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BIOCHEMICAL CHANGES INDUCED BY FLUBENDIAMIDE (39.35% SC) IN FRESHWATER FISH, *CATLA CATLA* (HAMILTON, 1822)

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ABSTRACT

The fish *Catla catla* fingerlings were exposed to the test toxicant Flubendiamide, a new lepidopteron insecticide, used to control important lepidopteron pests. The acute toxicity tests for 24, 48, 72 and 96 h were conducted and the LC_{50} values were calculated using Finneys probit analysis and were reported to be 3.566 mg.l^{-1}, 3.456 mg.L^{-1}, 3.0221 mg.l^{-1} and 2.892 mg.l^{-1}, respectively for 24, 48, 72 and 96 h.After determination of LC_{50}values the fingerlings were exposed to sub-lethal (0.289 mg.l^{-1}) and lethal (2.892 mg.l^{-1}) concentrations of Flubendiamide for 24, 48, and, 96 h, and 8 days exposure was made only to the sub-lethal concentration to study the alterations in different biochemical parameters viz, total glycogen, total proteins, and the changes in the contents of the nucleic acids such as DNA and RNA. The changes in the biochemical constituents of the vital organs viz, Gill, Liver, Brain, Muscle, and Kidney were studied. Significant changes in the biochemical constituents of these organs of fish were observed. All the biochemical constituents were found to be decreased in different tissues and the results obtained in all were discussed in the light of metabolic stress caused due to the exposure of the toxicant.

KEY WORDS : Flubendiamide, *Catla catla*, Toxicity, Biochemical constituents.

INTRODUCTION

The increasing use of pesticides in agriculture including commercial and household production of vegetables for the control of pests causes potential health hazards to live stock, especially to fish, frogs, birds and mammals. As a consequence, the structure and functions of communities, eco-system and populations are altered. Pesticides are credited with economic potential to enhance production of food and fibre and amelioration in vector-borne diseases, the long-term use has caused effects on human health and the environment including aquatic ecosystem that evolved new branch of aquatic toxicology (Igbedioh, 1991; Forget, 1993; Aktar *et al.*, 2009). Pesticides have been found to be highly toxic not only for fish but also to the other organisms which constitute the food chain. Agricultural run-off from near water bodies is the major cause of deposition of pesticides in aquatic ecosystems. The qualitative and quantitative chemical usage is of great concern ecologically. The discriminate use of chemicals is for the control of insect pests by elimination of target species whereas indiscriminate usage posed the problem on non-target organisms including human beings.

Wide and indiscriminate use of pesticides in modern agricultural practices throughout the world has indirectly created problem of pollution of aquatic ecosystems (Ganeshwade, 2012). These chemicals impair water quality which becomes unsuitable for all aquatic organisms due to their toxicity, persistence, bioaccumulation, and biomagnifications in food chain and ecological balance (Subramani Lavanya *et al.*, 2011). Fish accumulate these pollutants directly or indirectly from polluted waters and food chain (Jabeen *et al.*, 2016).

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Mass mortality of fish due to pesticide exposure is rare, and results only from accidents or direct spraying of the water bodies. Fish are the most often tested aquatic organisms because they are the most conspicuous as predominant and are economically important to man because they are linked in the food chain. Evaluation of LC_{50} is the pioneer step in toxicological assessment of any chemical. It helps to select sub-lethal concentrations to carry out several toxicity tests. Therefore, this knowledge is essential for exploring impacts of any chemical on physical and physiological status of exposed organisms. The present study was focused on a newly formulated lepidopteron insecticide Flubendiamide effect in freshwater fish *Catla catla* on exposure periods in sub-lethal and lethal concentrations at 24,48 and 96 hours and only sub-lethal for 8 days on biochemical constituents like glycogen, total protein, DNA and RNA.

The biochemical alterations in organisms are considered as most sensitive and earliest events of any pollutant damage. Effects of pesticides on biochemical processes in aquatic animals have been done earlier in India by Tripathi and Singh (2002). Exposure to pesticides causes severe alterations in tissue biochemistry of fish (Shrivastava and Singh, 2004). Hence, biochemical parameters are the best physiological indicators of the fish health. Therefore, they are important to be focused while studying the toxic effects of various pesticides and pollutants on fish. Hence in the present study an attempt has been made to study the impact of Flubendiamide on sub-lethal and lethal concentrations at 24,48 and 96 hours and only sub-lethal at 8 days of exposure period on glycogen, total protein, DNA and RNA contents of freshwater fish *Catla catla.*

MATERIALS AND METHODS

The fingerlings of the test fish *Catla catla* of size 6-8 ± ½ cm and weight 6-7 ± ½ g were procured from local fish hatcheries of Nandivelugu, Tenali mandal, Guntur district, Andhra Pradesh. The fish were acclimated at (28±2^{o}C) in the laboratory conditions for two weeks. All the precautions laid down on recommendations of the toxicity tests to aquatic organisms were followed. Fish were regularly fed with rice bran and one day prior to the experimentation feeding was stopped. Fish were exposed to sub-lethal (1/10th of 96 hrs LC_{50} value 0.030 mg.l^{-1}) and lethal (96 hrs LC_{50} value 0.289 mg.l^{-1}) concentrations of Flubendiamide for 24, 48, 72 and 96 h and only for sub-lethal concentration for 8 days. The hydrographical properties of water were estimated by the modified method followed by Golterman and Claimo (1969) method. Finney's probit analysis (Finney, 1971) as reported by Roberts and Boyce (1972) was followed to calculate the LC_{50} value. The 95% confidence limits of the LC_{50} values for each test were also calculated for different time period by using SPSS software. At the end of the exposure periods, the tissues like gill, brain, liver, kidney and muscle were taken out from exposed and control fish and processed for the estimation of glycogen, total proteins and nucleic acids (DNA & RNA). Glycogen was estimated by the method of Kemp *et al*. (1954), total protein by Lowry *et al*., (1951) and DNA and RNA by the methods of Searchy and Maclinnis (1970a and 1970b). The data obtained in the present work were expressed as means of four observations ± SD (standard deviation) and were statistically analysed using student "t" test (Pillai and Sinha, 1968) to compare means of treated data against their controls and the result was considered significant at ($P < 0.05$) level.

