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QUANTITATIVE ALTERATION IN THE NUCLEIC ACIDS AND PROTEIN DURING OVARIAN CYCLE OF COLISA FASCIATUS(BI. & SCHN.) , A TROPICAL FRESHWATER PERCH UNDER DISTILLERY EFFLUENT STRESS

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ABSTRACT

Studies were undertaken to record distillery effluent induced quantitative alterations in the nucleic acids (DNA & RNA) and protein during three principal phases of ovarian cycle (prespawning, spawning and post spawning) of *Colisa fasciatus* , a tropical freshwater fish. Observation were recorded under three different concentrations of the distillery effluent after 30 days of exposure. Observations reveal that 5% effluent concentration could produce least significant alterations ($P>0.05$) in the nucleic acids and protein content during any phase of ovarian cycle after 30 days of exposure. However, 10% and 20% distillery effluent concentration brought significant alterations in the nucleic acid (DNA & RNA) as well as protein content in all the phases in ovarian cycle after 30 days of exposure of *Colisa fasciatus* and was more pronounced under 20% effluent concentration . The declining trend in selected biochemical parameters was more pronounced during the spawning phase in comparison to the preparatory as well as post spawning phase of the ovarian cycle .

KEY WORDS : ***Distillery effluent, Phases of Ovarian cycle (preparatory ,spawning, and post spawning) Colisa fasciatus , Nucleic acids (DNA & RNA) , Protein.***

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INTRODUCTION

Liquid wastes (effluent) from food processing and other industries are the salient sources of water pollution. India stands as the major sugar producing country of the world having 579 sugar mills and 319 distilleries (Patil and Gholey,2010;). Besides sugar and alcohol, the sugar industries emit many bye products and waste materials (Raju and Manickan,1977; Fernando *et al.*,1999). Industries effluent in various developing countries are indiscriminately released into the near by aquatic bodies and even into the adjacent fields without any pretreatment. Distillery industry of GIDA, Gorakhpur (U.P.), India, discharges its effluent in the adjacent fields which during the rainy season gets its way into various major aquatic bodies including natural lakes and rivers. The industrial effluents contain varieties of pollutants such as organic and inorganic salts, heavy metals, detergents , pesticide etc., which directly or indirectly induce deleterious effects on non target aquatic fauna in general and fishes in particular (Ramakrishna *et al*.,1999; Fernando *et al*., 1999; Zsigmond *et al*., 2002; Ramakritinan *et al*.,2005; Shukla and Shukla 2011). The principal toxic constituents of distillery effluent include dissolved solids , sulphates, chlorides and higher percentage of dissolved inorganic matters (Chandra et al.,2008; Borreru *et al*., 2003; Ganeshan *et al*.,2010). In addition , the high BOD of the distillery effluent poses deleterious effects on the non-target aquatic fauna.

Numerous studies have been conducted on different biochemical parameters of the fishes (Khangarot,1985; Kumar *et al.*,1995; Pandey and Adholeya,2007) under pollutional stress of various industrial effluents. However,toxic effects of different concentrations of distillery effluent on nucleic acids and protein metabolism more particularly during different phases of ovarian cycle has yet not been worked out. Keeping this in view, present study has been undertaken to observe the effects of different concentrations of distilery effluent released from GIDA,Gorakhpur (U.P.) INDIA on the nucleic acids (DNA & RNA) and protein content during preparatory, spawning and post spawning phases of ovarian cycle of *Colisa fasciatus* (Bl .& Schn.), a tropical freshwater perch.

MATERIALS AND METHODS

The fish, *Colisa fasciatus* of an average total length 6.9±0.4 cm and weight 7.2±0.80 gm. were procured from local lake for experimental purpose. They were acclimatized for 14 days in laboratory tap water having the following physico-chemical properties as analysed by the procedure outlined by APHA, (2005).

Temperature	21.30 ± 1.640C
p^H	7.28 ± 0.22
Hardness as $CaCO_3$	128.30 ± 6.24 mg/l
Electrical conductivity	1296.6 µmho/cm

each of 20 fishes were exposed for thirty days under 5%, 10% and 20% distillery effluent concentration having the physico-chemical characteristics as shown in Table 1.

Table 1
Physico-chemical characteristics of undiluted distillery effluent of GIDA, Gorakhpur (U.P.)

