# Toxicity and Effect of Cypermethrin on Total Protein and Nucleic Acid Content in the Tissues of *Cirrhinus mrigala*

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Abstract— Effect of cypermethrin (25%EC) on total protein and nucleic acid content in different tissues of Cirrhinus mrigala was assessed by static renewal bioassay, using different sub-lethal concentrations (5, 10, 15 and 20% of 96h LC<sub>50</sub>) for 5, 10 and 15 days duration. There was a gradual decrease in protein content in all the tissues under sub-lethal concentrations at all exposure periods with maximum percentage of depletion (45.26%) in muscle and minimum (35.12%) in kidney at 15 days and at 20% 96hLC50. DNA and RNA contents were not altered much by cypermethrin at 5<sup>th</sup> day which later gradually decreased with increased exposure period. Decrement in DNA content is less in muscle when compared to the other tissues. Maximum percentage of depletion in DNA was (17.17%) in liver and minimum (13.94%) in muscle at 15 days and at 20% 96hLC50. RNA content decreased significantly in liver (29.90%), muscle (25.53%), brain (23.38%), kidney (21.82%) and gill (20.34%). This decrease was comparatively higher at 15 days and at 20% 96hLC50. Influence of cypermethrin was found to be time and exposure dependent for both the nucleic acids in the aquaculture practices used edible fish.

Keywords—Cypermethrin, DNA, Protein, RNA, Synthetic pyrethroids, Toxicity.

## I. INTRODUCTION

Contamination of aquatic ecosystems is the inevitable and burning issue of concern all over the world due to usage of chemicals both in agri and aqua practices. Such usage as environmental pollutants can cause alterations in the biochemical parameters of non-target organisms like fish and can react with each other as synergistic effect on aquatic biota [1]. Among them, pesticides are one of the toxicants take the lion's share known to affect fish and other aquatic fauna. Pesticides are the class of toxic compounds designed to kill unwanted organisms, despite of their benefits but when applied on land, they may be washed into the surface water and kill, or at least adversely influence, the life of aquatic organisms by producing wide range of toxic effects and pose potential hazards to the environment [2, 3]. The contamination of aquatic bodies by pesticides is mainly due to intensive agriculture combined with surface runoff and subsurface drainage, usually within a few weeks after application [4]. The continuous presence of pesticides in aquatic ecosystems is the consequence of their use (timing, rate, frequency) and the rainfall during the application period [5]. The use of pesticides in agriculture may also lead to contamination of surface and ground waters by drift, runoff, drainage and leaching [6]. Omnipresence of pesticides in surface waters due to application in disease management of aquaculture requires that sensitive biological tests be developed to study relative occurrence and toxicity to the ambient organisms [7]. Aquatic invertebrates and fish thus become targets of toxic substances at potentially hazardous concentrations and this is of special concern if sensitive larval and developmental stages are affected [8].

Pyrethroids are among the most widely used pesticides, due to their low environmental persistence and low mammalian and bird toxicity [9, 10], viable substitutes for organochlorine and organophosphate pesticides in pest-control programs. In fish farms they are applied to control ectoparasites and biological vectors especially lice, as well as insects in nursery and grow-out systems as alternatives for the more toxic organophosphates [11]. Fish are one of the most important aquatic organisms and ideal sentinels for a wide range of toxicity bioassays of various stress factors and toxic chemicals such as pesticide exposure due to their economic value of edibility and culturability, sensitivity to contaminants, ecological relevance in many

natural systems [9, 12]. Pyrethroids insecticides present low water solubility, low residence time in water and high absorbance into particulate matter [13, 14], which may decrease its toxicity in field conditions. However, these pesticides are very toxic even at low concentrations, posing risks to non-target aquatic populations. Fish are very susceptible to pyrethroid contamination [15] and the rate of toxicity to fishes is in a range of micrograms per liter [16]. The purpose of this study was to evaluate the acute toxicity of cypermethrin using a non-target aquatic organism, *Cirrhinus mrigala* in order to assess its sensitivity towards the toxicant with reference to total protein and nucleic acid content.

## II. MATERIALS AND METHODS

Cirrhinus mrigala of both sexes, weighing  $6.5 \pm 1.0$  g and measuring  $6.0 \pm 1.5$  cm in length, were obtained from a local fish farm. Fish were acclimated to laboratory conditions for 15 days in 200L plastic tubs before the experiments. They were kept in continuously aerated water with a static system and under a natural photoperiod (12 h light and 12 h dark). During the experimental period, under laboratory conditions, the average water parameters were as follows: temperature  $24.0 \pm 2.0$  C, pH  $7.8 \pm 0.3$ , dissolved oxygen  $8.5 \pm 2.0$  mg/L, chloride 35.5 mg/L and fluoride .6 mg/L. Fish were fed twice a day with rice bran and oil cake during acclimation period. Feeding was stopped one day prior to the acute toxicity test. All the precautions laid by committee on toxicity tests to aquatic organisms [17] were followed and such acclimatized fish only were used for the bioassay experiment. If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish was discarded. Commercial grade cypermethrin (25%EC) was obtained from United Phosphorus Ltd., Bombay, India. After the normal process of acclimatization, a group of ten fish each were transferred to polyethylene tubs (15L capacity) containing 10L of water. Fish were exposed to 4 sub-lethal concentrations i.e., 5, 10, 15 and 20 % of 96hLC<sub>50</sub> (4.23μg/L) for 5, 10 and 15 days along with the control. Control and exposed fishes were sacrificed at end of each day. The vital tissues like muscle, brain, liver, gill and kidney of the fish were taken for the estimation of total protein and nucleic acids (DNA & RNA). Estimation of total protein was done by the method of Lowry et al. [18]. The nucleic acids DNA, RNA were estimated by method of Searchy and Machnnis [19]. 5% homogenates of tissues were prepared in 5ml of 0.5N perchloric acid and heated at 90°C for 20 minutes after cooling the tissues homogenates were centrifuged at 3000rpm for 10 minutes. The supernatant was separated into two equal volumes and used for DNA and RNA analysis. The first half or one half of the homogenate was mixed with 5ml Biphenyl amine reagent and kept aside for 20 hours and the colour developed was read at 595 nm. The standard graph was plotted with standard DNA (Calf thymus) supplied by the sigma chemical company with the aforesaid method. The other part of the homogenate was mixed with Dischi-Orcinol and heated at 90°C for 5 minutes. After cooling at room temperature the colour developed was read at 655nm for RNA standard RNA [Baker yeast, sigma chemical] was dissolved in 0.5N perchloric acid and plotted standard graph and used for the present analysis. Statistical tools were applied to calculate the significance of the differences between control and experimental means. P values of 0.05 or less were considered statistically significant [20].

