BIOCHEMICAL ALTERATIONS INDUCED BY PHOSALONE (35%) EC IN DIFFERENT TISSUES OF FISH - *CTENOPHARYNGODON IDELLA*

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ABSTRACT

The nucleic acids (DNA and RNA) are informational molecules, set of directions by which they can duplicate themselves and guide the synthesis of proteins. The synthesis of proteins-most of which are enzymes-ultimately governs the metabolic activities of the cell (Lodish *et al.,* 2004). The stress induced biochemical changes are described as secondary responses of the fish. The biochemical analysis of DNA, RNA and proteins are considered as markers in the toxicity study. Inhibition of DNA synthesis, thus might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesis machinery. Pesticides are potential inhibitor of DNA synthesis, which might result in reduction of RNA level. The first half or one half of homogenate was mixed with diphenylamine reagent and kept aside for 20 hrs. After 20 hrs 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 homogenate was mixed with Dischi-orcinol and heated at 90^0C for 15 minutes. After cooling at room temp., the colour developed was read at 655 nm. The standard graph was plotted with standard RNA (Baker's yeast) supplied by Sigma chemicals company. The results in 4 and 8 days sublethal exposure of phosalone Table V.3 and Figure V.3 indicates heterogeneous levels of DNA in the tissue of liver, brain, muscle, gill and kidney. Under lethal and sublethal exposure to Phosalone for 24hr, the percentage of depletion was found in all the tissues of test fish, maximum percentage of depletion was in Gill (+26.17) and (-29.68), minimum depletion in liver(+1.42) and (-21.99) in brain. Under sublethal exposure to Phosalone for 4^{th} and 8^{th} days, the glycogen levels was found to decreased all the tissues of test fish *Ctenopharyngodon idella* and maximum depletion was in muscle (-43.20) and (-86.03), minimum decrease was observed in kidney (-0.47) and(- 40.23) in gill.

Keywords: nucleic acids, DNA, RNA, Ctenopharyngodon idella, Phosalone.

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1.INTRODUCTION:

Pesticides are useful tools in agriculture, but the gradual degrading of aquatic ecosystem. In addition to agriculture practices together with pest control programmes the surface runoff and aerial spraying forming the major source for translocation pesticides into aquatic ecosystems (Joseph and Raj *et al.,* 2011). The Improper management of pesticides in agriculture crops could result in contamination of water bodies (Candida Toni *et al.,* 2011; Capkin and Altinok, 2013). When pesticide reaches the aquatic environment, it may be present there for several days or weeks, depending upon its solubility, producing of mass mortality, morphological, physiological and behavioral changes in the organisms.

Analysis of Biochemical parameters helps to identify the target organs of toxicity and the general health status of animals Folmar(1993) In India, pesticides constitute an important component in agriculture development and protection of public health since the tropical climate is very conducive to pest breeding. Kumar and Prasad(2010) Contamination by pesticides in aquatic ecosystem is a serious problem and fishes are more frequently exposed to these pollutants and may be taken in through gills, skin and contaminated foods Ling and Zhang (2011). Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems Farkas and Salanki (2002). Although toxicant impairs the metabolic, physiological and histological analysis in vital tissues, Furthermore, pollutants may get into water in combination with each other, causing additive harmful effects on the fish, (Palmeira, *et al.,* (1994); Fatma, *et al.,* (2008). Food intake is one of the most important factors regulating the level of metabolism. Detection of derangements in metabolism is most sensitive parameters of stress; it integrates many processes, including enzyme activity and modulation, substrate pools and physiological response (Soucek, 2007). Fish experiencing acute exposures to sub lethal concentrations of pesticide exhibit significant feeding impairment, with potentially serve consequences for their ecological fitness**.** Some biochemical parameters also represent fine tools for evaluating the effects of contaminants and for environmental monitoring (Ahmad *et al.,* 2004). The cumulative toxicological impacts of pesticide mixtures are of particular concern for disorders the metabolic activity, alters physiological state and histological changes thereby changing the biochemical constituents of fish, Khan and Francis (2005).

