P: ISSN NO.: 2321-290X RNI : UPBIL/2013/55327 VOL-7* ISSUE-12* August- 2020
E: ISSN NO.: 2349-980X **Shrinkhla Ek Shodhparak Vaicharik Patrika**

Materials and Method Used for Studies on Atrazine Induced Changes in some Cat-fish

Paper Submission: 15/08/2020, Date of Acceptance: 26/08/2020, Date of Publication: 27/08/2020

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Abstract

As a Research Scholar I worked on Atrazine induced changes in some cat-fishes. During the process of my research work, I learned a lot about the impact of Atrazine induced changes in some cat-fishes within India and globally. Reviewed and studied a number of researchpapers and other important reports in reference to my research work. In fact study is very important to understand the impact of Atrazine induced changes in cat-fishes in our environmental conditions.

It was very interesting to know about the material and method used to understand the impact of Atrazine induced changes in cat-fishes as far as public health is concerned.

This paper is in continuation of the Ph.D. work on "Studies on Atrazine induced changes in some Cat-fishes."

After completion of this research work, author are continuously working on this issue and trying to understand more about the impact of Atrazine induced changes in Cat-fises.

The presented paper is also one more step to learn more about it. Author would like to discuss on this paper on the material and methods used in the study of Atrazine induced changes in some Cat-fishes. Paper will be very helpful in understanding about the impact of Atrazine induced changes in some Cat-fishes.

Keywords: Atrazine, Herbicides, Weedicides, Fresh Water Cat-Fish Hetropneustes Fossilis, Clarias Batrachus, Haematological Parameters.

Introduction

Chemical control of weeds by herbicides and weedicides are in common use throughout the world. It cannot be stopped because its use is very easy as well as economical, but at the same time it bad effects are also faced by agriculturists and pisculturist. Herbicides have been proved successful in the control of herbs in interrestrial agriculture system. Increased and indiscriminate use of herbicides flows down the aquatic body and ultimately casuses unpresidented ecological damages mainly through their effects on the non target organism including fish. The herbicide atrazine is used throughout the world for a varied range of uses. It is an environmental contaminant regularly found in rain, surface, marine and ground water.

Common Indian Cat Fish – Clarias batrachus and Heteropneustes fossilis (Bloch) were used as experimental animal. Fishes were collected from local market of Samastipur. Fishes were kept in aquarium and were acclimatized under laboratory condition at least for 15 days. Healthy and sexually mature fishes of more or less equal weight (the range of variation in weight no exceeding 15 grams) were selected for experiments. The fishes were fed daily with wheat pellets and ground dried shrimps (Srivastava, 1966). Aquaria were cleaned and the water was changed daily to eliminate contamination caused by the faecal matters.

Review of Literature

Certain reports are available on the efficacy of certain herbicides and weedicides on fishes. A survey of literature gives information about some of the works carried out in past by some investigators which includes the works of :-

1. Shrivasta and Gupta (1981) studied the effect of Soduum salt of 2, 4-D on Carbohydrate metabolism in Heteropneustes fossils.

2. Dhillon & gupta (1983 studies the effect of sublethal concentration of Aldrin & Swascofis CD-38 on certain haematological parameters of Clarias batrachus.
3. Shrivastava & Singh (1982) made a study on the effect of propozus on Carbohydrate metabolism on Heteropneustes fossilis.
4. Pathak S. K. (1986) studied the effect of simazine on common Indian Catfish Heteropneustes fossilis on some biochemical as well as heamaotological parameters and reported adverse effects.
5. Tkasew S. J. (1983) studied the effect of Heptachlor and Endrin on fish and reported adverse effect on the organism.
6. R. Clegg B. S., Sherman C. Champan ND (1990) studied on effect of Triazine and Chloroacetamide herbicides in Sydenham river water and municipal drinking water.
7. Advisory Committee of pesticides, International Programme on chemical safety (1993) have approved atrazine as product no. 71 and selective systemic herbicide.
8. Hicks B. Genetic Pesticide (1998) has reported that atrazine was introduced in 1958.
9. Co x C. (2001) has reported that atrazine ad atrazine containing herbicides reduces the ability to reproduce in human beings.
10. Sander R (2002) have reported the serious effects of atrazine on frogs sexual development.
11. De Sesso JM, Scialli AR, White TEK Breckenridge CB (2014). Multigeneration reproduction and male development toxicity stdies on Atrazine in Rats Birth defects. Res B. Dev Report Toxicol, 101, 237-53.
12. Corve M, Stanley K Petersen T, Kent M, Feists, LaDuj, et al 2012. Investigating the impact of Chronic Atrazine exposure on sexual development in Zebra fish. Birth defects, Res B 95, 276-88.

One of the crucial problems faced by pisciculturists is the control of aquatic weeds since its profusion causes imbalance in aquatic soluble nutrients and other essentials. Weeds severely restrict plankton production, limits the living spaces of Fish, upset the equilibrium of physico-chemical qualities of water, causes imbalance in dissolve O_2 budget, promote accumulation of deposit leading to siltation, provide shelter to predatory and weed-fishes, molluscan and aquatic insects and obstruct netting operation (Jhingran, 1983).

The aquatic word problems have been increasing day by day and have attracted the attention of pisciculturists. The extent of this problem can be realized from the fact that water hyacinth (Echornia crassipes) alone accounts for about 0.5 million hectare in India. The area infested with other aquatic weed is estimated to be an additional 3,20,000 hectare, which represents a potential annual loss of fish-crop of 1,60,000 MT per year. Recently another serious weed Salvinia Molesta has been found to be spreading very rapidly and is now considered the second major national weed problem. Among submerged weeds, Hydrilla Verticillata is the most important and difficult to control. Persistent algal-blooms and other blue-green algae seriously affect fish culture in ponds and become even more problematic in intensive culture programs (Ramprabhu and Ramchandran 1984).