Parameters	Units	Raw distillery effluent
Temperature	(C)	32.5±2.2
P^H		4.0-5.2
Oxygen	(mg/l^{-1})	ND
COD	(mg /l^{-1})	8000-12000
BOD	(mg /l^{-1})	1500-1800
Total Solids	(mg /l^{-1})	3600-4200
Suspended	(mg /l^{-1})	1800-2200

Solids	(mg /l^{-1})	6000-8000
Volatile Solids	(mg /l^{-1})	ND
Total Hardness	(mg /l^{-1})	ND
Free	(mg /l^{-1})	ND
Carbondioxide	(%)	0.80-120
Organic	(%)	0.034-1.02
Nitrogen	(mg /l^{-1})	1.16-1.28
Total Nitrogen	(mg /l^{-1})	3200-3800
Total	(mg /l^{-1})	260-340
Phosphours	(mg /l^{-1})	160-240
Potassium as	(mg /l^{-1})	180-260 (as
K_2O	(mg /l^{-1})	Ca^{++})
Sulphate as	(ppt)	50-680 (Cl^{-})
SO_4		ND
Ferrous		
Sulphide		
Calcium		
Chloride		
Salinity		

ND = not determined : Each value is the average of eight result ±SE.

During 14 days acclimatization, fishes were fed with dried shrimp powder on alternate days at the rate of 5% body weight. Though *Colisa fasciatus* is an air breathing fish, even then aeration facilities were provided for three hours daily in the control and experimental groups. Reproductive cycle has been divided into six phases, but only three principal phases viz., preparatory, spawning and post spawning have been selected for changes in biochemical parameters (DNA, RNA and protein) under control and distillery stress. Phases of ovarian cycle, their duration and period of experiment run were as follows:

Preparatory IInd week of March to IInd week of April

Spawning Ist week of June to Ist week of July.

Post spawning IInd week of November to IInd week of December.

The observations were recorded after 30 days of exposure. Experiment in different phases were performed at natural photoperiod and ambient water temperature. 20 fishes were kept each in experimental media and control. having 30 liters of water.

The DNA concentration was determined following the methods outlined by Dische (1930) based upon Diphenyl amine reagent and calf thymus DNA as standered.

Yeast RNA hydrolysate was used as the RNA standard for estimation of RNA concentration with the help of Orcinal reagent according the procedure outlined by Mejbaum, (1959).

Total protein content (sum of soluble and insoluble values) were estimated by applying the methods outlined by Lowery *et al*., (1951).

Student 't' test was used to test significant difference between experimental and their respective control groups with P<0.05 or less using the formula of Campbell (1974).

$$t = \frac{x_1 - x_2}{\sqrt{SE_1^2 + SE_2^2}}$$

x_1 = mean value of control
x_2 = mean value of experimental
SE_1 = Standard error of control mean
SE_2 = Standard error of experimental mean

RESULTS AND DISCUSSION

The DNA concentration in the control groups during three salient phases of ovarian cycle(preparatory,spawning and post

spawning) were 55.44±0.12, 52.26±0.28 and 33.32±0.18 µg/100mg wet weight of ovary respectively, The DNA concentration after 30 days of exposure during preparatory phase of ovarian cycle of *Colisa fasciatus* in 5%,10% and 20% distillery effluent concentration was 54.72±0.18, 53.26±0.16 and 50.48±0.20 µg/100mg wet weight of ovary respectively , as shown in Table 2 & Fig.1

Table2

Effect of distillery effluent(5%,10% and 20%) on nucleic acid (DNA & RNA and protein content during different phases of ovarian cycle of Colisa fasciatus. n=6 (*= P < 0.01 , **= P<0.001)