The nucleic acids (DNA and RNA) are informational molecules, set of directions by which they can duplicate themselves and guide the synthesis of proteins. The synthesis of proteins-most of which are enzymes-ultimately governs the metabolic activities of the cell (Lodish *et al.,* 2004). The stress induced biochemical changes are described as secondary responses of the fish. According to Tilak *et al.,* (2009), the biochemical analysis of DNA, RNA and proteins are considered as markers in the toxicity study. Inhibition of DNA synthesis, thus might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesis machinery. Pesticides are potential inhibitor of DNA synthesis, which might result in reduction of RNA level.

2.MATERIALS AND METHODS:

Biochemical analysis of biochemical components, Fish is important to estimate the physiological health of fish along with estimation of water pollution, discussed about the methods and materials to analysis the biochemical components of fish.

Fish *Ctenopharyngodon idella* of size 6±7 cm and 6±8 g weight were brought from a local fish farm kuchipudi, Guntur district of Andhra Pradesh, India and acclimatized at 28± 2^0 C in the laboratory for 15 days. Such acclimatized fish were exposed to sub lethal($1/10^{th}$ 96 hr LC_{50}) and lethal (96 hr LC_{50}) concentrations of Phosalone for 24 hr lethal and sub lethal, 4 day 8 days. The vital tissues like muscle, brain, liver, gill and kidney of the fish were taken for the estimation of total Glycogen, Proteins, Nucleic acids (DNA& RNA), Aspartate aminotransferase (SGOT/AAT), Alanine aminotransferase (SGPT/ALAT), lactate dehydrogenase (LDH) activity, Estimation of Succinate dehydrogenase (SDH) activity, Malate dehydrogenase (MDH) activity, Acid phosphatise (ACP), and Acetyl chlorine esterase (Ach.E) activity along with control exposures.

Estimation of Nucleic acids:

The nucleic acids, Deoxyribose (DNA) and Ribose (RNA) were estimated by the method of Searchy and Maclinnis 1970 (a&b). 5% homogenates of gill, brain, muscle, liver, and kidney were prepared in 5ml of 0.5 N perchloric acid(PCA) and heated at 90°C for 20 minutes. After cooling, the tissue homogenates were centrifuged at 3000 rpm for 10 minutes. The supernatant was separated into two volumes and used for DNA and RNA analysis.

DNA:
The first half or one half of homogenate was mixed with diphenylamine reagent and kept aside for 20 hrs. After 20 hrs 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.

RNA:
The other part of homogenate was mixed with Dischi-orcinol and heated at 90^0C for 15 minutes. After cooling at room temp., the colour developed was read at 655 nm.The standard graph was plotted with standard RNA (Baker's yeast) supplied by Sigma chemicals company.

Statistical analysis:
Student's t-test and one way analysis of variance (ANOVA) of SPSS (20.0 version), SPSS Chicago, USA, was employed to calculated the significance of the differences between control and experimental means. P values of 0.05 or greater than were considered statistically significant (Fisher, 1950). Significance level was based on $P<0.05$. Results were expressed as ±S.D of five individual**.**

3.RESULT AND DISCUSSION:
Biochemical biomarkers can provide information about the detoxification process of pesticides in living organisms. When it can content with an organisms, the toxic agent or substance can be transformed by enzymes, which act to make the xenobiotics substance or toxic compounds a less toxic compound and facilitate its excretion (Santhosh and Martinez, 2012).

