ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Biochemical and Oxidative Stress Response of Plant Nutrient Librel^{TM} In Oreochromis mossambicus and Labeo rohita

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Manuscript Info

Manuscript History:

Received: 15 March 2015
Final Accepted: 18 April 2015
Published Online: May 2015

Key words:

Micronutrient, ROS, Toxicity, Librel^{TM}

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Abstract

Soils deficient in their ability to supply micronutrients to crops are alarmingly widespread across the globe. Indian agriculture is now in an era of multiple plant nutrient deficiencies. Nutrients like N, P, K, Zn, Mn, Mg, Mo, B, S and Cu are now of widespread practical importance from an application point of view. To meet this deficiency application of trace elements in the form of fertilizers or micronutrients have been used rampantly whereas remediation of soils contaminated with metals is not addressed These chemicals though helpful to the plants, in excess enters the nearby aquatic systems through runoff affecting the non targeted aquatic species especially fishes. Fish have been largely used to evaluate the quality of aquatic systems as bio-indicators for environmental pollutants. Hence, the aim of the present study is to look into the toxicity of the plant nutrient Librel^{TM} on two freshwater edible fish, where, two freshwater teleost fish *O.mossambicus* and *L.rohita*, are taken as a experimental models to have an understanding of biochemical changes and oxidative stress response of plant nutrient element mixture at sub chronic level. In addition, species and tissue-specific (liver, kidney, muscle and gill) defenses were also studied.

INTRODUCTION

In agriculture, soils'inability to supply micronutrients to crops are alarmingly widespread issue of interest and concern across the globe due to the deficiency of trace elements such as zinc, iron, copper, manganese and boron (White and Zasoski, 1999). In contrast the dramatic increase in the agricultural production has resulted into the increased absorption of the nutrients from the soil boosting use of traditonal fertilizer practices, NPK, trace metals and micronutrients in the soil. These chemicals though helpful to the plants, in excess enters the nearby aquatic sytems through runoff affecting the non targeted aquatic species especially fishes (Jezierska and Witeska, 2006; Sadekarpawar and parikh, 2013). Inadvertently the ingestion of such metal contaminated fishes represents a human health risk (Mozaffarian and Rimm, 2006; Jomova and Valko, 2011).

ROS concentration is a dynamic parameter, i.e. they are continuously generated and eliminated. "Oxidative stress" is a situation when steady-state ROS concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents(Lushchak, 2011 and Nishida, 2011). Oxidative stress has become an important subject for aquatic toxicology (Livingstone, 2001). Biological systems have developed an adequate enzymatic and non-enzymatic antioxidant mechanisms to protect their cellular components from oxidative damage during their evolution. Enzymes are biochemical macro-molecules controlling the metabolic

processes of organisms whose analysis is widely used for rapid detection to predict early warning of toxicant toxicity (Dutta and Areids, 2003; Kumari *et al.*, 2011).

Fish have been largely used to evaluate the quality of aquatic systems as bioindicators for environmental pollutants (Adams and Greeley, 2000). Environmental pollutants such as pesticides, induce oxidative stress in aquatic organisms by generation of ROS (Banerjee et aI., 1999; Jyothi and Narayan, 2000; Schlenk et al 2004 Parikh et al., 2010). Plant nutrients are known to be available in various trade names. One of them is the Librel^{TM} , rapidly soluble chelated micronutrient mixture which because of its stability, solubility and its compatibility with wide range of herbicides, fungicides, insecticides and other crop care products has been used extensively. There are no toxicity reports available in fish or other aquatic organisms for Librel^{TM} plant nutrient. According to EU regulations it is classified as irritant and known to irritate eyes and respiratory system when brought to contact in human beings. However, there are no ecological toxicity data available for the same. Moreover, the studies conducted till date have been focused on the individual trace element Flora, *et al.*, 2008; Rauf, *et al.*, 2009; Malik, *et al.*, 2010) but their role in minute quantities through the fertilizers by way of plant nutrients are not well documented.