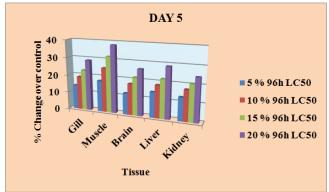
### III. RESULTS

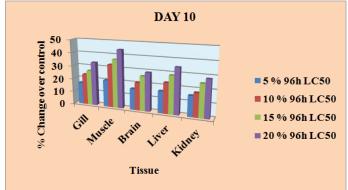
Calculated values for total protein and nucleic acids (DNA and RNA) along with standard deviations are given in tables 1-3. Percent change of total protein and nucleic acids in experimental fish over control is graphically represented as in figures 1-3. The values were expressed as mg/g body weight of the tissue. A significant decreased in proteins was observed in all the tissues under sub-lethal concentrations of cypermethrin (Table 1 & Figure 1). Total protein is comparatively low in the kidney followed by brain and gill and more in muscle followed by liver in control fish. Significant decrease was observed in protein content in all the five organs and all the test concentrations. However, the decrease was higher in 15 days exposed fishes at highest concentration (20% 96hLC<sub>50</sub>). The reduction in protein content in cypermethrin exposed fishes is comparatively less in kidney when compared to the other tissues studied. Maximum percentage of decrement was (45.83%) in muscle and minimum was (34.48%) in kidney at the longest exposure period (15 days) and highest sublethal concentration (20% 96hLC<sub>50</sub>).

TABLE 1
CHANGES IN THE TOTAL PROTEIN CONTENT (mg/g wet weight of the tissue) IN DIFFERENT TISSUES OF CIRRHINUS MRIGALA EXPOSED TO SUBLETHAL CONCENTRATIONS OF CYPERMETHRIN (25% EC)

CIRRINUS MRIGALA EAFOSED TO SUBLETHAL CONCENTRATIONS OF CITERMETHRIN (25 / 0 EC)								
Exposure period	Tissue	Concentration of Cypermethrin (% 96h LC <sub>50</sub> )						
in Days		Control	5	10	15	20		
5	Gill	$63.91 \pm 0.160$	$55.08 \pm 0.082$	$51.72 \pm 0.222$	$49.16 \pm 0.160$	$45.44 \pm 0.160$		
	Muscle	$81.46 \pm 0.240$	$67.03 \pm 0.162$	$60.90 \pm 0.140$	$55.52 \pm 0.200$	$50.28 \pm 0.228$		
	Brain	$61.25 \pm 0.103$	$53.84 \pm 0.226$	$50.43 \pm 0.163$	$48.11 \pm 0.126$	$45.15 \pm 0.177$		
	Liver	$69.51 \pm 0.190$	$59.52 \pm 0.103$	$56.72 \pm 0.190$	$54.25 \pm 0.240$	$49.43 \pm 0.240$		
	Kidney	$56.42 \pm 0.177$	$49.02 \pm 0.260$	$46.56 \pm 0.200$	$44.67 \pm 0.082$	$42.60 \pm 0.112$		
10	Gill	$65.48 \pm 0.225$	$54.54 \pm 0.135$	$50.02 \pm 0.162$	$47.99 \pm 0.103$	$43.63 \pm 0.103$		
	Muscle	$83.27 \pm 0.140$	$66.18 \pm 0.192$	$56.01 \pm 0.193$	$52.62 \pm 0.168$	$46.36 \pm 0.190$		
	Brain	$60.33 \pm 0.252$	$50.67 \pm 0.221$	$47.63 \pm 0.200$	$44.54 \pm 0.226$	$42.74 \pm 0.080$		
	Liver	$72.48 \pm 0.150$	$60.52 \pm 0.140$	$55.77 \pm 0.127$	$51.73 \pm 0.116$	$47.31 \pm 0.130$		
	Kidney	$59.14 \pm 0.180$	$49.97 \pm 0.165$	$48.55 \pm 0.200$	$44.42 \pm 0.126$	$42.34 \pm 0.123$		
15	Gill	$67.61 \pm 0.270$	$51.63 \pm 0.081$	$49.21 \pm 0.103$	$43.84 \pm 0.200$	$41.07 \pm 0.080$		
	Muscle	$86.05 \pm 0.226$	$61.86 \pm 0.120$	$56.40 \pm 0.173$	$51.57 \pm 0.170$	$46.61 \pm 0.120$		
	Brain	$58.46 \pm 0.140$	$45.62 \pm 0.103$	$42.84 \pm 0.081$	$39.19 \pm 0.192$	$36.36 \pm 0.210$		
	Liver	$75.15 \pm 0.260$	$55.66 \pm 0.266$	$52.48 \pm 0.220$	$48.09 \pm 0.150$	$44.52 \pm 0.240$		
	Kidney	$62.08 \pm 0.084$	$49.78 \pm 0.090$	$45.43 \pm 0.280$	$42.82 \pm 0.081$	$40.67 \pm 0.190$		

Mean $\pm$  standard deviation, n=5, Values are significant at p < 0.05





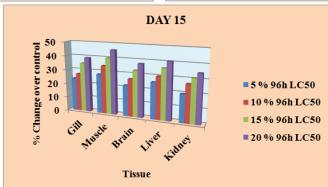


FIGURE 1: Per cent changes in the Total Protein content (mg/g wet weight of the tissue) in different tissues of *Cirrhinus mrigala* exposed to sublethal concentrations of cypermethrin (25%EC) over control

