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Impact of Herbicide (Atrazine) on the Biochemical Components of the Fish, *Labeo Rohita*

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A B S T R A C T

The biochemical components like protein, carbohydrate and cholesterol were estimated quantitatively in the tissues of liver, kidney, muscle and gill of control and herbicide treated fishes. The fishes were treated with the sub lethal concentration of 3ppm for 24, 48, and 72 hours. The protein, carbohydrate and lipid level of liver, kidney, muscle and gill of the control fish was very high when compared with the treated ones. In treated fish the protein content of tissues were greatly reduced. Maximum reduction was observed at 72 hours exposure. The carbohydrate and cholesterol content of the tissues are showed similar declining trend at different exposure periods. Studies on the toxic effects of atrazine on fish have shown varied responses according to the type of species and dosages.

Keywords: *Labeo rohita*, atrazine, protein, carbohydrate, Cholesterol

A R T I C L E I N F O

Contents

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1. Introduction

Fish can serve as bio-indicators of environmental pollution and can play significant roles in assessing potential risk associated with contamination in aquatic environment since they are directly exposed to chemicals resulting from agricultural production via surface run-off or indirectly through food chain of ecosystem. The use of herbicides to

control weeds has been recognized as a part of agricultural practices throughout the world. Unfortunately, the indiscriminate use of these herbicides to improve agricultural production and yield may have impacts on non-target organisms, especially aquatic life forms and their environment. (Verma and Raji, 2000). Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is one of the most commonly used herbicides found in the rural environments. It is extensively used on corn, sorghum, sugarcane, pineapples, and to some extent on landscape vegetation. Rated as moderately toxic to aquatic species. Biochemical changes induced by herbicides strain lead to disturb the metabolism, inhibition of an important enzyme, retardation of growth and reduction in longevity of the organs the persistence of toxic chemicals in aquatic environment became dangerous for the survival of fish and their food organisms. Therefore, it is essential to study the toxic effects of herbicide on living organisms.

2. Materials and methods

The freshwater fish, *Labeo rohita* (body length 5-7 cm, body weight 5-6 g) were collected from Aliyar Dam and acclimatized to laboratory condition for 2 weeks in a large cement tank at 24±3°C. The fish were fed regularly with conventional diet rice bran and oil cake in 1:1 ratio. Feeding was stopped one day prior to the start of the experiment. Technical grade of Atrazine herbicide was used in the investigation. Batches of 10 healthy fish were exposed to different concentration of the insecticide. LC50 value for 72 hrs was calculated by using probity analysis. Four groups of fish were exposed to 3 ppm concentration of the herbicide Atrazine for 24,48 and 72 hours respectively.

Another group was maintained as control. At the end of each exposure period, fishes were sacrificed and tissues such as liver, kidney, muscle and gill were dissected and removed. The tissues were homogenized with 80% methanol, centrifuged at 3500 rpm for 15 minutes, and the clear supernatant was used for analysis of different parameters. The total protein was estimated following the method of Lowery et al.(1951).Carbohydrate was estimated using the method of Hedge and Hofreiter (1962) and the cholesterol was estimated using the method of Richmond (1973). The results were expressed as mg/g wet weight of the tissue.

3. Results & Discussion

The changes in protein, carbohydrate and cholesterol levels in different tissues of fish after the treatment with Atrazine are presented in Tables 1 and 2 and 3 while analyzing the changes in the protein, chole sterol, and carbohydrate it became clear that they fluctuated during different intervals of treatment. The level of protein was found to be decreased in all tissues compared to control. The decrease in protein content was Liver > kidney > gill > muscle on 72 hours exposure (table 1). The percentage decrease ranged from 91.22 to 92 16 in liver, 61.90 to 65.57 in kidney; 19.65 to 33.33 in gill and 10.47 to 10.96 in muscle. The liver showed highest percentage decrease in protein content might be due to diversification of energy demands when fish under stress or altered enzyme activities (Jadhav and Lomate, 1982). Proteins are important organic substance required by organisms in tissue building. The amino acid is the building blocks of proteins which are synthesized in the body must be supplemented through diet. Since, the food value of fish is directly dependent on their protein content, the contamination by the toxic substance will reduce their nutritive value

Table 1: Changes in the level of protein (mg/g) different tissues of the fish, Labeo rohita exposed to different durations and concentration (3ppm) of the herbicide atrazine

Tissues (mg/g)	Exposed (hours)	Control	Treated	%	SE	't' test
Muscle	24	0.96 ±0.01	0.86*** ± 0.01	10.47↓	0.01	2.16
	48	0.95 ±0.01	0.85*** ± 0.01	10.58↓	0.01	2.16
	72	0.94 ±0.01	0.84*** ± 0.01	10.96↓	0.01	2.16
Liver	24	6.27 ±	0.55*** ± 0.04	91.22↓	0.04	7.78
	48	0.55	0.49*** ± 0.01	92.02↓	0.01	1.86
	72	6.15 ±0.05	0.47*** ± 0.01	92.16↓	0.004	2.73
		6.00±0.05				
Kidney	24	0.63 ±0.01	0.24*** ± 0.01	61.90↓	0.01	4.97
	48	0.64 ±0.03	0.23*** ± 0.01	64.06↓	0.01	3.58
	72	0.61 ±0.01	0.21*** ± 0.01	65.57↓	0.01	2.13
Gill	24	1.17±0.01	0.94*** ± 0.01	19.65↓	0.01	3.67
	48	1.16 ±0.01	0.93*** ± 0.01	19.82↓	0.01	6.49
	72	1.38 ±0.08	0.92*** ± 0.01	33.33↓	0.01	1.71

Results are mean (± SD) of 6 observations; %= Percent increase/ decrease over control;
*** = Significant at 0.05 level;**
***** = Significant at 0.01 level;**
***** = Significant at 0.001 level;**
NS = Not significant.

