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## IMPACT OF PYRACLOSTROBIN (20%WG) ON DNA AND RNA CONTENT OF THE FRESHWATER FISH CTENOPHARYNGODON IDELLA

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### ABSTRACT:

Contaminated food and water, either directly or indirectly, can lead to fish kills, reduce the fish productivity elevated concentrations of disagreeable, chemicals in edible fish tissue which can affect the health of humans consuming these fish. Lethal concentrations of Pyraclostrobin (20% WG) (24, 48, 72, 96 hrs of 7.2, 6.8, 6.4, 6.0mg/L) along with the controls. The 96hr LC<sub>50</sub> *Ctenopharyngodon idella* exposed of Pyraclostrobin found in 6.0mg/Lt. for sub-lethal concentrations 1/10 of lethal concentration of Pyraclostrobin was taken 4 and 8 days it was found that the tissues gill, liver, muscle, brain and kidney were isolated for the estimation of DNA & RNA content it was decreased in Fresh water fish *Ctenopharyngodon idella* by Pyraclostrobin (20% WG). The results were observed in the present study reveals that Pyraclostrobin caused variability in the nucleic acid content in different tissues and the grade of variability depends on Pyraclostrobin (20% WG). The decreasing order of DNA content in different tissues in the order of: Gill > Liver > Muscle > Brain > Kidney and RNA

**Key words:** DNA, RNA, Pyraclostrobin (20% WG), *Ctenopharyngodon idella*, and sub-lethal concentrations.

## INTRODUCTION

The aquatic environment is subject to an increasing range of man-made (anthropogenic or xenobiotic) pollutants, reflecting the ever more rapid innovations of our technology to manufacture goods to satisfy a perceived increase in consumer demand on which is our economy based. Some of the pollutants that are now present in the tissues of fish, wild life and humans also reflect past usage of chemicals, such as the organochlorine insecticides and PCBs, which have been banned or restricted in use for several decades. Measurements of tissue concentrations are, however, overwhelmingly limited to a range of pollutants such as pesticides, polyaromatic hydrocarbons (PAHs) and PCBs that are known to present in the aquatic environment and for which measurement methods do exist. (R. E. Hester, R. M. Harrison and David E. Kime, 1999). The natural physiological functioning of an organism gets disturbed on exposure to toxicant stress. It induces its effect first at cellular or even at molecular level, but ultimately causes physiological, pathological and biochemical alterations. It is, therefore necessary to focus attention on changes in biochemical composition of organisms, which are constantly under pollutant threat. When the pesticides come in contact with internal organs, irreversible changes in metabolic activities take place that eventually cause biochemical changes. Pesticide pollutants act as stress inducing agents, which affect the functional state of tissues of the exposed organisms, all pollutants are not toxic but all pesticides are toxicants. Many pesticides have been reported to produce a number of biochemical changes in fish both at lethal and more often at sublethal levels. Changes in ion concentrations, organic constituents, enzyme activity, endocrinal activity and chemo regulators in fish have been attributed to pesticides. Since aquatic environment is the ultimate sink for all pollutants, aquatic toxicity testing has become an integral part of the process of environmental hazard evaluation of the toxic chemicals. The objective of the present study to investigate the toxicity of Pyraclostrobin 20%(WG) on DNA and RNA levels of freshwater fish *Ctenopharyngodon idella*.

## MATERIAL AND METHODS:

Fish *Ctenopharyngodon idella* of size  $6 \pm 7$  cm and  $6.5 \pm 2$  g weight were brought from a local fish farm Kuchipudi, Guntur District of Andhra Pradesh, India and acclimatized at  $28 \pm 2^\circ\text{C}$  in the laboratory for 15 days. Such acclimatized fish were exposed to sublethal and lethal concentrations of Pyraclostrobin (20% WG) commercial grade for 24h, 4 and 8 days. Fish were washed with 0.1% KMnO<sub>4</sub> solution to avoid dermal infection. All the precautions laid down

by APHA *et al.*, (1998) are followed, for maintaining the fish. Pyraclostrobin (20%WG) (24, 48, 72, & 96 hrs of  $LC_{50}$  were 7.2, 6.8, 6.4, and 6.0mg/L) along with the controls. Mortality occurred during the experimental period, dead fish were removed immediately to avoid depletion of dissolved oxygen (DO) level which adversely affects other fish (Schreck and Brouna, 1975). The vital tissues like muscle, brain, liver, and kidney of the fish were taken for the estimation of Nucleic acids (DNA&RNA).