Weedicides and herbicides have proved successful in the control of weeds in aquaculture system. However, the production of increasing number of pesticides and herbicides has caused unprecedented ecological damage mainly through their effect of non-target organism including fish. The widespread use of herbicides has resulted in a steady increases in water pollution, evoking considerable damage of phytoplankton and zooplankton, thus depleting essential source of the food chain for fish (Montanes et al 1995).

Atrazine is one of the most commonly used herbicides in the United States. It is widely used in Asian continent and specially the India too. Atrazine is considered as a controversial organic compound due to its bad effects on the non-target organism. Tyrone Hayes a Scientist at UC Berkeley found evidence that it is a teratogen causing demasculinization in male frogs affected by atrazine could reach testosterone levels below females.

Atrazine is used to stop pre and post emergence and broad leaf and grassy weeds in major crops by binding to the plastoquinone binding protein in photosystem II, inhibiting electron transport. Atrazine and its derivatives are also used in many industrial processes, including the production of some dyes and explosives. Atrazine is the most widely used herbicides in conservation tillage system which are designed to prevent soil erosion.

Aim of the Study

1. To know about the impact of Atrazine on some cat fishes.
2. To understand the material and method used for the aforementioned study.
3. To understand the impact of herbicides on fishes.
4. To understand the impact of herbicides on aquatic environment.

Materials and Methods

Common Indian Cat Fish – Clarias batrachus and Heteropneustes fossilis (Bloch) were used as experimental animal. Fishes were collected from local market of Samastipur. Fishes were kept in aquarium and were acclimatized under laboratory condition at least for 15 days. Healthy and sexually mature fishes of more or less equal weight (the range of variation in weight no exceeding 15 grams) were selected for experiments. The fishes were fed daily with wheat pellets and ground dried shrimps (Srivastava, 1966). Aquaria were cleaned and the water was changed daily to eliminate contamination caused by the faecal matters.

Acclimatized fishes were divided into four groups :

Each group comprising of 60 fish specimens for various investigations at 12 mg/L Atrazine concentration.

Each of the above groups of fishes were divided into six subgroups of ten fish specimens each.

One group was selected for investigations of different biochemical parameters at control and the rest four groups were used for estimations of Blood Urea, Blood Glucose, Serum, Cholesterol and Plasma protein at different time intervals, i.e. controlled condition, 247 hr. 48 hr. 72 hr and 96 hr respectively after the treatment of Atrazine. One subgroup was maintained as reserve group.

The same p^H of water was maintained throughout the experiment to elude the effect due to its variation. In one aquarium only 10 litres of water was poured.

The experimental fishes were not given any food 24 hr. before the treatment of Atrazine.

Preparation of Atrazine Solution

The Atrazine supplied contained 50% active ingredient, thre-fore, just double amount of Atrazine were weighed and dissolved to obtain desired ppm solution.The ppm refers to one mg of the solute present in one kg. of solution.

Stock solution to obtain desired concentration of 120 mg/L was prepared.

The parameters under investigations comprised :-

(A) **Physico-Chemical properties of water**: It comprises the determination of pH, dissolved O_2 content and water temperature.

(B) Dissolved organic phosphorus and Nitrogen content of water.

(C) Biochemical Parameters : It comprised the estimations of :-

(a) Blood glucose (King et al varley 1970)
(b) Blood Urea (Natelson 1957)
(c) Serum Cholesterol (Sacketts Method)
(d) Plasma Protein (Sutherland et al 1949)

The fish was taken out of the aquarium and kept in dissecting tray. The head part of the fish was covered with a piece of cloth and was caught with a very little pressure to avoid any stress on the fish. The line of the lateral line system was located and the needle of a dry 2 ml syringe, rinsed with an anticoagulant (3.8%) Sol. of Sod. Citrate) was pierced gently into the muscle of the fish and was introduced into the lumen of caudadorsalis running just below the vertebral column keeping the syringe vertically at an angle of 45^0. The blood started coming into the syringe and in this way blood was collected and poured in a dry vial for various investigations.

Estimation of Blood Glucose

The blood glucose level was estimated by the modified Schaffer-Hariman Titrimetric technique of King et al (Varley, 1976).

Principle

The blood is added to an isotonic Sodium Sulphate-Copper sulphate solution from which the proteins are precipitated by the addition of Sodium tungstate. After centrifuging, reduction of Cupric Copper in an alkaline solution is followed by cooling and addition of Potassium Iodide to liberate iodine which then reoxidizes the Cuprous copper. The amount of iodine thus used up as determined by Thiosulphate titration of Test and Blank and is proportional to the amount of glucose originally present.

Reagents

Following reagents were used-

Isotonic Sodium Sulphate: Copper sulphate solution (t prevent glucolysis) :

A mixture of 320 ml of 3% Sodium sulphate and 30 ml of 7% Copper sulphate.

Sodium tungstate: 10% solution.

Copperreagent

Harding's modification of Schaffer – Hartman reagent. This reagent was made by mixing two solutions.

Solution 'A'

I contained 13 grams Copper sulphate crystals dissolved in water and the volume was made upto 1000 cc.

Solution 'B'

This solution was prepared by dissolving :

1. 24 gms Rochelle's salt (Sodium – potassium tartrate)
2. 40 gms Anhydrous sodium carbonate
3. 50 gms Sodium bicarbonate
4. 36.8 gms Pottasium Oxalate
5. Exactly 1.4 gms Pottasium Iodate

The solution 'B' was best prepared as described below:

The Bicarbonate was weighed out, washed into a litre flask with 700 ml distilled water and dissolved at room temperature. The Carbonate was then added and shaken well until it was dissolved. The Oxalate was weighed into a beaker, dissolved in 120 ml of warm distilled water and added to the main solution. The Rochelle's salt was dissolved in 100 ml distilled water in a beaker and was added to the mixture. Finally, the lodate was weighed out and washed directly into the solution, which was shaken well and made upto the mark and again thoroughly mixed.

