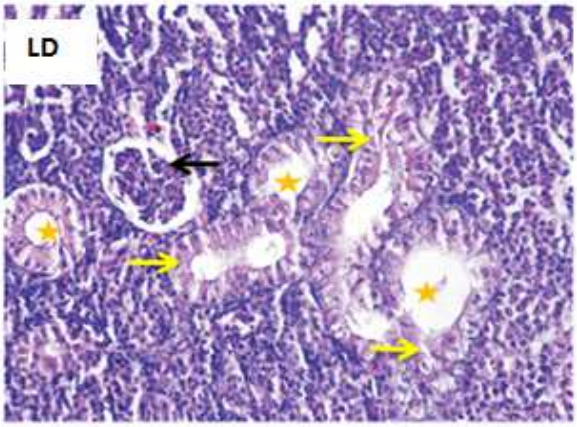
Photomicrograph 4A, 200X



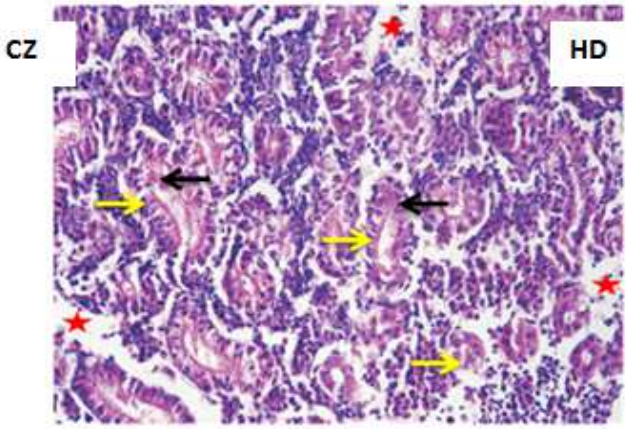
Photomicrograph 4B, 200X



Photomicrograph 4C, 200X



Photomicrograph 4D, 200X



Photomicrograph 4E, 200X

Photomicrograph 4A shows a normal structure of kidney of *L. rohita*. 4B showing inter-tubular degeneration (blak arrow), vacuolar degeneration of tubuler epithelial cells (yellow arrow), intra-cytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and lumen tubules diminished (green star). 4C shows mild haemorrhage (circle), vascular degeneration of tubular epithelial cells (yellow arrow), intercellular degeneration (black arrow) and hypertrophied renal tubular cells and lumen tubules diminished (star). 4D shows vascular degeneration of tubular epithelial cells (yellow arrow), shrinkage of glomeruliand expansion space inside the Bowman's capsule (black arrow) and severe intra-cytoplasmic vacuoles in epithelial cells of renal tubules with hypertrophied cells and lumen tubules diminished (green star). 4E showing severe intra-tubular degeneration (black arrow), increase in the intracellular space (red star) and necrosis and distortion of tubular epithelium (yellow arrow). IMI- Imidacloprid, CZ- Curzate, HD- High Dose, LD- Low dose

Figure 5

REFERENCES

1. Nicholsan, G.M. 2007. Fighting the global pest problem: Preface to the special Toxicon issue on insecticidal toxins and their potential for insect pest control. *Toxicon* 49(4): 413–422.
2. Minier, C., Abarnou, A., Jaouen-Madoulet, A. and Le Guellec, A.M., 2006, A pollution-monitoring pilot study involving contaminant and biomarker measurements in the Seine Estuary, France, using zebra mussels (*Dreissena polymorpha*). *Toxicol. Environ. Chem.,* 25: 112-119.
3. Oliveira Ribeiro, C. A., Vollaire, Y., Sanchez-Chardi, A. and Roche, H., 2005, Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the Eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France. *Aquatic Toxicol.,*74 (1): 53-69.
4. Miranda, A.L., Roche, H., Randi, M.A.F., Menezes, M.L. and Oliveira Ribeiro, C.A., 2008, Bioaccumulation of chlorinated pesticides and PCBs in the tropical freshwater fish Hopliasmalabaricus: Histopathological, physiological, and immunological findings. *Environ. Int.,* 34(7): 939-949.
5. Myers, M.S. and Fournie, J.W., 2002, Histopathological biomarkers as integrators of anthropogenic and environmental stressors. In: Biological indicators of aquatic ecosystem stress. *Am. Fish. Soc.*, 24: 221-287.
6. Ayas, Z., Ekmekci, G., Ozmen, M. and Yerli, S. V., 2007, Histopathological changes in the livers and kidneys of fish in Sariyar Reservoir, Turkey. *Environ. Toxicol. Pharmacol.*, 23 (2): 242-249.
7. Liebel, S., Tomotake, M.E.M., and Oliveira Ribeiro, C. A. 2013. Fish histopathology as biomarker to evaluate water quality. *Ecotoxicol. Environ. Contam*, 8(2): 09-15.
8. Kagabu S. 2003. Molecular design of neonicotinoids: past, present and future. In Chemistry of Crop Protection, Progress and Prospects in Science and Regulation, ed. G Voss, G Ramos, 193-212.
9. Li-tao, Q., Shao-long, F. and Yang, Y. 2006. Toxicity of a Novel Pesticide, Imidacloprid, on Tadpoles and Frogs of Rana Nigronaculata Hallowell, *Journal of Nanhua University*, 34(2): 181-184.
10. Siddiqui, A., Choudhary, M., Goriya, H.V., Bhavsar, S.K. and Thaker, A.M. 2007. Evaluation of immunotoxic effect of short-term administration of quinalphos and imidacloprid in white leghorn cockerels. *Toxicol Int.*, 14: 15-19.
11. Das, T., Pal, A.K., Chakraborty, S.K., Manush, S.M., Dalvi, R.S., Apte, S.K., Sahu, N.P. and Baruah, K. 2009. Biochemical and stress responses of *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) in relation to acclimation temperatures. *Journal of Fish Biology*, 74(7): 1487-1498.
12. Desai, B. and Parikh, P. 2014. Behavioural responses to acute exposure of Imidacloprid and Curzate on *Labeo rohita*(Hamilton, 1822). *International Journal of Open Scientific Research*, 2(1): 1 – 12.
13. Desai, B. and Parikh, P. 2013. Biochemical Alterations on Exposure of Imidacloprid and Curzate on Fresh Water Fish *Oreochromis Mossambicus* and *Labeo Rohita*. *Indian Journal of Forensic Medicine & Toxicology*, 7(2): 53-59.
14. Athikesavan, S., Vincent, S., Ambrose, T. and Velmurugan, B. 2006. Nickel induced histopathological changes in

the different tissues of freshwater fish, *Hypophthalmichthys molitrix* (Valenciennes). *J. Environ. Biol.,* 27: 391-395.

15. Fernandes, C., Fontainhas-Fernandes, A., Monteiro, S.M. and Salgado, M.A. 2007. Histopathological gill changes in wild leaping grey mullet (*Liza saliens*) from the Esmoriz-Paramos coastal lagoon. Portugal. *Environ. Toxicol.,* 22: 443-448.
16. Ortiz, J.B., González de Canales, M.L., and Sarasquete, C. 2003. Histopathological changes induced by lindane (?-HCH) in various organs of fishes. *Scientia Marina,* 67(1): 53-61.
17. Oliveria-Ribeiro, C.A., Belger, L., Pelletier, É. and Rouleau, C. 2002. Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinu salpinus*). *Environmental Research,* 90(2): 17-225.
18. Rabitto, I.S., Alves Costa, J.R.M., Silva de Assis, H.C., Pelletier, E., Akaishi, F.M., Anjos, A., Randi, M.A.F. and Ribeiro, O. 2005. Effects of dietary Pb(II) and tributylin an neotroptical fish *Hoplias malabarius*: Histopathological and biochemical findings. *Ecotoxicol. Environ. Saf.,* 60: 147-156.
19. Mohamed, F.A.S. 2009. Histopathological Studies on Tilapia *zillii* and *Solea vulgaris* from Lake Qarun, Egypt. *World Journal of Fish and Marine Sciences,* 1(1): 29-39.
20. Dobreva, V., Tsekov, A. and Velcheva, I. 2008. Study of the effect of zinc on gill functions of the crucian carp *Carassius gibelio* Bloch. *Bulgarian Journal of Agricultural Science,* 14(2): 182-185.
21. Arnaudova D., Tomova, E., Velcheva, I. and Arnaudov, A. 2008. A study on the lead, zinc and cadmium in various organs in fishes from Cyprinidae and Percidae families in "Studenkladenes" and "Kardzali" dam lakes. (eds: I.A. Velcheva and Tsekov) Anniversary scientific conference of ecology. 1 November 2008, Plovdiv, 327-336.
22. Parikh, P.H., Rangrez, A., Adhikari-Bagchi, R. and Desai, B.N. 2010. Effect of dimethoate on some histoarchitecture of freshwater fish *Oreocromis mossambicus* (Peters, 1852). *The Bioscan,* 5(1): 55-58.
23. Erkmen B., Caliskan M. and Yerli S.V. 2000. Histopathological effects of cyphenothrin on the gills of the *Lepistes reticulates*. *Vet Hum Toxicol.,* 42: 71-78.
24. Susithra, N., Jothivel, N., Jayakumar, P., Paul, V. I., Sen, M., Dastidar, M. G. and Ranade, P. 2007. Toxicopathological impact of cadmium chloride on the accessory respiratory organ of the air-breathing catfish *Heteropneustes fossilis*. *Iranian Journal of Environmental Health Science & Engineering,* 4(1): 1-8.
25. Thophon, S., Kruatrachue, M., Upatham, E.S., Pokethitiyook, P., Sahaphong, S., and Jaritkhuan, S. 2003. Histopathological alterations of white sea bass, *Lates calcarifer,* in acute and sub chronic cadmium exposure. *Environmental Pollution,* 121(3): 307-320.
26. Iqbal, F., Qureshi, I.Z. and Ali, M., 2004. Histopathological changes on the Kidney of common Carp, *Cyprinus carpio* following nitrate exposure. *Journal of Research in Science,* 15: 411-418.
27. Camargo, M.M.P. and Martinez, C.B.R. 2007. Histopathology of gills, kidney and liver of a Neotropical fish