### V.3.3. NUCLEIC ACIDS

#### V.3.3. DNA

DNA content in control fish *Ctenopharyngodon idella* in different tissues was in the order Under exposure to sub-lethal concentrations of Pyraclostrobin for 4 and 8 days it was found that the gill, liver, muscle, brain and kidney DNA content was decreased. The decreasing order of DNA content in different tissues in the order of: Gill > Liver > Muscle > Brain > Kidney

The calculated values of nucleic acids along with standard deviation and the cent per change over the control were given in Table.V.3.3.5; Table.V.3.3.6 and Fig.V.3.3.5; Fig.V.3.3.6 the of: Muscle > Gill > Brain > Liver > kidney

Under exposure to sub-lethal and lethal concentrations of Pyraclostrobin for 24 h, the DNA content decrease in gill, liver, muscle, brain and kidney. The decreasing order of DNA content in different tissues is in the order of: 24 h Sub-lethal: Gill > liver > Muscle > Brain > Kidney.

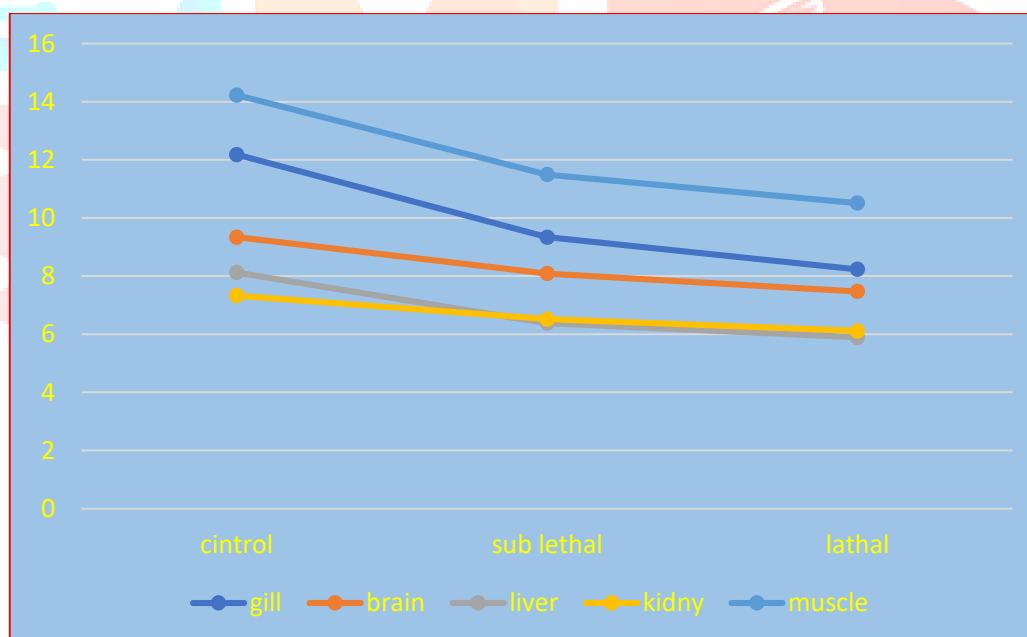
24 h lethal : Gill > liver > Muscle > Brain > Kidney.

**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 Pyraclostrobin (20% WG) for 24 h.

Tissues	Control (mg/g)	Sub-lethal (mg/g)	% Change (mg/g)	Lethal (mg/g)	% Change (mg/g)
Gill	12.18 ±0.011	9.34 ±0.018	23.31	8.23 ±0.10	32.43
Brain	9.34 ±0.014	8.09 ±0.022	13.38	7.47 ±0.023	20.02
Liver	8.12 ±0.023	6.38 ±0.019	21.42	5.89 ±0.043	27.46
Kidney	7.33 ±0.042	6.52 ±0.038	11.05	6.11 ±0.032	16.64
Muscle	14.23 ±0.065	11.49 ±0.010	19.25	10.51 ±0.021	26.14