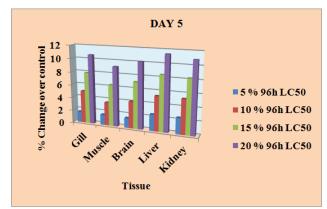
Decreased tendency was observed in both DNA and RNA in the vital tissues of test fish exposed to cypermethrin over control. Both the nucleic acids gradually decreased with increased exposure period and the decrease was observed to be directly proportional to increased sublethal concentrations. The DNA content is comparatively low in the muscle followed by brain and gill and more in liver followed by kidney in control fish. No significant changes were observed in DNA content in all the five organs at 5% 96hLC50 at 5 day exposure period. In 10 days and 15 days exposed fishes the DNA content decreased significantly in all the tissues studied at all the test concentrations. However, the decrease was higher in 15 days

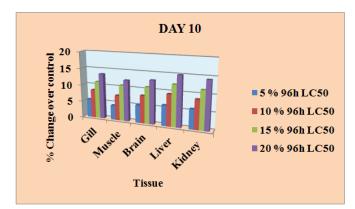
exposed fishes at highest concentration (20% 96hLC<sub>50</sub>). The reduction in the DNA content in cypermethrin exposed fishes is comparatively less in muscle when compared to the other tissues studied. Maximum percentage of decrement in DNA was 18.75% in liver and minimum was 14.74% in muscle at the longest exposure period of 15 days and at highest sub-lethal concentration of 20% in 96h LC<sub>50</sub> (Table 2 & Figure 2).

TABLE 2
CHANGES IN THE AMOUNT OF DEOXY RIBONUCLEIC ACID (DNA) (mg/g body weight of the tissue) IN THE TISSUES OF CIRRHINUS MRIGALA ON EXPOSURE TO SUB LETHAL CONCENTRATIONS OF CYPERMETHRIN (25%EC)

Exposure		Concentration of Cypermethrin (% 96h LC <sub>50</sub> )						
period in Days	Tissue	Control	5	10	15	20		
5	Gill	$6.56 \pm 1.12$	$6.45 \pm 1.38$	$6.23 \pm 1.21$	$6.04 \pm 1.54$	$5.86 \pm 1.13$		
	Muscle	$2.51 \pm 1.55$	$2.47 \pm 1.54$	$2.42 \pm 1.48$	$2.35 \pm 1.20$	$2.28 \pm 1.64$		
	Brain	$5.32 \pm 1.18$	$5.24 \pm 1.22$	$5.10 \pm 1.15$	$4.94 \pm 1.28$	$4.78 \pm 1.43$		
	Kidney	$7.71 \pm 1.29$	$7.52 \pm 1.37$	$7.30 \pm 1.24$	$7.07 \pm 1.34$	$6.87 \pm 1.57$		
	Liver	$9.52 \pm 1.44$	$9.28 \pm 1.52$	$9.01 \pm 1.33$	$8.71 \pm 1.59$	$8.43 \pm 1.61$		
10	Gill	$6.54 \pm 1.61$	$6.18 \pm 1.24$	$5.98 \pm 1.63$	$5.81 \pm 1.38$	$5.65 \pm 1.14$		
	Muscle	$2.52 \pm 1.25$	$2.41 \pm 1.88$	$2.33 \pm 1.42$	$2.25 \pm 1.11$	$2.21 \pm 1.51$		
	Brain	$5.33 \pm 1.14$	$5.05 \pm 1.18$	$4.89 \pm 1.26$	$4.75 \pm 1.31$	$4.64 \pm 1.44$		
	Kidney	$7.68 \pm 1.62$	$7.23 \pm 1.54$	$7.01 \pm 1.37$	$6.80 \pm 1.41$	$6.58 \pm 1.49$		
	Liver	9.51 ± 1.18	$8.93 \pm 1.34$	$8.61 \pm 1.22$	$8.34 \pm 1.48$	$8.08 \pm 1.15$		
	Gill	$6.53 \pm 1.42$	$6.04 \pm 1.77$	$5.79 \pm 1.40$	$5.60 \pm 1.60$	$5.41 \pm 1.24$		
	Muscle	$2.51 \pm 1.21$	$2.34 \pm 1.61$	$2.26 \pm 1.50$	$2.20 \pm 1.61$	$2.14 \pm 1.18$		
	Brain	$5.31 \pm 1.52$	$4.92 \pm 1.29$	$4.76 \pm 1.53$	$4.65 \pm 1.19$	$4.48 \pm 1.61$		
15	Kidney	$7.65 \pm 1.37$	$7.07 \pm 1.33$	$6.72 \pm 1.25$	$6.47 \pm 1.11$	$6.24 \pm 1.40$		
	Liver	$9.49 \pm 1.19$	$8.66 \pm 1.21$	$8.27 \pm 1.30$	$8.01 \pm 1.43$	$7.71 \pm 1.29$		

Mean $\pm$  standard deviation, n=5, Values are significant at p < 0.05





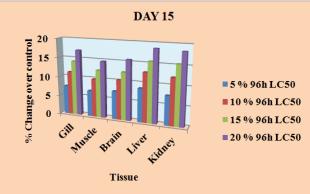


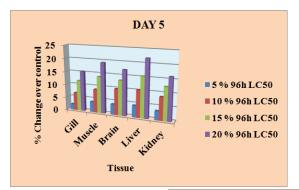
FIGURE 2: Percent change over control in the amount of DNA (mg/g body weight of the tissue) in the tissues of *Cirrhinus mrigala* on exposure to sub lethal concentrations of cypermethrin (25%EC)

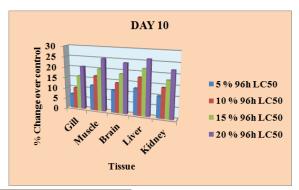
The control values of RNA in different tissues of the fish was in the order of Gill > Kidney > Brain > Muscle > Liver (Table 3 & Figure 3). Under sublethal exposure of cypermethrin the RNA content was found to decrease in all the tissues of the experimental fish. RNA content was not significantly altered by cypermethrin at 5 days exposure period at lowest test concentration. However, at both 10 days and 15 days exposure periods in all the test organs RNA content declined significantly. At 15 days exposure period the RNA content decreased significantly in liver 32.98%, muscle 30.22%, brain 29.35%, kidney 28.10% and gill 27.40%. The decrease was comparatively higher at 15 days exposure period at highest test concentrations (Table 3 & Figure 3).