RESULTS AND DISCUSSION

The calculated LC_{50} values for 24, 48, 72, and 96 h were 3.566 mg.l^{-1}, 3.456 mg.l^{-1}, 3.0221 mg.l^{-1} and 2.892 mg.l^{-1}, respectively.

Glycogen, total proteins and nucleic acids (DNA and RNA) decreased in various tissues, viz. gill, brain, liver, kidney and muscle of *Catla catla* exposed to sub-lethal and lethal concentrations of Flubendiamide for 24, 48, 96 h and only sub-lethal for 8 days were graphically represented in the following figures. In exposed fish percent decrease in glycogen, total protein, DNA and RNA is more apparent in lethal concentrations than at sub-lethal concentrations.

Glycogen

The changes in glycogen content observed in the various tissues of *Catla catla* after the Flubendiamide exposure along with the control are graphically represented in figures. In the tissues of control fish, *Catla catla* glycogen content was in the order of:

Liver > Muscle > Gill > Brain > Kidney

Under exposure to sub-lethal and lethal concentrations of Flubendiamide for 24,48,96 h and only sub-lethal for 8days, the amount of glycogen was found to decrease in all the tissue of *catla catla*. The lyotropic gradation series in terms of percent

Table 1. Changes in glycogen content (mg/g wet weight of the tissue) of the fish *Catla catla* exposed to the sub-lethal & lethal concentrations of Flubendamide for 24 and 48 h.

Tissue	24 h					48 h				
	Control	Sub-lethal	% Change	Lethal	% Change	Control	Sub-lethal	% Change	Lethal	% Change
Gill	43.71± 1.47	38.25± 0.81	13.444	26.45± .45	33.122 ·	46.61± 1.24	36.31± 0.91	24.63	24.63±1.77	43.5
Brain	31.15± 2.51	26.13± 0.96	11.4	20.23±0.85	22.072	32.03± 0.91	28.43± 1.21	50.58	18.95±0.58	30.95
Liver	81.23± 2.00	68.41± 2.06	24.315	48.16±1.18	42.858	81.23± 0.86	64.52± 0.81	33.31	42.79±0.97	54.79
Kidney	29.25± 1.61	24.21± 1.09	9.756	18.35±0.53	19.876	26.83± 0.87	22.83± 0.82	17.94	16.47±1.06	34.47
Muscle	47.63± 1.81	39.51± 0.87	17.74	30.27±1.27	34.659	46.71± 1.70	37.25± 1.23	37.36	25.95±0.92	43.95

Results are mean values of four observations
Standard Deviation is indicated as (±)
Values are significant at $p < 0.05$

decrement at 24, 48, 96h and 8 days exposure was:
Sub-lethal -24 h: Liver > Muscle > Gill > Kidney > Brain
Lethal -24 h: Liver > Muscle > Gill > Kidney > Brain
Sub-lethal- 48 h: Muscle > Liver > Gill > Brain > Kidney
Lethal- 48 h: Liver > Muscle > Gill > Kidney > Brain
Sub-lethal- 96 h: Liver > Muscle > Gill > Kidney > Brain
Sub-lethal- 8 days: Liver > Kidney > Gill > Brain > Muscle

Veeraiah *et al.* (2013a) observed that exposure to sub-lethal and lethal concentrations of cadmium chloride in the fish *Cirrhinus mrigala* for 96 h caused changes in the total glycogen level which may be attributed to toxic stress, resulting in the disruption of enzymes associated with carbohydrate metabolism. Bantu *et al.* (2013) observed depletion of glycogen in the liver of freshwater teleost *Channa punctatus* (Bloch) when exposed to sub-lethal concentrations of insecticide Chlorantraniliprole (18.5% SC) for period of 15 and 30 days respectively.

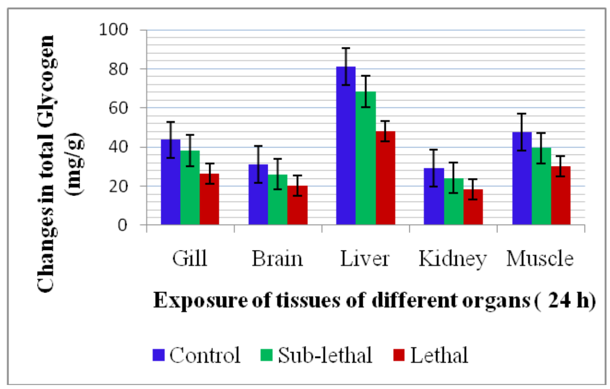

Fig. 1a. Changes in glycogen content of the fish *Catla catla* exposed to the sub-lethal & lethal concentrations of Flubendamide for 24 h.