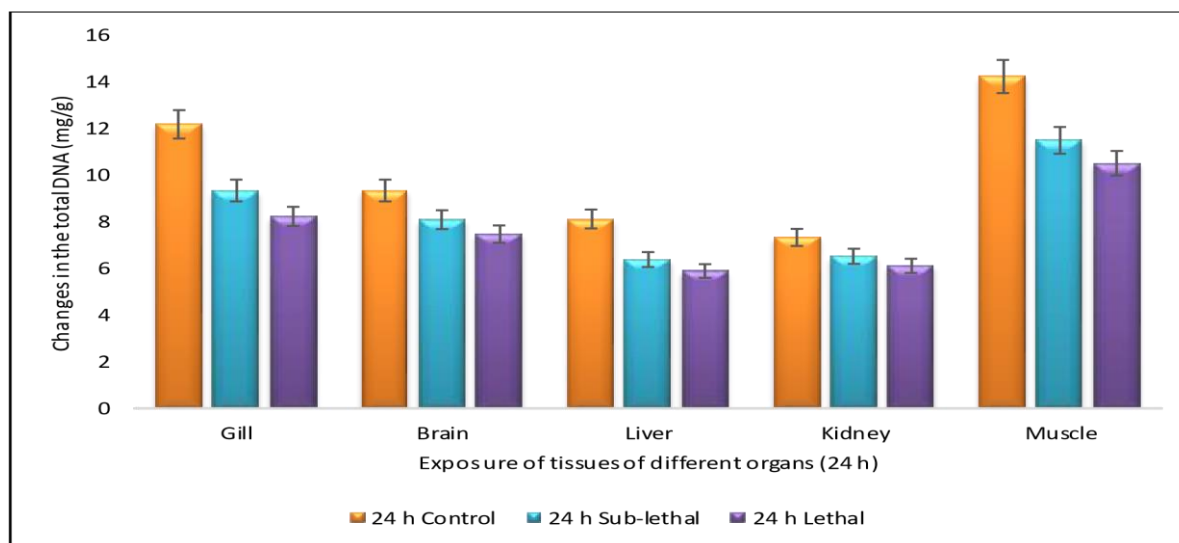
Values are the mean of five observations ;(±) indicates the standard deviation:

Values are significantly at  $P < 0.05$



**Fig: 1** Change in the Amount of DNA (mg/gr Body Wet Weight of the Tissue) and % Change over the Control in Different Tissues of Fish *Ctenopharyngodon idella* Exposed to Sublethal and Lethal Concentrations of Pyraclostrobin , Mean± S.E,n=5,P < 0.05

**Fig.2** Changes in the DNA (mg/g wet weight of the tissue) and per cent change over the control, in different tissue of the freshwater fish, *Ctenopharyngodon idella* exposed to sublethal and lethal concentrations of Pyraclostrobin (20% WG) for 24 h.



**Table.2** Changes in the DNA (mg/g wet weight of the tissue) and per cent change over the control, in different tissue of the freshwater fish, *Ctenopharyngodon idella* exposed to sublethal concentrations of Pyraclostrobin (20% WG) for 4 and 8 days.

Tissues	4 Days			8 Days		
	Control (mg/g)	Sub-lethal (mg/g)	% Change (mg/g)	Control (mg/g)	Sub-lethal (mg/g)	% Change (mg/g)
Gill	11.23 ±0.012	8.14 ±0.021	8.14	9.29 ±0.033	7.37 ±0.019	7.37
Brain	8.27 ±0.047	7.11 ±0.032	7.11	7.11 ±0.021	6.27 ±0.013	6.27
Kidney	7.76 ±0.022	5.88 ±0.014	5.88	6.02 ±0.019	5.21 ±0.031	5.21
Liver	6.43 ±0.032	5.71 ±0.088	5.71	5.22 ±0.022	4.11 ±0.015	4.11
Muscle	12.72 ±0.071	10.23 ±0.043	10.23	10.31 ±0.010	8.38 ±0.022	8.38

Values are the mean of five observations ;(±) indicates the standard deviation:

Values are significantly at  $P < 0.05$

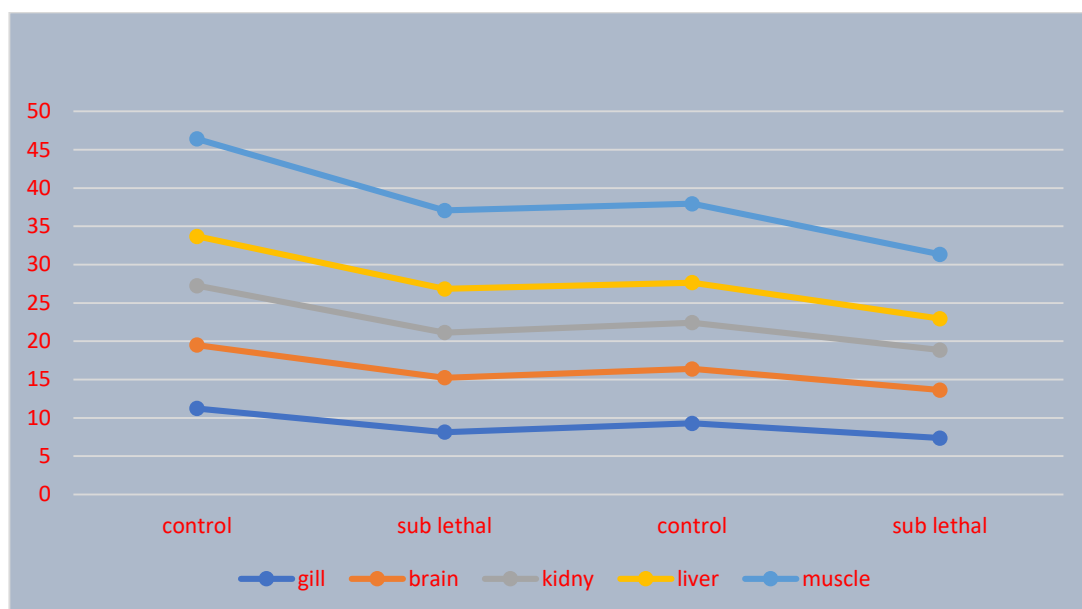
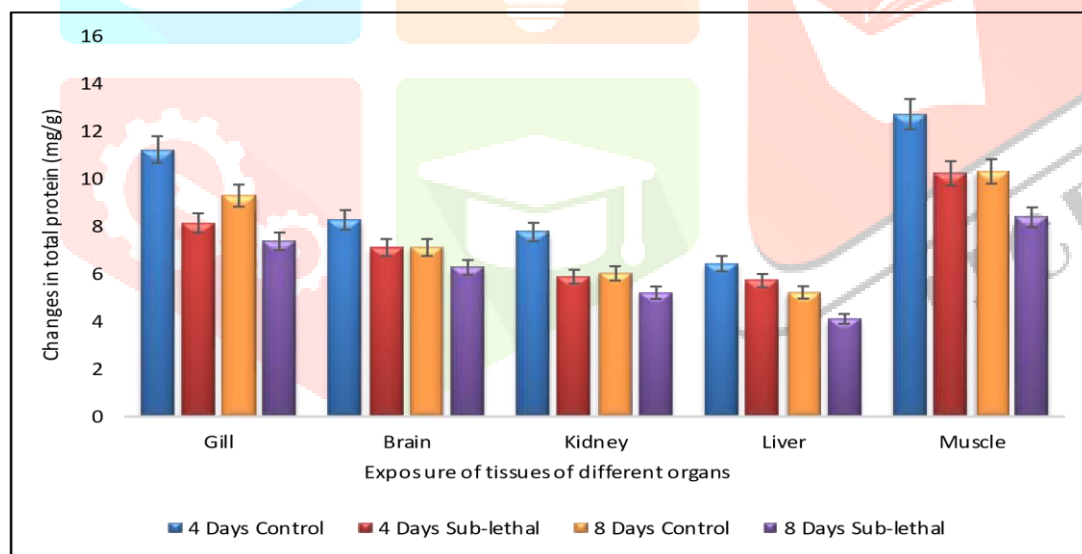


Fig:3 Change in the Amount of RNA (mg/gr Body Wet Weight of the Tissue) and % Change over the Control in different Tissues of fish *Ctenopharyngodon idella* Exposed to Sublethal and Lethal Concentrations of pyraclostrobin , Mean± S.E,n=5,P < 0.05

**Fig.4** Changes in the DNA (mg/g wet weight of the tissue) and cent per change over the control, in different tissue of the freshwater fish, *Ctenopharyngodon idella* exposed to sublethal concentrations of Pyraclostrobin (20% WG) for 4 and 8 days:



4days control                      muscle>gill>brain>kidney>liver

4days sub-lethal                  gill>kidney>muscle>brain>liver

8days control                      muscle>gill>brain>kidney>liver

8days sub-lethal                  liver>gill>muscle>kidney>brain

The results of 4 and 8 days sub-lethal exposure of Pyraclostrobin (20% WG) indicates heterogeneous levels of DNA in the tissue of liver, gill, muscle, kidney and brain. Under



Pyraclostrobin (20%WG) sub-lethal exposure, maximum per cent change (23.31) in gill and minimum percent change (11.05) in kidney was observed. Under lethal exposure of Pyraclostrobin (20%WG) for 24 hr, maximum cent per change (32.43) in gill and minimum cent per change (16.64) in kidney was noticed. Under sub-lethal exposure for 4th and 8th days, maximum cent per change (27.51) and (21.26) in gill and liver and minimum per cent change (11.19) and (11.81) in liver and brain. Variations in DNA and RNA contents in different tissues of fish treated with different type of toxicants have been reported by Shailendra Kumar Singh *et al.*, (2010) reported that decreased DNA-RNA levels in fish *Colisa fasciatus* exposed to cypermethrin at different seasons. DNA and RNA content were decreased in liver, brain and gill tissues of *Channa punctatus* treated with pyrethroid, due to inhibitory action of synthetic pyrethroid on DNA synthesis machinery or increased degradation, reported by Tripathi and Singh, (2013). decreased nucleic acids (DNA and RNA) content liver, muscle, gill tissues of freshwater fish *Ctenopharyngodon idella* 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. Thenmozhi *et al.*, (2011) Similar findings were observed by Kumar *et al.*, (2007) and Kishi *et al.*, (2019). The concentration of the extracted DNA in the tissue samples of both control and exposed groups was estimated using Nano Drop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA) (Salem *et al.*, 2014). The DNA and RNA content were increased in gill, liver, brain and kidney of fish *Chana punctuates* exposed to different concentrations of cypermethrin and L-cyhalothrin (Awasthi *et al.*, 2018). The decline DNA content could be due to the disturbance in the normal DNA synthesis. The level of DNA and RNA was found to decreased in brain, liver, gonads and kidney of *N.notopterus* fish due to exposure to copper sulphate indicates toxicity effect on nucleic acid synthesis (Ravikiran and kulakarni, 2015; Wistrand *et al.*, 2018). DNA damage studies have been performed in different fish species collected from upper and middle stretches of Adige river basin (Kracun-Kolarevic *et al.*, 2016).

