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RESEARCH ARTICLE

ACUTE TOXICITY OF CYPERMETHRIN (25%EC) ON NUCLEIC ACIDS (DNA AND RNA) IN *CYPRINUS CARPIO* (LINN.)

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ABSTRACT

In the present study, an attempt has been made to assess the acute toxic effect of cypermethrin (25%EC) on nucleic acids in different tissues of freshwater fish. Short-term acute toxicity tests were conducted by static renewal bioassay test, using different concentrations of cypermethrin. Fishes were exposed to sub lethal concentrations (5, 10, 15 and 20 per cent of 96h LC_{50} value) of cypermethrin for three different exposure periods, 5, 10 and 15 days. Decreased tendency was observed in both DNA and RNA in all the vital tissues of test fish exposed to cypermethrin over control. DNA and RNA contents were not altered by cypermethrin at 5 day exposure period. Both the nucleic acids gradually decreased with increased exposure period and the decrease was observed to be directly proportional to increased sublethal concentrations. 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 (17.17%) in liver and minimum was (13.94%) in muscle at the longest exposure period (15 days) and highest sublethal concentration (20% 96h LC_{50}). RNA content decreased significantly in liver (29.90%), muscle (25.53%), brain (23.38%), kidney (21.82%) and gill (20.34%). The decrease was comparatively higher at 15 days exposure period at highest test concentrations. In all the organs studied, the influence of cypermethrin was found to be time and exposure period dependent.

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INTRODUCTION

Biochemical markers are measurable responses to the exposure of an organism to xenobiotic compounds as well as good biosensors of contamination in the aquatic environment. They usually respond to the mechanism of toxic activity, detect the type of toxicity and in some of them, the magnitude of their response correlates with their level of the toxicant. The use of multiple biomarkers is more advantageous than the use of a single biomarker and offers an effective early warning system in biomonitoring of aquatic environment. The biochemical markers can detect early responses and prepathological alterations before other disturbances as disease, mortality or population changes occur. Biochemicals are the most assessable body constituents in fish for checking the toxicity of any chemicals. Any alteration in biochemical parameters can result in serious outcomes in the form of various diseases in both the fish and its consumer. The development and growth of the fishes depend upon the DNA and RNA which serve as biochemical indices (Buckley, 1980). Cellular enlargement and active protein synthesis are dependent on DNA and RNA content. Pesticides induce deoxyribonucleic acid damage (Vrhovae and Zeljezic, 2000) and structural chromosomal changes. Pesticides may attack DNA directly or modify other cellular process associated with the integrity of the genome. The physico-chemical interaction of the pesticides with the cellular DNA produces a variety of primary lesions such as single strand breaks, double strand breaks, DNA protein cross-link and damage to purine and pyrimidine bases. The intactness of the DNA is the important part of the normal cellular process. The changes in DNA, RNA ratio results in eventual losses of cell structure, proliferation and formation of new tissue and tissue degradation with a total loss of cellular control mechanism (Gowri *et al.*, 2013). Changes in biochemical parameters point to the development of sub lethal abnormalities, which limit potential of an animal population in effectively coping with the normal stress and strain for survival. Considering the role of above biomarkers in the field of eco-toxicology, the present study has been undertaken to understand the biochemical alterations induced by cypermethrin (25% EC) on exposure to sublethal and lethal concentrations to fish *Cyprinus carpio* in different tissues exposed.

MATERIALS AND METHODS

The freshwater fish *Cyprinus carpio* with length 6 - 8 cm, weight 6.5 to 7.5 g, irrespective of their sex, has been chosen as

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the test organisms for present investigation. Healthy and active fish were obtained from Ratna Singh Hatcheries, Kuchipudi, Guntur (A.P), India. Fish were washed with 0.1% $KMnO_4$ solution to avoid dermal infection. The fish were acclimatized to the laboratory conditions in large plastic water tanks for three weeks at a room temperature of 28 ± 1°C. Water was renewed every day with12-12 h dark and light cycle. During the period of acclimatization, the fish were fed with groundnut oil cake and rice bran. Feeding was stopped one day prior to the acute toxicity test. All the precautions laid by committee on toxicity tests to aquatic organisms (APHA, 1998) 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.