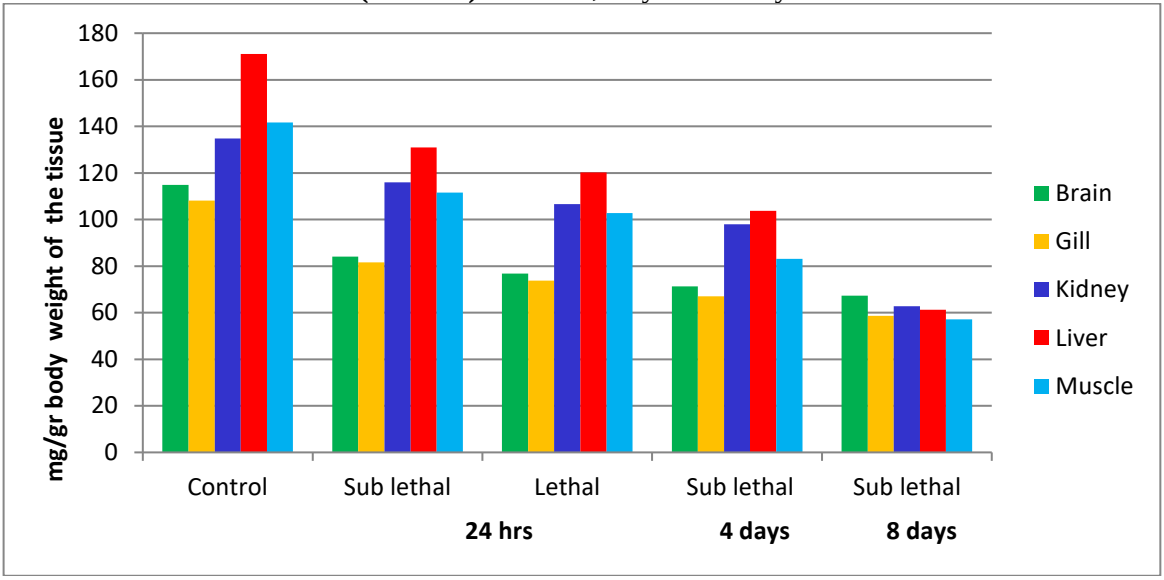
Nucleic acids DNA:
The calculated values for Nucleic acids along with percent over controls and standard deviations (SD) are given in TableV.3 and FigureV.3. In the tissues of control fish, *Ctenopharyngodon idella* DNA content was in the order of:
Liver> Muscle > Brain > Kidney >Gil
Under exposure to sublethal and lethal concentrations of Phosalone 35 % EC for 24hr, the present depletion of DNA content in the test tissues of *Ctenopharyngodon idella* was in the order of:
Phosalone sublethal 24hr: Liver> Muscle > Brain > Kidney >Gill
Phosalone lethal 24hr: Liver> Muscle > Brain > Kidney >Gill
Under exposure to sublethal concentrations of Phosalone 35 % ECfor 4days and 8days, the percent change depletion of DNA content in the test tissues of *Ctenopharyngodon idella* was in the order of:
Phosalone sub lethal 4 days: Liver>Kidney> Brain> Gill> Muscle
Phosalone sublethal 8 days: Liver > Kidney > Brain > Gill > Muscle
The results in 4 and 8 days sublethal exposure of phosalone Table V.3 and Figure V.3 indicates heterogeneous levels of DNA in the tissue of liver, brain, muscle, gill and kidney. Under lethal and sublethal exposure to Phosalone for 24hr, the percentage of depletion was found in all the tissues of test fish, maximum percentage of depletion was in Gill (+26.17) and (-29.68), minimum depletion in liver(+1.42) and (-21.99) in brain. Under sublethal exposure to Phosalone for 4^{th} and 8^{th} days, the glycogen levels was found to decreased all the tissues of test fish *Ctenopharyngodon idella* and maximum depletion was in muscle (-43.20) and (-86.03), minimum decrease was observed in kidney (-0.47) and(- 40.23) in gill.

Table:1. Changes in the DNA (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish,*Ctenopharyngodon idella* exposed to sublethal and lethal concentrations of **Phosalone (35%EC)** for 24hrs,4days and 8 days:

Tissues	Control	24hrs Sub lethal	% change	24hrs Lethal	% change	4days Sub lethal	% Change	8days Sub lethal	% Change
Brain	9.64± 0.38	7.52± 0.28	-21.99	10.62± 0.52	+10.16	6.92± 0.79	-28.21	3.4± 0.16	-64.73
Gill	5.12± 0.26	3.6± 0.32	-29.68	6.46± 0.52	+26.17	6.36± 0.55	+24.21	3.06± 0.20	-40.23
Kidney	8.5± 0.16	6.46± 0.35	-24.01	9.22± 0.34	+8.47	8.46± 0.30	-0.47	4.24± 0.38	-50.11
Liver	12.64± 0.38	9.7± 0.32	-23.25	12.82± 0.39	+1.42	9.5± 0.29	-24.84	5.7± 0.52	-54.90
Muscle	10.6± 0.31	8.2± 0.16	-22.64	11.3± 0.56	+6.61	6.02± 0.79	-43.20	1.48± 0.28	-86.03