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 (Stingele *et al.*, 2017). Pesticides are potential inhibitor of DNA synthesis, which might result in reduction of RNA level. Because of electrophilic nature, the carbamate compounds might attack many enzymes responsible for normal metabolic pathway. DNA damage in RBCs of four fish

*Ctenopharyngodon idella* species, showed an increase as the days of exposure to heavy metal increased (Kousar and Javed, 2015; Kurat, *et al.*, 2017). Thus, it is possible that the enzyme necessary for DNA synthesis might have been inhibited by toxicant. On compilations of the result, it appears that the disruption of DNA synthesis might have affected RNA synthesis and consequently protein synthesis (Thipathy *et al.*, 2003 and ravikiran *et al.*, 2012). Cypermethrin reduced the DNA and RNA content in the gill, liver and kidney of fish *Cirrhinus mrigala* (Vasanth raja *et al.*, (2014). The present study is compared with those of where red blood cells of carps showed significant ( $P < 0.05$ ) DNA damage due (Kousar and Javed, 2014).

Carbamate compounds exhibit strong mutagenic, genotoxic (Guilherme *et al.*, 2012) and clastogenic potentiality, which might responsible for the alteration of DNA level. Marc Andre (2008) a number of chemicals, associate with DNA damage, have been tested on liver of aquatic animals, isolated tissues or different cell types. Chemicals that act directly on DNA, chemicals whose metabolites cause DNA damage, chemicals that cause the production of reactive oxygen species that can damage DNA, chemicals that inhibit DNA synthesis and repair. Inhibition, many chemical contaminants damage DNA by multiple mechanisms. Increase in dietary 3 protein level up to 40% (D) the total protein content, 3 albumin content and globulin content increased significantly (Shyam Narayan Labh, 2015 and Thompson *et al.*, 2019).

## RNA

The calculated values of nucleic acid content RNA, along with standard deviation and the percent change over the control fish were presented in Table.V.3.4.7; Table.V.3.4.8 and Fig.V.3.4.7; Fig.V.3.4.8. The RNA content in 24 h control fish *Ctenopharyngodon idella* in different tissues was in the order of: Muscle > Liver > Brain > Gill > Kidney

Under exposure to sub-lethal and lethal concentration of Pyraclostrobin to fish *Ctenopharyngodon idella* for 24 h, the total RNA was found to decrease in most of the tissues and the lyotropic series in terms of decrement in the RNA content was in the order of:

24 hrs Sub-lethal : Muscle > Liver > Brain > Gill > Kidney

24 hrs lethal : Muscle > Liver > Brain > Gill > Kidney



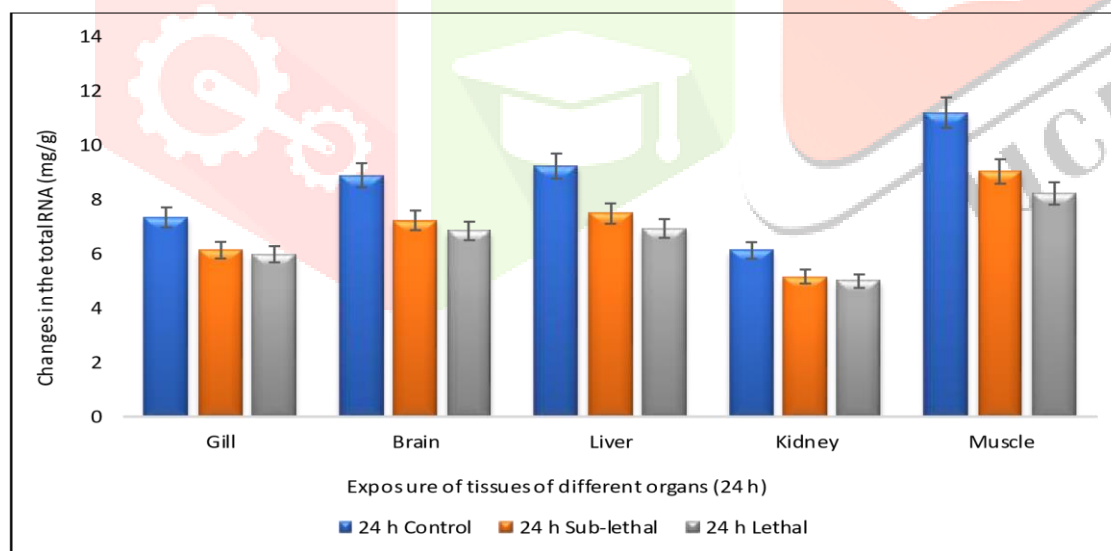
**Table.3.** Changes in the RNA (mg/g wet weight of the tissue) and per cent change over the control, in different tissue of the freshwater fish, *Ctenopharyngodon idella* exposed to sublethal and lethal concentrations of Pyraclostrobin (20% WG) for 24h.