Technical grade cypermethrin (25%EC) was obtained from United Phosphorus Ltd., Bombay. After the normal process of acclimatization, a group of ten fish each were transferred to plastic tubs (15L capacity) containing 10L of water. Fish were exposed to 4 sub-lethal concentrations i.e., 5, 10, 15 and 20 % of 96hLC_{50} (3.31µg/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 nucleic acids (DNA & RNA).

Estimation Of Nucleic Acid

The nucleic acids DNA, RNA were estimated by method of Searchy and Machnnis (1970).

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.

Estimation Of DNA

The first half or one half of the homogenate was mixed with 5ml Biphenyl amine reagent and kept aside for 20 hours after 20 hours the colour developed was read at 595 nm. The standard graph was potted with standard DNA (calf thymus) supplied by the sigma chemical company with the aforesaid method.

Estimation Of RNA

The other part of the homogenate was mixed with Dischi-Orcinol and heated at 90ºC 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 present analysis.

Students't-test was employed to calculate the significance of the differences between control and experimental means. P values of 0.05 or less were considered statistically significant (Fisher, 1950).

Table 1 Changes in the amount of Deoxy ribonucleic acid (DNA) (mg/g body weight of the tissue) in the tissues of *Cyprinus carpio* on exposure to sub lethal concentrations of cypermethrin (25%EC)

Exposure Period in Days	Tissue	Control	Concentration of Cypermethrin (% 96h LC_{50})			
			5%	10%	15%	20%
5	Gill	6.58 ± 1.18	6.51 ± 1.18 (1.06)	6.27 ± 1.25 (4.71)	6.07 ± 1.38 (7.75)	5.90 ± 1.60 (10.33)
	Muscle	2.53 ± 1.65	2.50 ± 1.94 (1.18)	2.45 ± 1.46 (3.16)	2.39 ± 1.48 (5.53)	2.31 ± 1.31 (8.69)
	Brain	5.34 ± 1.19	5.27 ± 1.22 (1.31)	5.13 ± 1.15 (3.93)	4.98 ± 1.21 (6.74)	4.82 ± 1.34 (9.73)
	Kidney	7.72 ± 1.24	7.61 ± 1.58 (1.42)	7.34 ± 1.21 (4.92)	7.10 ± 1.42 (8.03)	6.90 ± 1.17 (10.62)
	Liver	9.54 ± 1.82	9.37 ± 1.74 (1.78)	9.05 ± 1.52 (5.13)	8.74 ± 1.84 (8.38)	8.46 ± 1.37 (11.32)
10	Gill	6.56 ± 1.48	6.24 ± 1.56 (4.87)	6.03 ± 1.73 (8.07)	5.88 ± 1.98 (10.36)	5.71 ± 1.24 (12.95)
	Muscle	2.54 ± 1.65	2.44 ± 1.94 (3.93)	2.35 ± 1.46 (7.48)	2.29 ± 1.48 (9.84)	2.24 ± 1.31 (11.81)
	Brain	5.32 ± 1.19	5.09 ± 1.26 (4.32)	4.90 ± 1.35 (7.89)	4.79 ± 1.71 (9.96)	4.68 ± 1.48 (12.32)
	Kidney	7.70 ± 1.42	7.30 ± 1.78 (5.19)	7.05 ± 1.41 (8.44)	6.86 ± 1.26 (10.90)	6.64 ± 1.62 (13.76)
	Liver	9.51 ± 1.78	9.00 ± 1.49 (5.36)	8.65 ± 1.82 (9.04)	8.38 ± 1.82 (11.88)	8.14 ± 1.19 (14.40)
15	Gill	6.55 ± 1.85	6.07 ± 1.67 (7.32)	5.86 ± 1.25 (10.53)	5.72 ± 1.60 (12.67)	5.53 ± 1.31 (15.57)
	Muscle	2.51 ± 1.65	2.38 ± 1.94 (5.17)	2.28 ± 1.46 (9.16)	2.23 ± 1.48 (11.15)	2.16 ± 1.31 (13.34)
	Brain	5.33 ± 1.92	4.98 ± 1.63 (6.56)	4.80 ± 1.57 (9.94)	4.69 ± 1.19 (12.00)	4.55 ± 1.85 (14.63)
	Kidney	7.69 ± 1.65	7.10 ± 1.43 (7.67)	6.82 ± 1.22 (11.31)	6.63 ± 1.31 (13.78)	6.40 ± 1.80 (16.77)
	Liver	9.49 ± 1.85	8.68 ± 1.35 (8.53)	8.31 ± 1.49 (12.43)	8.12 ± 1.57 (14.43)	7.86 ± 1.25 (17.17)

