Vol 5, Issue 2, 2018 ISSN 2349-7041

Research Article

EFFECT OF AN INSECTICIDE CHLORANTRANILIPROLE ON BIOCHEMICAL CHARACTERISTICS OF MUDFISH, *CHANNA PUNCTATUS*

BANTU NAGARAJU[1*], K RAVI BABU[2], VAKITA VENKATA RATHNAMMA[2]

[1]Department of Chemistry, College of Natural and computational sciences, Aksum University, Ethiopia, Eastern Africa,2Department of Zoology, University College of Sciences, Acharya Nagarjuna University, Nagarjuna nagar-522510, India

Email: nagaraju.bantu301@gmail.com

ABSTRACT

Objective: The aims of the present study were to determine, the effects of chlorantraniliprole on biochemical characteristics of the mudfish, *Channa punctatus*. **Material and methods:** The freshwater Mudfish, *Channa punctatus,* size 12±13 cm and weight 18±20 g were used an experimental animal.The 96hr LC50 (14.424mg/L-1) and 1/10th of 96 hr LC50 (1.4424mg/L-1) were selected as lethal and sub lethal concentrations to study the behavioral responses and physiological alterations in the experimental animal.The soluble, the total proteins, free amino acids and lipid content in the organs were estimated by using the Standard methods. **Results and discussion:** Total proteins, and soluble protein, free amino acid, and lipid levels were exhausted in all the vital organs exposed to the lethal concentration of Chlorantraniliprole representative the breakdown of these proteins due to the severe pesticidal stress. Usually, the breakdown of proteins dominates over synthesis under enhanced proteolytic activity.Under the Chlorantraniliprole exposure, liver protein subunits showed more decreased intensity in banding pattern compared to the control sample. The result shows declined levels of biochemical parameters during all the exposure periods when compared with control. **Conclusion:** The results of the current study obviously show the toxic nature of the toxicant on the biochemical constituents of the fish, *Channa punctatus*. The changes in total, soluble proteins, free amino acids and lipid in the chlorantraniliprole treated fish.

Key words: contaminants,acclimatization,proteins,declined, proteolytic

INTRODUCTION

Pesticides were found to adversely affect a number of biological functions, thus causing harm to the non-target organisms and those compounds are known for this persistence in the environment and accumulation in the tissues for long periods for controlling the loss of produce due to pest attack and as a consequence of the demand for producing more food, there has been an increasing use of pesticides in developed countries [1]. Proteins are the most versatile biomolecules in living organisms, proteins are the main enzymes in a cell and regulate metabolism by selectively accelerating chemical reactions. They function as bio-catalysts, they conveyance and supply supplementary molecule oxygen, provide mechanical provision and defense mechanism against foreign substances, intercellular signaling and they transfer nerve impulses [2]. They are the abundant macromolecules in a biological organization and are the byproducts of high molecular weight polypeptides. They not only assist as a fuel to produce energy but also play an energetic role in the structural and functional physiognomies of the living organism. Functionally, proteins show a prodigious diversity, establish a heterogeneous group, having diverse physiological roles and are involved in major physiological activities [3]. Therefore, the valuation of the protein content can be measured as an analytical tool to determine the physiological levels of animals [4 and 5]. The concentration of proteins in tissue is a balance between the rate of their synthesis and degradation or catabolism [6] ; the overall protein turnover in an animal is the dynamic equilibrium between these two[7] .Hydrolysis of proteins is a quite common phenomenon wherein proteases split proteins stepwise into amino acids. Amino acids formed by protein degradation will also be utilized for energy production. Amino acids are vital intermediates in protein synthesis and degradation products appear in the form of different nitrogenous substances [8].[9]Reported that variations in lipids, phospholipids content in the *Heteropneustes fossilis* treated with endosulfan. The present investigation was intended to study the effect of chlorantraniliprole on soluble, and total proteins, free amino acids, and total lipid content of fish, *Channa punctatus* .

MATERIAL AND METHODS

Procurement and maintenance of fish

The freshwater Mudfish, *Channa punctatus* size 12±13 cm and weight 18±20 g were brought from a local freshwater body located at Kuchipudi, Guntur district of Andhra Pradesh, India. The fish were fed daily with commercial fish pellets and acclimatized to the laboratory conditions at 28±2^{o}C for 15 days. During the acclimatization period daily fed with fish meal. If in any batch, mortality exceeds 5% during acclimatization, that entire batch of fish was discarded. The water used for acclimatization and conducting experiments was clear unchlorinated ground water.The physical and chemical analyses of the water were carried out according to [10]. The containers of the test media are of 15-liter capacity, where in each test five containers were used, and each container consisted of ten fish. All the precautions were laid by [10] were followed. Hence, in the present investigation, 96 hr LC50 (14.424mg/L-1) and 1/10th of 96 hr LC50 (1.4424mg/L-1) were selected as lethal and sub lethal concentrations to study the behavioral responses and physiological alterations in the experimental animal.