(Solution "B" sometimes formed a deposit on standing This did not attest the oxidizing properties and was therefore, ignored)

The copper reagent used in the above method was a freshly made mixture of exactly equal volumes of solution "A" and "B".

Potassium iodide

2% solution prepared freshly.

2N Sulphuric acid

5 ml of the conc. H_2SO_4 was added to water and made upto 100ml with distilled water.

$\frac{N}{200}$Sodium thiosulphate

$\frac{N}{200}$ Solution of Sodium thiosulphate is prepared and kept was stock solution. 5 ml of this $\frac{N}{200}$Solution wasdiluted to 100 ml with distilled water as working $\frac{N}{200}$solution.

1% Starch solution

100ml of saturated benzoicacid was heated in a beaker to building. A paste of approximately 1 gm of Starch in cold distilled water was prepared and poured slowly into the boiling saturated benzoic acid solution. The solution was cooled.

Standard Glucose Solution

1.5 gms of benzoic acid solution was weighed on a watch-glass and washed into a beaker by means of distilled water. The solution was heated to boiling. When it was dissolved, 0.75 gms of Glucose was added to it The mixture was stirred well. The solution was then cooled and was transferred into a 500 ml of volumetric flask. The volume was made upto the mark with distilled water.

Diluted solution for use

2.5 ml of stock glucose solution was taken on the day of use in a 50 ml volumetric flask and the volume was made upto the mark with distilled water.

Procedure

In a centrifuge tube 3.5ml of isotonic Sodium sulphate Copper sulphate solution was taken and into it, 0.2 ml of bloodwas added. Then 0.3 m of 10% Sodium tungstate was added to the mixture and the whole mixture was then shaken well and centrifuged at 7000 R.P.M. After getting centrifuged, 2 ml of Supernatant fluid was taken in a hard glass test - tube marked T (Test). 2 ml of distilled water was taken in another hard glass test - tube marked 'B (Blank). Then 2 mi of Alkaline copper reagent was added to both the test - tubes, and then these test-tubes were stoppered lightly with cotton and P acid in a boiling water - bath exactly for ten minutes. They were then immediately cooled under the tap water.

To each test - tube. 1 ml of 2% Potassium iodide and 1 ml of 2N Sulphuric acid were added. After 1 minute, each test- tube was titrated with $\frac{N}{200}$Sodium thiosulphate using 1% solublestarch as an indicator.

The blood glucose in mg per 100 ml of blood was calculated from the following formula :-
(Reading of Blank - Reading of Test) x 116.)

Estimation of Blood-Urea

Diacetyl Monoxime Method described by Natelson (1957) was adopted for determination of Blood-Urea.

Reagents

1. 10% Sodium tungstate
2. 2/3 N Sulphuric Acid
3. Diacetyl monoxime - 2% solution in 2% acetic acid.

i. It was prepared as follows:
ii. 2 gm of solid diacetyl monoxime was added to about 60 ml of water and 2 ml of Glacial acetic acid was added to it. The mixture was taken and slightly warmed to dissolve the mixture and it was made 100 ml with water

4. Stock standard solution of Urea - It was preparedby dissolving 250 mg Urea in 100 ml of distilled water.
5. Working Standard. It was prepared by diluting 1 ml of stock standard solution to 200 ml and 100 ml withdistilled water so that each solution contained 0.0125 and 0.025 mg Urea/ml respectively.

Procedure

01 mi o freshly sampled blood was washed into 3.3. ml distilled water. 0.3 ml of 10% sodium tungstate and 0.3 ml of 2/3 NH_2SO, were added to it. It was mixed well and centrifuged at 3000 RPM to obtain clear supernatant. To 1 ml of Supernatant fluid 1 ml of water, 0.4 ml diacetyl monoxime were added in hard glass test-tube. The tubes were placed in a boiling water bath for 30 minutes. The tube was cooled and the colour developed was read against a water-blank at 480 millimicron At the same time the colour was developed from 1 ml of each of the two standards in the same way.

The calculation was made from the following formula:

$$\text{mg Urea/100mi of blood} = \frac{\text{Reading of unknown}}{\text{Reading of known}} \times \frac{100}{0.025} \times 0.0125 \text{ or } 0.025$$

$$= \frac{\text{Reading of unknown}}{\text{Reading of known}} \times 50 \text{ for standard I and}$$

$$= \frac{\text{Reading of unknown}}{\text{Reading of known}} \times 100 \text{ for standard II}$$

Estimation of Serum Cholesterol

Following Serum Cholesterol was estimated bySackett's method (Varley, 1976)

Reagents

Following reagents were used:

Alcohol - Ether mixture

3 volumes of alcohol and 1 volume of ether weremixed. (9 ml Abs. alcohol & 3 mi of Ether).

Acetic anhydride - Sulfuric and mixture

20 ml of Acetic anhydride mixed with 1 ml of conc. H SO It was prepared freshly just before use.

Chloroform

Stock standard solution of Cholesterol: 200 ml of pure Cholesterol was dissolved in Chloroform and was made upto 100ml with Chloroform. It was kept well stoppered and was usedas a standard solution.

1 mi of Stock standard solution was diluted to 25 ml with Chloroform 5mi of that solution contained0.4 mg of Cholesterol.