caged in an urban stream. *Neotrop. ichthyol.*, 5(3): 327-336.

28. Velmurugan, B., Selvanayagam, M., Cengiz, E.I., and Unlu, E. 2007. The effects of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala*. *Journal of Environmental Science and Health Part B,* 42(2), 157-163.
29. De Bravo, M.I., Medina, L., Marcano, S., Finol, H.J. and Boada-Sucre, A. 2005. Effects of herbicide on the kidneys of two Venezuelan cultured fish: *Caquetaia kraussii* and *Colossoma macropomum* (Pisces: Ciclidae and Characeae). *Revista de Biologia Tropical,* 53(1): 55-60.
30. Peebua, P., Kruatrachue, M., Pokethitiyook, P., and Singhakaew, S. 2008. Histopathological alterations of Nile tilapia, *Oreochromis niloticus* in acute and subchronic alachlor exposure. *Journal of Environmental Biology*, 29(3) 325-331.
31. Zapata, A.G. and Cooper, E.L. 1990. Cells of the immune system. (eds: A.G. Zapata and Cooper, G.L.) The immune system: comparative histophysiology, Wiley, New York, 15-30..
32. Duffy, J.E. and Zelikoff, J.T. 2006. The relationship between noncoplanar PCB-induced immunotoxicity and hepatic CYP1A induction in a fish model. *Journal of immunotoxicology*, 3(1): 39-47.
33. Reynaud, S. and Deschaux, P. (2006). The effects of polycyclic aromatic hydrocarbons on the immune system of fish: a review. *Aquatic Toxicology*, Vol.77, No.2, pp. 229-238, ISSN 0166-445X.
34. Costa, P.M., Caeiro, S., Diniz, M.S., Lobo, J., Martins, M., Ferreira, A.M., Caetano, M., Vale, C., DelValls, T,A. and Costa, M.H. 2009. Biochemical endpoints on juvenile *Soleasene galensis* exposed to estuarine sediments: the effect of contaminant mixtures on metallothionein and CYP1A induction. *Ecotoxicology*, 18(8) 988-1000.
35. Bravo, C.F., Curtis, L.R., Myers, M.S., Meador, J.P., Johnson, L.L., Buzitis, J., Collier, T.K., Morrow, J.D., Laetz, C.A., Loge, F.J. and Arkoosh, M.R. 2011. Biomarker responses and disease susceptibility in juvenile rainbow trout *Oncorhynchus mykiss* fed a high molecular weight PAH mixture. *Environmental Toxicology & Chemistry*, 30(3): 704-714.

View publication stats

Evaluating the toxicological effects of agrochemicals on glucocorticoid receptor and serum cortisol level in Mozambique tilapia.

Parikh Pragna, Pandya Parth, Upadhyay Ankur and Thakkar Bhumi

Accepted Manuscript Version

This is the unedited version of the article as it appeared upon acceptance by the journal. A final edited version of the article in the journal format will be made available soon.

As a service to authors and researchers we publish this version of the accepted manuscript (AM) as soon as possible after acceptance. Copyediting, typesetting, and review of the resulting proof will be undertaken on this manuscript before final publication of the Version of Record (VoR). Please note that during production and pre-press, errors may be discovered which could affect the content.

© 2018 The Author(s). This open access article is distributed under a Creative Commons Attribution (CC-BY) 4.0 license.

Publisher: Cogent OA

Journal: *Cogent Biology*

DOI: http://doi.org/10.1080/23312025.2018.1480338

ACCEPTED MANUSCRIPT

Title Page:

Evaluating the toxicological effects of agrochemicals on glucocorticoid receptor and serum cortisol level in *Mozambique tilapia.*

Parth Pandya[a], Ankur Upadhyay[b], Bhumi Thakkar[b] , Pragna Parikh[b]
[a]*Division of Life Science, School of Science and Engineering, Navrachana University*
[b]*Department of Zoology, Faculty of Science, The M.S.University of Baroda, Vadodara*

Authors Information:

Name: Dr. Parth Pandya
Designation: Assistant Professor
Address: Division of Life science, School of Liberal Studies and Education, Navrachana University
Email ID: parthp@nuv.ac.in

Name: Prof. Pragna Parikh (Corresponding Author)
Designation: Professor
Address: Department of Zoology, Faculty of Science, The M.S.University of Baroda, Vadodara
Email ID: php59@yahoo.co.in

Name: Mr. Ankur Upadhyay
Designation: Research Scholar
Address: Department of Zoology, Faculty of Science, The M.S.University of Baroda, Vadodara
Email ID: ankur_upadhyay1659@yahoo.com

Name: Ms. Bhumi Thakkar
Designation: Research Scholar
Address: Department of Zoology, Faculty of Science, The M.S.University of Baroda, Vadodara
Email ID: bhumi.thakkar91@yahoo.co.in

ACCEPTED MANUSCRIPT

About The Authors Group:

Prof. Pragna Parikh

Dr. Parth Pandya

Ms Bhumi Thakkar

Mr Ankur Upadhyay

Prof. Parikhs works dynamically in the field of ecotoxicology from past 20 years and their research group has provided vital insights into the mechanism and toxicity of various groups of agrochemicals on non-target organisms. The studies in their lab are ranging from basic biochemistry to endocrine disruption of agrochemicals, where they have shown a sub chronic and sub acute toxicological potential of various toxicants.

Public Interest Statement:

This article is one of the novel, depicting the negative effects of 4 different class of agrochemicals on fishes. The main aim of the authors group is to check the environmental bioaccumulation and it ultimate effects in humans. Here, Cortisol one of the vital regulator of stress in an organism's body is being evaluated, when a toxicant enters into the body of it. Thus this article will help the policy makers to deduce certain policys for the reduce use of agrochemicals so as to minimize its deleterious effects on the environment.

ACCEPTED MANUSCRIPT

Evaluating the toxicological effects of agrochemicals on glucocorticoid receptor and serum cortisol level in *Mozambique tilapia.*

Abstract:

The agricultural sector is considered to be the backbone of a countries economy. To increase the crop yield there is an extensive use of agrochemicals in the form of insecticides, herbicides, nutrient mixture, fungicides, etc. This is necessarily involved in controlling the unwanted organisms; however, it leads to toxicity in a nearby ecosystem. Aquatic habitats being a complexed ecosystem are distressed with the increasing concentration of agrochemicals which leads to an alteration in non-target species at various trophic levels. Fishes are considered to be the dynamic players of this ecosystem and are the first one to be acquainted with any contaminants present in it. In this context, the present study was aimed to find the sub-lethal effects (1/10th of the LC_{50} value) of four different agrochemicals (Imidacloprid-Insecticide, Pyrazosulfuron Ethyl-herbicide, Curzate-fungicide and micronutrient mixture) on the stress physiology of *Oreochromis mossambicus*. The hormonal titer of cortisol and gene expression of its receptor was carried out to understand the toxicity of diverse agrochemicals. The results suggested that there was a significant decrease in hormonal titer of cortisol upon the exposure of curzate and imidacloprid, with a significant down-regulation of glucocorticoid receptor under the exposure of imidacloprid. Thus, the novel findings suggest that fishes were found to be devoid of stress response which may be due to hypothalamus pituitary inter-renal exhaustion.

Keywords: Cortisol, Glucocorticoid receptor, sub-lethal, Agrochemicals, Aquatic ecosystem.