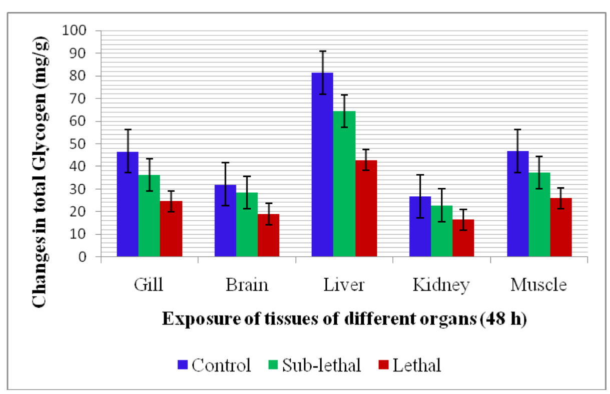

Fig. 1b. Changes in glycogen content of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubedamide for 48 h.

Dhanalakshmi (2013) noticed decrement in the tissue glycogen concentration in fish *Cirrhinus mrigala* when exposed to 0.25 $mg.l^{-1}$ concentration of the metal chromium sulphate for 24, 48, 72 h and 10, 20 and 30 days which may be due to its enhanced utilization, since glycogen forms the immediate source of energy to meet energy demands under metallic stress caused by test toxicant.

Nagaraju and Rathnamma (2013) reported a decline in glycogen, protein and nucleic acids content of freshwater fish *Channa punctatus* exposed for 45 days to $1/10^{th}$ and $1/20^{th}$ of LC_{50} 96 h of Chlorantraniliprole 18.5% SC (Suspension concentration). Reduction in glycogen content was also noticed by Naik *et al.*, (2016) in all the tissues of *Labeo rohita* when exposed to sub-lethal concentrations of cypermethrin for 1, 2 and 3 weeks. Exposure of sub-lethal doses (40% and 80 % of LC_{50} of 24 h) of Glyphosate for 24 or 96 h against the freshwater non-target fish *Channa punctatus* caused significant ($P < 0.05$) alteration in biochemical parameters in liver and muscle tissues of the fish *Channa punctatus* (Bloch) was reported by Singh *et al*. (2017). Vani *et al.* (2020) reported decrease in glycogen content in all the tissues of the fish *Cirrhinus mrigala* on exposure to lethal and sub-lethal concentrations of carbamate pesticide Cartap hydrochloride (50% SP).

According to Dezwaan and Zandee (1973) depletion of glycogen in tissues may be due to direct utilization of the compound for energy generation, a demand caused by pesticide-induced hypoxia. Under hypoxia condition, the fish derives its energy from anaerobic breakdown of glucose which is available to the cell by increased glycogenolysis (Chandravathy and Reddy, 1996; Rajamannar and Manohar, 1998; Rajamanickam, 1992). Susan *et al.*, (1999) observed drastic decreased glycogen content in liver of *Catla catla* under fenvalerate toxicity stress. Das and Mukherjee (2003) reported that the sub-lethal exposure of cypermethrin up to 45 days alterated blood glucose level in *Labeo rohita*.

Liver suggested as an organ for detoxification. During exposure to Flubendiamide exposure fishes came under stress condition and need more energy to cope with the toxicants. Glycogen serves as reserve material. It is utilized when the body came

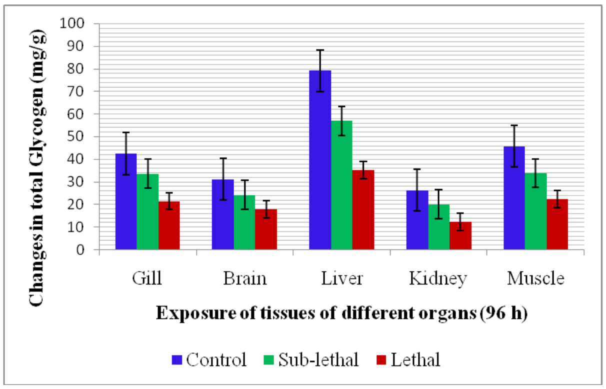

Fig. 2a. Changes in glycogen content of the fish *Catla catla* exposed to the sub-lethal & lethal concentrations of Flubendamide for 96 h

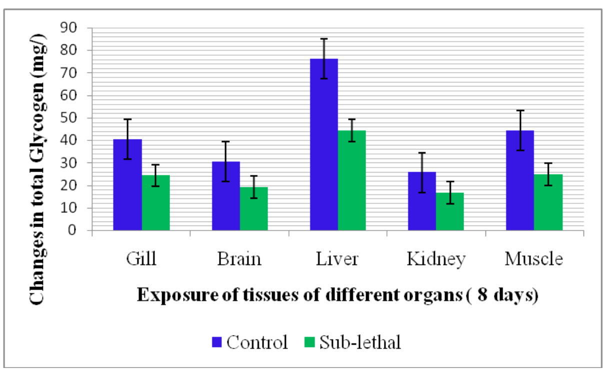

Fig. 2b. Changes in glycogen content of the fish *Catla catla* exposed to the sub-lethal concentration of Flubendamide for 8 days

Table 2. Changes in glycogen content (mg/g wet weight of the tissue) of the fish *Catla catla* exposed to the sub-lethal & lethal concentrations of Flubendamide for 96 h and only sub-lethal for 8 days.