Values are the mean of 5 observations Standard Deviation is indicated as (±) Values are significant at $p < 0.05$
Percent changes over control are given in Parenthesis

RESULTS

Calculated values for nucleic acids (DNA and RNA) along with standard deviation are given in tables 1 and 2. Percent change of nucleic Acids (DNA and RNA) in experimental fish over control is graphically represented in figures 1 and 2. The values were expressed as mg/g body weight of the tissue. Decreased tendency was observed in both DNA and RNA in all 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.

DNA

Table 1 and figure 1 represent the DNA content in the tissues of experimental fish exposed to sublethal concentrations of cypermethrin. The DNA content is comparatively low in the muscle followed by brain and muscle 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% 96h LC_{50} 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% 96h LC_{50}). 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 (17.17%) in liver and minimum was (13.34%) in muscle at the longest exposure period (15 days) and highest sublethal concentration (20% 96h LC_{50}).

RNA

Table 2 and Figure 2 shows the RNA content in the control and experimental fishes, exposed to cypermethrin under sublethal test concentrations. The control values of RNA in different tissues of *Cyprinus carpio* was in the order of Gill > Kidney > Brain > Muscle > Liver. Under sublethal exposure of cypermethrin the RNA content was found to decrease in all the tissues of the experimental fish.

Table 2 changes in the amount of Ribonucleic acid (RNA) (mg/g body weight of the tissue) in the tissues of *Cyprinus carpio* on exposure to sub lethal concentrations of cypermethrin (25%EC)

Exposure period in Days	Tissue	Control	Concentration of Cypermethrin (% 96h LC_{50})			
			5%	10%	15%	20%
5	Gill	4.12 ± 0.121	4.02 ± 0.125 (2.42)	3.85 ± 0.225 (6.55)	3.66 ± 0.211 (11.16)	3.52 ± 0.152 (14.56)
	Muscle	7.47 ± 0.236	6.85 ± 0.173 (3.48)	6.54 ± 0.140 (12.44)	6.23 ± 0.181 (16.59)	6.01 ± 0.146 (19.54)
	Brain	6.04 ± 0.019	5.58 ± 0.141 (3.97)	5.39 ± 0.167 (10.76)	5.25 ± 0.184 (13.07)	5.07 ± 0.141 (16.05)
	Liver	8.58 ± 0.324	7.69 ± 0.216 (4.31)	7.32 ± 0.321 (14.68)	7.01 ± 0.211 (18.29)	6.67 ± 0.217 (22.26)
	Kidney	5.29 ± 0.145	5.03 ± 0.173 (3.59)	4.85 ± 0.229 (8.31)	4.63 ± 0.120 (12.47)	4.46 ± 0.132 (15.68)
10	Gill	4.08 ± 0.152	3.82 ± 0.225 (6.37)	3.69 ± 0.211 (9.55)	3.58 ± 0.152 (12.25)	3.40 ± 0.154 (15.68)
	Muscle	7.45 ± 0.157	6.50 ± 0.157 (12.75)	6.21 ± 0.138 (16.64)	6.00 ± 0.172 (19.46)	5.75 ± 0.140 (22.81)
	Brain	6.03 ± 0.131	5.34 ± 0.167 (11.44)	5.20 ± 0.184 (13.76)	4.98 ± 0.141 (17.41)	4.79 ± 0.141 (20.56)
	Liver	8.56 ± 0.214	7.28 ± 0.167 (14.95)	6.94 ± 0.228 (18.92)	6.61 ± 0.214 (22.78)	6.33 ± 0.141 (26.05)
	Kidney	5.27 ± 0.173	4.78 ± 0.229 (9.29)	4.60 ± 0.120 (12.71)	4.46 ± 0.132 (15.37)	4.27 ± 0.132 (18.97)
15	Gill	4.08 ± 0.158	3.62 ± 0.211 (11.27)	3.51 ± 0.152 (13.97)	3.38 ± 0.154 (17.15)	3.25 ± 0.219 (20.34)
	Muscle	7.44 ± 0.217	6.18 ± 0.138 (16.93)	5.97 ± 0.172 (19.75)	5.79 ± 0.140 (22.17)	5.54 ± 0.140 (25.53)
	Brain	6.03 ± 0.165	5.25 ± 0.184 (12.93)	5.00 ± 0.141 (17.08)	4.81 ± 0.138 (20.32)	4.62 ± 0.174 (23.38)
	Liver	8.56 ± 0.291	6.99 ± 0.172 (18.34)	6.52 ± 0.125 (23.83)	6.27 ± 0.214 (26.75)	6.00 ± 0.188 (29.90)
	Kidney	5.27 ± 0.134	4.63 ± 0.120 (12.14)	4.46 ± 0.134 (15.37)	4.27 ± 0.162 (18.97)	4.12 ± 0.194 (21.82)