Phases	Parameters	Control	5% effluent	%change	10% effluent	% Change	20% effluent	% change
	DNA (µg100mg)	55.44±0.12	54.72±0.18	-1.29	53.26±0.16**	-3.93	50.48±0.20***	-8.94
	RNA(µg/100mg)	92.12±0.32	90.04±0.38	-2.25	87.88±0.46**	-4.60	82.14±0.38***	-10.83
	Protein(mg/gm)	93.12±0.48	90.56±0.52	-2.74	88.84±0.58**	-4.59	83.14±0.46***	-11.79
	DNA (µg100mg)	52.26±0.28	50.32±0.28	-3.71	48.84±0.32**	-6.54	44.36±0.28***	-15.11
	RNA(µg/100mg)	82.38±0.28	80.66±0.42	-2.08	78.76±0.40**	-4.39	74.12±0.38***	-10.02
	Protein(mg/gm)	102.24±0.68	99.86±0.68	-2.32	97.72±0.56**	-4.42	90.52±0.46***	-11.46
Post Spawning phase	DNA (µg100mg)	33.32±0.18	32.68±0.16	-1.92	31.04±0.24**	-6.85	28.12±0.26***	-15.60
	RNA(µg/100mg)	60.34±0.38	58.66±0.38	-2.78	78.76±0.40**	-5.50	54.22±0.28***	-10.14
	Protein(mg/gm)	82.28±0.36	80.32±0.52	-2.38	97.72±0.56**	-4.15	73.52±0.44***	-10.64

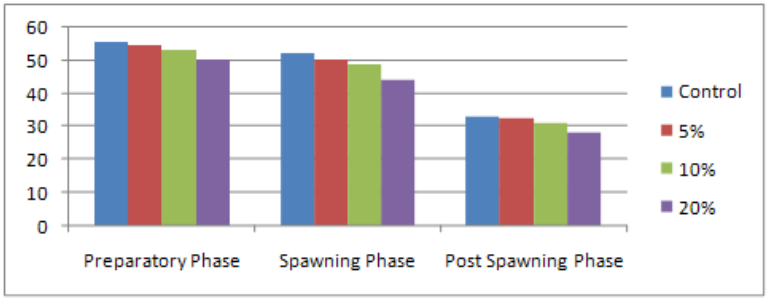

Figure.1

Fluctuation showing DNA content in different concentration (5%, 10% & 20 %) as compared to control in Distillery effluent stress for 30 days exposure

During spawning phase ,the DNA concentration after 30 days of exposure in 5,10 & 20% effluent concentration was 50.32±0.28; 48.84±0.32 and 44.36±0.28 µg/100mg of wet weight of ovary respectively when compared with control(table2 & Fig.1). During post spawning phase of ovarian cycle as compared to control, the DNA concentration after 30 days of exposure in 5%,10% and 20% was 32.68±0.16, 31.04±0.24 and 28.12±0.26 µg/100mg wet weight of ovary respectively (Table 2 and Fig1).

The RNA in control during preparatory, was 92.12±0.28 which in experimental lots containing 5%,10% and 20% effluent concentration was 90.04 ±0.38, 87.88±0.46 and 82.14±0.38 µg/100mg respectively(Table2 &Fig2).The RNA content during spawning phase of ovarian cycle was 82.38±0.28 in control, where as in 5%,10% and 20% selected effluent concentration, it

was 80.66±0.42, 78.76±0.40, and 74.12±0.38 µg/100mg wet weight of ovary after 30 days of exposure respectively. During post spawning phase, under aforesaid concentration(5,10&20%) it was 58.66±0.38, 57.02±0.32 and 54.22±0.28 µg/100mg wet weight of ovary as compared to control (60.34±0.38) µg/100mg.

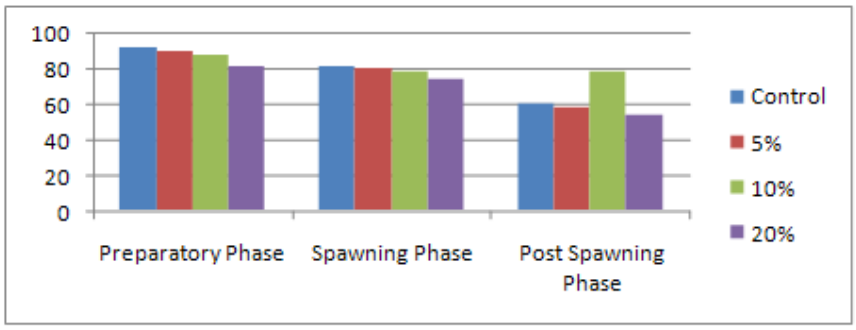

Figure.2

Fluctuation showing RNA content in different concentration (5%, 10% & 20 %) as compared to control in Distillery effluent stress for 30 days exposure

The protein content in the control group during preparatory , spawning and post spawning phases of ovarian cycle were 93.12±0.48, 102.24±0.68 and 82.28±0.36 mg/gm wet of ovary respectively. Under 5%,10%&20% concentrations of distillery effluent, these were 90.56±0.52, 88.84±0.58 and 83.14±0.46 mg/gm during preparatory; 99.86±0.68, 97.72±0.56 and 90.52±0.46mg/gm during spawning phase respectively and during post monsoon concentrations as compared to control as shown in Table 2 and Fig 1.