In the present study the maximum reduction of carbohydrate content was observed upon 72 hours exposure (Table2). In the tissues, the trend of decrease in carbohydrate content was Kidney> muscle > gill > liver. The percentage decrease ranged from 76.26 to 77.16 in kidney, 54.01 to 55.57 in muscle, 39.82 to 40.18 in gill, 35. 39 to 35.66 in liver. The kidney showed the highest percentage decrease (77.16) in carbohydrate content.

Table 2: Changes in the level of carbohydrate (mg/g) in different tissues of the fish, *Labeo rohita* exposed to different durations and concentration (3ppm) of the herbicide atrazine

Tissues(mg/g)	Exposed (hours)	Control	Treated	%	SE	't' test
Liver	24	23.65 ± 0.55	15.28*** ± 0.01	35.39↓	0.01	2.45
	48	23.60 ±0.05	15.23*** ± 0.01	35.46↓	0.01	2.84
	72	23.58±0.08	15.17*** ± 0.01	35.66↓	0.01	1.79
Kidney	24	11.33 ±0.82	2.69*** ± 0.01	76.26↓	0.01	1.52
	48	10.50 ±0.55	2.59*** ± 0.01	76.29↓	0.01	3.47
	72	9.33 ±0.52	2.51*** ± 0.01	77.16↓	0.01	5.27
Muscle	24	16.95 ±0.55	7.79*** ± 0.01	54.01↓	0.01	1.98
	48	16.80 ±0.11	7.49*** ± 0.02	55.44↓	0.02	8.39
	72	16.67 ±0.08	7.51*** ± 0.01	55.57↓	0.01	1.16
Gill	24	13.95 ±0.55	8.39*** ± 0.01	39.82↓	0.01	2.41
	48	13.85 ±0.05	8.29*** ± 0.01	40.11↓	1.16	3.23
	72	13.70 ±0.06	8.19*** ± 0.01	40.18↓	0.01	4.39

Carbohydrate represents the principal and immediate energy precursor for fish exposed to stress condition. This reduction might be the result of the effects of the toxin atrazine on the kidney of the fish (Braunbec et al. 1982) the present observation indicated that the extent of decrease in cholesterol was more in high concentration upon 72 hrs exposure (Table 3). In the tissus, the trend of decrease in cholesterol content was kidney > liver > gil l> muscle, The percentage decrease ranged from 99.73 to 99.89 in kidney, 93.76 to 95.87 in liver, 78,.24 to 80.39 in gill and 59.82 to 60.61 in muscle. The kidney showed the highest percentage decrease (99.90) in cholesterol content). Cholesterol is the base material for all steroid hormones. When it increases due to cortisol synthesis, then a large amount of cholesterol areneeded (Kazemi et al. 2010). Therefore, the reduction in the amount of cholesterol may be relatedto its utilization in the manufacture of Cortisol arising from stress created by the toxin atrazine. Lipids serve as energy source for fish metabolism and hence reveal their importance during stress condition (Jezreiska et al., 1982). Decrease in muscle lipid indicates that lipid hydrolysis might be accelerated to derive energy to overcome toxic stress (Rao et al., 1985). The disturbance of fat metabolism is an indication impaired pancreatic functions (Jayantharao et al., 1984). A similar declining trent of lipid control of the brain,gill, kidney, liver and muscle on exposure to carbamate in fish, Oreochromis mossambicus was observed by Arockiya Reta and John Milton (2006).

Table 3: Changes in the level of cholesterol (mg/g) in different tissues of the fish, *Labeo rohita* exposed to different durations and concentration (3ppm) of the herbicide atrazine

Tissues(mg/g)	**Exposed (hours)**	**Control**	**Treated**	**%**	**SE**	**'t' test**
Liver	24	49.55 ± 0.55	3.09*** ± 0.01	93.76↓	0.01	4.73
	48	49.45 ±0.05	2.55*** ± 0.49	94.84↓	0.53	2.77
	72	49.03±0.08	1.99*** ± 0.01	95.87↓	0.01	1.84
Kidney	24	37.85 ±0.55	0.099*** ± 0.01	99.73↓	8.23	9.34
	48	37.73 ±0.05	0.07*** ± 0.01	99.81↓	0.004	1.73
	72	37.55±0.05	0.04*** ± 0.01	99.89↓	0.01	1.41
Muscle	24	19.65 ±0.05	7.90*** ± 0.12	59.82↓	0.12	5.82
	48	16.55 ±0.05	7.75*** ± 0.01	60.38↓	0.01	5.56
	72	19.53 ±0.05	7.69*** ± 0.01	60.61↓	0.01	3.50
Gill	24	42.25 ±0.05	9.19*** ± 0.01	78.24↓	0.01	2.38
	48	42.13 ±0.05	8.95*** ± 0.06	78.77↓	0.06	1.68
	72	42.08 ±0.08	8.25*** ± 0.27	80.39↓	0.30	6.44

On the whole, it has been observed that the atrazine, is highly toxic to fish, labeo rohita, leading to effect the nutritive value of the fish and all the metabolites studied are found to be sensitive change in the normal indictors which reflect changes in the normal indictors which reflect changes in the normal activities of various functional systems. The random use of atrazine must be avoided for preserving our aquatic resources.

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