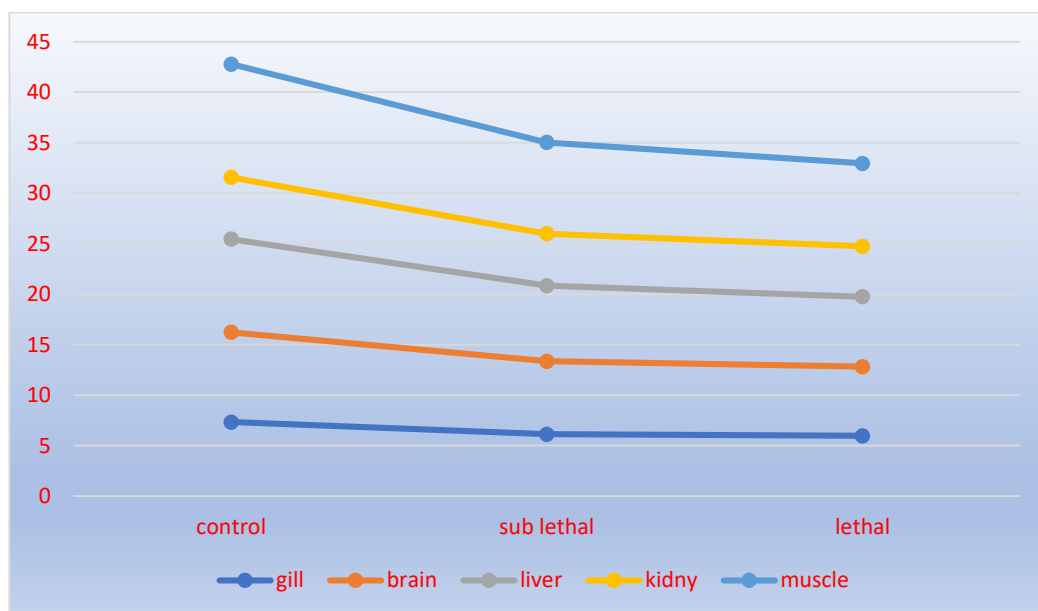
Tissues	Control (mg/g)	Sub-lethal (mg/g)	% Change (mg/g)	Lethal (mg/g)	% Change (mg/g)
Gill	7.34 ±0.034	6.13 ±0.067	16.48	5.98 ±0.90	18.52
Brain	8.89 ±0.055	7.23 ±0.062	18.67	6.84 ±0.012	23.05
Liver	9.23 ±0.011	7.48 ±0.078	18.95	6.93 ±0.032	24.91
Kidney	6.12 ±0.023	5.16 ±0.012	15.68	4.99 ±0.021	18.46
Muscle	11.2 ±0.017	9.03 ±0.067	19.37	8.22 ±0.011	26.60

Values are the mean of five observations ;(±) indicates the standard deviation:

Values are significantly at  $P < 0.05$ .

**Fig. 5.** Changes in the RNA (mg/g wet weight of the tissue) and per cent change over the control, in different tissue of the freshwater fish, *Ctenopharyngodon idella* exposed to sublethal and lethal concentrations of Pyraclostrobin (20% WG) for 24hrs.