Also, the percent decling was in increasing order in

spawning phase protein content was 80.32±0.52, 78.86±0.54 and 73.52±0.44 mg/gm respectively in three different concentrations as compared to control as shown in table 2 and Fig. 3 . Also the percent declining was in increasing order in the selected three biochemical parameters during all the phases of ovarian cycle. Significant declining was more pronounced during three different phases under 20% ,less in 10% and least in 5% effluent concentration in all phases of ovarian cycle.

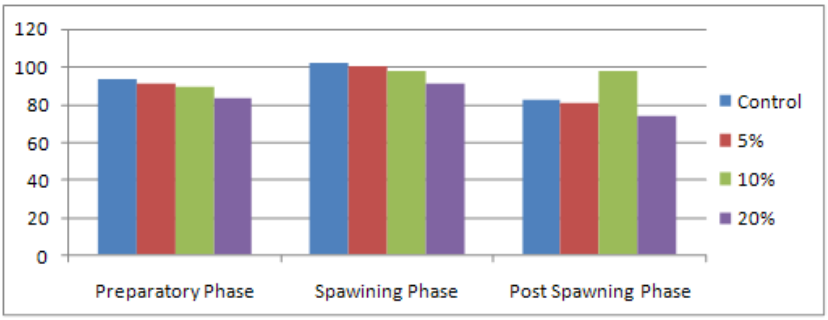

Figure. 3

Fluctuation showing Protein content in different concentration (5%, 10% & 20 %) compared to control in Distillery effluent stress for 30 days exposure

Xenobiotics cause acute and chronic toxicity depending upon the dose and duration of exposure. Gonads have a high cell turnover during reproductive cycle and are especially

vulnerable to a wide variety of stimuli (Drobeck and Coulston,1962). Our study demonstrates that the fish *Colisa fasciatus* gonads are highly sensitive to various distillery effluent concentration possibly due to their constant contact with the body surface because of the aquatic environment.

Distillery effluent contains various pollutants as reported by various workers including (Ramkritinan *et al*.,2005; Srivastava *et al*., 2007; Krishna and Prakash , 2010 ; Patil and Gholey,2010; Shukla and Shukla 2011)as shown in Table 1 . Many workers studied on certain biochemical alterations, more particularly enzymatic alterations but very few reports are available on the alterations in the nucleic acid (DNA & RNA) and protein particularly during different phases of the reproductive cycling of freshwater fishes. Keeping this in view, present study has been conducted. Our study reveals that less concentration of the effluent (5%) after long term exposure (30 days) could produce least significant alterations in the nucleic acid content when compared to control.

However, higher concentration (10% &20%) brought about significant alterations in nucleic acids (DNA & RNA) content. Recently more or less similar findings have been noticed by Shukla & Shukla,(2011) in the testicular cycle of a freshwater fish, *Colisa faciatus* under distillery effluent stress. Studies reveal that industrial pollutants containing metallic constituents may break intrastand cross links and strand breaking in the fish sperms indicating impairment in the spermatogenesis (Loyd *et al*., 1997). In case of ovarian cycle the pollutants present in the distillery effluent may interfere during oogenesis and consequently declining in the nucleic acids content under high concentration of effluent stress is obvious.

Further, the distillery effluent containing complex mixture of pollutants may impair the enzyme reactivity which bring transcription and hence quantitative decling in the RNA content was observed during three salient phases of ovarian cycle of *Colisa fasciatus more* particularly during the preparatory and spawning phases in which the process of oogenesis is highly active and fast. Decrease in the nucleic acid content during post spawning phase of ovarian cycle may be due to the exhaustion of ova.