Physico-chemical analysis of water

Turbidity–8 Silica units, Electrical conductivity at 28oC -816 micro ohms/cm,Alkalinity-1, Phenolphthalein-Nil,Methyl orange-472, Total hardness (as CaCO3) - 232, Non carbonate hardness (as CaCO3)-Nil, Calcium Hardness (As N) -Nil, Sulphate (as SO4) -Trace, Chloride (as Cl)- 40, Fluoride (as Fl)- 1.8, Iron (as Fe) -Nil , Dissolved oxygen- 8-10 ppm, Temperature- 28±2oC. All the precautions laid by the committee on toxicity tests to aquatic organisms [10] were followed.

Estimation of soluble, total proteins, free amino acids, and lipid content

The soluble and the total proteins in the organs were estimated using the folin-phenol reagent method as described by [11]. Free

amino acid levels in the tissues were estimated by the ninhydrin method as described by [12]. Lipids were extracted as described by [13], and estimated by the method of [14].

Protein electrophoresis

A change in protein fractions was done using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) according to [15] method.

Statistical analysis

Student's t-test was employed to calculate the significance of the differences between control and experimental means. P values of0.05 or less was considered statistically significant.

RESULTS AND DISCUSSION

The data is presented on the levels of soluble, total proteins, free amino acids and lipids in the organs of the fish *Channa punctatus* on exposure to lethal 24 hr and 1, 10, 20 and 30 days of sublethal concentrations of Chlorantraniliprole. All results are presented in the tables from 1-4 and figure 1-4, a substantial decrease comparative to controls is seen in the soluble, and total proteins, amino acids, and lipids of all the vital organs of fish, *Channa punctatus* at all the exposure periods in the lethal and sub lethal concentrations of Chlorantraniliprole. These protein levels also recorded a significant decrease in the organs of fish on day 1 and 10 on exposure to sub lethal concentration but on further exposure gradual reduction in the increase was observed at 20 and 30 day (Tables 1-2 and figure 1&2). During the exposure periods, the levels of soluble, total proteins, amino acids and lipids significantly decreased in the kidney, muscle and liver compared to control fish. The lowest decrease was observed in kidney (12.49%) at 24 h and maximum (121.78%) in the liver at 96 h on exposure to the lethal concentrations. The similar observation was not the circumstance at sub lethal concentration, among the tissues of fish, the reduction in protein content was greater in liver than kidney and muscle exposed to the lethal and sublethal concentrations of Chlorantraniliprole. The data presented in table 4, corresponding to the reduction in protein content a rapid increase in free amino acid levels in all the organs of fish at all the exposure periods in the lethal concentration of Chlorantraniliprole was observed. Also in under sublethal concentration, however free amino acid levels were decreased, it is mainly more in the tissues of the fish exposed to lethal than the sub lethal concentration.

The relative mobility's of the protein fractions of the freshwater fish, *Channa punctuates* exposed to Chlorantraniliprole in sublethal concentrations for 30days are given in the Table 5and Figure 5. The electrophoretogram (Fig.5) represents the decrease in the intensity of liver protein subunits compared to control. Under the Chlorantraniliprole exposure, liver protein subunits showed more decreased intensity in banding pattern compared to the control sample. The Rm value of protein subunit 0.66 nearer to 87 daltons (Kda) was absent in 10 days exposure and Rm value of protein subunit 0.92 in between molecular weight of 21 daltons and 43 daltons was absent in both 20 and 30 days treated fish tissue samples when compared to control.

Table 1: Total protein content (mg/g wet wt) in the organs of fish, *Channa punctatus* on exposure to the lethal and sub lethal concentrations of Chlorantraniliprole