Procedure

The sampled blood was taken in a Centrifuge-tube and centrifuged for 10 minutes at 7000 RPM 0.2 ml of serum from the centrifuged blood was added to 10ml of Absolute alcohol - Ether mixture taken in another Centrifuge tube. The tube was then corked tightly and shaken thoroughly for a minute. Thereafter, the tube was laid horizontally for half an hour for even distribution of the precipitate. The mixture in the tube was then centrifuged for 10 minutes and the Supernatant fluid was decanted in a hard-glass test-tube marked T (Test). Than test- tube was placed in a boiling water bath and the Supernatant fluid was then allowed to evaporatecompletely up to dryness. Then 5ml of Chloroform was added to dry substance i.e. Cholesterol residue. Meanwhile working standard solution was prepared by mixing 1 m stock standard solution of Cholesterol with 25 ml Chloroform of the prepared working solution 5 ml was taken in another hard glass test-tube marked s (Standard). Then 2 ml of a mixture of acetic anhydride and conc H_2SO_4 (ratio 2:1) was added in both the test-tubes marked 'T'&'S'. The solution in the test-tubes was mixed and kept in the dark for 15 minutes tor the complete development of colour.

The Photoelectric Colorimeter was switched on and left for five minutes and Chloroform as Blank solution was used to set for 100 percent transmission, i.e. '0' optical density using the red filter (680 nm). Then the readings of the optical density of the standard and unknown solution were taken and the result was finally calculated by the following formula 1mg of Cholesterol per 100 ml of blood = $= \frac{\text{Reading of unknown}}{\text{Reading of known}} = 200$

Estimation of Plasma Protein

The Plasma protein was determined by Biuret method as described by Varley (1980).

Reagents

Biuret Reagent

3 gm of Copper sulphate ($CuSO_4 + 7H_2O$) and 9 gms of Sodium - Potassium tartrate were dissolved in 500ml of 0.2N Sodium hydroxide. 5 gms of Potassium iodine was added to it and it was made 1000 cc with 0.2 N Sodium hydroxide.

Procedure

A measured volume of blood plasma was washed in Ethanol. Ether mixture and protein fractions were precipitated out forming pellets. The protein pellets were dissolved in 2ml of distilled water and 2ml of Biuret reagent was added to it. The solution was allowed to stand for 10 minutes at 37^0 C.

The Photoelectric Colorimeter was switched on and let for 5 minutes. The color developed in the solution (Violet) was read in Photoelectric Colorimeter at 540 nm using Biuret solution as blank**.**

Protein concentration was calculated by standard curve which was drawn by using different concentrations of the Bovine serum albumin

Standards

Standard solution of pure bovine serum albumin was prepared freshly by dissolving 5 mg/ml. The colour of the standards was developed at the same time and in the same manner.

Calculation

Protein gm / 100 ml of Blood

$$= \frac{\text{Reading of unknown}}{\text{Reading of known}} \text{ x} = \frac{\text{Conc .of Std .}}{\text{Plasma taken}} \text{ x} =$$

The accuracy of the results was checked by the help ofStandard curve.

Statistical Analysis

Statistical analyses of all the recorded data were made by simple analysis of variance and Bar notations of recorded datawere made at 0.01 level of P.

Model of Analysis of Variance Table

Source of Variation	Degree of freedom (D.F.)	Sume of Square S.S.	Mean Square M.S.S.	F. Value
Treatment T	K – K = No. of treatment	Tr. S.S.	$\frac{\text{Tr. S. S.}}{\text{K} - 1}$	
Error	N – K N = Total no. of observation	E.S.S.	$\frac{\text{E. S. S.}}{\text{N} - \text{K}}$	$\frac{\text{Tr. M. S.}}{\text{E. M. S.}}$
Total	N – 1	Total S.S.		

Observation Tables

Table 1

Showing Blood glucose (mg/100 ml of blood) of Clarias batrachus at 120 ng/L concentration and different time intervals of treatment of Atrazine.

Replication	TREATMENT				
	Control	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.
1	34.17	51.74	70.85	83.73	81.22
2	32.77	50.35	72.44	91.14	79.75
3	36.65	55.60	70.30	92.39	94.57
4	35.82	50.30	70.40	89.22	54.93
5	33.6	50.58	65.54	90.00	57.46
6	31.11	49.98	59.80	88.50	62.31
7	35.53	46.32	71.80	92.50	51.20
8	39.39	52.41	67.82	91.40	77.01
9	37.81	52.25	61.34	97.32	78.80
10	38.22	53.25	63.25	92.44	76.35
Mean	35.3	51.67	37.35	91.56	70.52

CD = 6.01 (at 1%)
SE (Mean) = 1.65

Graph – 1
Graph Showing Blood glucose (mg/100 ml of blood) of Clarias batrachus at 120 ng/L concentration and different time intervals of treatment of Atrazine.