TABLE 3
CHANGES IN THE AMOUNT OF RIBONUCLEIC ACID (RNA) (mg/g body weight of the tissue) IN THE TISSUES OF CIRRHINUS MRIGALA ON EXPOSURE TO SUB LETHAL CONCENTRATIONS OF CYPERMETHRIN (25%EC)

Exposure		Concentration of Cypermethrin (% 96h LC <sub>50</sub> )						
period in Days	Tissue	0	5	10	15	20		
5	Gill	$4.09 \pm 0.120$	$4.00 \pm 0.129$	$3.81 \pm 0.229$	$3.61 \pm 0.218$	$3.46 \pm 0.182$		
	Muscle	$7.45 \pm 0.232$	$7.15 \pm 0.150$	$6.78 \pm 0.131$	$6.41 \pm 0.186$	$6.01 \pm 0.148$		
	Brain	$6.01 \pm 0.015$	$5.77 \pm 0.137$	$5.41 \pm 0.164$	$5.21 \pm 0.157$	$4.97 \pm 0.191$		
	Liver	$8.55 \pm 0.224$	$8.17 \pm 0.212$	$7.66 \pm 0.317$	$7.22 \pm 0.253$	$6.67 \pm 0.257$		
	Kidney	$5.26 \pm 0.115$	$5.08 \pm 0.170$	$4.80 \pm 0.229$	$4.60 \pm 0.128$	$4.41 \pm 0.172$		
10	Gill	$4.06 \pm 0.157$	$3.78 \pm 0.221$	$3.65 \pm 0.218$	$3.41 \pm 0.152$	$3.22 \pm 0.154$		
	Muscle	$7.42 \pm 0.136$	$6.53 \pm 0.155$	$6.18 \pm 0.135$	$5.93 \pm 0.144$	$5.56 \pm 0.140$		
	Brain	$6.02 \pm 0.131$	$5.37 \pm 0.142$	$5.16 \pm 0.154$	$4.90 \pm 0.163$	$4.59 \pm 0.161$		
	Liver	$8.53 \pm 0.214$	$7.45 \pm 0.165$	$7.01 \pm 0.218$	$6.67 \pm 0.217$	$6.30 \pm 0.108$		
	Kidney	$5.24 \pm 0.179$	$4.71 \pm 0.219$	$4.50 \pm 0.140$	$4.32 \pm 0.130$	$4.08 \pm 0.172$		
15	Gill	$4.05 \pm 0.148$	$3.58 \pm 0.215$	$3.37 \pm 0.156$	$3.18 \pm 0.174$	$2.94 \pm 0.211$		
	Muscle	$7.41 \pm 0.237$	$6.22 \pm 0.108$	$5.88 \pm 0.182$	$5.47 \pm 0.140$	$5.17 \pm 0.160$		
	Brain	$6.03 \pm 0.160$	$5.21 \pm 0.154$	$4.86 \pm 0.128$	$4.50 \pm 0.188$	$4.26 \pm 0.104$		
	Liver	$8.55 \pm 0.251$	$7.12 \pm 0.178$	$6.68 \pm 0.149$	$6.24 \pm 0.274$	$5.73 \pm 0.188$		
	Kidney	$5.23 \pm 0.154$	$4.60 \pm 0.127$	$4.32 \pm 0.115$	$4.04 \pm 0.162$	$3.76 \pm 0.184$		

Mean $\pm$  standard deviation, n=5, Values are significant at p < 0.05





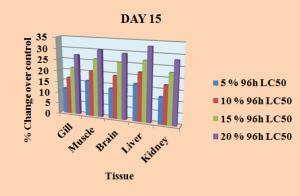


FIGURE 3: Percent change over control in the amount of RNA (mg/g body weight of the tissue) in the tissues of *Cirrhinus mrigala* on exposure to sub lethal concentrations of cypermethrin (25%EC)

#### IV. DISCUSSION

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 [21]. The quantity of protein is dependent on the rate of protein synthesis, or on rate of its degradation. The quantity of protein may also be affected due to impaired incorporation of amino acids into polypeptide chains [22]. The decreased tendency of the protein content as observed in the present study in the fish tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose; or due to the directing of free amino acids for the synthesis of necessary proteins, or for the maintenance of osmotic and ionic regulation [23].

Since fish have a very little amount of carbohydrates, the next alternative source of energy to meet the increased energy demand is protein only. The decrease in protein level in tissues may be a result of a physiological mechanism trying to meet higher energy demands for metabolic purposes providing energy to cope with the stress situation caused by cypermethrin intoxication. Under stress condition fish will mobilize proteins as an energy source via the oxidation of amino acids. The decrease in total protein content in tissues of test fish indicates active degradation of proteins under cypermethrin stress. Decreased protein level may be attributed to stress mediated immobilization of these compounds to fulfill an increased element of energy by the fish to cope with environmental condition exposed by toxicant [24]. The depletion of total protein content is may be due to augmented proteolysis and possible utilization of degraded products for metabolic purposes as reported by Begum [25]. Depletion might also be attributed to the destruction or necrosis of cellular function and consequent impairment in protein synthetic machinery [26, 27]. Decrement of proteins might be due to the blocking of protein synthesis, protein denaturation, or interruption in the amino acid synthesis. Neff [28]explained the depletion in protein content may be related to impaired food intake, increased energy cost of homeostasis, tissue repair and detoxification mechanism during toxic stress.

The depletion of protein content observed in this investigation can be correlated with the earlier report of David et al. [27] who reported similar situation in *Cyprinus carpio* and *Labeo rohita* exposed to cypermethrin. Depletion of tissue protein in fish exposed to cypermethrin has been reported by several workers [29-39] and revealed that the toxic stress influences the conversion of tissue protein into soluble protein fraction reaching in the blood for its utilization. The decrease in proteins quantity may be due to increased energy demand during stress or it could be due to altered enzymatic activities. In long term exposure to cypermethrin much of the energy must have been used up to compensate the stress, hence the depleted levels of protein were observed.