	96 h				8 days			
Tissue	Control	Sub-lethal	% Change	Lethal	% Change	Control	Sub-lethal	% Change
Gill	42.46± 1.66	33.63± 1.21	27.853	21.51± 1.45	46.96	40.42± 1.90	24.40± 1.06	47.055
Brain	31.25± 1.06	24.37± 0.83	31.604	17.93± 1.05	39.41	30.62± 1.69	19.15± 1.58	43.975
Liver	79.11± 1.01	56.95± 1.24	40.646	35.33± 0.92	64.179	76.35± 0.84	44.31± 1.72	46.673
Kidney	26.36± 0.96	20.11± 1.03	19.907	12.45± 0.83	45.163	25.71± 0,80	16.72± 0.57	34.952
Muscle	45.73± 0.91	33.85± 1.03	43.462	22.37± 0.83	59.815	44.23± 0.79	24.84± 0.56	55.129

Results are mean values of four observations
Standard Deviation is indicated as (±)
Values are significant at $p < 0.05$

under stress condition. Depletion of glycogen in liver and tissues may be due to increment in the glycolysis pathway. During stress conditions, the glycogen reserves depleted to meet energy demand (Rawat *et al.*, 2002). Fall in glycogen levels indicates its rapid utilization to meet the enhanced energy demands intoxicant treated animals through glycolysis or hexose monophosphate pathway as observed by Cappon and Nicholas (1975). The above findings support the alterations of glycogen in the present study.

Proteins

The changes in protein content observed in the various tissues of *Catla catla* after Flubendiamide exposure along with the control was graphically represented in Figures. The Protein content in different tissues of control fish *Catla catla*was in the order of:

Muscle > Liver > Brain > Kidney > Gill

Under exposure of sub-lethal and lethal concentrations of Flubendiamide for 24 h, the amount of protein was found to decrease in all the tissue of *Catla catla*.

The lyotropic gradation series in terms of per cent decrement at 24, 48, 96 h and 8 days exposure was:

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

Lethal-24 h- Liver > Muscle > Gill > Kidney > Brain

Sub-lethal-48 h: Liver > Gill > Muscle > Kidney > Brain

Lethal-48 h: Liver > Muscle > Gill > Kidney > B

Sub-lethal -96 h: Liver > Gill > Muscle > Kidney > Brain

Lethal-96 h: Liver > Gill > Muscle > Kidney > Brain

Sub-lethal-8d: Liver > Kidney > Gill > Brain > Muscle

Protein is the most primary biochemical ingredient present in large quantities in the body of fish. Liver is rich in protein and centre for various metabolism of the fish. In the present study maximum decrease of total protein in the liver is due to the increased rate of proteolytic activity or repeated break down of protein to yield energy due to stress caused due to pesticide exposure. Anitha & Rathnamma (2016) noticed decreased protein levels in all the tissues like liver, kidney, brain, gill and muscle of *Labeo rohita* exposed to lethal and sub-lethal concentrations of Pyraclostrobin 20%WG (carbamate) for 24 h and sub-lethal concentrations

Table 3. Changes in protein content (mg/g wet weight of the tissue) of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 24 h and 48h.

	24 h					48 h				
Tissue	Control	Sub-lethal	% Change	Lethal	% Change	Control	Sub-lethal	% Change	Lethal	% Change
Gill	210.30± 3.13	178.20± 2.16	27.576	108.58± 2.06	43.174	206.62± 2.72	168.23± 1.18	35.15	134.16± 1.29	51.63
Brain	146.42± 3.01	128.12± 1,85	19.809	98.16± 2.00	33.53	142.40± 1.08	124.15± 1.37	28.14	98.80± 2.59	45.15
Liver	237.63± 3.16	176.37± 1.89	34.307	154.11± 3.39	47.81	235.70± 3.78	186.45± 1.51	39.74	134.12± 3.09	55.87
Kidney	119.45± 2.04	92.91± 1.86	25.181	76.33± 1.23	36.27	118.15± 1.37	82.16± 0.85	52.48	52.48± 3.10	49.9
Muscle	215.81± 3.63	176.74± 2.33	21.169	142.07± 2.09	38.082	213.63± 1.40	174.45± 1.39	33.63	134.52± 1.08	55.24

Results are mean values of four observations
Standard Deviation is indicated as (±)
Values are significant at $p < 0.05$

for 5 and 10 days. Thiamethoxan affected liver total protein of *Oreochromis niloticus* (Bose *et al.*, 2011) whilepropiconazole and mancozeb induced changes in protein content in *Clarias batrachus* (Srivasatav and Singh, 2013a,c). Kumar *et al.* (2017) reported a reduction in proteins in the liver and kidney of freshwater fish, *Channa punctatus* exposed to different sub-lethal concentrations of pesticide Carbaryl for a period of 15, 30, 45, 60, and 75 and up to 90 days. Muddassir (2015) observed significant decrease value in Total protein in the liver of *Channa punctatus* was treated with 0.1 mg.l^{-1} Carbofuran and 0.09 mg.l^{-1} Malathion pesticides at different time intervals 7, 14, 21 and 28 days. The decrement of total protein may be due to the inhibition of RNA synthesis disturbing the protein metabolism or this may be due to liver damage where most protein synthesis usually occurs, these results agreed with that of Singh and Sharma (1998). The depletion of

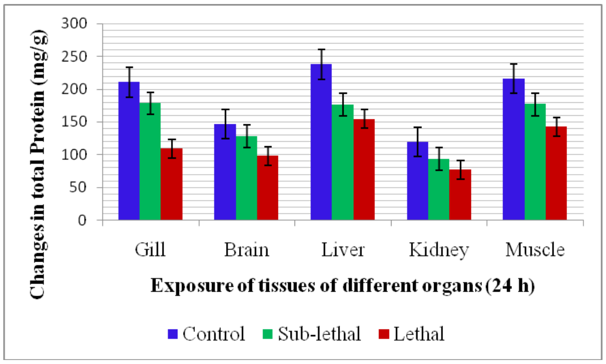

Fig. 3a. Changes in protein content of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 24 h.