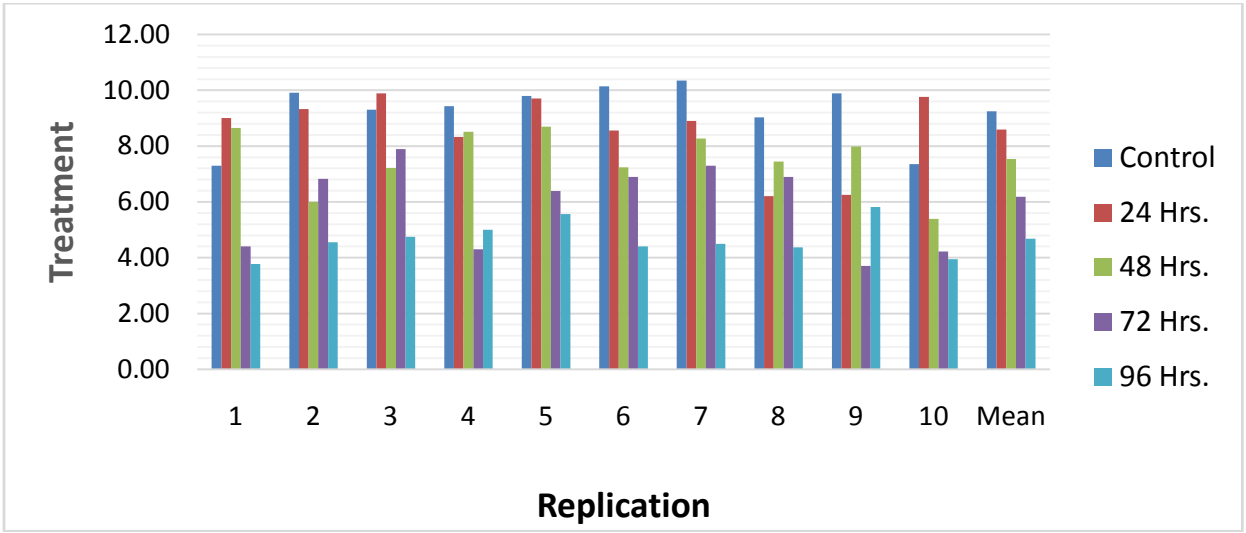


Table – 2
Showing Blood glucose (mg/100 ml of blood) of H. fossilisat 120 ng/L concentration and different time intervals of treatment of Atrazine.

Replication	Treatment				
	Control	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.
1	28.14	41.64	41.55	51.18	48.74
2	36.75	49.50	40.83	59.32	49.35
3	35.23	46.15	48.56	52.82	53.60
4	34.14	46.70	50.10	52.81	48.30
5	31.5	44.55	54.71	51.79	48.58
6	29.76	39.10	52.12	55.15	47.98
7	35.84	40.29	58.34	52.78	44.32
8	37.15	49.55	48.27	47.00	50.40
9	30.34	45.73	48.20	52.35	50.01
10	32.67	47.34	52.80	56.15	51.50
Mean	33.47	44.63	48.27	52.81	49.67

CD = 5.98 (at 1%)
SE (Mean) = 1.59

Graph – 2
Graph showing Blood glucose (mg/100 ml of blood) of H. fossilisat 120 ng/L concentration and different time intervals of treatment of Atrazine.

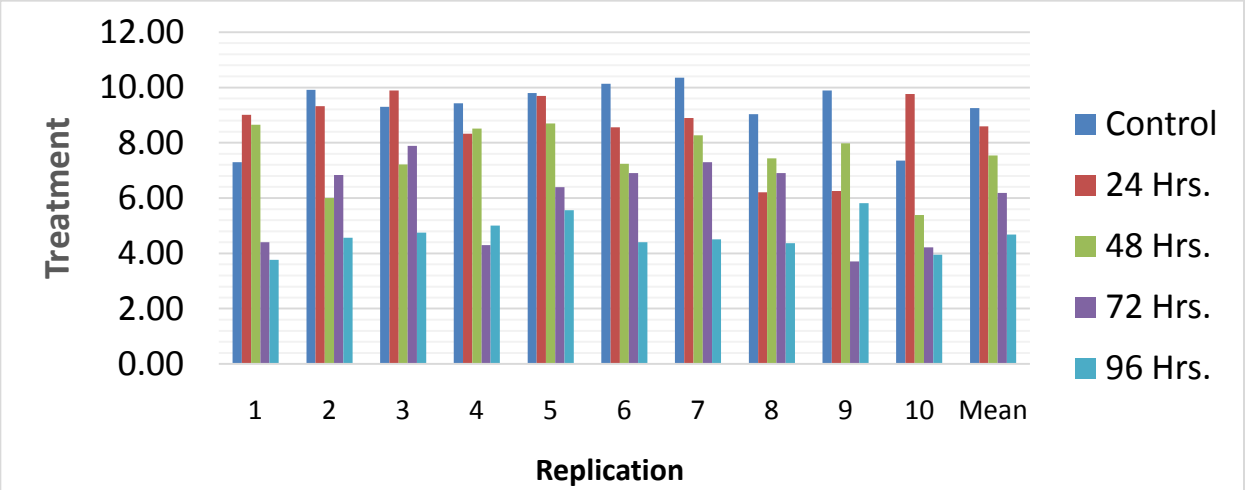
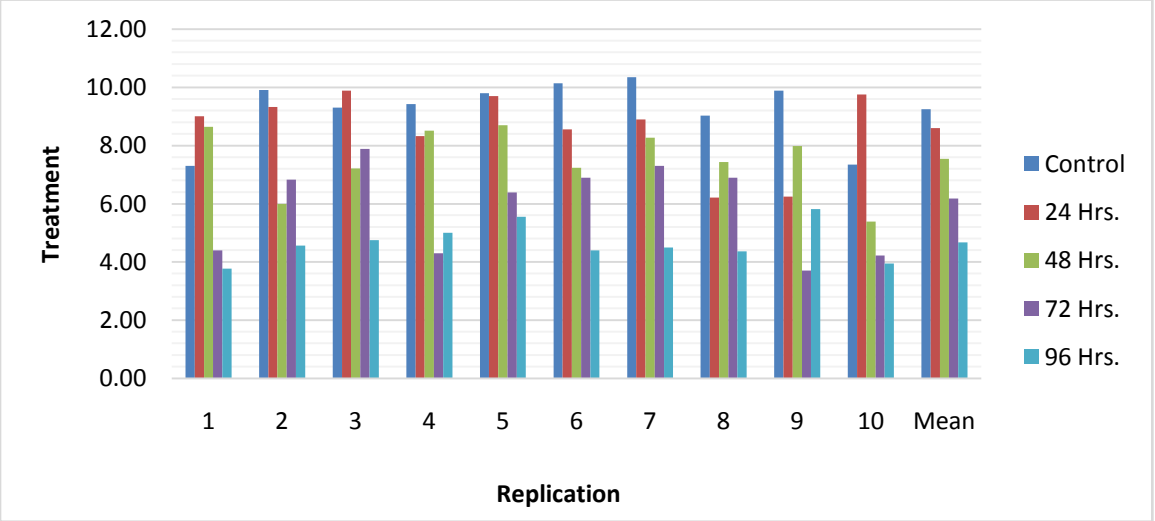


Table – 3
Showing Blood Urea (mg/100 ml of blood) of Clarias batrachusat 120 ng/L concentration and different time intervals of treatment of Atrazine.

