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Fluctuations of certain Biochemical constituents as a consequence of Chlorantraniliprole toxicity in the Mud Fish, *Channa punctatus* (Bloch, 1974)

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ABSTRACT

The Mudfish, Channa punctatus were exposed to two sublethal (1/10th and 1/20th 96h of LC50) concentrations of insecticide Chlorantraniliprole. The biochemical constituents like proteins, glycogen, free amino acids and total lipids was induced by pesticide stress due to disturbed metabolism, even though, the pesticides have their own target site of action; most of them are metabolic depressors. They affect the activity of biomolecules such as proteins, carbohydrates and lipids proteins are indeed of primary and paramount importance in the living world not only because of their peculiars but also because of the fact that they appear to confer their biological specificity among various type of cells. The results of the present study revealed that there was overall decrease in protein, glycogen, free amino acids, and total lipids in the test samples compared to control.

Keywords: *Insecticide, Gills, Freshwater, and Protein.*

INTRODUCTION

The continuous releases of chemicals impair water quality and become unsuitable for aquatic organisms due to their persistence, bio accumulation, toxicity and biomagnifications in the food chain (Palaniappan and Karthikeyan, 2009).Aquatic ecosystem is the final sink for many chemicals used in industry and agriculture and has become a global problem (Ghosh *et al*, 2006). Although the emerging pollutants introduced into the aquatic environment by discharges from sewage treatment plants, disposal use and so on (Kasprzyk-Hordern *et al*, 2009).

Pesticides have brought tremendous benefits to mankind by increasing food production and controlling the vectors of man and animal diseases. At the same time use of these pollutants has posed potential health hazards to the life of fishes. Long term exposure of organisms to pesticides means a continuous health hazard for the population. So, human population is at high risk by consuming these toxicated fish (Baby Joseph and Justin Raj, 2011**).** Pesticides are major cause of concern for aquatic environment because of their toxicity, persistency and tendency to accumulate in the organisms (Joseph and Raj, 2010).The pesticides are posing a great threat to aquatic fauna especially to fishes, which constitute one of the major sources of protein rich food for mankind (Sharma and Singh, 2006, 2007).The impact of these pesticides on aquatic organisms is due to the movement of pesticides from various diffuse or point sources. Chemical pesticides with persistent molecules pose a threat to fish and also to the human population consuming effected fish. (Mohsen Khalili *et al* ,2012).Fish responds to toxicants by altering their enzyme activities and the inhibition of these enzyme activities has been used to indicate the tissue damage (Webb *et al*, 2005).The effectiveness of biomarkers has been demonstrated in several studies on the toxicity of pesticides to fish(Ramesh *et al;* 2009). The aim of the present study to estimate the biochemical constituents in gill, muscle, liver, and kidney of fish *Channa punctatus* (Bloch) exposed to the insecticide chlorantraniliprole 18.5% SC (Suspension concentrate).

MATERIALS AND METHODS

The Mudfish, *Channa punctatus* (Bloch), size 12-13 cm and weight 18-20 g were brought from a local water bodies at Kuchipudi, Guntur district of Andhra Pradesh, India. The fish, *Channa punctatus* (Bloch) were acclimatized to the laboratory conditions at 28 $\pm$ 2^{o}C for 15 days. If in any batch, mortality exceeds 5% during acclimatization, that entire batch of fish was discarded. Insecticide was purchased from local market in Guntur of Andhra Pradesh. The

water used for acclimatization and conducting experiments was clear unchlorinated ground water; the sample water is clear, colorless and odorless. Chemical analysis of water was analyzed by (APHA, 2005).

The containers of the test media are of 35 L capacity, where in each test five containers were used and each container consisted of 30 fish. The mortality rate was taken into consideration and while taking the data, dead fish was removed immediately. The experiments were conducted to determine the toxicity in different concentrations of the toxicant for 24 h, 48 h, 72h and 96 h in semi static system to calculate the LC 50 values. The 96h LC50 value of chlorantraniliprole (Coragen) on the fish, *Channa punctatus* was found to be 14.424mg/l.

The fish were exposed to two sub lethal concentration ($1/10^{th}$, $1/20^{th}$96 h LC of 1.442mg/l, 0.721mg/l) for a period of 45 days. Water in the test containers renewed daily and was aerated. Nourishing of fishes with fish pellets were done uniformly throughout the experiment. The natural photo period of 13:11 light: day h was maintained. The physicochemical analysis of water used for experiments were shown in (Table 1). At the end of 45 days exposure, the tissues such as liver, muscle, kidney and gill tissues were collected by dissecting the animal and stored at – 20°C, for biochemical studies.