As fish production had always been associated with agriculture, it is mandatory to explore and understand the effects of plant nutrient supplementation on fish health so that one can take remedial actions to improve fish health, both in terms of ecology as well as economics. Hence, the aim of the present study is to look into the toxicity of the plant nutrient Librel^{TM} on two freshwater edible fish, where, two freshwater teleost fish *O.mossambicus* and *L.rohita*, are taken as a experimental models to have an understanding of biocehmical changes and oxidative stress response of plant nutrient element mixture at subchronic level. In addition, species andtissue-specific (liver, kidney, muscle and gill) defences were also studied.

Materials And Methods

Experimental fish*:* *Labeo rohita* and *oreochromis mossambicus* were obtained from the local ponds of Vadodara district and transferred to the laboratory. They were acclimated for 10 days at 27 ± 4^{0}C, pH 7.4 ± 0.05, 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. Healthy fishes having weight and length, *O. mossambicus* (12 ± 2 cm, 25 ± 1.9 g) and *L. rohita* (20± 2 cm, 125 ± 5 g) were selected. 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).

Experimental chemical: The Trace element mixture used was a commercial formulation of Librel^{TM}, Chelated Micronutrient mixture(Nutrient % by Wt.Min., Zn-4.0, Mn-0.5, Cu-0.3, Fe-2.0 and B-0.5). Adult fishes were exposed to sub-lethal concentrations of micronutrient mixture of 300 mg/L (1/20thof LC_{50} value) for 15,30 and 45 days after acclimatization period. Experimental and control water was refreshed every third day to minimize loss of mixture concentration. No mortality occurred under any of these conditions. After each experimental periods, the fishes from the control and experimental groups were euthenised by decapitation and the tissue samples (gills, muscles, liver and kidney) were dissected out blot free and were store at -80ºC for further biochemical analysis.

Methods of Oxidative stress markers and biochemical estimations: All enzyme activities were measured spectrophotometrically. Frozen tissue were quickly weighed and then homogenized in a homogenizer. ALP Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were done using standard diagnostic kits purchased from Reckon Diagnostics. Oxidative stress markers, Catalase (CAT) was estimated following the method of Maehly and Chnace, (1955); Lipid peroxidation(LPO) was estimated following Niehaus and Samuealson, (1968); Reduced glutathione (GSH) was estimated following Ellman and Fiches (1959); (superOxide diamutase (SOD) was assayed following the method of Kakkar *et al*., (1984) and Ascorbic acid (AA) content was estimated by the method described by Roe J.H. (1967)

Statistical analysis

The significance of Wilk's Lambda test, $p < .001$ and the insignificance of Levene's test Equality of Error Variances, $p > .05$ were considered for testing MANOVA. Multivariate analysis of variance (MANOVA) was performed to test for significant differences between the control and exposed groups by SPSS software. Means were compared and statistical significance was established by Dunnett's test ($p<0.05$).

Results :

The results of this investigation are as presented in Table I (Wilk's lambda test), II (Levene's test of Equality of Error variances) and III a, b (Dunnett's test) respectively. Graphs showing the alterations in the stress and bioochemical parameters are presented in Fig. I. Significant increase in GSH, LPO and AA ($p<0.001$) was obtained in all the tissues of both the fishes compared to control. When species specific changes were compared the ROS parameters of *O.mossambicus* were seen to be altered more compared to *L.rohita*. Significant increase in the ALP

($p<0.001$) was observed in kidney, muscle and gills while in liver though there was an increase at 30th day, at 45th day the decrease was seen in both fishes. ALT ($p<0.05$) increased in all tissues in *O.mossambicus* while in *L.rohita* it increased significantly in gills and muscle and decreased in kidney and liver. Again in liver at the 30th day an increase was seen. AST showed an significant increase ($p<0.001$) in gills, muscle and liver, whereas kidney showed a significant ($p<0.05$) decrease. In contrast to the differential trend in all the parmeters, CAT expressed significant ($p<0.001$) decrease in all the tissues except in gill in *L.rohita* whereas O.mossambicus a significant increase in liver and gills and decrease in the intial 30 days of exposure and an increase at 45th day. (Table III).