ACCEPTED MANUSCRIPT

1. Introduction:

Agrochemicals (insecticide, Fungicide, and Herbicide etc) have brought tremendous benefits to mankind by increasing production and controlling the vector of man and animal diseases. At the same time use of these agrochemicals has affected the health of aquatic organisms as it has posed potential health hazards to the life of fishes (Osman *et al*., 2011). Agrochemicals are the major cause of concern for the aquatic environment because of their toxicity, persistence, and tendency to accumulate in the organisms (Joseph and Raj, 2010). The impacts of these, on aquatic organisms is due to their movement from various diffuse or point sources and are posing a great threat to aquatic fauna especially to fishes, which constitute one of the major sources of protein-rich food for mankind (Ray *et al*., 2015). Agrochemicals in the form of synthetic compounds are commonly referred as EDCs (Kojima *et al*., 2004) have hormone-like activity. Of the agrochemicals proved to have endocrine disrupting property, 46% are insecticides, 21% herbicides and 31% fungicides (Mnif *et al*., 2011). The endocrine effects of these agrochemicals are believed to be due to their ability to: mimic the effect of endogenous hormones, antagonize the effect of endogenous hormones, disrupt the synthesis and metabolism of endogenous hormones as well as of hormone receptors (Raun *et al*., 2002; Blair *et al*., 2002; Tabb, and Blumberg, 2006). They may also bind to these receptors without activating them, these antagonistic accomplishment blocks the receptors and inhibits their action. Finally, they may also interfere with the synthesis, transport, metabolism, and elimination of hormones, thereby decreasing the concentration of natural hormones (Cocco, 2002).

The intricate relationship between the brain (hypothalamus and pituitary) and endocrine action allows adaptability of an organism to the environment while maintaining homeostasis. In vertebrates, the hypothalamus represents a master regulator of homeostasis and is the critical nexus between the nervous and endocrine systems. The hypothalamus mediates responses to homeostatic imbalance mainly through regulation of the pituitary gland, which, in turn, produces hormones that are able to affect systemic change, for example within the Inter-renal gland. The central role of the hypothalamic-pituitary axis makes it particularly susceptible and sensitive to perturbation by a variety of environmental contaminants. The response to stress in fish is characterized by the stimulation of the hypothalamus, which results in the activation of the neuroendocrine system and a subsequent cascade of metabolic and physiological changes (Lowe & Davison 2005; Martínez-Porchas *et al*. 2009). These changes enhance the

ACCEPTED MANUSCRIPT

tolerance of an organism to face an environmental variation or an adverse situation while maintaining a homeostasis status (Martínez-Porchas *et al*. 2009). Under conditions of stress, the body of the fish emits immediate responses recognized as primary and secondary responses. The primary response is the perception of an altered state by the central nervous system (CNS) and the release of the stress hormones, cortisol and catecholamines into the bloodstream by the endocrine system (Martínez-Porchas *et al*. 2009). Secondary responses occur as a consequence of the released stress hormones (Martínez-Porchas *et al*. 2009), causing changes in the blood and tissue chemistry, e.g. an increase of plasma glucose (Begg & Pankhurst 2004; Martínez-Porchas *et al*. 2009). Cortisol is a hormone secreted from the internal gland and is used in many studies as the stress indicator (Flik *et al*., 2006; Gagnon *et al*., 2006; Sepici-Dinçel *et al*., 2009). The effects of prolonged exposures to agrochemicals on cortisol production have been examined in both the laboratory and in wild populations. Many studies have indicated HPI axis exhaustion as a common phenomenon. Prolonged cortisol elevation eventually creates negative feedback on the HPI axis, down-regulating receptors and causing atrophy of cells e.g., pituitary corticotrophs (Basu *et al*. 2002; Laflamme *et al*. 2000; Levesque *et al*. 2003; Gravel *et al*. 2005; Marentette *et al*., 2012). Hence, the present study is focused on the alterations in the cortisol hormonal profile and its receptor as an adaptive change of the freshwater teleost, *Oreochromis mossambicus* exposed to the sub-lethal concentration of four different agrochemicals (Imidacloprid-IMI, Pyrazosulfuron Ethyl-PE, Curzate-CZ, and Micronutrient mixture-MN). The rationale behind selecting these agrochemicals was their widespread used in the country and its residues found in nearby aquatic ecosystem in our study (unpublished data) and their unexplored effect on inter-renal axis. Similarly, *O.mossambicus* was selected as a model due to its wide availability in aquatic ecosystem and its use as a edible fish.

2. Materials and Method:

2.1 Animal Maintenance:

Mature Tilapia *O.mossambicus* (12 ± 2 cm, 25 ± 1.9 g) of similar size in length and weight were obtained from the pure brooders of the Vadodara district and transferred to the laboratory. The acclimation period was for 15 days at 27 ± 40C, pH 7.4 ± 0.5, dissolved oxygen 8 ± 0.3 mg/L, total hardness 188 mg/L $CaCO_3$ with a 12:12 light:dark photoperiod. Fish were supplied daily with commercial fish food during acclimation. Animal maintenance and experimental procedures were in accordance with the guideline of A.P.H.A., A.W.W.A., and W.P.C.F. (1998).

ACCEPTED MANUSCRIPT

2.2 Experimental protocol:

On basis of the LC_{50} value (Patel *et al.,* 2016; Sadekarpawar *et al.,* 2016; Upadhyay *et al.,* 2015.) of each agrochemical sub-lethal study dose $LC_{50/10}$ (IMI-0.74 mg/L, PE-500 mg/L, CZ-49.61 mg/L, MN-5000 mg/L) was chosen (on the basis of their effective concentration found in pond ecosystem) to find its mechanistic action on HPI axis for which, hormonal assay and gene expression studies were conducted. The experimental regime was maintained in the laboratory for 14 days with a control group having three replicates in each group. The experiment was performed semi statically with a group of 10 fishes (5 male and 5 females) in experimental aquaria. Hormonal assays of the experimental, as well as the control fish, were carried out at 15th day of exposure. All the groups were kept under continuous observation during the experimental period. After the completion of the exposure, fish were caught very gently using a small dip net, one at a time with least disturbance. They were slowly released in the tough containing 1% clove oil to make it immobile, blotted dry and blood was collected by tail ablation.

2.3 Hormonal assays:

The blood was collected and serum was separated and kept in cold storage for hormonal assays. The hormonal titer of cortisol (Cyaman Cat # 500360) was performed. Each sample was assayed in triplicates where 100 µL of ELISA buffer was added to all the wells, followed by addition of 50 µL of cortisol standard to each well. Cortisol standard was made using serial dilution from the stock solution (400ng/mL) in 8 tubes.50µL of the sample were added to each triplicate trailed by 50µL AChE tracer in each well except for blank and TA (total activity). Finally, 50µl of cortisol antiserum were added to each well except for the well of TA and NSB (Non-Specific binding). The plate was incubated at room temperature on an orbital shaker for 1 hr and was developed using Ellman's reagent. The standard curve and sample concentration were determined using the following formula:

$$\text{logit}(B/B0) = \ln [B/B0/(1 - B/B0)]$$

2.4 Total RNA isolation and PCR Amplification:

On 15th-day fishes were removed and washed with fresh water. Control, as well as treated groups, were euthanized by decapitation and blood was allowed to drain and organs (Hypothalamus, Liver, Gills, Kidney, Thyroid, Ovary) were dissected out and gene expression pattern of the glucocorticoid receptor (GR) was analyzed. Testis was not taken into account as, upon dissection it was found to be ruptured in 7 fishes of two groups (CZ & IMI). Total RNA

ACCEPTED MANUSCRIPT

was isolated by Trizol Invitrogen according to the method of Peterson and Freeman (2009) and concentration was measured spectroscopically by Perkin Elmer. cDNA was reverse transcribed from 50ng total isolated RNA using Thermo Verso cDNA synthesis kit (AB-1453/B) and PCR for GR was performed with its specific primers (FP-5'-TTTCGGTAATTGGTTGCTGATGAT-3', RP-5'- AGTGCTCCTGGCTGTTTCTAAGT-3') and with standardized condition i.e. denaturation was performed at 95^0C for 1 min, annealing was carried out for 30 sec at 59^0C and extension was carried out at 72^0C for 7 mins with 18srRNA (FP:5′-TATTGTGCCGCTAGAGGTGAA3′, RP:5′-CCTCCGACTTTCGTTCTTGA-3′) as the reference gene. A total of 35 cycles were carried out for all the genes (described in materials and method) and finally the amplicon obtained were checked on 2% agarose gel and images were taken using ABI gel documentation system. Relative quantification analysis of the PCR products was done using Image J software.

2.5 Statistical Analysis:

The difference between the mean of control and the exposed fishes was determined by One-way ANOVA using Graph Pad Prism software version 6. If there was any significant difference, post hoc test was carried out where Dunnett's multiple comparison test were employed to recognize difference in the alterations found in between the control and the exposed groups. The significant level of the tests was set at 5% ($p<0.05$).