Table1. Physico-chemical analysis of water used for experiments

Temperature	:	28 + 32oC
Turbidity		8 silica units
Electrical conductivity at 28oC	:	816 micro ohms/cm
pH value at 28oC	:	7.20±12°C
Dissolved oxygen		8. 24 ± 0.19 mg/L
Hardness		32.6 ± 2.8 mg as CaCO3/L
Total alkalinity		27.2± 7.1 mg as CaCO3/L
Conductivity		< 12 µS/cm
Specific gravity	:	2.009
Non-carbonate Hardness (as CaCO3)	:	Nil
Total pesticide residue		Not detectable (N.D)
Particular matter	:	< 25 mg/L
Unionised ammonia	:	< 0.72 µg/L
Residual chlorine	:	< 0.62 µg/L
Iron (as fe)	:	Nil

Biochemical studies

The protein content of the sample was determined according to the method of (Lowry *et al*, 1951) using crystalline bovine serum albumin standard. The glycogen by (Kemp *et al*, 1954) for estimation of the glycogen glucose was used as standard. The free amino acids were determined by Ninhydrin method (Moore and Stein, 1954).The total lipids were extracted based on the procedure of (Folch *et al*., 1957).

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test using the SPSS 20.0 v. The significance of difference was set up at (p< 0.05). The values were expressed as mean ± SD.

Results and Discussion

The results of the present study as presented in Table 2 and 3, the protein content of the various tissues like liver, kidney, gills, and muscle decreased by 41%, 37%, 63%, and 50% respectively, at both sub lethal concentrations, as compared with control. In the fish exposed to two sub lethal concentrations of insecticide chlorantraniliprole for period of 45 days, the protein, glycogen, free amino acids and total lipids was significantly decreased when compared to the control. Biochemical variations induced by pesticide stress due to disturbed metabolism, even though, the pesticides have their own target site of action; most of them are metabolic depressors.

They affect the action of biomolecules such as proteins, carbohydrates and lipids proteins are indeed of primary and paramount importance in the living biosphere not only because of their peculiars but also because of the fact that they appear to deliberate their biological specificity among various type of cells. Hence, the protein content of the cell considered to be a vital tool for the evaluation of physical standards. The muscle protein is metabolized to produce glucose by the process of gluconeogenesis and it

is utilized for energy invention under pesticide stress condition (Elumalai *et al*, 1999).The protein content is reduced due to chlorantraniliprole stress may be attributed to the consumption of amino acids in the various catabolic reactions.

Amino acid content in the liver, kidney, gills, muscles howed a continuous decrease as the pesticide concentration was increased (Table 1 and 2). The maximum reduction of amino acid content in kidney, liver, gills, and muscle of exposed fish were 60%, 63%,62%, and 51% respectively at sub lethal concentrations. The initial increase in free amino acids in tissues and later their sudden decline shows that these are utilized in the glycogenesis to compensate the energy demand under chemical stress. Thus, the pesticides intoxication has disturbed the normal functioning of cells with the resultant alterations in the fundamental biochemical mechanisms in fish. This would in turn result in the mortality of fish on long-lasting exposure to the pesticide (Thenmozhi *et al*, 2011).

The glycogen content of liver, gill, kidney, and muscle tissues displayed a decreasing trend as pesticide concentration increased (Table 1and 2). Reduction of glycogen in the matters is symbol of typical stress response in fish confronted with pesticides. A reduction in glycogen levels shows its fast utilization to encounter the improved energy stresses in pesticide treated animals through glycolysis (Swarna kumara *et al*, 2008); Glycogen depletion in liver and muscles after toxic stress has been reported in numerous studies with aquatic animals (Bhavan *et al*,1997),The fish, *C.punctatus* after exposure to two sublethal concentrations of chlorantraniliprole showed overall decrease in the content of glycogen in the fish organs.

The extreme decrease observed in liver, muscle, slight variations were recorded in kidney and gill. Between the biochemical profile's plasma glucose has been extensively used as a parameter to evaluate stress and also used as a subtle indicator of environmental stress in aquatic animals (Kavitha *et al*; 2010).The reduction level of glucose during sub lethal treatments may be due to hypoxic disorder initiated by the toxicant, which reflects an additional utilization of stored carbohydrates throughout the experimental period. These results are agreement with the finding of (Ghosh *et al*.1992).