Table I: Wilks' lambda test for *O.mossambicus and L.rohita*

	Tissues	Wilks' Lambda	F	Hypothesis df	Error df
O. mossambicus	**Gills**	.003	51197.02	12.000	13.520
	Muscles	.014	2894.22	12.000	13.520
	Liver	.011	5352.10	12.000	13.520
	Kidney	.002	125851.43	15.000	11.444
L.rohita	**Gills**	.000	524219.544	12.000	13.520
	Muscles	.000	746.659	12.000	13.520
	Liver	.000	17290.6	12.000	13.520
	Kidney	.000	166396	15.000	11.444

Table II: Levene's Test of Equality of Error Variances *O.mossambicus and L.rohita*

Species	Parameters	Gills		Muscles		Liver		Kidney	
		F	Sig.	F	Sig.	F	Sig.	F	Sig.
O.mossambicus	**SOD**	6.265	.017	6.435	.016	.639	.611	1.084	.410
	CAT	0.768	.543	.418	.745	3.630	.064	.601	.632
	GSH	4.427	.041	.473	.709	6.134	.018	.623	.620
	AA	2.583	.126	.556	.658	2.076	.182	.779	.538
	LPO	10.929	.470	13.849	.002	.354	.788	.498	.694
	ALP	1.488	.290	.982	.448	.143	.931	1.115	.399
	ALT	0.645	.608	1.564	.272	1.280	.345	2.928	.100
	AST	1.588	.267	.765	.545	2.349	.149	.885	.489
L.rohita	**SOD**	2.281	.156	2.281	.156	3.108	.089	2.281	.156
	CAT	1.544	.277	1.544	.277	3.108	.089	1.544	.277
	GSH	2.281	.156	2.281	.156	1.461	.296	2.281	.156
	AA	2.281	.156	1.461	.296	2.281	.156	2.281	.156
	LPO	1.461	.296	1.461	.296	1.461	.296	2.281	.156
	ALP	3.108	.089	3.108	.089	2.281	.156	3.108	.089
	ALT	1.461	.296	1.461	.296	2.281	.156	2.333	.150
	AST	1.461	.296	1.461	.296	2.281	.156	1.461	.296

Table IIIa: Post Hoc tests (Dunnetts' t test) showing comparison between the exposed groups and control in tissues of *O.mossambicus*