2.6 Pathway representation:

The amplification of target gene resulted in multiple action site of the agrochemicals. Thus, bioinformatic tools were used for representation of the pathways and to investigate which pathways were being affected by the tested agrochemicals. Candidate gene (GR) network maps were generated using Pathway Common and wiki pathways and were visualized in the open-source software platform Cytoscape.

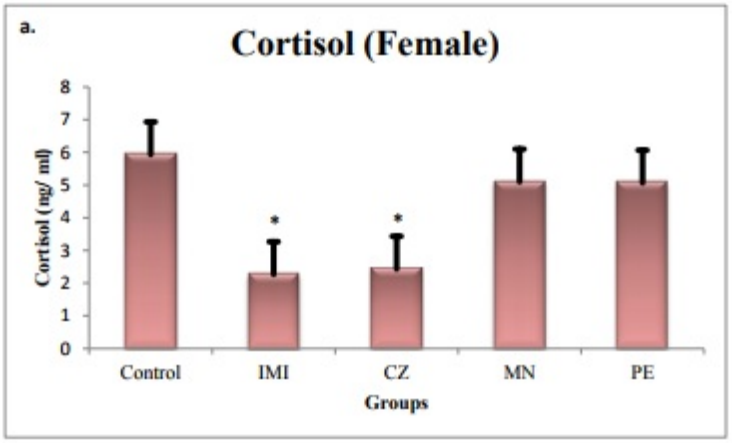
3. Results:

Hormonal Assay:

Sublethal exposure of agrochemicals resulted in a overall decrease in cortisol concentration ranging from 2.2 ± 0.15 ng/mL to 5.9 ± 0.6 ng/mL in response to agrochemical exposure. At individual level, however, the significance (*$p<0.05$) was found only with exposure to CZ (-61%), a fungicide and IMI (-58%) an insecticide in comparison to

ACCEPTED MANUSCRIPT

the control group. While there was no significance reported in case of PE and MN exposed fishes (Fig 1). There were little alterations found in sex-specific cortisol titers of male and female, however, it was non-significant.



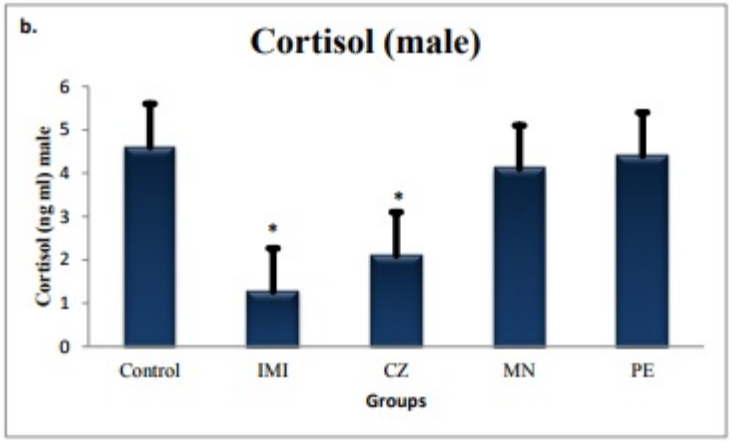


Figure 1: Serum Cortisol level in female and male *O.mossambicus. Significance is reported at *p<0.05.*

Gene Expression studies:

The candidate gene studied for the HPI axis was GR in vital tissues (liver, brain, kidney, gills, thyroid and ovary). Of all the agrochemicals, only IMI exposure resulted in a significant (*p<0.05) down-regulation of GR in all the tissues along with a significant (*p<0.05) down-regulation in testis and ovaries of fish exposed to CZ (Fig 2,3). There was an alteration in the expression in other tissues also but it was non-significant on exposure of PE and MN. Data of the testes has not been presented as in 7 fishes it was found to be ruptured in CZ and IMI exposed group, thus we postulate, that the fishes had initiated the process of undergoing sex reversal (Pandya and Parikh 2016).

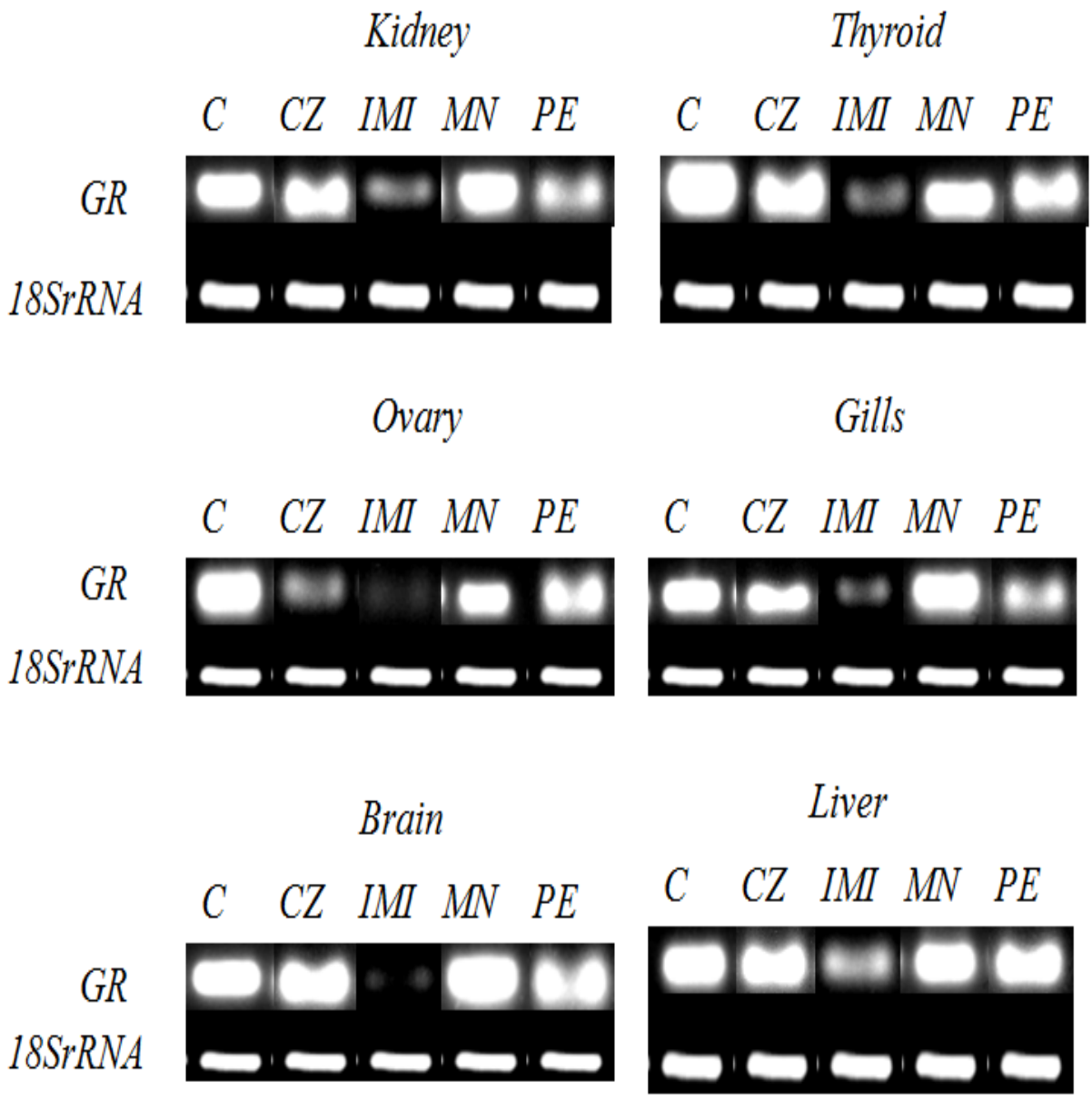


Fig 2: 2% Agarose Gene expression images of GR-Glucocorticoid receptor in kidney, thyroid, ovary gills, brain and liver. C-Control, CZ-Curzate, IMI-Imidacloprid, MN-Micronutrient Mixture, PE-Pyzosulphuron ethyl