Organs	Control	Exposure periods							
		Lethal (h)				Sublethal(days)			
		48	72	96	1	10	20	30	
Kidney	131.78	115.32^{b}	99.76^{a}	85.72^{d}	61.44^{a}	102.51^{c}	97.26^{a}	95.79^{d}	108.42^{d}
SD±	0.22	0.29	0.35	0.44	0.22	0.51	0.59	0.44	0.32
%Change	---	12.49	17.04	34.95	53.37	22.21	26.19	27.31	17.72
Muscle	146.87	121.39^{d}	113.45^{d}	98.77^{b}	82.36^{d}	119.43^{a}	114.89^{d}	128.22^{b}	131.92^{c}
SD±	0.51	0.32	0.39	0.44	0.33	0.22	0.52	0.59	0.29
% Change	---	17.34	22.75	32.75	43.92	18.68	21.77	12.69	10.17
Liver	193.31	166.29^{c}	153.92^{a}	130.75^{d}	121.78^{c}	173.52^{d}	120.65^{c}	179.32^{a}	186.29^{b}
SD±	0.01	0.22	0.32	0.35	0.51	0.52	0.44	0.22	0.29
% Change	---	13.97	25.39	32.36	56.11	10.23	0.3758	0.07.23	0.3.63

Means are SD± (n=5)fora parameter in a row, different letters indicate significant differences between the values of control and pesticide Chlorantraniliprole exposed groups are based on 24, 48, 72, 96 h &1, 10,20 and 30 days exposure. a) $p \leq 0.02$ denotes significant when compared with control values, b) $p \leq 0.05$ denotes significant when compared with control values, c) $p \leq 0.005$ denotes significant when compared with control values, d) $p \leq 0.01$ denotes significant when compared with control values.

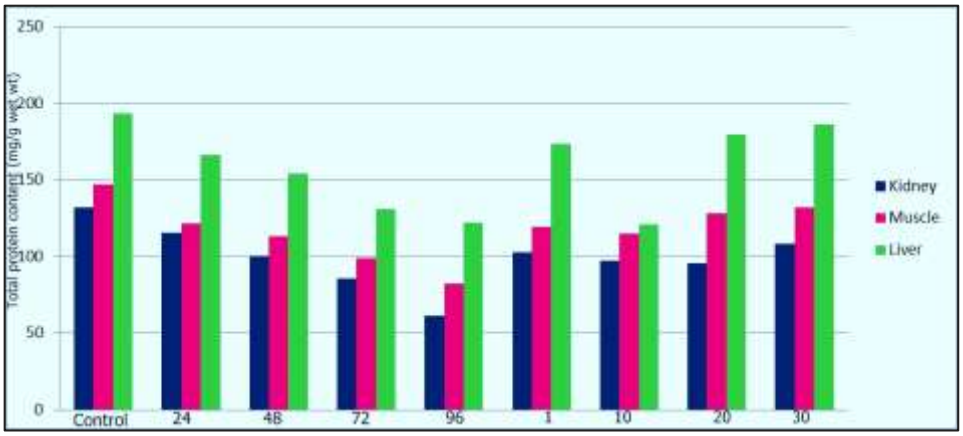

Fig. 1: Total protein content (mg/g wet wt) in the organs of fish, *Channa punctatus* on exposure to the lethal and sublethal concentrations of Chlorantraniliprole for 10, 20, and 30days

Innoriginal International Journal of Sciences | Volume 5 | Issue 2 | Mar-Apr 2018 | 10-15

Table 2: Soluble protein content (mg/g wet wt) in the organs of fish, *Channa punctatus* on exposure to the lethal and sublethal concentrations of Chlorantraniliprole

Organs	Control	Exposure period in days							
		Lethal (h)				Sublethal(days)			
		24	48	72	96	1	10	20	30
Kidney	61.5b	54.76^{d}	51.93^{a}	46.25^{a}	42.88^{a}	57.33^{b}	49.72^{b}	45.25^{c}	53.88^{b}
SD±	0.05	0.01	0.12	0.05	0.12	0.16	0.3	0.29	0.01
% Change	---	-11.08	-15.68	-24.9	-30.37	-6.91	-19.27	-26.53	12.51^{a}
Muscle	84.65	79.20^{a}	74.11^{d}	72.99^{d}	68.29^{d}	82.90^{d}	77.29^{d}	73.84^{a}	79.99
SD±	0.29	0.61	0.29	0.45	0.31	0.41	0.2	0.45	0.21
% Change	---	-6.43	-12.45	-13.77	-19.32	-2.09	-8.69.	-12.77	-5.5
Liver	108.5a	92.29^{d}	87.66^{c}	82.21^{b}	61.77^{c}	95.99^{c}	82.85^{a}	95.04^{b}	103.22^{d}
SD±	0.05	0.05	0.01	0.41	0.29	0.45	0.29	0.41	0.29
% Change	---	-14.97	-19.23	-23.75	-43.09	-11.56	-23.66	-12.43	-4.9

Means are SD± (n=5)fora parameter in a row; different letters indicate significant differences between the values of control and pesticide Chlorantraniliprole exposed groups are based on 24, 48, 72, 96 h & 1, 10,20 and 30 days exposure. a) $p \leq 0.02$ denotes significant when compared with control values, b) $p \leq 0.05$ denotes significant when compared with control values, c) $p \leq 0.005$ denotes significant when compared with control values, d) $p \leq 0.01$ denotes significant when compared with control values.