Channa punctatus exposed to sub lethal concentration, 0.04 mg/L (1/10<sup>th</sup> of LC<sub>50</sub>=0.4 mg/L) of cypermethrin for a period of 15, 30 and 45 days showed a significant (p<0.05) decrease in protein content in gill, liver and kidney when compared to control with the increase in the period of exposure to the toxicant [29]. Jipsa et al. [30] also reported sublethal concentration of cypermethrin in *Tilapia mossambica* decreased the protein in the tissues at 24, 48, 72 and 96h. Kannan et al. [40] investigated the effects of cypermethrin (10% EC) in the concentration of 0.0006ml/L to Catla catla for 24h and reported a significant (P<0.01) decrease. Olalekan [41] reported biochemical responses of Clarias gariepinus exposed to cypermethrin (20μg/L) for 5 days and the total protein decreased in liver and muscle. Shruti and Tantarpale [42] observed at sub-lethal concentration of 0.0007µg/L of cypermethrin caused decrement in protein level in muscle and liver of Ophiocephalus orientalis. Veeraiah et al. [43] reported Cirrhinus mrigala exposed to lethal and sublethal concentration of 96hLC<sub>50</sub> as 2.28ppm and 1/10<sup>th</sup> of 96hLC<sub>50</sub> of cypermethrin (10% EC) for 96h also reported similarly. Patil and Patole [44] reported significant fluctuation in protein over the control in *Lepidocephalichthys guntea* exposed to sublethal concentrations, i.e  $1/4^{th}$ and 3/4<sup>th</sup> of LC<sub>50</sub> of cypermethrin for 96h. Laboratory evaluation of cypermethrin toxicity made by Tiwari et al. [32] revealed sublethal doses of cypermethrin, 0.129µg/L, 0.258µg/L for 24h and 0.082µg/L, 0.164µg/L for 96h exposure period caused significant (P<0.05) change in protein of Labeo robita in both liver and muscle tissues. Vasantharaja et al. [45] studied acute toxicity of cypermethrin (30µg/L) and its impact on biochemical alteration in Cirrhinus mrigala for 24, 48, 72, 96 and 120h and dose dependent alterations in the level of total protein were observed. Firat et al. [12] reported the effect of cypermethrin (0.05µg/L) in Oreochromis niloticus which showed decreased total protein levels at 21 days. Tantarpale [46] observed sublethal concentration of cypermethrin, at 0.00078µl/L for 24, 48, 72, and 96h reported a decrease in total protein level in Channa striata. Total protein was found to decrease 58.99, 41.12, 38.11, 21.81mg/L in muscle and 23.62, 20.77, 19.17, 12.67mg/L in liver tissues at different exposure periods. In common carp, cypermethrin resulted in a significant (P<0.01) decrease in total protein compared to controls [33]. Several other investigations also revealed a decrease in protein with cypermethrin exposure [47-50]. All these investigations support the present study of decreasing trend of proteins in the

tissues of the fish *Cirrhinus mrigala* exposed to sublethal concentrations of cypermethrin (25%EC). Toxicity response generally depends on the toxicant concentration and the duration of exposure in the tissue [51]. The time dependent and tissue specific response in the present study could be attributed to the concentration of cypermethrin in the tissue and also due to its distribution and elimination.

Nucleic acids which serve as biochemical indices play a major role in all biological activities including growth and development and regulate biosynthesis of proteins [52, 53]. RNA/DNA ratio indicates the degree of metabolic (protein) synthesis and is an index of fish growth [54, 55]. Such an indices is based on the fact that the DNA content per cell is constant within the same species, and the RNA is mainly ribosomic and varies with the rate of protein synthesis. It has been traditionally used as a growth check in ecological studies, in aquaculture and as a biomarker in long-term exposure experiments in ecotoxicology [56]. The maintenance of DNA integrity is vital to the protection of genetic diversity in natural populations [57]. The detection of structural/functional disturbances in the DNA enables the assessment of organism's health and can assist in the prevention of DNA damage [58].

The amount of DNA, the carrier of genetic information, remains stable under changing environmental situations and has been used as an indicator of biomass. It is also known that the DNA functions as primer in DNA and RNA polymerase reactions and the inhibition in the DNA content caused inhibition their synthesis. The inhibition of DNA synthesis affects protein content by decreasing the content of RNA. Therefore, it is possible that the enzyme necessary for DNA synthesis might have been inhibited by pesticide, cypermethrin toxic stress. As RNA synthesis plays an important role in protein synthesis and its inhibition at transcription level may affect the protein content [34]. Significant decrease in RNA content observed in different organs of test fish in the present study might have caused protein depletion in the organs too and because, the fish requires more energy to overcome the stress upon exposure to the toxicant.

Decrease in the nucleic acid content observed in the present study in all the tissues exposed to cypermethrin were in accordance with the previous studies [29, 59-65]. *Channa punctatus* exposed to sub lethal concentration, 0.04mg/L ( $1/10^{\text{th}}$  of  $LC_{50}$ =0.4mg/L) of cypermethrin for a period of 15, 30 and 45 days showed a significant (p<0.05) increase in DNA content in gill and liver tissues, but in the kidney, it was found to be in a decreased trend. Whereas, the RNA content of all the tissues (gill, liver and kidney) have decreased significantly (p<0.05) when compared to control with the increase in the period of exposure [29]. *Cyprinus carpio* exposed to sub lethal concentration (0.6mg/L) of cypermethrin for 7, 14 and 21 days showed decrease in DNA and RNA content in brain, gills and liver with diminished RNA/DNA ratio which was fund to be exposure period dependent. The reduction is comparatively less in liver when compared to the brain and gills [60]. Cypermethrin induced reduction in DNA and RNA content was observed in gill and liver and kidney of *Cirrhinus mrigala* [59]. Sublethal doses of cypermethrin (0.129µg/L, 0.258µg/L for 24h and 0.082µg/L, 0.164µg/L for 96h) caused significant (P<0.05) reduction in DNA and RNA contents in fingerlings of *Labeo rohita* in both liver and muscle tissues [32]. Sublethal concentration of cypermethrin resulted in a significant decline in DNA at 96h exposure to 40 and 60% of 24h LC<sub>50</sub> resulted in a decrease of DNA level in gonadal tissue to value of 54 and 31% at 16°C and 58 and 36% of the controls at 28°C water temperature respectively. For RNA the decrease noted to 55 and 31% at 16°C and 58 and 35% of the level of control at 28°C water temperature respectively [34].