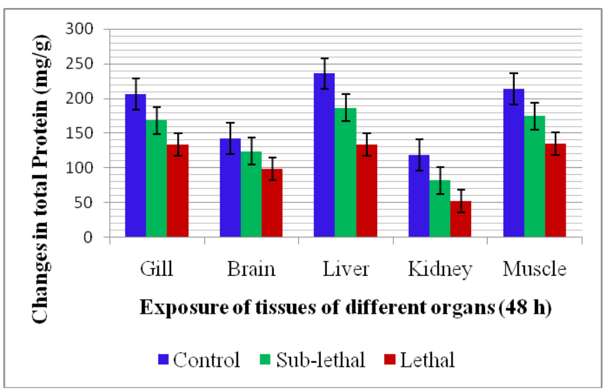

Fig. 3b. Changes in protein content of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 48 h.

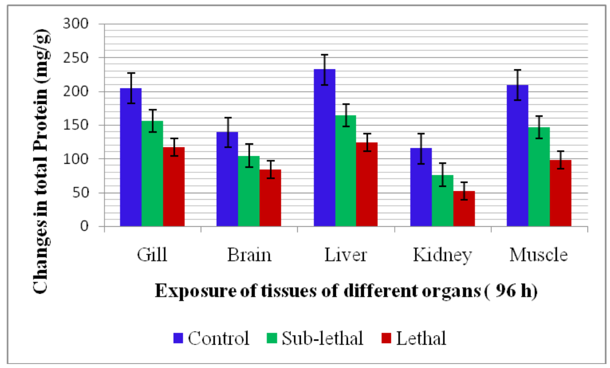

Fig. 4a. Changes in protein content of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 96 h.

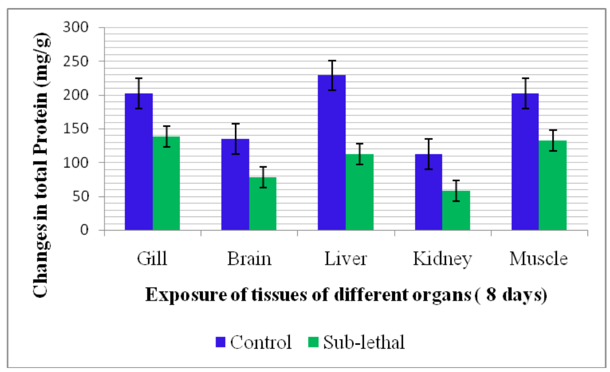

Fig. 4b. Changes in protein content of the fish *Catla catla* exposed to the sub-lethal concentration of Flubendamide for 8 days.

Table 4. Changes in protein content (mg/g wet weight of the tissue) of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 96 h and only sub-lethal for 8 days.

	96 h				8 days			
Tissue	Control	Sub-lethal	% Change	Lethal	% Change	Control	Sub-lethal	% Change
Gill	204.47± 2.72	156.18± 1.18	33.456	116.86± 1.36	44.86	202.22± 1.19	138.01± 1.11	34.299
Brain	138.95± 2.39	104.51± 1.69	29.264	84.35± 1.33	42.35	134.33± 0.82	78.24± 2.57	31.87
Liver	232.16± 3.78	164.41± 1.49	34.11	124.30± 1.93	51.3	228.7± 0.87	112.23± 1.43	37.69
Kidney	115.27± 3.10	76.06± 1.78	37.01	52.41± 1.28	52.41	112.07± 1.37	58.27± 1.65	34.5
Muscle	208.93± 1.40	146.76± 1.39	35.276	98.392± 1.08	47.92	202.20± 2.04	132.50± 1.67	22.81

Results are mean values of four observations
Standard Deviation is indicated as (±)
Values are significant at $p < 0.05$

protein might also be attributed to spontaneous utilization of amino acids in various catabolic reactions inside the organism to combat the stress condition (Borah and Yadav, 1996). Veeraiah *et al*. (2018) observed a decrease in protein content in all tissues exposed to lethal and sub-lethal concentrations of Cyhalothrin 2.5% EC, in the fish *Ctenopharyngdon idellus* for 24 and 96 h.

Proteins are important organic constituents of the animal cells. Understanding the protein components of the cell becomes necessary in the light of the radical changes taking place in protein profiles during pesticide intoxication (Anitha and Rathnamma, 2016). The decreased trend of the protein content as observed in the present study in most of the fish tissues may be due to metabolic utilization of the ketoacids through 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 (Schmidt Neilson, 1975).