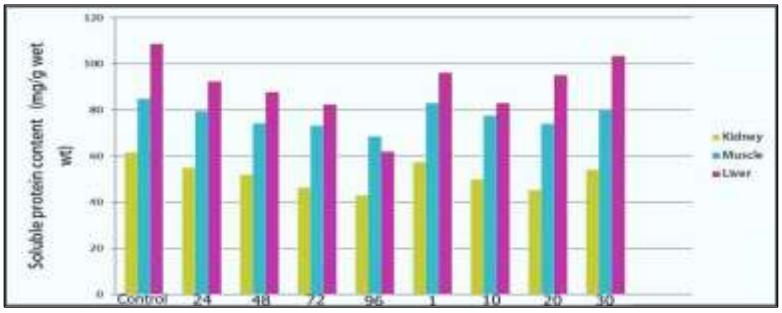

Fig. 2: Soluble protein content (mg/g wet wt) in the organs of fish, *Channa punctatus* on exposure to the lethal and sublethal concentrations of Chlorantraniliprole for 10, 20, and 30days

Table 3: Lipid content (mg/g wet wt) in the organs of fish, *Channa punctatus* on exposure to the lethal and sublethal concentrations of Chlorantraniliprole

Organs	Control	Exposure periods							
		Lethal (h)				Sublethal(days)			
		24	48	72	96	1	10	20	30
Kidney	45.44	57.99^{a}	52.66^{d}	39.33^{b}	25.96^{d}	55.65^{a}	51.99^{c}	57.54^{d}	51.20^{a}
SD±	0.92	0.51	0.52	0.41	0.29	0.27	0.45	0.05	0.29
% Change	---	-27.61	-15.88	-13.44	-42.86	-22.46	-14.41	-26.64	-12.76
Muscle	34.32	29.57^{d}	27.65^{a}	25.22^{c}	21.33^{b}	28.43^{d}	29.98^{a}	21.76^{a}	19.66^{d}
SD±	1.06	0.44	0.39	0.31	0.25	0.46	0.42	0.49	0.51
% Change	---	-13.84	-19.43	-26.51	-37.84	-17.16	-12.64	-36.59	-42.71
Liver	27.54	21.96^{a}	19.72^{b}	18.99^{a}	16.44^{d}	24.75^{b}	21.97^{c}	15.65^{c}	12.78^{c}
SD ±	1.45	0.51	0.47	0.41	0.32	0.29	0.11	0.51	0.29
% Change	---	-10.43	-28.39	-31.04	-40.31	-10.14	-20.22	-43.17	-53.59

Means are SD± (n=5)fora parameter in a row, different letters indicate significant differences between the values of control and pesticide Chlorantraniliprole exposed groups are based on 24, 48, 72, 96 h & 1, 10,20 and 30 days exposure. a) $p \leq 0.02$ denotes significant when compared with control values, b) $p \leq 0.05$ denotes significant when compared with control values, c) $p \leq 0.005$ denotes significant when compared with control values, d) $p \leq 0.01$ denotes significant when compared with control values.

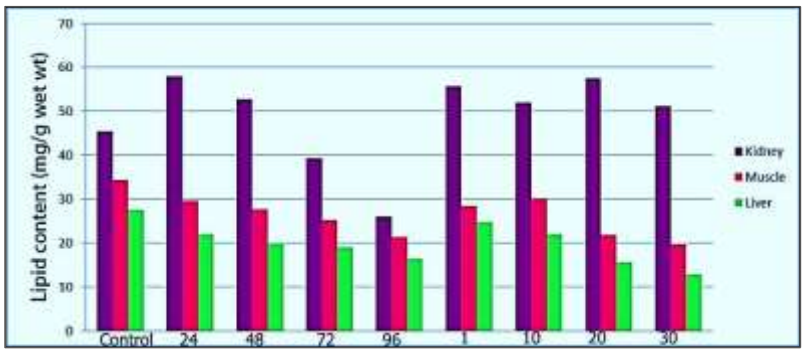

Fig. 3: Lipid content (mg/g wet wt) in the organs of fish, *Channa punctatus* on exposure to the lethal and sub lethal concentrations of Chlorantraniliprole for 10, 20, and 30days