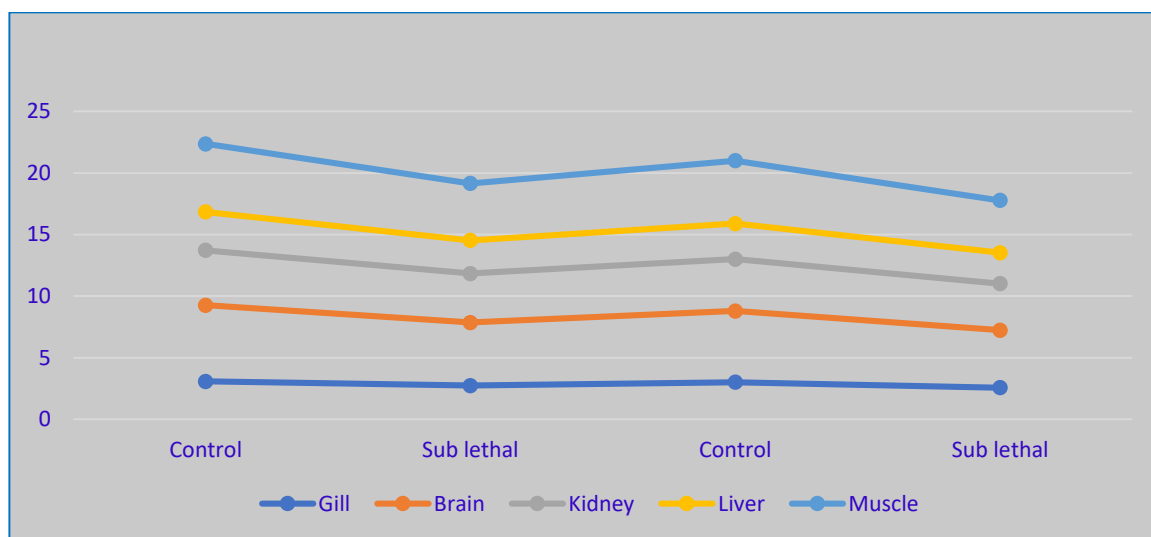


**Fig: 6:**Change in the Amount of RNA (mg/gr Body Wet Weight of the Tissue) and % Change over the Control in different Tissues of fish *Ctenopharyngodon idella* Exposed to Sublethal and Lethal Concentrations of Pyraclostrobin , Mean $\pm$  S.E,n=5,P < 0.05

**Table.4.** Changes in the RNA (mg/g wet weight of the tissue) and per cent change over the control, in different tissue of the freshwater fish, *Ctenopharyngodon idella* exposed to sublethal concentrations of Pyraclostrobin (20% WG) for 4 and 8 days.

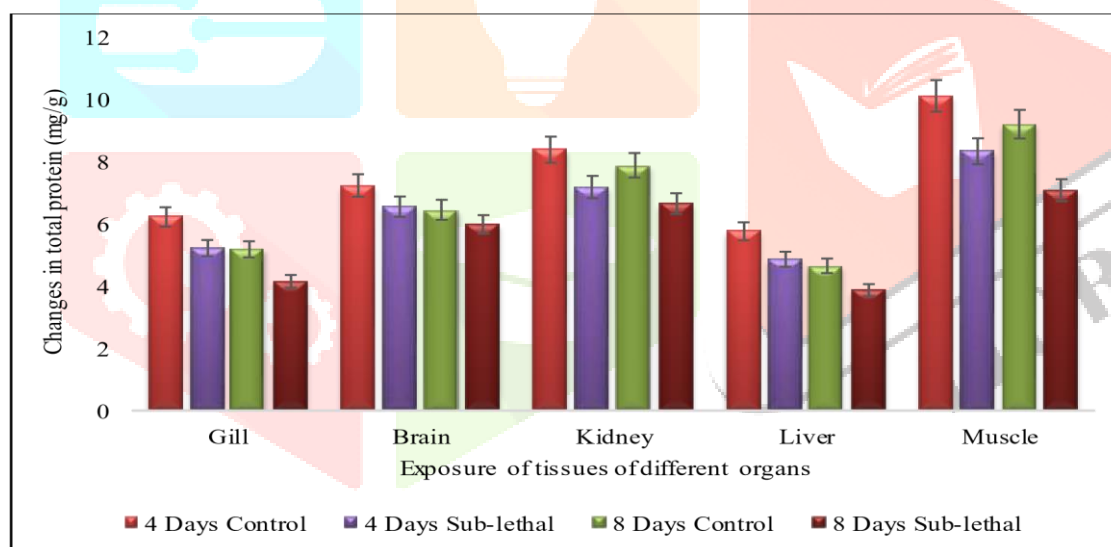
Tissues	4Days			8Days		
	Control (mg/g)	Sub-lethal (mg/g)	% Change (mg/g)	Control (mg/g)	Sub-lethal (mg/g)	% Change (mg/g)
Gill	3.08 $\pm 0.011$	2.74 $\pm 0.087$	11.03	3.01 $\pm 0.014$	2.57 $\pm 0.012$	14.61
Brain	6.18 $\pm 0.025$	5.12 $\pm 0.023$	17.15	5.78 $\pm 0.023$	4.67 $\pm 0.017$	19.2
Kidney	4.46 $\pm 0.015$	3.98 $\pm 0.017$	10.76	4.21 $\pm 0.066$	3.78 $\pm 0.043$	10.21
Liver	3.12 $\pm 0.034$	2.68 $\pm 0.098$	14.1	2.89 $\pm 0.012$	2.51 $\pm 0.087$	13.14
Muscle	5.52 $\pm 0.056$	4.63 $\pm 0.023$	16.12	5.11 $\pm 0.002$	4.25 $\pm 0.033$	16.82