Values are the mean of five observations ;(±) indicates the standard deviation:
Values are significantly at $P< 0.05$

Fig:1. Changes in the DNA (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish, *Ctenopharyngodon idella* exposed to sublethal and lethal concentrations of **Phosalone (35%EC)** for 24hrs,4days and 8 days:



The nucleic acids play a major role in all biological activities and are regulators of all biological 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. Pesticide appears as a potential inhibitor of DNA synthesis, which might result in reduction of RNA level. Because of electrophilic nature, the organophosphate (OP) compounds may attack many enzymes responsible for normal metabolic pathway Tripathi, and Verma, (2004).

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 crosslink 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). Furthermore, inhibition of DNA synthesis, thus, might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesis machinery. The regulatory roles of nucleic acid metabolism as observed in the different animals when treated with the different pesticides were reported earlier Das and Mukherjee, (2003), Asif Zaidi, *et al.*, (1990).

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 Seljezic, 2000) and structural chromosomal changes. Pesticides may attack DNA directly or modify other cellular process associated with the integrity of the genome.

Gautam *et al.*, (2002) reported the histo-chemical observations on nucleic acids (RNA and DNA) in the stomach and intestine of *Channa punctatus* (Bloch) after the treatment with endosulfan and diazinon pesticides, and significant decrease in nucleic acides of gastrointestine tract was reported. However, the decrease in nucleic acides content after diazinon treatment was not significant. The treatment with endosulfan in mucosa and submucosal tissues show very little impact on nucleic acid content, and in diazinon treatment the DNA was completely decreased. The effect of fenvalerate on DNA content of the gill but not on DNA content of kidney may be due to efficient uptake of the toxicant across the gill. This tissue specific difference may be further assigned to the differential effects of fenvalerate or its metabolites on the synthesis or degradation of DNA in gill and kidney cells of the fish (Bradbury *et al.*, 1987).

The stress induced biochemical changes were described as secondary responses of the fish. According to Abou Donia *et al.*, (1998), the

biochemical analysis of DNA, RNA and protein are considered as markers in the toxicity study (Tilak *et al.*, 2009). Inhibition of DNA synthesis, thus might affect both protein as well as amino acid levels by decreasing the level of RNA in Protein machinery. Pesticides are potential inhibitor of DNA synthesis, which might result in reduction of DNA level. Because of electrophonic nature, the carbamate compounds may attack many enzymes responsible for normal metabolic pathway. Thus, it is possible that the enzyme is necessary for DNA synthesis might have been inhibited by bothe toxicants. On compilation of the result, it appears that the disruption of DNA synthesis might have affected RNA synthesis and consequently protein synthesis (Tripathy and Singh, 2003; Ravikiran *et al.*, 2012).

The effects of sublethal concentration of Fenvalerate on DNA and RNA, the RNA/DNA ration and protein contents were estimated in gill and kidney tissues of an air breathing fish, *Clariasbatrachus*. Fenvalerate reduced the DNA content in gills, whereas it dose not produce any significant effect of Fenvalerate or its metabolit(s) on synthesis and/degradation of DNA in gill and kidney cells of the fish Tripathi, *et al.*, (2002). The reason for decreased nucleic acids levels in liver under the influence of carbosulfan treatment in mice might caused genotoxic action by decreased mitotic index and disturbed cell division (Topktas, *et al.*, (1996); Tripathi, *et al.*, (2003), also reported that fish exposed to Dimethoate (organophosphate) exhibited a decrease in nucleic acid (DNA and RNA) content.