The lipid content of liver, kidney, gills, and muscle showed decreased levels in the fish *C.punctatus* exposed to chlorantraniliprole for 45 days period at both sub lethal concentrations (Table 2,3, figure 1 and 2). In the low accessibility of carbohydrates, lipids serve as source of energy for supporting physiological roles of the body. Similar findings were observed by Ramand Sathyanesan, 1984) a declined lipid levels in the liver tissue of fish, *Channa punctuates* treated with Mercuric chloride. The deterioration in the lipid content due to be the utilization of lipids for assembly the energy demand under the pesticide stress.

Table 2: Variation in the Biochemical constituents of Liver, Kidney, Gill, and Muscle of *C. Punctatus* for 45 days

Organ	Control	Sub lethal 45 days Exposure ($1/10^{th}$96 hrLC50)	Biochemical constituents expressed in mg/g wet weight of the tissue
Muscle	127.41±0.81	98.32±0.39d (29.09%)	Protein
	23.54±0.05	17.87±0.06b (5.67%)	Glycogen
	9.78±0.08	8.62±0.02c (1.16%)	Free amino acids
	196.84±2.35	182.98±3.0a (13.86%)	Total lipids
Liver	149.56±0.01	46.41±4.10b (103.15%)	Protein
	48.24±0.12	39.59±0.6d (8.65%)	Glycogen
	13.84±0.02	11.16±0.03a (2.68%)	Free amino acids
	87.28±2.29	79.12±.09d (8.16%)	Total lipids
Kidney	132.24±0.03	85.28±0.05a (47.03%)	Protein
	24.55±0.05	21.39±0.01a(3.16%)	Glycogen
	7.88±0.09	6.16±0.01d (1.72%)	Free amino acids
	84.47±5.17	73.10±2.03b (11.37%)	Total lipids
Gills	35.54±0.24	26.22±0.03a (9.32%)	Protein
	5.46±0.02	3.89±0.08a (1.57%)	Glycogen
	10.28±0.02	8.34±0.01c (1.94%)	Free amino acids
	79.57±6.30	67.22±0.16d (1.36%)	Total lipids

The values were as means ± SD (n=6); a $p \leq 0.05$; b $p \leq 0.02$; c $p \leq 0.01$; d $p \leq 0.005$.

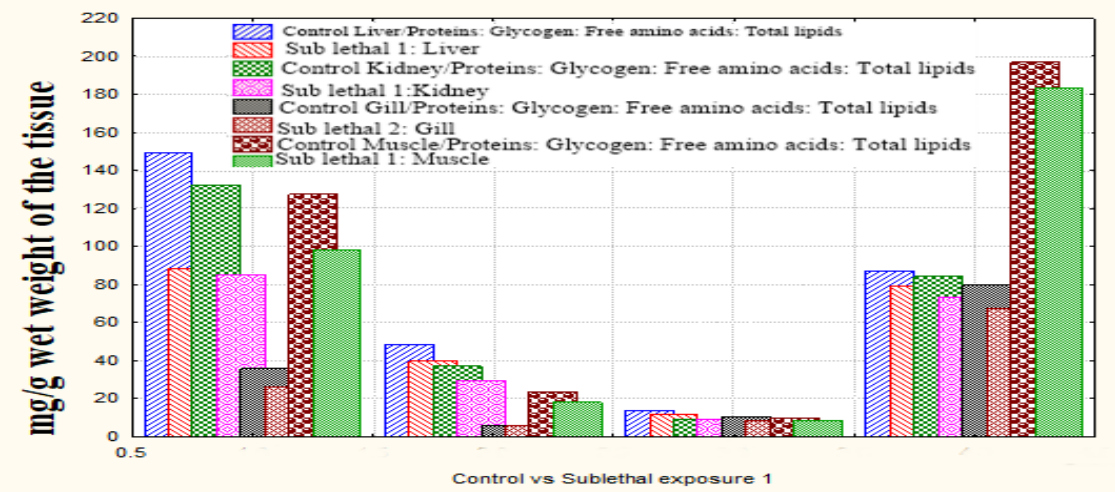

Figure1. Changes in the Biochemical constituents of fish *C.Punctatus* exposed to sub lethal concentration 1 of Chlorantraniliprole for 45 days.