Our findings differ with that of Das and Mukherjee [63] who reported an increase in DNA and RNA content of liver, brain, kidney of the fish *Labeo rohita* response to sublethal concentration of 0.014ppm cypermethrin for 96h. Similar trend was also noticed in DNA and RNA profile of gill, brain, liver and kidney of *Channa punctatus* treated with 40-60µg/L cypermethrin by Kumar et al. [36]. *Lepidocephalicthys thermalis* exposed to cypermethrin also showed a decline in the RNA content of the liver and muscle tissues [64]. Ansari and Kumar [65] reported a significant decline in the DNA and RNA content of the liver tissue of *Brachydanio rerio* exposed to cypermethrin.

## V. CONCLUSION

Based on the results obtained from the present investigation, it is concluded that cypermethrin causes deleterious effects on fishes and much alters the DNA and RNA contents of certain fish tissues. Significant decrease in both protein and nucleic acids levels would suggest that cypermethrin impairs the process of protein synthesis in the tissues of the test fish. Therefore it is recommended that care must be taken to prevent its entrance into aquatic ecosystems as the sub-lethals are real lethals in the long run.

#### ACKNOWLEDGEMENTS

We are thankful to the Head, Department of Zoology & Aquaculture and the authorities of Acharya Nagarjuna University for the encouragement and support by providing the laboratory facilities. Authors would like to thank Dr. G. Simhachalam, Assistant Professor, Department of Zoology & Aquaculture for his valuable suggestions and allowing us to use laboratory facilities in Biodiversity and Parasitology Laboratory.

#### REFERENCES

- [1] A. Moore, and C.P. Waring, "The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (Salmo salar L.)", Aquat. Toxicol., 52, 1-12, 2001.
- [2] E. Georgieva, S. Stoyanova, I. Velcheva, and V. Ancheva, "Histopathological alterations in common carp (*Cyprinus carpio L.*) gills caused by Thiamethoxam", Braz. Arch. Biol. Tech., 57(6), 991-996, 2014.
- [3] M. Atamanalp, and O. Erdoğan, "Alterations of HSP70 gene expression in rainbow trout (*Oncorhyncus mykiss*) exposed to deltamethrin", Turk. J. Vet. Anim. Sci., 34(4), 359-363, 2010.
- [4] M. Banaee, and K. Ahmadi, "Sub-lethal toxicity impacts of endosulfan on some biochemical parameters of the freshwater crayfish (*Astacus leptodactylus*)", Res. J. Env. Sci., 5, 827-835, 2011.
- [5] B. K. Hope, "Using legacy data to relate biological condition to cumulative aquatic toxicity in the Willamette River Basin (Oregon, USA)", Arch. Env. Contam. Toxicol., 62, 424-437, 2012.
- [6] M. J. Cerejeira, P. Viana, S. Batista, T. Pereira, E. Silva, M. J. Vale'rio, A. Silva, M. Ferreira, and S.A.M. Fernandes, "Pesticides in Portugal surface and ground waters", Wat. Res., 37, 1055-1063, 2003.
- [7] P. Saglio, S. Bretaud, E. Rivot, and K. H. Olse'n, "Chemobehavioral changes induced by short-term exposures to Prochloraz, Nicosulfuron, and Carbofuran in Goldfish", Arch. Env. Contam. Toxicol., 45, 515-524, 2013.
- [8] B. Sebastian, I. Werner, E. C. Richard, and P. G. Juergen, "Sublethal toxicity of commercial insecticide formulations and their active ingredients to larval fathead minnow (*Pimephales promelas*)", Sci. Tot. Env., 408, 3169-3175, 2010.
- [9] Y. Huang, J. Zhang, X. Han, and T. Huang, "The use of zebrafish (*Danio rerio*) behavioral responses in identifying sublethal exposures to Deltamethrin", Int. J. Env. Res. Pub. Heal., 11, 3650-3660, 2014.
- [10] D. M. Soderlund, J. M. Clark, L. P. Sheets, L. S. Mullin, V. J. Piccirillo, D. Sargent, J. T. Stevens, and M. L. Weiner, "Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment", Toxicology, 171, 3-59, 2002.
- [11] I. El-Elaimy, M. F. F. Bayomy, A. G. Khallaf, K. M. Geba, G. M. Rodríguez, and J. M. Mancera, "Development of sensitive molecular markers for detecting the genotoxicity induced by two pyrethroids insecticides, cypermethrin and permethrin, to the Meditnerranean sole, *Solea senegalensis* (Kaup 1858)", J. Appl. Pharma. Sci., 4(2), 34-42, 2014.
- [12] Ö. Fırat, H. Y. Cogun, T. A. Yüzereroğlu, G. Gök, Ö. Fırat, F. Kargin, and Y. Kötemen, "A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus*", Fish Physiol. Biochem., 37(3), 657-666, 2011.
- [13] C. M. Bajet, A. Kumar, M. N. Calingacion, and T. C. Narvacan, "Toxicological assessment of pesticides used in the Pagsanjan-Lumban catchment to selected non-target aquatic organisms in Laguna Lake", Philip. J. Agri. Wat. Manag., 106, 42-49, 2012.
- [14] J. J. Rasmussen, N. Friberg, and S. E. Larsen, "Impact of lambda-cyhalothrin on a macroinvertebrate assemblage in outdoor experimental channels: Implications for ecosystem functioning", Aquat. Toxicol., 90, 228-234, 2008.
- [15] F. D. Moraes, F. P. Venturini, L. R. X. Cortella, P. A. Rossi, and G. Moraes, "Acute toxicity of pyrethroid-based insecticides in the Neotropical freshwater fish *Brycon amazonicus*". *Ecotoxicol. Env. Contam.*, 8(2), 59-64, 2013.
- [16] U. Güner, "Determination of lambda-cyhalotrin (Tekvando 5EC) 96 hour lethal concentration 50 at Gambusia affinis (Baird & Girard, 1853)". J. Fish. Sci., 3, 214-219, 2009.
- [17] APHA In: Standard methods for the examination of water and waste water. APHA/AWWA/WPCF, 1998, Washington, DC.
- [18] O. H. Lowry, N. H. Rosenbrough, A. L. Farr, and R. J. Randal, R.J. "Protein measurement with folin-phenol reagent", J. Biol. Chem., 173, 807-814, 1951.
- [19] D. G. Searchy, and A. J. MacInnis, "Determination of RNA by Dische-Orcinol technique. In: Experiments and techniques in parasitology", Eds: Mac Inis A.J. and Voge M.W.H. freeman & Co., San Fransisco. p. 189-190, 1970.
- [20] R. A. Fisher, "Statistical Methods for research workers", 11th edition, Edinburgh UK: Oliver and Boyd Ltd, 1950.
- [21] P. B. Bhushan, K. M. Singh, and M. Rani, "Dimethoate and Monocil toxicity on the concentration of protein and amino acid in the serum and liver of *Channa marulius* (Ham.)", Nat. Env. Poll. Tech., 1(2), 147-150, 2002.
- [22] P. Y. Ram, S. K. Digvijay, and A. Singh, "Metabolic changes in freshwater fish *Channa punctatus* due to stem-barck extract of *Crton tiglium*". Pak. J. Biol. Sci., 6, 1223-1228, 2003.
- [23] Schmidt, and B. Nielson, "Osmoregulation: Effect of salinity and heavy metal", Federation Proceedings, 33, 2137-2146, 1975.
- [24] F. Jenkins, J. Smith, B. Rajanna, U. Shameem, K. Umadevi, V. Sandhya, and R. Mahadevi, "Effect of sublethal concentration of endosulfan on haematological and serum biochemical parameters in the carp *Cyprinus carpio*", Bull. Env. Contam. Toxicol., 70(5), 993-997, 2003.
- [25] G. Begum, "Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (Linn) and recovery response", Aqua. Toxicol., 66(1), 83-92, 2004.