Replication	TREATMENT				
	Control	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.
1	23.5	20.6	24.9	21.4	40.9
2	18.5	26.1	36.7	22.7	4.8
3	27.6	30.2	29.2	28.1	35.4
4	21.5	25.6	21.2	27.0	31.5
5	17.7	20.3	23.2	18.6	33.5
6	13.0	20.0	23.6	19.3	36.5
7	25.2	26.8	31.5	31.2	41.8
8	29.4	23.6	32.3	22.1	34.3
9	13.8	16.8	30.1	16.6	29.4
10	15.7	20.5	29.3	22.8	33.1
Mean	19.30	23.05	28.05	23.03	35.82

CD = 5.82 (at 1%)
SE (Mean) = 1.602

Graph – 3
Graph showing Blood Urea (mg/100 ml of blood) of Clarias batrachusat 120 ng/L concentration and different time intervals of treatment of Atrazine.



P: ISSN NO.: 2321-290X RNI : UPBIL/2013/55327 VOL-7* ISSUE-12* August- 2020
E: ISSN NO.: 2349-980X **Shrinkhla Ek Shodhparak Vaicharik Patrika**

Table – 4
Showing Blood Urea (mg/100 ml of blood) of H. fossilisat 120 ng/L concentration and different time intervals of treatment of Atrazine.

Replication	Treatment				
	Control	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.
1	26.6	32.4	36.2	27.7	48.5
2	24.5	23.4	39.3	31.0	55.9
3	28.7	37.5	40.8	29.3	52.4
4	21.5	39.2	38.3	30.1	51.4
5	23.5	29.9	27.2	25.5	45.1
6	32.7	37.2	47.4	35.1	57.6
7	34.9	39.2	41.2	35.4	56.3
8	25.6	30.5	34.3	27.4	58.9
9	29.3	28.8	43.0	33.8	49.2
10	37.9	33.8	49.4	38.5	52.5
Mean	28.44	33.19	39.30	31.34	52.76

CD = 5.980 (at 1% level)
SE (Mean) = 1.643

Graph – 4
Graph showing Blood Urea (mg/100 ml of blood) of H. fossilisat 120 ng/L concentration and different time intervals of treatment of Atrazine

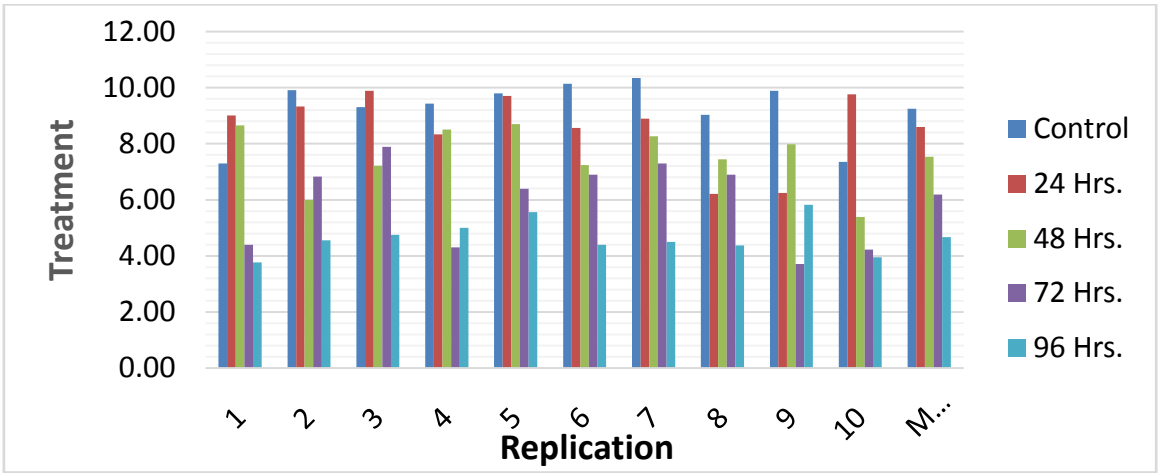


Table – 5
Showing Serum Cholesterol (mg/100 ml of blood) of Clarias batrachusat 120 ng/L concentration and different time intervals of treatment of Atrazine.

Replication	Treatment				
	Control	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.
1	391	422	382	416	455
2	428	460	479	513	546
3	319	428	488	556	638
4	411	452	490	512	675
5	402	436	483	571	625
6	406	345	514	490	566
7	415	473	453	415	433
8	306	452	363	592	638
9	309	430	540	562	607
10	405	391	508	545	584
Mean	384.2	426.8	470	517.2	576.7

CD = 59.15 (at 1% level)
SE (Mean) = 16.11

Graph – 5
Graph showing Serum Cholesterol (mg/100 ml of blood) of Clarias batrachusat 120 ng/L concentration and different time intervals of treatment of Atrazine.