ACCEPTED

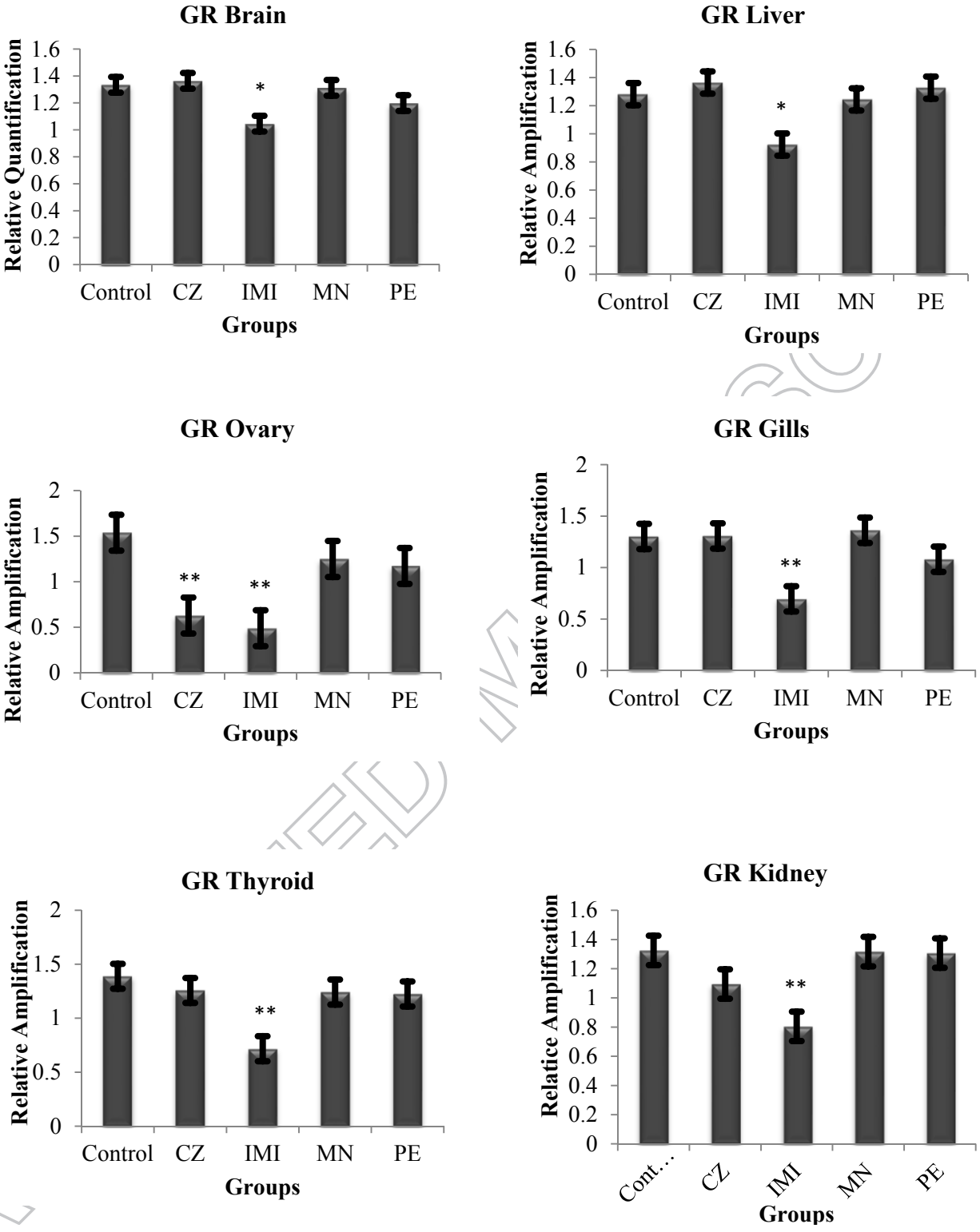


Fig 3: Graph showing relative expression pattern of the Glucocorticoid receptor (GR) in four different groups of *O.mossambicus* exposed to agrochemicals.(*) denotes the significance of *p<0.05.

Pathway Analysis:

To know the multiple actions of the chosen agrochemicals, the bioinformatic approach was used, where 32 genes (fig 4) were found to interacting with GR and were mostly the downstream signaling molecules. Further, the analysis also conveyed the fact, that it had its association with the xenobiotic metabolism enzyme *cyp 450* oxygenases and can be thought to be regulated by these canonical genes.

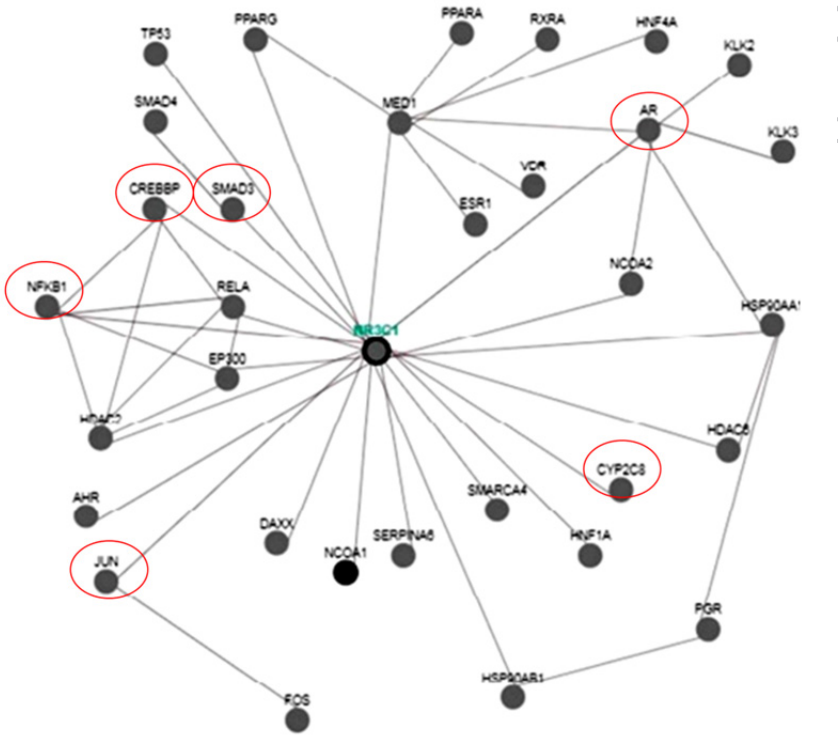


Fig 4. Illustrates the interaction of various genes with glucocorticoid receptor (NR3C1) using pathway commons and cytoscape software.

4. Discussion:

The chemical coordination by endocrine system in organisms is known to regulate a number of hormone-dependent physiological functions which are essential for the survival of the organism both in normal as well as in an altered environmental condition. This endocrine system is a potential target of xenobiotics, and its vulnerability resides in part in the finely tuned mechanisms through which the endocrine control system operates in animals (Hontela,

ACCEPTED MANUSCRIPT

1997). The xenobiotics that enter into the body can have either direct adverse effects on the endocrine gland and tissues or indirect through alterations of homeostasis and damaging activities of nonendocrine organs (Harvey,1996).

The hypothalamic–pituitary interrenal (HPI) axis is responsible for releasing corticosteroids and catecholamines in response to a stressor. Cortisol is the major corticosteroid in teleost and most mammals (Miller, 2006). When a fish perceives a stimulus as a stressor, the hypothalamus releases corticotropin-releasing hormone (CRH) that stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH), which in turn enters the bloodstream and stimulates the steroidogenic interrenal cells in the anterior head kidney to synthesize cortisol (Hontela 2005). ACTH binds to its receptor in the cell membrane, and cyclic adenosine monophosphate (cAMP) activates protein kinase A. Protein kinase A activates cholesterol ester hydrolase releasing free cholesterol from cholesterol esters within the cellular matrix (Stocco 2000). At the outer mitochondrial membrane, steroidogenic acute regulatory (StAR) protein regulates the passage of cholesterol to the inner membrane where cytochrome P450 side-chain cleavage (cP450scc) mediates the transformation of cholesterol to pregnenolone (Stocco 2000). This is a rate-limiting step in cortisol synthesis and can be disrupted by contaminants (Walsh *et al*. 2000). Pregnenolone moves to the cytoplasm where cytochromes P45017α and P450C21 create 11- deoxycortisol, that is shunted back to the mitochondria to be transformed into cortisol by cytochrome P45011ß (Hontela 2005). Cortisol regulates its own production through a negative feedback loop by altering ATCH secretion at the pituitary and hypothalamus (Hontela, 2006). Cortisol affects a variety of systems that regulate homeostasis (Dorval *et al*., 2003). High serum cortisol levels indicate the animal is under acute or sub-chronic stress, while low levels indicate no stress, interrenal exhaustion or impairment of the HPI axis (Martinez-porchas *et al*., 2009; Miller and Hontela, 2011). Corticosteroids can also be influenced by factors other than stressors.

Some fish are more sensitive to stress during molting and may decrease their cortisol production as they age (Barton 2002; Jasmin Christal *et al*., 2003). Several studies have corroborated the impairment in cortisol synthesis and secretion due to the action of agrochemicals (Aluru *et al*., 2004-06; Hontela, 2006; Gravel and Vijayan, 2006). The impairment is either at the level of CRH, ACTH or at the level of StAR protein and N6, 2'-dibutyl adenosine 3': 5'-cyclic monophosphate (dbc-AMP) (Leblond *et al*., 2001) which is the common target of many environmental pollutants ranging from pesticides to pharmaceuticals (Hontela, 2006; Martinez-porchas *et al*., 2009). Therefore

ACCEPTED MANUSCRIPT

many pollutants halt cortisol secretion and even if the fish is under stress this will probably not be reflected in the cortisol response. In the present study cortisol level in *O. mossambicus* was found to be lower than that of control level on exposure to IMI and CZ, suggesting an impairment of the interrenal exhaustion or impairment of the HPI axis (Miller, 2006; Martinez-porchas *et al*., 2009). Similar results were reported in *Fundulus heteroclittus* where, a decreased level of serum cortisol under toxic conditions on exposure to naphthalene as a result of severe necrosis of interrenal tissues (Swarnalatha, 2015).