Table3: Variation in the Biochemical constituents of Liver, Kidney, Gill, and Muscle of *C.Punctatus* for 45 days

Organ	Control	Sub lethal 45 days Exposure(1/20th 96 h LC 50)	Biochemical constituents expressed in mg/g wet weight of the tissue
Muscle	127.41±0.81 23.54±0.05 9.78±0.08 196.84±2.35	64.54±0.0.7^{d} (49.34%) 12.65±0.05^{a} (46.26%) 5.062±0.005^{d} (48.24%) 102.62±1.59^{b} (47.86%)	Protein Glycogen Free amino acids Total lipids
Liver	149.51±0.01 48.24±0.12 13.84±0.02 87.19±2.29	62.41±4.10^{b} (87.10%) 32.21±0.3^{a} (16.04%) 8.43±0.08c (5.41%) 57.54±1.8d (29.65%)	Protein Glycogen Free amino acids Total lipids
Kidney	132.24±0.03 24.55±0.05 7.88±0.09 84.47±5.17	49.82±0.01^{b} (82.42%) 19.74±0.08c (4.81%) 4.28±.84^{d} (3.6%) 32.18±0.03^{b} (52.29%)	Protein Glycogen Free amino acids Total lipids
Gills	35.54±0.24 5.46±0.02 10.28±0.02 79.57±6.30	18.84±1.86^{d} (10.07%) 4.05±0.09^{c} (1.14%) 6.55±0.026^{a} (3.73%) 56.10±0.08^{c} (23.27%)	Protein Glycogen Free amino acids Total lipids

The values were as means ± SD (n=6); a $p \leq 0.05$; b $p \leq 0.02$; c $p \leq 0.01$; d $p \leq 0.005$.

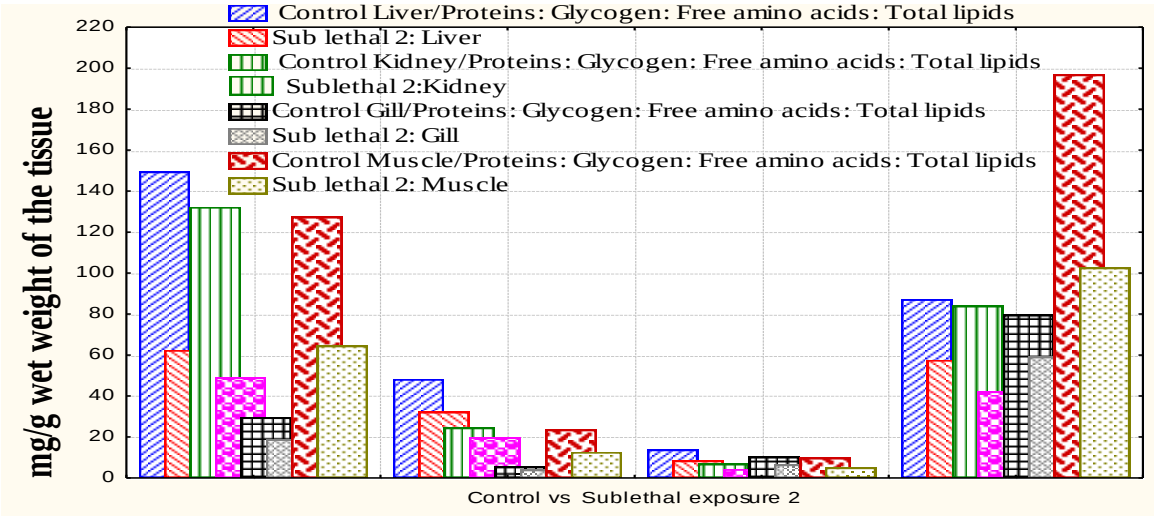

Figure2. Changes in the Biochemical constituents of fish *C.Punctatus* exposed to Sub lethal concentration 2 of Chlorantraniliprole for 45days.

CONCLUSION

In summary, the results obtained in this work showed that chlorantraniliprole was toxic to the fish *C.punctatus* even at low concentrations, since it promoted significant alterations in the in the biochemical parameters evaluated. The biochemical constituent depletion mainly in the liver, which is primarily responsible for metabolism, and which caused the accumulation of this pesticide in the hepatocytes of fish liver.

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