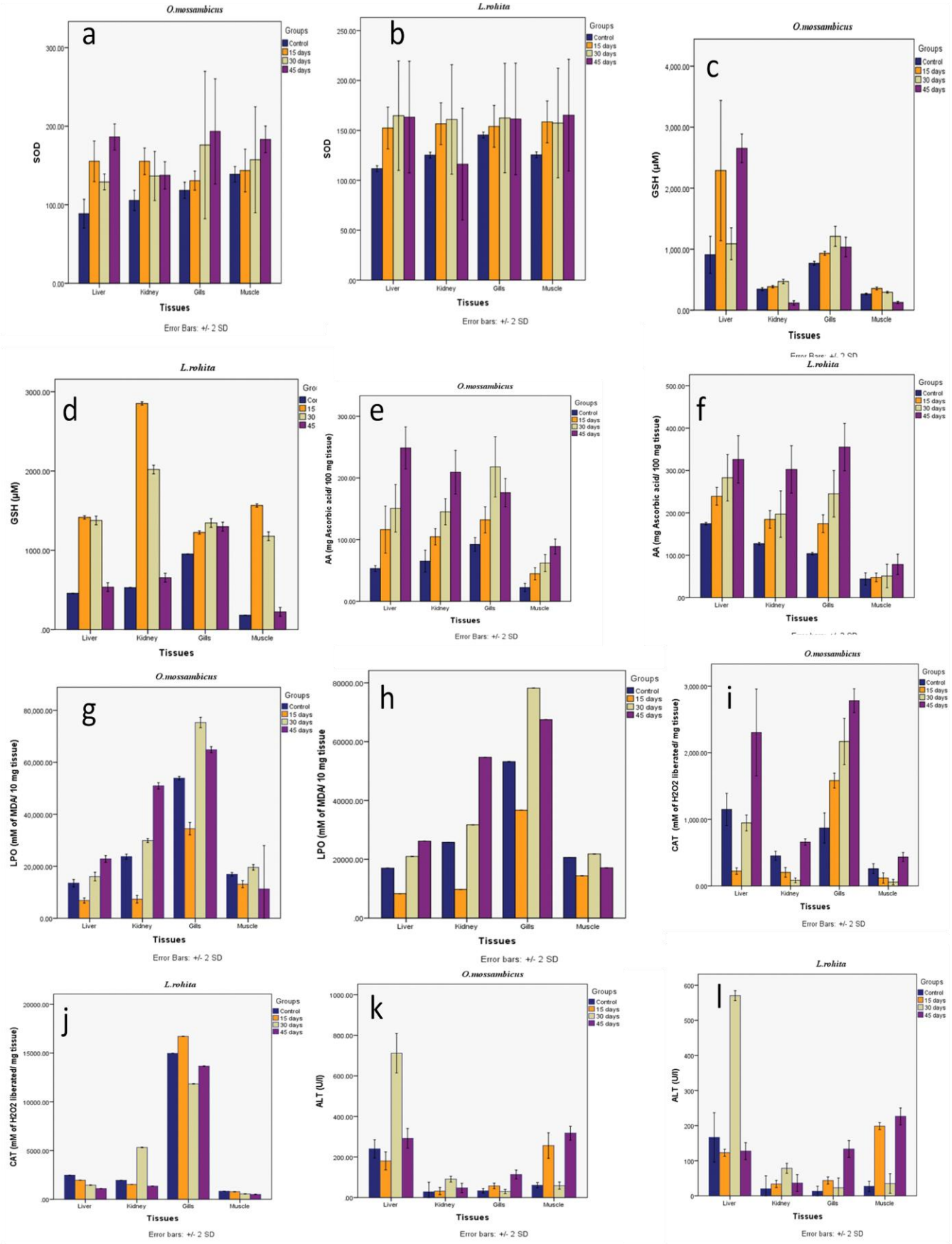
Multiple Comparisons		SOD	CAT	GSH	AA	LPO	ALP	ALT	AST
		MD ± SE	MD ± SE	MD ± SE	MD ± SE	MD ± SE	MD ± SE	MD ± SE	MD ± SE
GILLS	15d vs C	12.29 ±23.73	713.0* ± 95.03	161.84* ± 26.57	39.84* ± 16.57	-19422.6* ± 362.33	-34.50 ± 19.78	24.0* ± 6.13	178.0* ± 23.31
	30d vs C	57.32 ± 23.73	1300.00* ± 95.03	441.57* ± 46.57	125.83* ± 16.57	21414.3*± 216.57	394.00* ± 15.60	-3.33 ± 2.31	-54.66 ± 8.31
	45d vs C	75.00 ± 23.73*	1912.87* ± 96.33	265.20* ± 47.57	83.92* ± 16.57	10951.3* ± 16.57	326.00* ± 15.60	80.33* ± 8.31	192.6* ± 31.55
MUSCLE	15d vs C	4.76 ± 16.57	-139.9* ± 8.28	91.26* ± 16.57	22.27* ± 8.31	-3768.6 ± 8.31	17.00± ± 15.60	195.66* ± 8.31	-88.66* ± 8.31
	30d vs C	18.57 ± 16.57	201.79* ± 8.28	29.10* ± 16.57	39.63* ± 8.31	2695.33 ± 8.31	53.66* ± 8.31	-1.66 ± 8.31	-159.33* ± 8.31
	45d vs C	44.42 ± 16.57	175.40 ± 8.28	-137.90* ± 16.57	66.42* ± 8.31	-5637.66 ± 8.31	77.00* ± 8.31	257.00* ± 8.31	117.66* ± 8.31
LIVER	15d vs C	4.76 ±16.57	-139.95* ± 8.31	-91.26* ±16.57	22.27* ±16.57	-3768 ±16.57	17.00 ±15.60	195.66 ±15.60	-88.66 * ± 8.31
	30d vs C	18.57 ±16.57	-201.79* ± 8.31	29.10* ±16.57	39.63* ±16.57	2695 ±16.57	53.66 ±15.60	-1.66 ±15.60	-159.33* ± 8.31
	45d vs C	44.42* ±16.57	-201.79* ± 8.31	-137.90* ±16.57	66.42* ±16.57	-5637 ±16.57	77.00 ±15.60	257.00 ±15.60	117.66* ± 8.31
KIDNEY	15d vs C	49.76 *± 16.57	-248.47 ± 8.29	40.62* ± 16.57	39.45* ± 16.57	-16361.6* ± 16.57	188.00 ± 15.60	4.00 ± 9.68	-218* ± 8.31
	30d vs C	31.05* ± 16.57	-367.97* ± 8.29	126.57* ± 16.57	80.00* ± 16.57	6199.33* ± 16.57	112.00 ± 15.60	63.00** ± 9.68	-525*** ± 8.31
	45d vs C	31.96* ± 16.57	205.99* ± 8.29	-226.23* ± 16.57	144.20* ± 16.57	27227.66* ± 16.57	607.33*± 15.60	19.33 ± 9.68	-564.66 ± 8.31