Hontela and her co-workers (2006), while investigating the impact of toxicants on fish interrenal tissues, based on the in vitro studies, also reported inhibiting effects of pollutants on interrenal function and cortisol secretion. *Oreochromis mossambicus*, under sub-lethal thiodon exposure, registered significant reductions in serum cortisol levels following 1, 6 and 12 hours of exposures (Parvatham *et al*., 2004). Similar reductions in serum cortisol level were also reported in *O.mossambicus* exposed to sub-lethal concentration of dimecron (Karthikeyan *et al*., 2004), in *Channa striatus* exposed to lethal and sub-lethal concentrations of sevin (Sumathiral, 2006), in *Labeo rohita* fingerlings exposed to endosulfan and dietary pyridoxine (Akhtar *et al*., 2010), in *Clarias gariepinus* exposed to varying concentrations of endosulfan (Ezemonye and Ikpesu, 2010) and in *Sarotherodon mossambicus* under endosulfan toxicity (Thangavel *et al*., 2010). Wedemeyer and Yasutake (1997) stated that too low levels of cortisol are indicative of interrenal exhaustion from severe stress, whereas, too high levels indicated the fish to be under chronic or acute stress. Similar reductions in cortisol level as a function of interregnal exhaustion were also reported in fish exposed to various pesticides (Parvatham *et al*., 2004; Karthikeyan *et al*., 2004; Sumathiral, 2006 and Thangavel *et al*., 2010; Swarnalatha, 2015) and during in vitro exposure of head kidney tissues of fish to pesticides (Leblond *et al*., 2001) and to heavy metals (Brodeur *et al*., 1997). The reduced cortisol level in the present study, together with similar reports of interrenal exhaustion and reduction in cortisol level in other fish under different types of toxic conditions could be considered as an adaptive response by the fish by way of maintaining low metabolic rate under pesticide.

Genome duplication event occurs in teleost fish (Jaillon *et al*., 2004), leading to two distinct GR genes (Bury *et al*., 2003; Greenwood *et al*. 2003; Bury & Sturm 2007; Stolte *et al*., 2008; Alsop *et al*.,2008). In the present work, the focus was mainly on the GR, as it has high affinity to bind its steroid cortisol (Basu *et al*. 2003; Cruz *et al*.,2013). Indeed, several studies have shown that the xenobiotics disrupt cortisol and its receptor response to stress by

ACCEPTED MANUSCRIPT

targeting multiple sites along the HPI axis, including impaired steroidogenesis and brain glucocorticoid signaling (Aluru *et al*., 2004; Hontela, 2005; Vijayan *et al*., 2005; Aluru and Vijayan, 2006).In the present study, tissue-specific receptor expression was studied under the exposure of agrochemicals, among which, significant damage was encountered by IMI, which down-regulated the mRNA of GR in all the organs followed by CZ in the ovary and testis only. The exposure of IMI and CZ resulted in sensitization of cortisol receptor and maybe its steroid (cortisol), substantiating the receptor downregulation probably due to its self-regulation (Sathiyaa and Vijayan 2003). Thus, the mechanism that may be operative is the increase in the cortisol, which binds to its receptor leading to activation of other growth regulated transcriptional factors and thus maintaining the physiological stress caused by the agrochemicals (Gravel and Vijayan 2006).

To analyze interactions between the specific and non-specific genes involved at the transcriptional level an attempt was made to interconnect with the help of Cytoscape software, pathway common and wiki pathways were used which helped in understanding the possible role of the association of genes with the target genes. GR signaling governs many metabolic pathways through more than 100 genes. The data of the present work was applied to Cytoscape and pathway common, which resulted in the interaction of various genes. A total of 32 genes was found to be interacting with the glucocorticoid receptor among which major transcription factors like jun, CREBP, Smad's may be the downstream activators which initiate the physiological response upon pesticide exposure. Moreover, CYP2C8 (cytochrome-p450) may be activated due to the toxic exposure of IMI and CZ which may lead to degradation of this xenobiotics and maintaining the adaptive response (Fig 4). Further, the androgen receptor (AR) was also found to be interacting with it, which suggests that it also aids in combating the agrochemical stress and maintains its reproductive potency in its proper state.

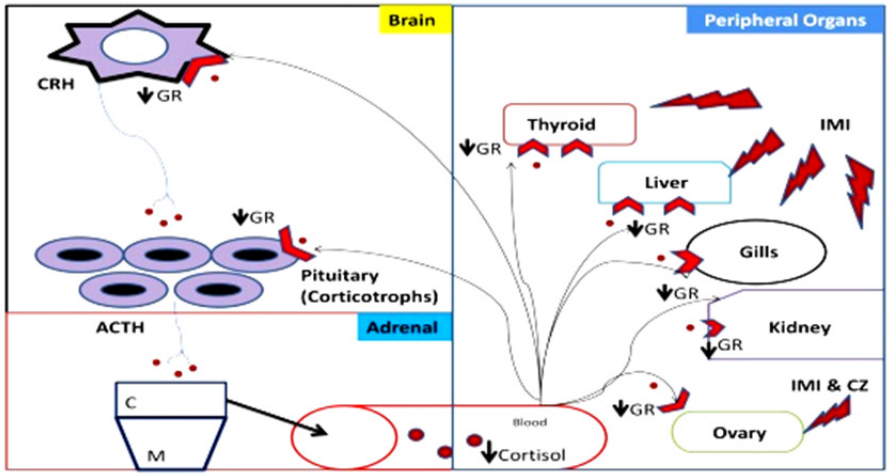
5. Conclusion:

So from the present work, it can be concluded, agrochemicals exposure invoked alterations in the expression of genes associated HPI axis. The observed reductions in the serum cortisol level in IMI and CZ group is an indication of interrenal exhaustion and also an adaptive stress response of the fish by way of maintaining low basal metabolic rate (BMR) under agrochemical toxicity (Fig 5). A strong link is thus determined between the measured upregulation/down regulation of GR and indicates the potential of using gene expression in toxicological studies as markers. However, immunohistochemistry, microarray and cloning of the upstream (ACTH, StA, 17-β hydroxylase)

ACCEPTED MANUSCRIPT

and downstream genes (signaling molecules) will provide insights into a better understanding of the mechanisms governing the effect of agrochemicals on the canonical pathways of this axis at each level.

Fig 5. Conclusion- Schematic representation of Hypothalamus-pituitary interenal Axis (HPI) under the exposure of Agrochemicals (PE-Pyzosulphuroethyl, IMI-Imidacloprid, MN-Micronutrient Mixture and CZ-Curzate). CRH-Cortocotropin Releasing Hormone, ACTH-Adrenocorticotropic Hormone, GR-Glucocorticoid Receptor, C-cortical region, M-medullary region.



Abbreviations:

HPI-hypothalamic–pituitary interrenal axis

GR-Glucocorticoid Receptor

IMI-Imidacloprid.

CZ- Curzate.

MN- Micronutrient Mixture

PE- Pyzosulphuroethyl

CRH-Cortocotropin Releasing Hormone

ACTH-Adrenocorticotropic Hormone

C-cortical region

M-medullary region.

AR-Androgen receptor

ACCEPTED MANUSCRIPT

Acknowledgment:

The authors are thankful to the Head, Department of Zoology, Faculty of Science, The M.S.University of Baroda, Vadodara for providing instrument facilities.

Compliance with ethical standards

Conflict of interest

The author declares that the present work has no conflict of interest.

Ethical Approval:

All the procedures were in accordance with guidelines of A.P.H.A., A.W.W.A. and W.P.C.F. (1998) for the care and use of animals were followed.

ACCEPTED MANUSCRIPT

References:

Alsop, D., Vijayan, M. M. (2008). Development of the corticosteroid stress axis and receptor expression in zebrafish. *American Journal of Physiology, Regulatory, Integrative and Comparative Physiology*. 294: 711–719. doi: 10.1152/ajpregu.00671.2007

Aluru, N., and Vijayan, M. M. (2006). Aryl hydrocarbon receptor activation impairs cortisol response to stress in rainbow trout by disrupting the rate-limiting steps in steroidogenesis. *Endocrinology*. 147: 1895–1903. doi:org/10.1210/en.2005-1143

Aluru, N., Jorgensen, E. H., Maule, A. G., and Vijayan, M. M. (2004). PCB disruption of the hypothalamus-pituitary-interrenal axis involves brain glucocorticoid receptor downregulation in anadromous arctic charr. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*. 287: 787–793. doi:10.1152/ajpregu.00091.2004

Aluru, N., Renaud, R., Leatherland, J. F. and Vijayan, M. M. (2005). An Receptor-mediated impairment of interrenal steroidogenesis involves StAR protein and P450scc gene attenuation in rainbow trout. *Toxicological Science*. 84(2):260-269.doi:org/10.1093/toxsci/kfi077.