Nucleic Acids (DNA and RNA)

The changes in DNA content observed in various

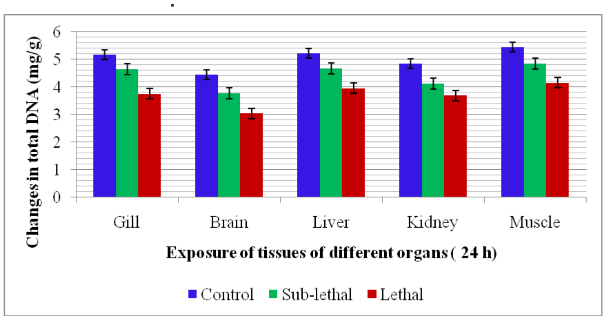

Fig. 5a. Changes in DNA content in different tissues of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 24 h.

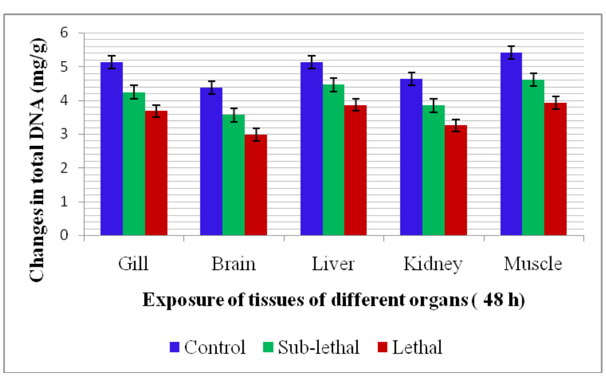

Fig. 5b. Changes in DNA content in different tissues of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 48 h.

Table 5. Changes in the amount of deoxyribonucleic acid (DNA) mg/g wet weight of the tissue) in different tissues of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 24 h and 48 h.

	24 h					48 h				
Tissue	Control	Sub-lethal	% Change	Lethal	% Change	Control	Sub-lethal	% Change	Lethal	% Change
Gill	5.18±0.04	4.65± 1.03	19.689	3.76± 0.15	43.2	5.12± 0.06	4.24± 0.04	25.357	3.68± 0.16	46.6
Brain	4.45± 0.15	3.78± 1.04	18.73	3.04± 0.66	30.83	4.38± 0.14	3.56± 0.60	24	2.98± 0.66	36.94
Liver	5.23± 0.16	4.68± 0.60	16.924	3.96± 0.54	31.54	5.12± 0.74	4.46± 0.76	23.494	3.86± 0.82	36.97
Kidney	4.85± 0.15	4.12± 0.41	12.553	3.69± 0.64	29.58	4.64± 0.11	3.84± 0.26	13.006	3.25± 0.84	29.78
Muscle	5.45± 0.68	4.84± 0.45	14.699	4.16± 0.64	28.01	5.42± 0.58	4.61± 0.86	17.939	3.92± 0.32	30.31

Results are mean values of four observations
Standard Deviation is indicated as (±)
Values are significant at $p < 0.05$

tissues of *Catla catla* after the Flubendiamide exposure along with the control was graphically represented in Figures.

In control fish, DNA content present in different organs was in the order of:

Muscle > Gill > Brain > Liver > Kidney

Under exposure to sub-lethal and lethal concentrations of Flubendiamide, for 24 h the amount of DNA was found to decrease in all the tissue of *Catla catla*. The lyotropic gradation series in terms of percent decrement at 24,48, 96 h and 8 days exposure was:

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

Lethal-24 h-Gill > Liver > Muscle > Brain > Kidney

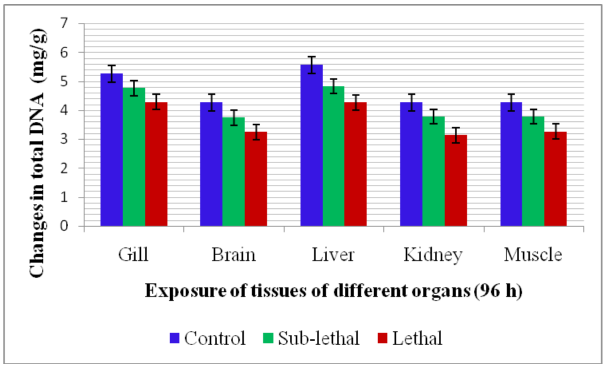

Fig. 6a. Changes in DNA content in different tissues of the fish *Catla catla* exposed to thesub-lethal and lethal concentrations of Flubendamide for 96 h.

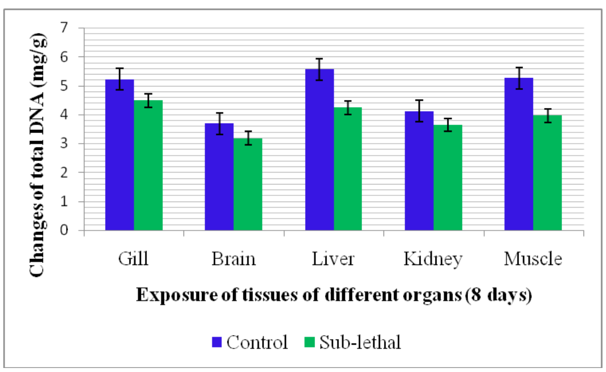

Fig. 6b. Changes in DNA content in different tissues of the fish *Catla catla* exposed to the sub-lethal concentration of Flubendamide for 8 days.