Table4: Free amino acid levels (mg amino acid nitrogen/g wet wt.) in the organs of fish, *Channa punctatus* on exposure to the lethal and sublethal concentrations of Chlorantraniliprole

Organs	Control	Exposure periods						
		Lethal (h)			Sublethal(days)			
		48	72	96	1	10	20	30
Kidney	16.45	21.93[b]	23.38[c]	25.22[a]	28.61[d]	19.20[b]	18.76[a]	16.65[c]	12.24[b]
SD±	0.05	0.01	0.29	0.10	0.11	0.12	0.11	0.08	0.10
%Change	---	33.31	42.12	53.31	73.92	16.71	14.04	12.15[a]	25.59[a]
Muscle	18.45	21.96[d]	24.78[a]	26.21[d]	28.92[d]	20.55[a]	23.62[d]	11.49	9.85
SD±	0.01	0.05	0.11	0.12	0.14	0.12	0.08	0.01	0.05
% Change	---	19.02	34.30	42.05	56.74	11.38	28.02	37.72[b]	46.61[d]
Liver	33.18	29.27[a]	25.75[c]	28.53[a]	22.98[a]	21.34[c]	24.32[c]	27.93	19.28
SD±	0.05	0.10	0.12	0.29	0.12	0.22	0.11	0.01	0.0 5
% Change	---	11.78	22.39	14.01	30.74	35.68	26.70	15.82	41.89

Means are SD± (n=5)for a parameter in a row; different letters indicate significant differences between the values of control and pesticide Chlorantraniliprole exposed groups are based on 24, 48, 72, 96 h & 1, 10,20 and 30 days exposure. a) $p \leq 0.02$ denotes significant when compared with control values, b) $p \leq 0.05$ denotes significant when compared with control values, c) $p \leq 0.005$ denotes significant when compared with control values, d) $p \leq 0.01$ denotes significant when compared with control values.

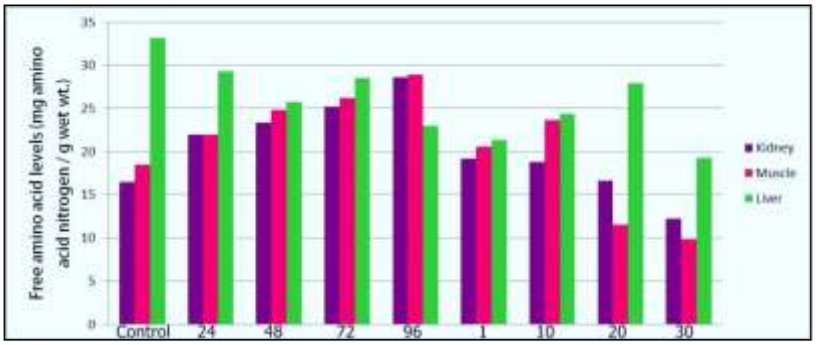

Fig. 4: Free amino acid levels (mg amino acid nitrogen / g wet wt.) in the organs of fish, *Channa punctatus* on exposure to the lethal and sublethal concentrations of Chlorantraniliprole for 10, 20, and 30days

Table 5: Relative mobility values for fish , *Channa punctatus* exposure to the lethal and sublethal concentrations of Chlorantraniliprole for 10, 20, and 30 days.

Marker	Lane1 Control	Lane 2 Chlorantraniliprole exposed liver for 10days	Lane 3 Chlorantraniliprole exposed liver for 20days	Lane 4 Chlorantraniliprole exposed liver for 30days
-	0.16±0.01	0.11±0.21	0.10±0.45	0.09±0.05
-	0.18±0.09	0.13±0.5	0.12±0.21	0.15±0.49
0.39	-	0.15±0.03	-	0.17±0.01
-	0.22±0.51	-	0.18±0.29	0.19±0.29
-	0.35±0.40	0.22±0.22	0.24±0.14	0.20±0.22
0.42	-	0.24±0.21	-	-
-	0.42±0.01	0.39±0.41	-	0.25±0.05
0.58	-	-	0.28±0.05	0.22±0.08
-	0.49±0.29	0.41±0.01	0.53±0.29	0.44±0.14
0.64	-	-	-	-
-	0.55±0.14	0.45±0.03	0.69±0.45	0.59±0.01
0.77	-	0.58±0.05	0.78±0.22	0.66±0.55
-	0.66±0.55	0.74±0.59	-	-
0.82	0.72±0.03	0.81±0.14	0.89±0.03	0.79±0.22
-	0.89±0.21	0.83±0.01	0.92±0.25	0.85±0.29
-0.89	-	0.91±0.03	-	-
-0.97	0.91±0.08	0.96±0.29	0.94±0.05	0.91±0.45
-	-	0.94±0.21	0.96±0.49	0.94±0.22