RNA:

The calculated values forNucleic acids along with percent over controls and standard deviations (SD) are given in Table V.4 and Figure V.4 In the tissues of control fish,*Ctenopharyngodon idella* RNA content was in the order of:

Liver> Gill > Brain > Kidney > Muscle

Under exposure to sublethal and lethal concentrations of Phosalone 35 % EC for 24hr, the present depletion of RNA content in the test tissues of *Ctenopharyngodon idella* was in the order of:

Phosalone sublethal 24hr: Liver> Brain > Kidney > Gill > Muscle

Phosalone lethal 24hr: Liver> Gill > Brain > Kidney > Muscle

Under exposure to sublethal concentrations of Phosalone 35 % ECfor 4days and 8days, the percent depletion of RNA content in the test tissues of *Ctenopharyngodon idella* was in the order of:

Phosalone sub lethal 4 days: Liver> Gill > Brain > Kidney > Muscle

Phosalone sublethal 8 days: Liver > Brain > Gill > Muscle > Kidney

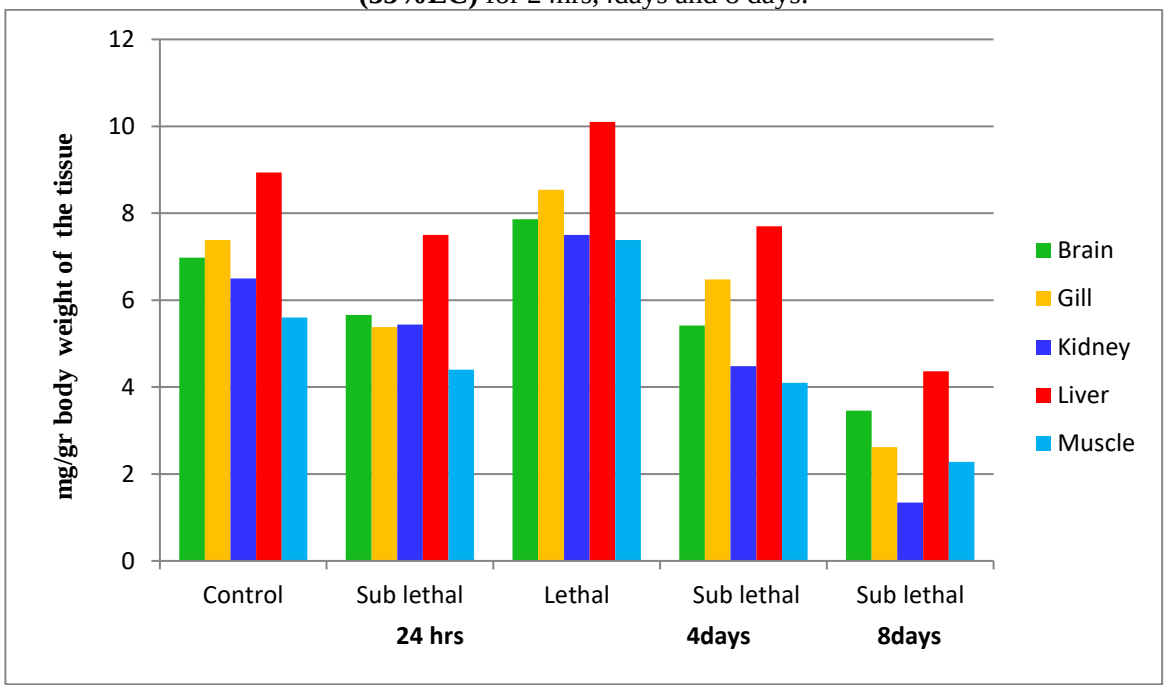
The results in 4 and 8 days sublethal exposure of phosalone TableV.4 and .Figure. indicates heterogeneous levels of RNA in the tissue of liver, brain, muscle, gill and kidney. Under lethal and sublethal exposure to Phosalone for 24hr, the percentage of depletion was found in all the tissues of test fish, maximum percentage of depletion was in muscle (+31.78) and (-27.10) in gill, minimum depletion in brain(+12.61) and (-16.11) in liver.Under sublethal exposure to Phosalone for 4th and 8th days, the glycogen levels was found to decrease in all the tissues of test fish *Ctenopharyngodon idella* and maximum depletion was in kidney (-31.07) and (-79.38), minimum decrease was observed in gill (-12.19) and(- 50.42) in brain.