Barton, B. A. (2002). Stress in Fishes: A Diversity of Responses with Particular Reference to Changes in Circulating Corticosteroids. *Oxford Journals*. 42(3): 517-525.doi: 10.1093/icb/42.3.517

Basu, N., Kennedy, C.J. and Iwama, G.K. (2003). The effects of stress on the association between hsp70 and the glucocorticoid receptor in rainbow trout. *Comparative Biochemistry and Physiology*. 134A (3): 655-663. doi:10.1016/S1095-6433(02)00372-0

Basu, N., Kennedy, C.J., Hodson, P.V. and Iwama, G.K. (2002). Altered stress responses in rainbow trout following a dietary administration of cortisol and beta-napthoflavone. *Fish Physiology Biochemistry*. 25:131–140.

Begg, K. and Pankhurst, N.W. (2004). Endocrine and metabolic responses to stress in a laboratory population of the tropical damselfish *Acanthochromis polyacanthus*. *Journal of Fish Biology*. 64: 133-14. doi**:** 10.1111/j.1095-8649.2004.00290.x

ACCEPTED MANUSCRIPT

Blair, R.M., Fang, H., Branham, W.S., Hass, B.S., Dial, S.L., Moland, C.L., Tong, W., Shi, L., Perkins, R., Sheehan, D.M., (2000). The estrogen receptor relative binding affinities of 188 natural and xenochemicals: structural diversity of ligands. *Toxicological Science.* 54, 138–153.

Book on Standard methods for the examination of water and wastewater. (1998). APHA-AWWA-WEF, 20th Edition. National government publication.

Brodeur, J.C., Sherwood, G., Rasmussen, J.B. and Hontela, A. (1997). Impaired cortisol secretion in Yellow perch (*Perca flavescenes*) from lakes contaminated by heavy metals: In vivo and in vitro assessment. *Canadian Journal Fisheries and Aquatic. Science*. 54: 2752-2758. doi:10.1139/cjfas-54-12-2752.

Bury N.R., Sturm, A., Le Rouzic, P., Lethimonier C, Ducouret B, Guiguen Y, Robinson-Rechavi M, Laudet V, Rafestin-Oblin ME, Prunet P., (2003). Evidence for two distinct functional glucocorticoid receptors in teleost fish. *Journal of Molecular Endocrinology*. 31:141–156.

Bury, N.R and Sturm, A. (2007). Evolution of the corticosteroid receptor signalling pathway in fish. *General and Comparative Endocrinology*. 153: 47–56. doi: 10.1016/j.ygcen.2007.03.009

Cocco, P. (2002). On the rumors about the silent spring. Review of the scientific evidence linking occupational and environmental pesticide exposure to endocrine disruption health effects. *Cadernos De Saúde. Pública*. 18(2): 379-402. doi:org/10.1590/S0102-311X2002000200003

Colborn T. (2002). Clues from wildlife to create an assay for thyroid system disruption. *Environmental Health Perspectives*. 3:363-7

Cruz SA, Lin C-H, Chao P-L, Hwang P-P. (2013). Glucocorticoid Receptor, but Not Mineralocorticoid Receptor, Mediates Cortisol Regulation of Epidermal Ionocyte Development and Ion Transport in Zebrafish (*Danio Rerio*). *PLoS ONE* 8(10): e77997. doi:org/10.1371/journal.pone.0077997.

Dorval, J., Leblond, V.S., Hontela, A. (2003). Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) exposed in vitro to endosulfan, an organochlorine pesticide. *Aquatic Toxicology*. 63: 229-241. doi:org/10.1016/S0166-445X(02)00182-0

ACCEPTED MANUSCRIPT

Ezemonye, L. I. N. and Ikpesu, T. O. (2010). Evaluation of sublethal effects of endosulfan on cortisol secretion, glutathione s-transferase and acetycholinesterase activities in *Clarias gariepinus*. *Food and Chemical Toxicology*. 49 (9):1898-1903. doi:org/10.1016/j.fct.2010.10.025

Flik, G., Klaren, P.H.M., Van den Burg, E.H., Metz, J.R., Huising, M.O. (2006). CRF and stress in fish. *General and Comparative Endocrinology*. 146: 36-44. doi:10.1016/j.ygcen.2005.11.005

Gagnon,A., Jumarie,C. and Hontela, A. (2006). Effects of Cu on plasma cortisol and cortisol secretion by adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*, 78(1): 59-65. doi:org/10.1016/j.aquatox.2006.02.004

Gagnon,E., Hontela,A. and Jumarie, C. (2007). Reciprocal inhibition of Cd and Ca uptake in isolated head kidney cells of rainbow trout (*Oncorhynchus mykiss*). *Toxicology in vitro*. 21(6): 1077-1086. doi:10.1016/j.tiv.2007.03.019

Gravel, A. and Vijayan, M. M (2006). Salicylate Disrupts Interrenal Steroidogenesis and Brain Glucocorticoid Receptor Expression in Rainbow Trout. *Toxicological sciences*. 93(1): 41–49. doi:org/10.1093/toxsci/kfj166

Gravel, A. and Vijayan, M. M. (2007). Salicylate impacts the physiological responses to an acute handling disturbance in rainbow trout. *Aquatic Toxicology*. 85(2):87-95. doi: 10.1016/j.aquatox.2007.07.001

Gravel, A., Campbell, P.G.C., Hontela, A. (2005). Disruption of the hypothalamo-pituitaryinterrenal axis in 1+ yellow perch (*Perca flavescens*) chronically exposed to metals in the environment. *Canadian Journal Fisheries and Aquatic. Science*. 62:982–990. doi:org/10.1139/f05-011

Greenwood, A.K., Butler, P.C., White, R.B., DeMarco, U., Pearce, D., Fernald, R. D. (2003). Multiple corticosteroid receptors in a teleost fish: distinct sequences, expression patterns, and transcriptional activities. *Endocrinology*. 144:4226–4236. doi:org/10.1210/en.2003-0566

Hontela A. (2006). Corticosteroidogenesis and StAR Protein of Rainbow Trout Disrupted by Human-Use Pharmaceuticals: Data for Use in Risk Assessment. *Toxicological sciences*. 93(1), 1–2. doi: org/10.1093/toxsci/kfl054

ACCEPTED MANUSCRIPT

Hontela, A. (1997). Endocrine and physiological responses of fish to xenobiotics: Role of glucocorticosteroid hormones. *Critical Reviews in Toxicology*. 1: 1-46. doi:org/10.1006/taap.1999.8660

Hontela, A. (2005). Stress and the hypothalamo-pituitary-interrenal axis: Adrenal toxicology— Effects of environmental pollutants on the structure and function of the HPI axis. *Environmental Toxicology* 331–363. doi:org/10.1016/S1873-0140(05)80015-3.

Jaillon, O., Aury, J. M., Brunet, F., Petit, J. L., Thomann, N. S., Mauceli, E., Bouneau, L., Fischer, C., Ozouf-Costaz, C., Bernot, A., Nicaud, A., et al. (2004). Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype. *Nature*. 431: 946.

Jasmin Christal, C., Sumathiral, K. and Ramaswamy, M. (2003). Changes in serum cortisol and electrolyte levels in a freshwater teleost, *Sarotherodon mossambicus* (Peters) under individual and synergistic exposures to dimecron and cuman L. National Conference on Zoology, (NACOZ— ,2003), held on 21st to 23rd December 2003 at Vivekananda College, Agastheeswaram, Kanyakumari, Tamil Nadu, India.

Joseph, B., S.J. Raj, B.T. Edwin, P. Sankarganesh, M.V. Jeevitha, S.U. Ajisha and S.R. Sheeja, (2010). Toxic effect of heavy metals on aquatic environment. *International Journal of Biological and Chemical Sciences.* 4: 939-952. doi:org/10.4314/ijbcs.v4i4.62976

Karthikeyan, S., Sumathiral, K. and Ramaswamy, M. (2004). Dimecron-induced endocrine responses in a freshwater, edible, teleost fish, *Oreochromis mossambicus* (Peters). National Symposium on Recent Trends in Applied Biology, held on 28th & 29th January, 2004 at Avinasilingam Institute for Home Science and Higher Education for Women Deemed University, Coimbatore, Tamil Nadu, India.

Kojima H., Katsura E., Takeuchi S., Niiyama K., Kobayashi K.(2004). Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. *Environmental Health Perspective*.112:524–531.