MD ± SE – Mean difference ± Standard error
The mean difference in significant at the .05 level.
**** indicates mean difference is significant at 0.05 level***

Table IIIb: Post Hoc tests (Dunnetts' t test) showing comparison between the exposed groups and control in tissues of *L.rohita*

Multiple Comparisons		SOD	CAT	GSH	AA	LPO	ALP	ALT	AST
		MD ± SE	MD ± SE	MD ± SE	MD ± SE	MD ± SE	MD ± SE	MD ± SE	MD ± SE
GILLS	15d vs C	8.62 ±16.57	1738.47* ±8.28	271.84* ± 16.57	70.74 * ± 16.57	-16500.3* ± 8.31	-119.6*** ± 15.6	30.66* ± 8.31	198.66* ± 8.31
	30d vs C	16.96 ± 16.57	3138-.89* ± 8.28	391.57* ± 16.57	141.32* ± 16.57	25000.0 * ± 16.57	394.00* ± 15.60	10.00 ± 8.31	-91.00* ± 8.31
	45d vs C	16.00 ± 16.57	-1315.2* ± 8.28	345.20* ± 16.57	251.49* ± 16.57	14300 ± 16.57	326.00* ± 15.60	120.33* ± 8.31	83.33* ± 8.31
MUSCLE	15d vs C	33.100 ± 16.57	-52.68 *± 8.28	1385.13* ±16.57	3.66 ± 8.31	-6240.33* ± 8.31	18.33 ± 15.60	171.66 *± 8.31	-74.33* ± 8.31
	30d vs C	31.91 ± 16.57	-271.66* ± 8.28	996.43** ± 16.57	7.000 ± 8.31	1160.00 * ± 8.31	53.00* ± 8.31	8.000 ± 8.31	-199.0* ± 8.31
	45d vs C	39.76 ± 16.57	-327.71* ± 8.28	43.15 ± 16.57	34.33** ± 8.31	-3539.66* ± 8.31	33.00 ± 8.31	199.33* ± 8.31	86.33* ± 8.31
LIVER	15d vs C	40.72 ±16.57	-514.33* ± .31	957.95 * ±16.57	64.63* ±16.57	-870.00 *±16.57	-56.3* ±15.60	-43.66 ±15.60	101.66* ± 8.31
	30d vs C	53.10* ±16.57	-1021.00 *± 8.31	919.11 *±16.57	108.51* ±16.57	3965.00 *±16.57	308.66* ±15.60	404.00* ±15.6	243.00* ± 8.31
	45d vs C	51.67 ±16.57	-1373.66 ***± 8.31	78.26 **±16.57	151.74** ±16.57	9200.0 *±16.57	-59.33* ±15.60	-39.00 ±15.60	174.33* ± 8.31
KIDNEY	15d vs C	31.43 ± 16.57	-421.40* ± 8.29	2321.50* ± 16.57	57.14* ± 16.57	-16060.00 ± 16.57	261.00*** ± 15.60	13.66 ± 9.68	-147.3* ± 8.31
	30d vs C	35.72 ± 16.57	3371.77* ± 8.29	1488.70* ± 16.57	69.72** ± 16.57	5925.00* ± 16.57	70.00* ± 15.60	58.33* ±9.6	-62.0* ± 8.3
	45d vs C	-9.04 ± 16.57	-593.19* ± 8.29	124.59* ± 16.57	175.18* ± 16.57	28900.0*± 16.57	644.00* ± 15.60	16.33 ± 9.68	-37.66* ± 8.31