Table.2: Changes in the RNA (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish, *Ctenopharyngodon idella* exposed to sublethal and lethal concentrations of **Phosalone (35%EC)** for 24hrs,4days and 8 days:

Tissues	Control	24hrs Sub lethal	% change	24hrs Lethal	% Change	4days Sub lethal	% change	8days Sub lethal	% Change
Brain	6.98± 0.54	5.66± 0.48	-18.91	7.86± 0.73	+12.61	5.42± 0.33	-22.34	3.46± 0.40	-50.42
Gill	7.38± 0.19	5.38± 0.42	-27.10	8.54± 0.29	+15.71	6.48± 0.28	-12.19	2.62± 0.19	-64.49
Kidney	6.5± 0.32	5.44± 0.29	-16.31	7.5± 0.50	+15.38	4.48± 0.28	-31.07	1.34± 0.51	-79.38
Liver	8.94± 0.20	7.5± 0.34	-16.11	10.1± 0.60	+12.97	7.7± 0.38	-13.87	4.36± 0.42	-51.23
Muscle	5.6± 0.54	4.4± 0.35	-21.42	7.38± 0.82	+31.78	4.1± 0.37	-26.78	2.28± 0.32	-59.28

Values are the mean of five observations ;(±) indicates the standard deviation:
Values are significantly at $P< 0.05$

Fig.2: Changes in the RNA (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish, *Ctenopharyngodon idella* exposed to sublethal and lethal concentrations of **Phosalone (35%EC)** for 24hrs,4days and 8 days:



Toxicants that cause genetic effects may be present at very low, sub-lethal concentrations Anderson *et al.,* (1994). The animal requires more energy to overcome the stress upon exposure to the pesticides. As a result, the animal prefers glucognesis to protein synthesis and diverts all the metabolites for carbohydrate biosynthesis. Tripathi, *et al.,* (2003), reported that significant declines in RNA level were observed in treated fish might also be any obstruction in RNA level were observed in treated fish might also be any obstruction in RNA synthesis. The decrease in RNA may be suggested that the daily addition of pesticides results in the swelling and chromatolysis of Nissle bodies which are rich in RNA. RNA plays significant role in prkotein synthesis hence depletion in RNA contents also results in depletion in protein level Tripathi, *et al.,*(2002).

Hence, there is decrease in RNA level thus reducing protein synthesis. Maruthanayagam and Sharmila, (2004) reported similar results.Thenomozhi *et al.,* (2011) reported that decreased nucleic acids (DNA and RNA) content liver, muscle, gill tissues of freshwater fish *Labeo rohita* treated with malathion, decline in nucleic acid content due to decreased protein synthesis and damage to liver, which is the major tissue for detoxification mechanism. Similar findings were observed by Kumar *et al.,* (2007), the DNA and RNA content was increased in gill, liver, brain and kidney of fish *Channa punatatus* exposed to different concentrations of cypermethrin and L-cyhalothrin. The decline DNA content could be due to the disturbance in the normal DNA synthesis. Increase in RNA content of gill was reported by Gracy and Rajasekar (2012).

Akhtar *et al.,* (2012), reported that there is no significant changes in DNA levels in liver and muscle but RNA level were significantly increased in liver and muscle tissues of *L.rohita* treated with dietary pyridoxine.Thus, from the present investigation, it can be concluded that the marked decrease in DNA and RNA content upon exposure to pesticides may be due to decrease in protein synthesis, impairment of nucleic acid metabolism, the degradation of cells, resulting in the reduction in the DNA and RNA content. Thus, it is possible that these pesticides will have inhibited the enzyme necessary for DNA synthesis. On compilation of the results, it appears that the disruption of DNA synthesis might have affected RNA synthesis.

4.CONCLUSION

The nucleic acids play a major role in all biological activities and are regulators of all biological 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. Pesticide appears as a potential inhibitor of DNA synthesis, which might result in reduction of RNA level.

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