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



**Fig:7:** Change in the Amount of RNA (mg/gr Body Wet Weight of the Tissue) and % Change over the Control in different Tissues of fish *Ctenopharyngodon idella* Exposed to Sublethal and Lethal Concentrations of Pyraclostrobin , Mean $\pm$  S.E,n=5,P < 0.05

**Fig.5.** Changes in the RNA (mg/g wet weight of the tissue) and per cent change over the control, in different tissue of the freshwater fish, *Ctenopharyngodon idella* exposed to sublethal concentrations of Pyraclostrobin (20%WG) for 4 and 8 days.



Under exposure to sub-lethal concentrations of pyraclostrobin for 4<sup>th</sup> and 8<sup>th</sup> days, the cent per depletion of protein content in the tissues of *Ctenopharyngodon idella* was in the order of: 4days control :muscle

The results in indicate heterogeneous levels of RNA in the tissue of gill, brain, kidney, liver and muscle. Under Pyraclostrobin 24 h sub-lethal exposure, maximum cent per change (19.37) in muscle and minimum cent per change (15.68) in kidney was perceived. Under lethal exposure of Pyraclostrobin for 24 h, maximum percentage of depletion was (26.60) in muscle and minimum cent per change was (18.46) in kidney. Under sub-lethal exposure for 4<sup>th</sup> and

8<sup>th</sup> days, maximum cent per of depletion was (17.60) and (23.04) in muscle and minimum cent per change (9.40) and (7.28) in brain. In the present study decrease in levels of RNA was observed in all the tissues except brain of fish exposed whereas RNA followed by damage to neuron cells (Mcilwain and Bachelard,1971) resulting in demyelination (Health,1961). Increase in RNA content of gill by Gracy and Rajasekar (2012) & Akhtar *et al.*, (2012), reported that there are no significant changes in DNA levels in liver and muscle but RNA level was significantly increased in liver and muscle tissues of *Ctenopharyngodon idella* treated with dietary pyridoxine. Ravikiran and kulakarni (2015), the level of DNA and RNA was found to decreased in brain, liver, gonads and kidney of *N.notopterus* fish due to exposure to copper sulphate indicates toxicity effect on nucleic acid synthesis. The RNA content in control fish *Labeo rohita* in different tissues are in the order of: muscle, gill, liver, kidney and brain content were decreased (Monali Chakraborty1 and Deepronil Roy,2017). According to Mukhopadhyay and Dehadrai (1980), the decrease of RNA might also be due to interference in the incorporation of precursor in the nucleic acid synthesis or inhibition of the RNA polymerase function (De Paula Brandao *et al.*, 2020). The present observations might be supported by the pesticide-mediated reduction in protein contents of various tissues including blood of other species of fish reported by Thripathi and Priyanka Verma, (2004) Jin Y *et al.*, (2011). DNA damaging agents capable of inducing strand breakage, cross-links and alkali-labile sites (Pandey *et al.*, 2008). The DNA and RNA contents have been studied in gill, liver and brain of a common carp, *Cyprinus carpio* exposed to cadmium chloride and lead acetate Muley *et al.*, (2000) that decreased DNA content in all the tissues along with RNA content in liver and brain, it was increased in gill due to cadmium and lead toxicity (Neelima *et al.*, 2017). The present study reveals that Pyraclostrobin caused variability in the nucleic acids content in different tissues and the degree of variability or extent of alterations caused by the pyraclostrobin. RNA: DNA ratio also followed the same increasing trend as RNA content along with the increase in dietary protein level during the whole experimental period (Zhang *et al.*, 2017; Ma *et al.*, 2019).

## Conclusion:

This toxicity test on the effect of Pyraclostrobin on *Ctenopharyngodon idella* a rapid method for assessing the Pyraclostrobin impact on this fish. This type of preliminary investigations can be useful for deriving the safe level of Pyraclostrobin concentration that can be released into the aquatic environments. . A number of chemicals, associate with DNA damage, have been tested on live marine animals, isolated tissues or different cell types. These chemicals were grouped into four classes: (1) chemicals whose metabolites cause DNA damage. (2) Chemicals act directly on DNA. (3) Chemicals prevent DNA synthesis and repairs. (4). Chemicals that cause the production of reactive oxygen species that can damage DNA. (5) Chemicals that prevent DNA synthesis and repair, many chemical and toxins. DNA damage by multiple mechanism. The results observe in present-day study reveals Pyraclostrobin.(20%WG) caused variability in the nucleic acid content in changed tissues and the grade of inconsistency by the Pyraclostrobin (20%WG) was less compared to and found to the dose dependent.

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