Values are the mean of 5 observations Standard Deviation is indicated as (±) Values are significant at $p < 0.05$ Percent changes over control are given in Parenthesis

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 (29.90%), muscle (25.53%), brain (23.38%), kidney (21.82%) and gill (20.34%). The decrease was comparatively higher at 15 days exposure period at highest test concentrations.

DISCUSSION

The nucleic acids play a major role in all biological activities and are regulators of all biological synthesis of proteins which are structural and functional units of the biological systems. The decreased DNA content might have been caused by the direct action of toxicant. It is known that DNA functions as a primer in DNA and RNA polymerase reactions (Haqqi and Adhami 1979), and the inhibition in DNA content can result in

the inhibition of both DNA and RNA synthesis. Any alteration in nucleic acid content leads to variations in protein profile (Durai Raj and Selvarajan, 1992; Abou Donia *et al*., 1988).

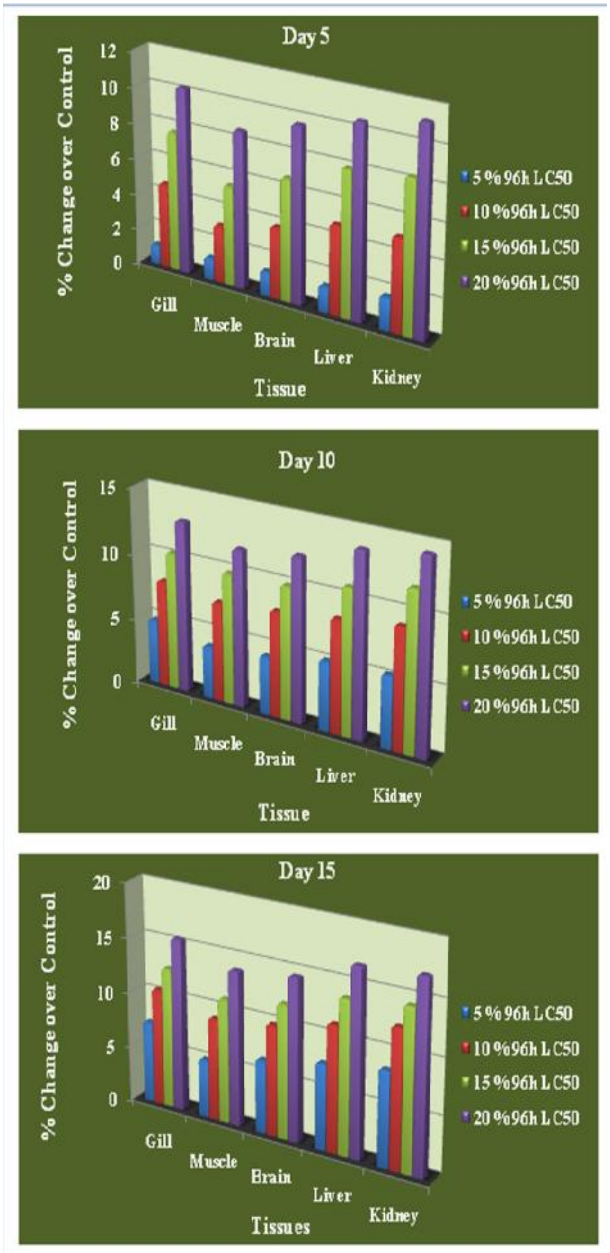

Figure 1 Percent change over control in the amount of DNA (mg/g body weight of the tissue) in the tissues of *Cyprinus carpio* on exposure to sub lethal concentrations of cypermethrin (25%EC)

In the present study the decrease in the DNA and RNA content of the tissues of the test fish exposed to cypermethrin may be due to inhibition of the enzymes in DNA synthesis. All the enzyme activities are controlled by the process of transcription. When the transcription process is curtailed, no mRNA and no protein synthesis occur. As a result, metabolism is impaired.

The synthesis of RNA plays an important role in protein synthesis. The inhibition of RNA synthesis at transcription level may affect the protein content (Singh *et al*., 2010). Significant decrease of RNA observed in the presen study might have caused protein depletion in these organs. Pesticides may influence DNA directly or modify other cellular process associated with the integrity of the genome. The physio-chemical interaction of the pesticides with the cellular DNA produces a variety of primary lesions such as single strand breaks, double strand breaks, DNA protein cross-link and damage to purine and pyrimidine bases (Van Loon *et al*., 1991).