Values are the mean of five observations; Standard deviation in dicatedas (±), Values are significant at $p<0.05$

Innoriginal International Journal of Sciences | Volume 5 | Issue 2 | Mar-Apr 2018 | 10-15

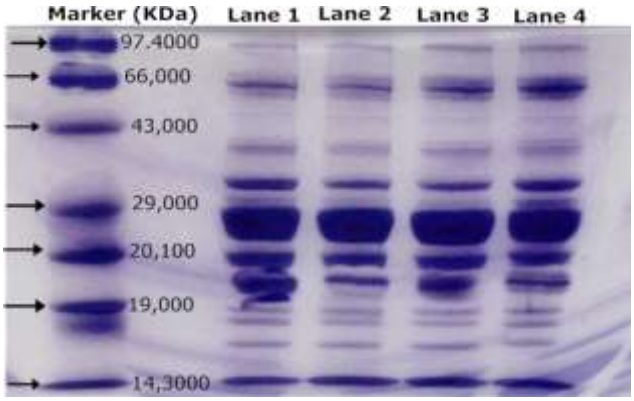

Fig. 5: Changes in protein subunits in Liver tissue of fish, *Channa punctatus* exposure to the lethal and sublethal concentrations of Chlorantraniliprole for 10, 20, and 30 days

In the present study, the toxic effects of chlorantraniliprole on the total protein content, free amino acid levels and lipids content in the tissues of the fish, *Channa punctatus* showed time-dependent alterations. Total proteins and soluble protein, free amino acid, and lipid levels were exhausted in all the vital organs exposed to the lethal concentration of Chlorantraniliprole representative the breakdown of these proteins due to the severe pesticidal stress. Usually, the breakdown of proteins dominates over synthesis under enhanced proteolytic activity [16]. It is evident in the current study that the hypoproteinemia is related with the sudden rise free amino acid levels in the tissues of the fish exposed to the lethal concentrations. The maintenance of proteins in a highly organized state requires an active and incessant supply of energy. Similar observations were recorded with other pesticides in numerous fishes, as reported in Cirrhinus mrigala[17] exposed toRogor. Depletion of proteins in tissues may constitute a physiological mechanism and may play a vital role of compensatory mechanism below pesticidal stress, to distribute intermediates to the Kreb's cycle or to enhance osmolarity, by retaining free amino acid content in hemolymph, to compensate osmoregulatory problems encountered due to the seepage of ions and other indispensable molecules, during the pesticide stress[18,19]. The depletion of protein endorses increased proteolysis and possible utilization of the products of their degradation for metabolic purposes [20]. The reduction of protein level induces to the diversification of energy to meet the imminent energy demands during the toxic stress [21].

The depletion of protein level induces the diversification of energy to meet the impending energy demand during toxic stress [22]. Under proteolysis, enhanced breakdown dominates over synthesis while in the case of the anabolic process; increased synthesis dominates the protein breakdown [16]. This is further corroborated by the increased levels of free amino acids in all the tissues. These amino acids might be fed into the TCA cycle as keto acids by way of transamination since transaminases are known to be elevated during pesticide intoxication [22]. The increased levels of free amino acids might also be due to increased synthetic potentiality. This possibility might exist in the tissues of toxicant exposed fish.

It appears that protein degradation is in active phase over synthesis in the kidney, muscle, and liver of fish at sub lethal concentration of toxicant as evidenced from the decrease in soluble and total proteins with the significant increase in protease activity and amino acid levels. Similar reports were observed in Mus boodoja on exposure to BHC [23]. But the reduced decrease in soluble and total proteins along with the gradual rise in protease activity and free amino acid levels in the kidney, muscle, and liver of fish at day 10 and 15 indicates the onset of the acceleratory phase of protein synthesis over the breakdown. The reduced decrease in total proteins could be helpful to the animal to fortify its organs for developing resistance to the imposed sub lethal toxic stress; further, the reduced magnitude of the decrease in soluble protein fraction could indicate the synthesis of enzymes necessary for detoxification. Protein synthesis being an energetically expensive process, the increase in oxidative metabolism of the fish during sub lethal toxicant stress also strengthens the increase in its protein synthetic potentials. Degradation of proteins by proteolytic enzymes results in the increased amino acid pool. Further, prevalence of pathological conditions in the organ systems of an animal may decrease protein synthetic acid pool. The above two factors could be responsible for the increase in free amino acid levels in the organs of fish exposed to the lethal concentration of toxicant. High concentrations of amino acids in tissues can lead to hyper amino academia which in turn can cause a number of side effects on the physiological conditions of the cell. The increase in the free amino acids in the organs of fish exposed to sublethal concentrations can be partly due to the increased proteolytic activity and partly due to certain transaminases reported to be indicators of protein degradation in salmonoids and liver intoxication in rainbow trout [24].