- [26] O. E. Ogueji, and J. Auta, "The effects of sublethal doses of λ-cyhalothrin on some biochemical characteristics of the African catfish *Clarias gariepinus*", J. Biol. Sci., 7(8), 1473-1477, 2007.
- [27] M. David, S. B. Mushigeri, R. Shiva Kumar, G. H. Philip, "Response of *Cyprinus carpio* (Linn.) to sublethal concentration of cypermethrin: alterations in protein metabolic profiles", Chemosphere, 56, 347-352, 2004.
- [28] J. M. Neff, "Polycyclic aromatic hydrocarbons" In: Fundamentals of aquatic toxicology, Rand, G.M. and Petrocelli, S.R. Editors, Hemisphere, New York, pp 416-420, 1985.
- [29] K. Jaya, and N. Shettu, "Changes in the biochemical constituents of the freshwater fish, *Channa punctatus* (Bloch) exposed to the toxicity of cypermethrin". J. Chem. Pharma. Res., 7(9), 695-697, 2015.
- [30] J. R. Jipsa, R. Kalavathi, P. Y. Dhanya, and S. Logaswamy, "Studies on the impact of a Cypermethrin insecticide on oxygen consumption and certain biochemical constituents of a fish *Tilapia mossambica*". Int. J. Fish. Aquat. Stud., 1(5), 93-97, 2014.
- [31] O. O. Ojutiku, F. P. Asuwaju, I. O. Ayanda, R. A. Obande, and O. O. Agbelege, "Effect of acute toxicity of cypermethrin on some biochemical parameters of juveniles of *Clarias gariepinus* (Burchell, 1822)". Int. J. Eng. Sci. Inv., 2(3), 1-7, 2013.
- [32] S. Tiwari, R. Tiwari, and A. Singh, "Impact of cypermethrin on fingerlings of common edible carp (*Labeo rohita*)". The Sci. World J., 1, 1-7, 2012.
- [33] J. Velisek, A. Stara, and Z. Svobodova, "The effects of pyrethroid and triazine pesticides on fish physiology", In: Stoytcheva. M., ed. Pesticides in the modern world: pests control and pesticides exposure and toxicity assessment. Rijeka: InTech, 377-402, 2011.
- [34] K. S. Singh, S. K. S. Singh, and R. P. Yadav, "Toxicological and biochemical alterations of cypermethrin (synthetic pyrethroid) against freshwater teleost *Colisa fasciatus* at different seasons", World J. Zool., 5(7), 25-32, 2010.
- [35] N. Korkmaz, E. I. Cengiz, E. Unlu, E. Uysal, and M. Yanar, "Cypermethrin induced histopathological and biochemical changes in Nile tilapia (*Oreochromis niloticus*) and the protective and recuperative effect of ascorbic acid", Env. Toxicol. Pharmacol., 28(2), 198-205, 2009.
- [36] A. Kumar, B. Sharma, and R. S. Pandey, "Cypermethrin and λ-cyhalothrin induced alterations in nucleic acids and protein contents in a freshwater fish, *Channa punctatus*", Fish Physiol. Biochem., 34, 331-338, 2008.
- [37] M. S. Prashanth, "Cypermethrin induced protein metabolism in the freshwater fish *Cirrhinus mrigala* (Hamilton)", The *J. Basic Clin. Physiol. Pharmacol.*, 18(1), 49-63, 2007.
- [38] M. S. Prashanth, "Impact of cypermethrin on protein metabolism in freshwater fish *Cirrihinus mrigala*". Nat. Env. Poll. Tech., 5(2), 321-332, 2006.
- [39] M. Atamanalp, M. S. Keles, H. I. Haliloglu, and M. S. Aras, "The effects of cypermethrin (A synthetic pyrethroid) on some biochemical parameters (Ca, P, Na and TP) of rainbow trout (*Oncorhynchus mykiss*)", Turk. J. Anim. Sci., 26, 1157-1160, 2002.
- [40] M. Kannan, P. Muthusamy, and U. Venkatachalam, "Response of synthetic pyrethroid cypermethrin (10% EC) induced stress in biochemical and hematological parameters of Indian major carp *Catla catla* (Hamilton, 1822)", World J. Pharma. Res., 3(4), 1976-1996, 2014.
- [41] A. Olalekan, "Biochemical response of Clarias gariepinus to cypermethrin", J. Env. Anal. Toxicol., 5(1), 1-4, 2014.
- [42] S. G. Shruti, and V. T. Tantarpale, "Protein and amino acid modulation in freshwater fish *Ophiocephalus orientalis* exposed to cypermethrin". J. Pharm. Sci. Inno., 3(4), 344-347, 2014.
- [43] K. Veeraiah, Ch. Vivek, P. Srinivas Rao, and G. Venkatrao, "Biochemical changes induced by cypermethrin (10% EC), a pyrethroid compound in sublethal and lethal concentrations to the freshwater fish *Cirrhinus mrigala* (Hamilton)". J. Atoms Mol., 3(6), 625-634, 2013.
- [44] M. U. Patil, and S. S. Patole, Effect of malathion and cypermethrin on biochemical constituents of freshwater fish, *Lepidocephalichthys guntea* (Ham-Buch). Sodh Samik. Mulya., 4(39), 33-35, 2012.
- [45] C. Vasantharaja, K. Pugazhendy, S. Venkatesan, M. Meenambal, S. Prabakaran, and K. Jayachandran, "Acute toxicity of cypermethrin and its impact on biochemical alteration in the freshwater fish *Cirrhinus mrigala* (Hamilton) and protective effect of *Cardiospermum helicacabum* (Linn.)", Int. J. Pharma. Biol. Arch., 3(1), 146-152, 2012.
- [46] A. Tantarpale, "Cypermethrin impact on total protein in muscle and liver of the freshwater fish *Channa striatus*", Sci. Res. Report, 1(3), 155-158, 2011.
- [47] S. Logaswamy, and K. M. Remia, "Impact of cypermethrin and ekalux on respiratory and some biochemical activities of a freshwater fish, *Tilapia mossambica*", *Curr. Biot.*, 3(1), 65-73, 2009.
- [48] R. Dobsikova, J. Velisek, T. Wlasow, P. Gomulka, Z. Svobodova, and L. Novotny, "Effects of cypermethrin on some haematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.)", Neuroendocrin. Lett., 27, 101-105, 2006.
- [49] G. Begum, "In vivo biochemical changes in liver and gill of *Clarias batrachus* during cypermethrin exposure and following cessation of exposure", Pesticide Biochemistry and Physiology, 82, 185-196, 2005.
- [50] M. K. Durga Prasad, and K. Veeraiah, "Effect of cypermethrin on protein metabolism of the fish, *Labeo rohita* (Hamilton)", Bull. Pure Appl. Sci., 21(1), 27-32, 2002.
- [51] Q. H. Pickering, and C. Henderson, "The acute toxicity of some heavy metals to different species of warm water fishes", Proceedings of 19th Indian Waste Conference, Purdue University, West Lafayette, Ext, Ser. 117, 578-591, 1964.
- [52] M. Massimo, V. Milena, S. G. Scassellati, and P. Rossana, "Pesticide induced primary DNA damage in peripheral blood leucocytes of farm workers evaluated by the computerized comet assay", Biomarkers, 5, 192-204, 2000.
- [53] G. V. Vrhovae, and D. Zeljezic, "Evaluation of DNA damage in workers occupationally exposed to pesticide using SCG assay. Pesticides genotoxicity revealed by comet assay", Mut. Res., 469, 279-285, 2000.