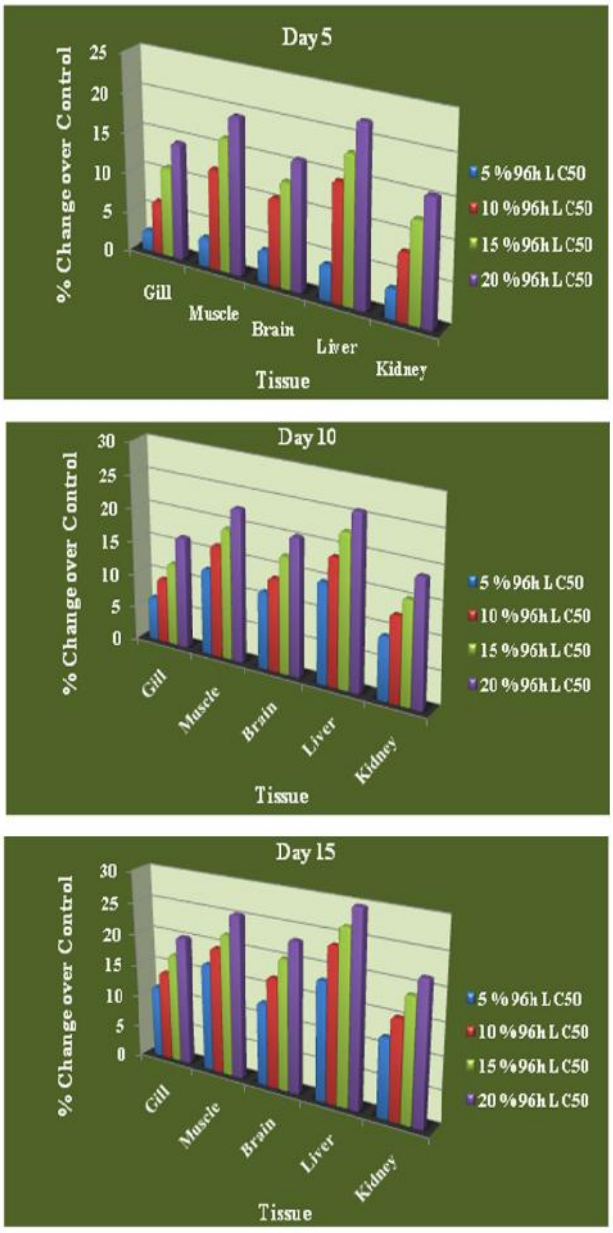

Figure 2 Percent change over control in the amount of RNA (mg/g body weight of the tissue) in the tissues of *Cyprinus carpio* on exposure to sub lethal concentrations of cypermethrin (25%EC)

Significant reduction in RNA and DNA content in different organs of cypermethrin exposed fish in the present investigation may be due to decrease in protein synthesis, defective nucleic acid metabolism and also degradation of cells. Similar results were also reported by various investigations: Gowri *et al*., 2013 (*Cyprinus carpio*); Veeraiah *et al*., 2013 (*Cirrhinus mrigala*); Thakur and Kakde, 2012 (*Channa punctatus*); Tiwari *et al*., 2012 (*Labeo rohita*); Singh *et al*., 2010 (*Colisa lalia*); Vasantharaja *et al*., 2013 (*Cirrhinus mrigala*); Raksheskar, 2012 (*Channa striatus*); Kumar *et al*., 2007 (*Channa punctatus*); Ansari and Kumar, 1998 (*Cyprinus*

carpio); Sheela and Muniandi, 1992 (*Lepidocephalichthyes thermalis*) which substantiate the results of this study suggesting that cypermethrin is a potent inhibitor of nucleic acid synthesis even at sub lethal concentrations.

Gowri *et al*. (2013) studied the effect of sublethal concentrations cypermethrin for 7, 14 and 21 days in *Cyprinus carpio* in which DNA and RNA content decreased at 14 and 21 day significantly in brain, gills and liver. However, the decrease was more in 21 day. The reduction in the DNA and RNA content in cypermethrin exposed fishes is comparatively less in liver when compared to the brain and gills. Thakur and Kakde (2012) reported DNA, RNA and RNA/DNA ratio in muscle of *Channa punctatus* under the influence of sub lethal concentrations of cypermethrin (0.00078µl/l) for 24, 48, 72 and 96h and observed a decreased trend in DNA, RNA levels. While the RNA /DNA ratio significantly changed respectively at different periods. Tiwari *et al*. (2012) 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) reduction in nucleic acids (DNA and RNA) in fingerlings of *Labeo rohita* in both liver and muscle tissues.