The slow increase in soluble protein in the fish exposed to the sub lethal stress could also support the elevation in these enzyme activities. The increase could be due to the stepwise induction of these enzymes greater and eater association of their oligomers [25]. The increase in these enzyme activities could be helpful to the fish for structural reorganization of proteins and incorporation of keto acids into the TCA cycle to favor gluconeogenesis or energy production.

Lipids support as energy reserves to meet the metabolic requirement for more energy to mitigate toxic stress. [26]Reported that decreased lipid content in Tilapia mossambica exposed to atrazine. The decreases in lipid contents in kidney, muscle, and liver tissues were found to be increased with the time of exposure. The decline in the lipid levels may be due to the inhibition of cholesterol biosynthesis in the liver or due to reduced absorption of dietary cholesterol as reported by [27].[28] have shown decreasing trend of lipid content in the brain, gill, kidney, liver and muscle tissues upon exposure to lannate in the fish Oreochromismossambicus. Various authors studied the similar reduction of lipids content in different tissues.[29] Observed a reduced lipid content in the liver tissue of fish *Channa punctuatus* exposed to emisan. A decrease in the lipid content of the liver, muscle and kidney tissues exposed to chlorantraniliprole recommends that lipid might have been directed for energy production for other metabolic function in which these products play a vital role during toxicant stress condition.

[30] reported that plasma protein band pattern in fish Nile tilapia by using SDS-PAGE, the number of protein bands declined in fish on exposure to 0.20 ppm, 0.002 ppm, 0.004 ppm, 0.008 ppm and 0.02 ppm concentrations of butataf .The number of plasma protein bands decreased due to the toxicity of butataf, when compared to control. SDS-PAGE was performed for the liver tissue of *Channa punctatus* exposed to chlorantraniliprole, Similar results also reported by [31], in freshwater fish, *Labeo rohita* on exposure to profenofos and carbosulfan. The protein subunits of liver showed a decrease in intensity and some protein subunits were disappeared. Inhibition of proteins may be due to tissue necrosis which leads to losses of intracellular enzymes or other proteins.

CONCLUSION

An influence of chlorantraniliprole and its effect at the cellular, molecular level and ultimately cause physiological and biochemical changes. The results of the current study obviously show the toxic nature of the toxicant on the biochemical parameters of the fish, Channa punctuatus. The changes in total, soluble proteins, free amino acids and lipid in the chlorantraniliprole treated fish will unusually affect the nutritive value of these fishes and all the metabolites studied are found to be sensitive changes in the normal indictors, which reflect changes in the normal activities of various functional systems.

REFERENCES

1. Bonansea R.I, Marino d.J.Wunderlin D.A. AMÉ, M. V. Tissue-specific bioconcentration of cypermethrin, chlorpyrifos and its mixture in a native fish (Jenynsiamultidentata). Environ ToxicolChem, 2016.doi:10.1002/etc.3613
2. Berg, JM. Tymoczko, JL, and Stryer, L. Biochemistry, fifth edition, W. H. Freeman and Company and Sumanas, Inc, 2005.