MD ± SE – Mean difference ± Standard error
The mean difference in significant at the .05 level.
**** indicates mean difference is significant at 0.05 level***


O.mossambicus
a
SOD
Groups
Control
15 days
30 days
45 days
Liver
Kidney
Gills
Muscle
Tissues
Error Bars: +/- 2 SD
L.rohita
b
SOD
Tissues
Error bars: +/- 2 SD
O.mossambicus
c
GSH (µM)
Tissues
L.rohita
d
GSH (µM)
Tissues
O.mossambicus
e
AA (mg Ascorbic acid/ 100 mg tissue)
Tissues
Error Bars: +/- 2 SD
L.rohita
f
AA (mg Ascorbic acid/ 100 mg tissue)
Tissues
O.mossambicus
g
LPO (mM of MDA/ 10 mg tissue)
Tissues
Error Bars: +/- 2 SD
h
LPO (mM of MDA/ 10 mg tissue)
Tissues
Error bars: +/- 2 SD
O.mossambicus
i
CAT (mM of H2O2 liberated/ mg tissue)
Tissues
Error Bars: +/- 2 SD
L.rohita
j
CAT (mM of H2O2 liberated/ mg tissue)
Tissues
Error bars: +/- 2 SD
O.mossambicus
k
ALT (U/l)
Tissues
Error bars: +/- 2 SD
L.rohita
l
ALT (U/l)
Tissues
Error bars: +/- 2 SD