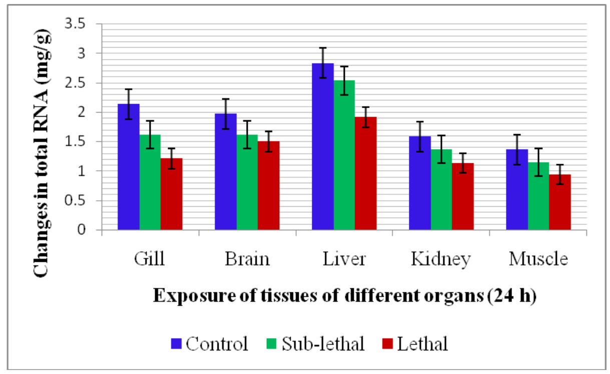

Fig. 7a. Changes in RNA content in different tissues of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 24 h.

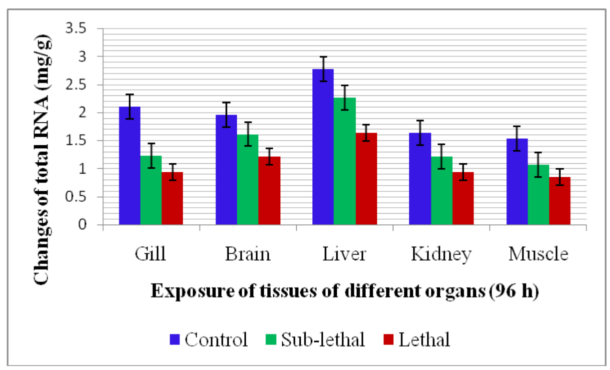

Fig. 7b. Changes in RNA content in different tissues of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 48 h.

Table 6. Changes in deoxyribonucleic acid (DNA) (mg/g wet weight of the tissue) in different tissues of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 96 h and only sub-lethal for 8 days.

	96 h					8 days		
Tissue	Control	Sub-lethal	% Change	Lethal	% Change	Control	Sub-lethal	% Change
Gill	5.26± 0.28	4.76± 0.96	34.143	4.28± 0.74	50.91	5.22± 0.43	4.48± 0.62	34.03
Brain	4.26± 0.56	3.74± 0.43	27.947	3.24± 0.38	41.1	3.68± 0.82	3.18± 0.78	28.91
Liver	5.56± 0.65	4.82± 0.72	32.771	4.26± 1.04	49.43	5.56± 1.05	4.24± 0.86	33.72
Kidney	4.26± 0.77	3.78± 0.78	17.631	3.14± 0.84	31.69	4.12± 0.88	3.64± 0.96	20.95
Muscle	4.26± 1.26	3.78± 0.73	23.829	3.26± 0.83	43.55	5.26± 0.96	3.96± 0.69	26.15

Results are mean values of four observations
Standard Deviation is indicated as (±)
Values are significant at $p < 0.05$

Table 7. Changes in the amount of ribonucleic acid (RNA) in different tissues of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 24 h and 48 h.

Tissue	24 h					48 h				
	Control	Sub-lethal	% Change	Lethal	% Change	Control	Sub-lethal	% Change	Lethal	% Change
Gill	2.13± .05	1.61± 0.14	26.678	1.21± 0.02	44.53 ·	2.17± 0.62	1.37± 0.13	35.697	1.21± 0.6	43.036
Brain	1.97±0.92	1.61± 0.15	17.181	1.50± 0.72	22.73	1.81± 0.42	1.41± 0.16	20.978	1.10± 1.36	38.01
Liver	2.83± 0.49	2.53± 0.45	9.563	1.91± 0.72	31.39	2.67± 0.94	2.32± 0.83	12.059	2.0± 0.45	20.64
Kidney	1.58± 0.04	1.37± 0.54	12.207	1.13± 0.46	27.3	1.5± 0.34	1.31± 0.47	16.5	0.97± 0.43	37.75
Muscle	1.36± 0.19	1.15± 0.48	14.328	0.94± 0.22	29.65	1.41± 0.52	1.07± 0.82	22.943	0.81± 0.09	41.25

Results are mean values of four observations
Standard Deviation is indicated as (±)
Values are significant at $p < 0.05$

Sub-lethal-48 h: Gill > Brain > Liver > Muscle > Kidney
Lethal-48 h: Gill > Liver > Brain > Muscle > Kidney
Sub-lethal-96 h: Gill > Liver > Muscle > Kidney > Brain
Lethal-96 h: Gill > Liver > Muscle > Kidney > Brain
Sub-lethal-8 d: Gill > Liver > Brain > Muscle > Kidney

The changes in RNA content observed in the various tissues of *Catla catla* after Flubendiamide exposure along with the control was graphically represented in Figures. The RNA content in different tissues in control fish *Catla catla*was in the order of:

Muscle > Brain > Gill > Liver > Kidney

Under exposure to lethal and sub-lethal concentrations of Flubendiamide for 24, 48, 96 h and 8 days, the amount of RNA was found to decrease in all the tissues. The lyotropic gradation series in terms of per cent decrement at 24,48, 96 h and 8 days exposure was:

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

Lethal-24h: Muscle > Gill > Kidney > Liver >

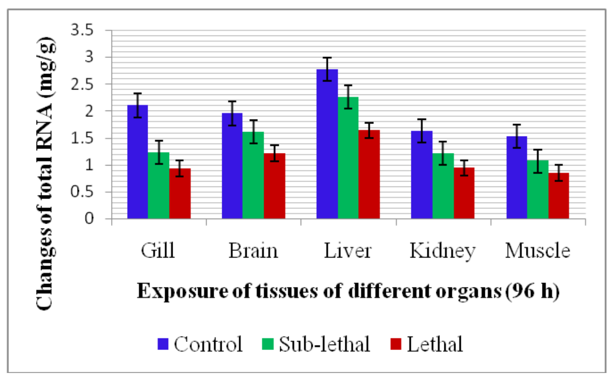

Fig. 8a. Changes in RNA content in different tissues of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 96 h.