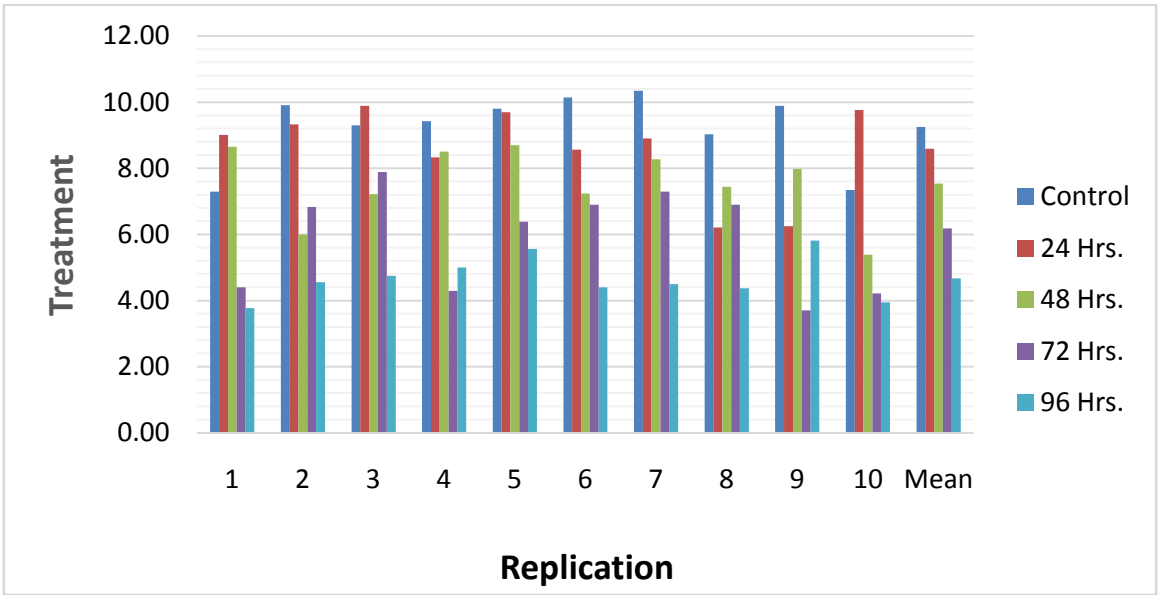
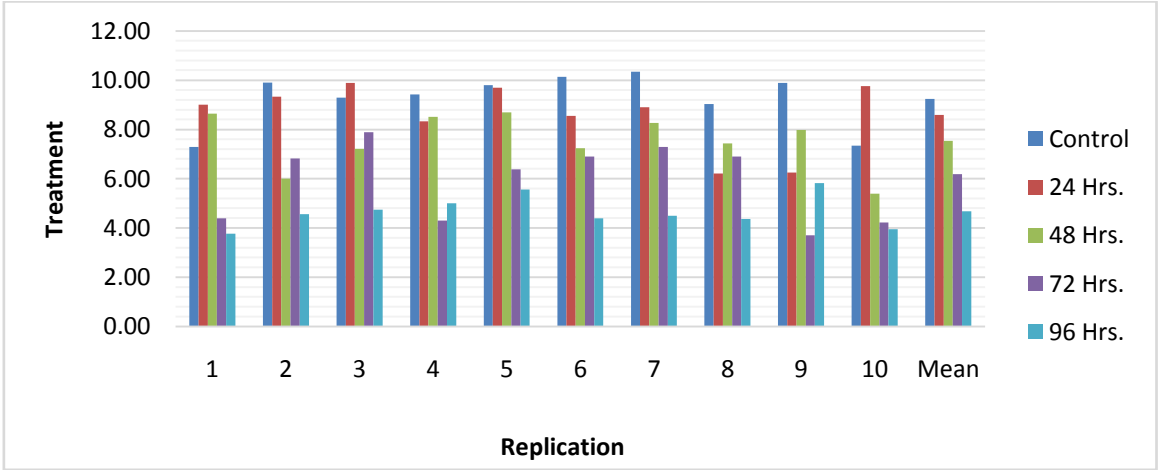


Table – 6
Showing Serum Cholesterol (mg/100 ml of blood) of H. fossilisat 120 ng/L concentration and different time intervals of treatment of Atrazine.

Replication	TREATMENT				
	Control	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.
1	411	425	441	427	535
2	412	421	454	481	503
3	302	360	511	479	612
4	329	372	468	495	496
5	320	388	408	485	451
6	338	374	423	537	519
7	405	412	441	576	502
8	453	465	476	483	537
9	466	472	480	475	518
10	625	428	502	479	615
Mean	366.1	410.6	460.4	491.7	528.8

CD = 58.55 (at 1% level)
SE (Mean) = 16.10

Graph – 6
Graph showing Serum Cholesterol (mg/100 ml of blood) of H. fossilisat 120 ng/L concentration and different time intervals of treatment of Atrazine.



P: ISSN NO.: 2321-290X RNI : UPBIL/2013/55327 VOL-7* ISSUE-12* August- 2020
E: ISSN NO.: 2349-980X **Shrinkhla Ek Shodhparak Vaicharik Patrika**

Table – 7
Showing Plasma Protein (mg/100 ml of blood) of Clarias batrachusat 120 ng/L concentration and different time intervals of treatment of Atrazine.

Replication	TREATMENT				
	Control	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.
1	8.33	10.00	9.55	8.86	6.54
2	9.25	10.20	7.00	7.75	8.34
3	9.77	9.36	8.67	8.95	6.90
4	8.47	7.80	8.26	6.75	8.40
5	8.50	9.21	8.31	6.80	8.42
6	9.35	9.20	9.15	7.85	6.50
7	7.22	8.58	8.90	8.10	6.55
8	10.41	8.34	7.51	9.00	7.64
9	10.45	9.30	8.94	8.65	8.00
10	9.45	7.25	9.70	6.80	8.85
Mean	9.12	8.924	8.609	7.948	7.614

CD = 1.32 (at 1% level)
SE (Mean) = 0.39

Graph – 7
Graph showing Plasma Protein (mg/100 ml of blood) of Clarias batrachusat 120 ng/L concentration and different time intervals of treatment of Atrazine.