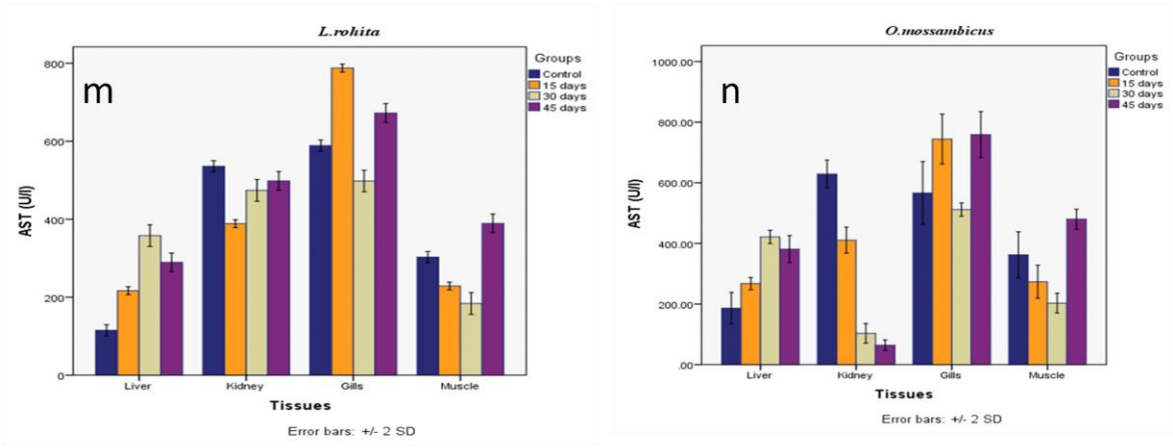


Fig I:Biochemical Alteration in *O mossambicus and L. rohita in different tissues Exposed to Plant nutrient Mixture a)* Activity of superoxide dismutase (SOD*)* in *O. mossambicus.b)* Activity of superoxide dismutase (SOD*)* in *L.rohita. c) Alterations in the Reduced Glutathione (GSH) O. mossambicus. d) Alterations in the Reduced Glutathione (GSH) L.rohita. e) Alterations in the Ascorbic Acid (AA) Oreochromis mossambicus.f)* Alterations in the Ascorbic Acid (AA*) L.rohita. g)* Alterations in LPO *O.mossambicus. h) Alterations in LPO L.rohita. i) Alterations in the Catalase (CAT) in O. mossambicus. j) Alterations in the Catalase (CAT) in L.rohita.k)* Alterations in Alkaline Phosphatase (ALP) in *O. mossambicus. l)* Alterations in Alkaline phosphatase (ALP) in *L.rohita. m)* Alterations in Alanine transaminase (AST) in *O.mossambicus* . n) Alterations in Alanine transaminase (AST) in *L.rohita*

Discussion

In the present study the alterations in the ROS parameters, LPO, transaminases and phosphatases were evident. The oxidative stress is known to be caused due to an increase in ROS, or impairment of antioxidant defence systems, or a default to repair oxidative damage (Buege and Aust, 1978; Ahmad *et al.*, 2000) threatening the integrity of cellular macromolecules such as membrane lipids, proteins and DNA. An increase in the ROS such as superoxide anion radical (O^{2-}), hydroxyl radical (OH^{-}), and hydrogen peroxide ($H_2O_2^{-}$) are known to be removed by the enzymes SOD, GSH and CAT respectively (Cao, *et al.*, 2010). The activities of the enzymes usually increases as an adaptive response to free radical overload during moderate oxidative stress by means of an increased synthesis. However, a severe oxidative stress suppresses glutathione levels due to the mutilation of adaptive mechanism (Sevcikova *et al.*, 2011; Zhang *et al.*, 2004). GSH depletion may reduce the cellular ability to scavenge free radicals which affects the general oxidative potential of the tissue. Our results are in agreement with the earlier reported work(Sevcikova *et al.*, 2011) where an increase in GSH and SOD was clearly evident on day 15^{th} and 30^{th} in all the tissues and decrease on day 45^{th}. The increase in GSH and SOD in the study suggests their role in combating the build up in the ROS content. At the same time the decrease in the CAT activity is probably due to the cell damage and its inability to counter effect the agrochemical toxicity. Charge of AA is well established as a scavenger of free radical and is regarded as are forceful antioxidant. To compensate this, the body's non-enzymatic protective mechanisms is taken over by AA which has a well defined role as a scavenger whose inherent trait of performing redox reactions contributes in filling of an electron in the outer shell of R and thereby neutralising it to a nonreactive species. AA content in the present study was found to be increased significantly ($p<0.001$) compared to control in liver and kidney. As AA has a central position in curing the impaired condition occurred during the agrochemical exposure, the increased AA content thereby is a self explanatory mechanism adopted by the teleost fish. Furthermore, as stated by Mahajan and Zambare (2001) Sometimes vitamin C and vitamin E acts in combination for detoxification, hence, increased AA content is probably performing the detoxicification task.