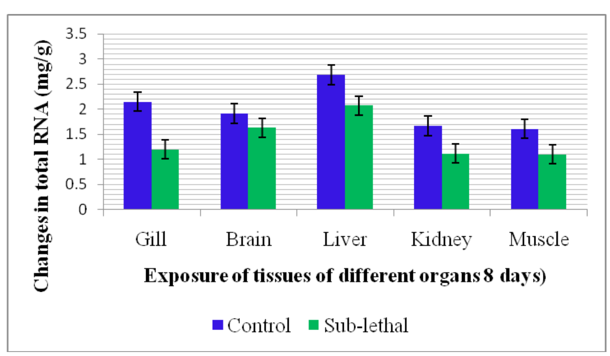

Fig. 8b. Changes in RNA content in different tissues of the fish *Catla catla* exposed to the sub-lethal concentration of Flubendamide for 8 days.

Brain

Sub-lethal- 48 h: Gill > Muscle > Brain > Kidney > Liver

Lethal- 48 h: Gill > Muscle > Brain > Kidney > Liver

Sub-lethal -96h: Gill > Muscle > Kidney > Liver > Brain

Lethal-96h: Gill> Muscle > Liver > Kidney > Brain

Sub-lethal-8 d:Gill > Kidney > Muscle > Liver > Brain

The results indicated that the DNA and RNA content in all the tissues of test fish were decreased compared to controls and the decreasing trend was more pronounced in lethal concentrations than in sub-lethal concentrations. In maintaining the physiological configuration of the fish Nucleic acids plays a vital role. As Nucleic acid and protein play the main role in regulating different activities of cells they are regarded as important biomarkers of the metabolic potential of cells (Veeriah *et al.,* 2013a).

Several other investigators also observed decrease in nucleic acid content in fish exposed to pesticides. The decrease in nucleic acid content was found by Dasu (2014) in fingerlings of *Labeo rohita* were exposed to Thiocarb (Larvin 75% WP) a thiocarbamate pesticide. Anitha and Rathnamma (2016) noticed decreased DNA and RNA levels in all the tissues like liver, kidney, brain, gill and muscle of *Labeo rohita* exposed to lethal and sub-lethal concentrations of Pyraclostrobin 20%WG (carbamate) for 24 h and sub-lethal concentrations for 5 and 10 days. Vani *et al.*, 2020 found decrease in nucleic acid content in fish *Cirrhinus mrigala* exposed to sub-lethal and lethal concentrations of Cartap hydrochloride (50% SP) for 24 and 96 h. Chengbin xu *et al.* (2020) reported that the new generation insecticide Cyantraniliprole at sub-lethal doses damaged the DNA of liver cells of fish *Oreochromis mossambicus.*

Tilak *et al*. (2009) noticed a decreased level of DNA and RNA content in Alachlor treated freshwater fish, *Channa punctatus* (Bloch). The decrease of RNA may be due to inhibiting the function of RNA polymerase or due to interference in the incorporation of precursor in the nucleic acid synthesis. The alterations in DNA levels may be due to disturbances in DNA synthesis and its turnover rate besides degenerative changes caused by pesticides.

From the present study, it can be concluded that exposure of *Catla catla* to Flubendiamide caused a decline in the glycogen, total protein and Nucleic acids (DNA, RNA) content which is more pronounced in lethal exposure than in sub-lethal exposure. The alterations caused during pesticide exposure may be due to the decreased catabolism of the bio molecules to meet the energy demand of test organism under stress or their reduced synthesis due to impaired tissue function. Therefore, the results of this study suggest a serious concern towards the potential danger of Flubendiamide for the aquatic environment and organisms suggesting judicious and careful use of this pesticide in the agricultural area.

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Table 8. Changes in the amount of ribonucleic acid (RNA) (mg/g wet weight of the tissue) in different tissues of the fish *Catla catla* exposed to the sub-lethal and lethal concentrations of Flubendamide for 96 h and only sub-lethal for 8 days.

	96 h					8 days		
Tissue	Control	Sub-lethal	% Change	Lethal	% Change	Control	Sub-lethal	% Change
Gill	2.1± 0.47	1.23± 0.25	42.63	0.93± 0.22	56.27	2.15± 0.47	1.20± 0.32	42.981
Brain	1.95± 0.92	1.61± 0.76	16.34	1.21± 0.47	36.75	1.91± 0.56	1.63± 0.58	13.583
Liver	2.77± 0.62	2.26± 0.54	17.34	1.64± 0.73	39.64	2.68± 0.53	2.07± 0.62	21.676
Kidney	1.63± 0.52	1.21± 1.04	24.6	0.94± 0.86	41.07	1.67± 0.94	1.12± 0.47	31.738
Muscle	1.53± 0.39	1.07± 0.17	28.87	0.85± 0.26	43.15	1.61± 0.28	1.10± 0.19	30.481

Results are mean values of four observations

Standard Deviation is indicated as (±)

Values are significant at $p < 0.05$

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