3. Lehninger principles of biochemistry. Albert L., David L. Nelson, and Michael M. Cox. New York: Worth Publishers, 2013.
4. Kapilamanoj and G. Ragathaman. Mercury, copper and cadium induced changes in total protein level in muscle tissue of an edible estuarine fish, Boleophthalmusussumieri (CUV). J. Environ. Biol., 1999, 20(3): 231-234
5. Ramadan, A. A. Genotoxic Effects of Butataf Herbicide on Nile Tilapia. Journal of the Arabian aquaculture society, 2007, 2 (1), 70-89;
6. Schimke, RT. In neuro sciences (Eds) F.O. Schmitt and F.G. worder, Cambridge, Massachusetts III, 1974, 813-825.
7. Grainde, B. Seglen, PO. Effects of amino acid analogues on protein degradation in rat hepatocytes. Biochemica. Ada. Biophysica. Acta., 1981, 676, 43-50.
8. Nagaraju Bantu, VenkatarathnammaVakita, somaiahkarra.Effect of Chlorantraniliprole on Biochemical and Certain Biomarkers in Various Tissues of Freshwater Fish,Labeorohita(Hamilton).Environment and Ecology Research, 2013, 1(4): 205-215, 2013.
9. Singh, PB and Singh, V. impact of endosulfan on the profiles of phospholipids at sublethal concentration in the male Heteropneustesfossilis Bloch. Journal of Environmental Biology, 2006, 27(3), pp. 509-514.
10. APHA. Standard Methods for the Examination of Water and Wastewater. 21st ed., American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington DC, 2005, USA.
11. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the folin phenol reagent. J BiolChem, 1951, 193: 265-275.
12. Moore, S. Stein, WH. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J BiolChem, 1954, 211: 907-913.
13. Folch, J.M. a simple method for isolation and purification of total lipids from animal tissues. Journal of Biological and Chemistry, 1957, 226, pp. 497- 509.
14. Barnes, H. and Blackstock, Z.J. estimation of lipids in marine animals and tissues. Detailed investigation of the phosphovanilin method for total lipids. Journal of. Experimental Marine Biology and Ecology, 1973, 12, pp.103-118.
15. Laemmli, U.K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 1970, 227, 680–685;
16. Harper, HA. In : Harper's Review of Biochemistry (eds.) D.W.Martin, P.A. Mayes and V.W.Rodwell,, Longe Medical Publications, MaruzenAsia, 1985,Singapore.
17. Pechiammal.K andKiruthika, K. Changes in biochemical parameters of freshwater fish, cirrhinus mrigala exposed to rogor (insecticide). World journal of pharmacy and pharmaceutical sciences, 2006, volume 5, issue 3, 723-728.
18. Rafat, Y. Physiological responses of freshwater fish, Anabas scandens to the toxicity of endosulfan. Ph.D. Thesis, 1986, Osmania University, Hyderabad, India.
19. Rajeshwari, K. Effect of endosulfan toxicity on ion regulation in the freshwater field crab, Oziotelphusasenexsenex (Fabricius). M. Phil. dissertation, S.V. University, Tirupathi, 1996, India.
20. Klassan, CD. Heavy metal and heavy antagonists. In: Gilman AG, Goodman LS and Gilman. Eds. Pharmacological Basis of Therapeutics. London, Baillie, Tindall, 1991, 1625
21. Jha, BS. Verma, BP. Effect of pesticides mixture on protein content in the freshwater fish, Clariasbatrachus. J.Ecotoxicol.Environ.Moint, 2002, 12(3): 177-180.
22. Jagadeesan, G. Mathivanan, A. Organic constituent changes induced by 3 different sub lethal concentrations of mercury and recovery in the liver tissues of Leaeorohita fingerlings. Poll. Res, 1999, 18(1): 177-181.
23. Philip, G.H; Mallareddy, P and Ramamurthi. Changes in protein metabolism in liver and kidney of Mus boodurga Gray after BHC feeding. Bull. Environ. ContamToxicol, 1988,41: 822-827.
24. Gingerich, WH. Weber, LJ. Assessment of clinical procedures to evaluate liver intoxication in fish. Ecological Res. Ser. G.P.A. , 1976, 600 P-118.
25. Kulkarni, B.G and Kulkarni, R.C. Effect of mercury exposure on enzymes in the clam, Ketelysiaopima. Indian. I. Mar. Sci., 1987. 16: 256-266.
26. Srinivas, T., T.A.V. Prasad, G.M.D. Rafi and D.C. Reddy. Effect of atrazine on some aspects of lipid metabolism in freshwater fish. Biol. Inter., 1991, 23: 603-609.
27. Mishra, S.K., J. Padhi AND L. Sahoo. Effect of malathion on lipid content of liver and muscles of Anabas testudineus. J. Appl. Zool. Res., 2004, 15: 81-82.
28. Arockia, J.J. and J.M.C. Mitton. Effect of carbamate pesticide lannate (methomy1) on the biochemical components of the freshwater cichlid Oreochromismossambicus (Peters). Ind. J. Environ Ecoplan, 2006,12: 263-268.
29. Ram, R.H. and A.G. Sathyanesan, . Mercuric chloride induced changes in the protein lipid and cholesterol levels of the liver and ovary of fish, *Channa punctatus* . Environ. Ecol., 1984, 2: 113-117.
30. Ramadan, A. A. Genotoxic Effects of Butataf Herbicide on Nile Tilapia. Journal of the Arabian aquaculture society, 2007, 2 (1), 70-89.
31. Bantu Nagaraju, ZenebeHagos. Lethal and sublethal effects of profenofos and carbosulfan on protein pattern of Indian major carp, Labeorohita (hamilton). Scientific Study & Research – Biology, 2016, 25/2, 77-83.