- [54] I. Miglavs, and M. Jobling, "Effect of feeding regime on food consumption, growth rates and tissue nucleic acids in juvenile Arctic charr (*Salvenius alpinus*) with particular respect to compensatory growth", J. Fish Biol., 34, 947-957, 1989.
- [55] F. J. Bulow, "RNA-DNA ratios as indicators of growth in fish; a review" In: Summerfelt, R. C., Hall, G. E, 1987.
- [56] H. Miliou, N. Zaboukas, and M. N. Apostolopoulou, "Biochemical composition, growth and survival of the guppy, *Poecilia reticulata*, during chronic sublethal exposure to cadmium", Arch. Env. Contam. Toxicol., 35, 58-63, 1998.
- [57] M. A. Farah, B. Atteq, M. N. Ali, and W. Ahmad, "Evaluation of genotoxicity of PCP and 2,4-D by micronucleus test in freshwater fish, *Channa punctatus*". Ecotoxicol. Env. Safe., 54, 25-29, 2003.
- [58] R. D. Handy, A. N. Jha, M. H. Depledge, "Biomarker approaches for ecotoxicological biomonitoring at different levels of biological organization", In Burden, F., McKelview, I., Forstner, U., Guenther, A (eds). Handbook of Environmental Monitoring, MCGraw Hill, New York, 9.1-9.32, 2001.
- [59] C. Vasantharaja, C. Pugazhendy, M. Meenaambai, S. Prabukaran, N. A. Dar, "Properties of Cardiospermum halicacabum against the toxic effect of cypermethrin in the quantification of nucleic acids in the freshwater fish Cirrhinus mrigala (Hamilton)", Int. Res. J. Pharm., 4(1), 280-283, 2013.
- [60] B. Gowri, P. Govindassamy, and V. Ramalingam, "Influence of cypermethrin on DNA and RNA content in different organs of freshwater fish Cyprinus carpio", Iran. J. Pharma. Sci., 9(3), 1-10, 2013.
- [61] A. C. Thakur, and V. R. Kakde, "Influence of Cypermethrin on DNA, RNA and RNA/DNA ratio in muscles of the freshwater fish Channa Punctatus". Int. Interdisc. Res. J., 2, 38-42, 2012.
- [62] G. Raksheskar, "Influence of cypermethrin on DNA, RNA and RNA/DNA ratio in gills of the freshwater fish *channa striata*", Biosci. Disc., 3(1), 17-19, 2012.
- [63] B. K. Das, and S. C. Mukherjee, "Toxicity of cypermethrin in *Labeo rohita* fingerlings; biochemical, enzymatic and haematological consequences", Comp. Biochem. Physiol., 134, 109-121, 2003.
- [64] M. Sheela, and S. Muniandi, "Impact of cypermethrin on protein conversion efficiency, RNA, protein, glycogen content and protease enzyme activity in different tissues of the fish, *Lepidocephalichthyes thermalis*. Env. Ecol., 10, 829-832, 1992.
- [65] B. A. Ansari, and K. Kumar, "Cypermethrin toxicity: Effect on the carbohydrate metabolism of the Indian catfish, *Heteropneustes fossilis*". Sci. Tot. Environ., 72, 161-166, 1988.