Singh *et al*. (2010) reported toxicological and biochemical alterations of cypermethrin against *Colisa fasciatus* at different Seasons. Cypermethrin after 96h exposure significantly altered the levels of nucleic acids (DNA and RNA) in time and dose dependent manner. Kumar *et al*., 2007 observed increasing effect on DNA and RNA profile of gill, brain, liver and kidney of *Channa punctatus* treated with cypermethrin (40-60µg/l). Das and Mukherjee (2003) in *Labeo rohita* observed sublethal exposure to cypermethrin (0.014ppm for 96h) induced changes in DNA, RNA in muscle, liver, brain and kidney under and reported an increase in both the nucleic acids. Ansari and Kumar (1988) reported a significant decline in the DNA and RNA content of the liver tissue of zebra fish, *Brachydani orerio* by the exposure to cypermethrin. *Lepidocephalicthys thermalis* exposed to cypermethrin also showed a decline in the RNA content of the liver and muscle tissues (Sheela and Muniandi, 1992).

CONCLUSION

From the present study, it can be concluded that the sublethal exposure of cypermethrin can alter the nucleic acid content in vital tissues of the fish. Variations in nucleic acid content in tissues serve as indices in monitoring the pathological status of the pesticide treated fish. These variations were found to be tissue specific and hence, can be used as meaningful indicators of pesticide pollution. The above said parameters can be examined more critically to develop more meaningful indicators or markers to assess or to characterize a particular pollutant and its potential toxicity.

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