The direct consequence of failure of antioxidant system is the accumulation of ROS in the system leading to the higher rate of formation of lipid peroxides. The significant increase in the levels of LPO at 30^{th} and 45^{th} day suggests malfunctioning of tissue. In addition, LPO is considered an important indicator of oxidative damage of cellular components due to excess generation of ROS which can lead to several biological effects ranging from alterations in signal transduction to gene expression and apoptosis and oxidative stress development (Kannak and Jain, 2000; Babusyte *et al.*, 2009; Cayir *et al.*, 2009). Increase on LPO is always known to be parallel with SOD increase, which

is again due to an enhanced production of superoxide anion radical. These alterations in the antioxidant enzymes and scavengers clearly depicts that oxidative stress was obvious in the exposed fishes which in turn damaged lipids which is proved by the alterations in the LPO levels. Our results are in agreement with the earlier reported work of Ahmad *et al.,* 2000; Favari *et al.,* 2002; Sayeed *et al.,* 2003; Abdollahi*et al.,* 2004;Roberts and Oris, 2004;Bagnyukova *et al.*, 2006;Wang *et al.*, 2006;Abbas and Ali, 2007;Farombi *et al.,* 2007;Gabriel *et al.,* 2007;Xing *et al.,* 2012)

Transaminases play an important role at the junction between the carbohydrate and protein metabolism by interconverting the strategic compounds viz; ketoglutarate, pyruvate and oxaloacetate on one hand and alanine, aspartate and glutamate on the other hand. Alterations in transaminases has been reported exhibiting an important role in carbohydrate and amino acid metabolism in various tissues of fish [Lushchak, *et al.,* 2001;Jomova and Marian, 2011; Dhanapakiam *et al.*,2006]. The increased transaminase activity in the gills, muscles and kidney might be due to increase in transamination reaction i.e. transferring of NH_2 group from amino acid to a ketoacid. Documented evidences showed that transamination and transdeamination reactions are prominent under stress condition (Dhanapakiam *et al.,* 2006). In most of the cases, it has been observed that different enzymes behave differently and even the same enzyme behave in different ways in different species (Kalele and Dhande, 2005; Devlin, 2006; Gupta and Kumar, 2006;Kumar *et al.,* 2011;Salahuddin and Khola, 2013). The differential expression of ALP and AST in the tissues as well as the species reported in the present study thus is supported by the work of these scientists.

Alkaline phosphatase is an ubiquitous transport enzyme present in almost all tissue of an organism especially in cell membrane. It catalyses the hydrolysis of monophosphate esters and also has wide substrate specificity. Firat *et al.*, (2011) have reported that ALP may increase due to the cellular damage in the liver and that high levels of these enzymes usually in an indicative of necrosis in the liver of animals. Time dependent increase in the ALP activity is in agreement with the earlier reported work of Das and Mukherjee 2003 in *Labeo rohita*, Jee *et al.,* (2005) in Korean rockfish (*Sebastes schlegeli*), Borges *et al.,* (2007) in fish Bagre(*Rhamdia quelen*) and El-Sayed and Saad, (2008) in Nile tilapia(*Oreochromis niloticus*).

Conclusion:

Thus from the present studies it can be concluded that the plant nutrient exposure has led to the alterations in the antioxidants, lipid peroxidation, transaminases and phosphatase, and that the alterations in the parameters in the target tissue (i.e., liver, gill, kidney and muscles) is due to the damage leading to dysfunction. The results of the present studies also indicate that the activities of certain biomarkers in *O.mossambicus*are are more sensitive to the plant nutrient than those in *L. rohita* suggesting the differences in the defense capacity of the teleost fish and that the uptake and elimination pathways differ substantially among tissues.

Acknowledgement:

The authors are highly thankful to UGC Major research Project for financial support of the study.

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