Laflamme, J-S., Couillard, Y., Campbell, P.G.C., Hontela, A. (2000). Interrenal metallothionein and cortisol secretion in relation to Cd, Cu and Zn exposure in yellow perch, *Perca flavescens*, from Abitibi lakes. *Canadian Journal Fisheries and Aquatic. Science.*57:1692–1700. doi:org/10.1139/f00-118

ACCEPTED MANUSCRIPT

Leblond, V. S., Bisson, M. and Hontela, A. (2001). Inhibition of cortisol secretion in dispersed head kidney cells of rainbow trout (*Oncorhynchus mykiss*) by endosulfan, an organochlorine pesticide. *General and Comparative Endocrinology.* 121: 48-56. doi:10.1006/gcen.2000.7556

Levesque, H.M., Dorval, J., Hontela, A., Van Der Kraak, G.J., Campbell, P.G.C. (2003). Hormonal, morphological and physiological responses of yellow perch (*Perca flavescens*) to chronic environmental metal exposures. *Journal of Toxicology and Environmental Health.* 66:657–676. doi: 10.1080/15287390309353773

Lowe, C. J. and Davison, W. (2005). Plasma osmolarity, glucose concentration and erythrocyte responses of two .Antarctic nototheniid fishes to acute and chronic thermal change. *Journal of Fish Biology*. 67: 752–766. doi:10.1111/j.0022-1112.2005.00775.x

Marentette, J. R., Tong, S. and Balshine, S. (2012). The cortisol stress response in male round goby (*Neogobius melanostomus*): effects of living in polluted environments. *Environmental Biology of Fishes.* 96 (6):723–733.

Martínez-Porchas, M., Martínez-Córdova, L.R and Ramos-Enriquez, R. (2009). Cortisol and Glucose: Reliable indicators of fish stress, *Pan-American Journal of Aquatic Sciences*. 4: 158-178.

Miller, L. (2006). The effects of selenium on the physiological stress response in fish. Thesis submitted to Department of Biological Sciences University of Lethbridge Lethbridge, Alberta, Canada.

Miller, L.L. and Hontela, A., (2011). Species-specific sensitivity to selenium-induced impairment of cortisol secretion in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). *Toxicology and Applied Pharmacology*. 253: 137–144. doi:org/10.1016/j.taap.2011.03.021

Mnif, W., Hassine, A.I.H., Bouaziz, A., Bartegi, A., Thomas, O. and Roig, B. (2011). Effect of Endocrine Disruptor Pesticides: A Review. *International Journal of Environmental Research and Public Health*. 8(6): 2265– 2303. doi:10.3390/ijerph8062265

Osman, A. G., Abd El Reheema, A. M., Moustafa, M. A., Mahmoud, U. M., Abuel-Fadld, K. Y., Kloas, W. (2011). *In Situ* Evaluation of the Genotoxic Potential of the River Nile: I. Micronucleus and Nuclear Lesion Tests of Erythrocytes of *Oreochromis niloticus* (Linnaeus, 1758) and *Clarias gariepinus* (Burchell, 1822). *Toxicological and Environmental Chemistry*. 93: 1002-1017. doi:org/10.1080/02772248.2011.564496

ACCEPTED MANUSCRIPT

Pandya P and Pairkh P (2016). Agrochemicals Induced Gene Expression Alterations In *O.mossambicus*. *International Journal of Advanced Research.* 4 (6), 830-840

Parvatham, M., Sumathiral, K. and Ramaswamy, M. (2004). Adaptive endocrine responses in a freshwater teleost, *Oreochromis mossambicus* (Peters) exposed to organophosphate pesticide, endosulfan. National Symposium on Recent Trends in Applied Biology held on 28th and 29th January, 2004 at Department of Life Sciences, Avinashilingam Institute for Home Science and Higher Education for Women, Deemed University, Coimbatore, Tamil Nadu, India.

Patel, B., Pandya P. and Parikh, P. (2016). Effects of Agro-Chemicals On Antioxidant Enzymes And Lipid Peroxidation In *Oreochromis Mossambicus* and *Labeo Rohita. International Journal of Zoology and Applied Biosciences.*1(3).163-172

Patel, B., Upadhyay, A. and Parikh, P. H. (2016). Histological changes in the tissues of *Oreochromis mossambicus and Labeo rohita* on exposure to imidacloprid and curzate. *International Journal of Research in Applied Natural and Social Sciences.* 4(5):149-160.

Peterson, S. M. and Freeman, J. L. (2009). RNA Isolation from Embryonic Zebrafish and cDNA Synthesis for Gene Expression Analysis. *Journal of Visualized Experiments.* 30: 1470. doi: 10.3791/1470.

Raun Andersen, H., Vinggaard, A.M., Hoj Rasmussen, T., Gjermandsen, I.M., Cecilie Bonefeld Jorgensen, E., (2002). Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity in vitro. *Toxicology and Applied Pharmacology*. 179: 1–12. doi: 10.1006/taap.2001.9347

Ray, S. N. C., Mohan, C. and Sinha, R. C. (2015). Effect of Methyl Parathion on TSH, T4 and T3 in Fish, *Labeo Rohita*. *International journal of Applied Research*. 5 (3): 554-557.

Sadekarpawar, S., Pandya P., Upadhyay, A. and Parikh P. (2015). A comparative assessment of trace metal accumulation in Oreochromis mossambicus and labeo rohita exposed to plant nutrient librel TM. International Journal of Current Advanced Research. 4(10): 441-449.

ACCEPTED MANUSCRIPT

Sathiyaa, R., and Vijayan, M. M. (2003). Autoregulation of glucocorticoid receptor by cortisol in rainbow trout hepatocytes. *American Journal of Physiology-Cell Physiology*. 284: 1508–1515. doi: 10.1152/ajpcell.00448.2002

Sepici-Dinçel, A., Çağlan Karasu Benli, A., Selvi, M., Sarıkaya, R., Şahin, D., AyhanÖzkul, I., Erkoç, F. (2009). Sublethal cyfluthrin toxicity to carp (*Cyprinus carpio L*.) fingerlings: Biochemical, hematological, histopathological alterations. *Ecotoxicology and Environmental Safety*. 72: 1433- 1439. doi: 10.1016/j.ecoenv.2009.01.008

Stocco, D. M. (2000). The role of the StAR protein in steroidogenesis: challenges for the future. *Journal of Endocrinology.* 164: 247-253.

Stolte, E. H., de Mazon, A. F., Leon-Koosterziel, K. M., Jesiak, M., Bury, N. R., Sturm, A., Savelkoul, H. F. J. Lidy, B. M., van Kemenade, V and Flik, G. (2008). Corticosteroid receptors involved in stress regulation in common carp, *Cyprinus carpio*. *Journal of Endocrinology*. 198: 403–417. doi: 10.1677/JOE-08-0100.

Sumathiral, K. (2006). Endocrine and immunological responsiveness in a freshwater, edible, airbreathing teleost, *Channa striatus* (Bloch) exposed to a carbamate pesticide, sevin. Ph.D., Thesis, Bharathiar University, Coimbatore, Tamil Nadu, India.

Swarnalatha, M. (2015). A comprehensive study on hormone response ionic regulation selected enzyme levels and immuneresponsiveness in a freshwater air breathing teleost mystus vittatus bloch exposed to an organophosphate pesticide quinalphos. A Thesis Submmited to Department of Zoology, Bharathiar University. India.

Tabb, M. M. and Blumberg, B. (2006). New modes of action for endocrine-disrupting chemicals. *Molecular Endocrinology*. 20: 475–482. doi: 10.1210/me.2004-0513

Thangavel, P., Sumathiral, K., Maheswari, S., Rita, S., Ramaswamy, M. (2010). Hormone profile of an edible, freshwater teleost, *Sarotherodon mossambicus* (Peters) under endosulfan toxicity. *Pesticide Biochemistry and Physiology*. 97: 229-234. doi : 10.1016/j.pestbp.2010.03.001.

ACCEPTED MANUSCRIPT

Upadhyay, A., Pandya, P and Parikh, P. (2014). Acute exposure of Pyrazosulfuron Ethyl induced Haematological and Blood Biochemical changes in the Freshwater Teleost fish *Oreochromis mossambicus*. *Int.J.Adv. Res.Biol.Sci*. 1(2):79-86.

Vijayan, M.M., Prunet, P., Boon, A.N. (2005). Xenobiotic impact on corticosteroid signalling. In: Mommsen TP, Moon TW (eds) Environmental toxicology. *Biochemistry and molecular biology of fishes.* 6.:365–396.

Walsh, L. P. and Stocco, D. M. (2000). Effects of Lindane on steroidogenesis and steroidogenic acute regulatory protein expression. *Biology of Reproduction.* 63: 1024–1033.

Wedemeyer, G. A. and Yasutake, W. T. (1977). In: Clinical methods for assessment of the effects of environmental stress on fish health. *U. S. Fish and Wild Life Service.* 9: 1-19.

ACCEPTED MANUSCRIPT