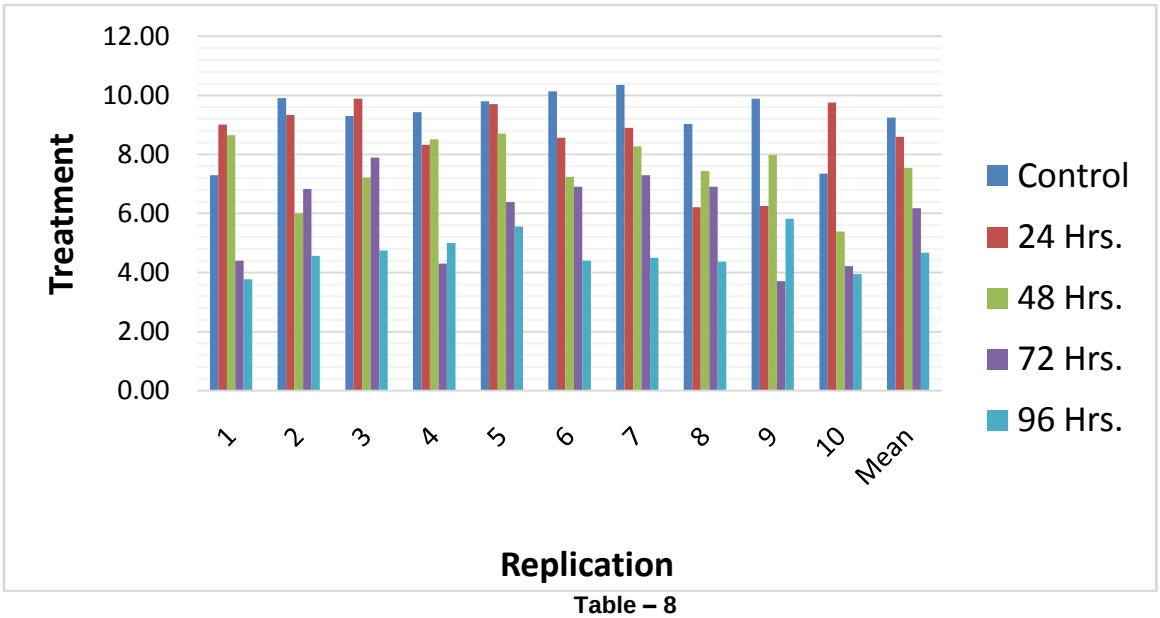
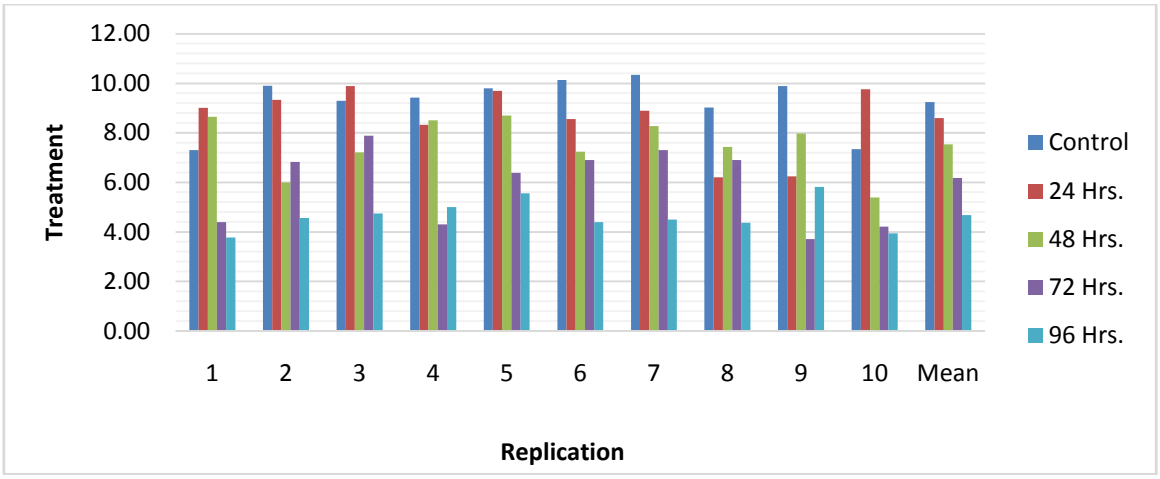


Table – 8
Showing Plasma Protein (mg/100 ml of blood) of Heteropneustes fossilisat 120 ng/L concentration and different time intervals of treatment of Atrazine.

Replication	TREATMENT				
	Control	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.
1	7.30	9.01	8.65	4.40	3.77
2	9.91	9.33	6.00	6.83	4.56
3	9.30	9.89	7.22	7.89	4.75
4	9.43	8.33	8.51	4.30	5.00
5	9.80	9.70	8.70	6.39	5.56
6	10.14	8.56	7.24	6.90	4.40
7	10.35	8.90	8.27	7.30	4.50
8	9.03	6.21	7.44	6.90	4.37
9	9.89	6.25	7.98	3.71	5.82
10	7.35	9.76	5.39	4.22	3.95
Mean	9.250	8.598	7.540	6.184	4.675

CD = 1.3 (at 1% level)
SE (Mean) = 0.38

Graph – 8
Graph showing Plasma Protein (mg/100 ml of blood) of Heteropneustes fossilis batrachusat 120 ng/L concentration and different time intervals of treatment of Atrazine.



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