The estimated protein content in *Colisa fasciatus* under control reveals increasing trend from preparatory to spawning phase which gradually declines and becomes lowest during the post spawning phase. The reason for being higher protein content from preparatory to spawning may be attributed to the proliferation in the ovarian tissue and increase and increase in ovarian activity which consequently gives rise to the development of new crop of germ cells. Soon after the spawning, the oogenesis becomes static and thus, lowest protein content was observed during the post spawning phase as shown in Table2 & Fig 3. The declining in the protein content in the different phases of ovarian cycle after 30 days of exposure under distillery effluent stress particularly under 10%and 20% may be attributed to the fact that distillery effluent may bring impairment in the intracellular protein synthesis. So much so, various constituents of the effluent may block the metabolism of proteiogenic amino acids thus, the declining in the protein content during ovarian cycle is obvious. Further, the key component of central dogma (DNA &RNA) reveals significant declining during preparatory and spawning phase in particular as evident from Table 2 hence, protein synthesis may be hampered in increasing distillery effluent concentrations.

Present study may thus be regarded as suggestive of the fact that distillery effluent may exert deleterious effects in the fishes in general and ovarian cycle in particular.

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REFRENCES

1. APHA (2005): Standard methods for the examination of water and waste water Washington DC.
2. Borrero, M.A.V.,J.T.V. Pereria, E.E. Miranda (2003): An environmental management method for sugarcane alcohol production in Brazil. *Biomass Bioenergy* , 25:287-299.
3. Campbell, R.C. (1974): Statistics for biologist, pp 385, Combridge University Press, London.
4. Chandra , R.,S. Yadav, D. Mohan (2008): Effect of distillery sludge on seed germination and growth parameters of green gram (*Phaseolus mungo* L.) :*Journal of Hazardous Materials*; 152: 431-439.
5. Dische, Z (1930): Cited in colowick S.P. and Kaplan N.O. Ed. Methods in Enzymology. Vol III : 680-684.
6. Drobeck,H.P.& Coulston, F (1962): Inhibition and recovery of spermatogenesis in rats, monkeys and dogs medicated with bis (dichloroacetyl diamines): *Exp. Melec. Path*.: 1: 251.
7. Fernando, J., Beltran, Juan F. Garcia – Araya and Pedro M. Alarez (1999): Wine distillery waste water degradation. 1. Oxidative treatment using ozone and its effect on the waste water biodegradability: *J. Agric. Food. Chem*; 47; 3911-3918.
8. Ganesan S., Natarazan A., Karuppiah P. S., Khandhasamy P., Balamuthu K. (2010): Recycling a distillery sludge from sugarcane industry using bioresource technology; *J. of App. Sci.*; 6 (3) : 218-223.
9. Joshi, H.C. (1999): Bio-energy potential of distillery effluents. Bioenergy News 3 (3).
10. Khnangarot, B.S. P.S. Rao, S.S. Shekhawat and V.S. Durgve (1985): Acute toxicity of distillery -effluent on a freshwater fish and Thwere pond, Shails; *Acta Hydrochim. Hydrobiol :*143: 53-58.
11. Kumar, S, S.S. Sahay and M.K. Sinha (1995): Bioassay of distillery effluent on common guppy, *Lebistes reticulatus :Bull. Environ. contam. toxicol*. ;54; 309.
12. Krishna, R. and Prakash, S. (2010): Effect of sugar mill effluent on the serum biochemical parameters of a freshwater catfish, *Heteropneustes ; Sci. Tech.*; 5 (2) : 81-82
13. Lowery, O.H., Resbrough, N.J., Furs, A.L. and Randall., R.J. (1951): cited in colowick, S.P. and Kalpan, N.O. Ed. Methods in Enzymology, Vol III 448-450.
14. Lolyd, D.R. , Phillips, D.H; Carmichad, P.L. (1997):Generation of putative intrastand cross-link and staric break in DNA by transition metalion medicated oxegen radical attack; *Chem. Res. Toxicol*. 10:393-400
15. Majbaum, W. (1959); Cited in colowick, S.P. and Kaplan N.O. Edition Methods in Enzymology. Vol. III 558-560.
16. Pant, D. and A. Adholeya (2007) : Comparative toxicological studies of distillery effluent treatment such as UASB reactor followed by on oxidizer unit using *Cyprinus carpio* fish : *Environmental Technology* : 31 (2) : 185-191.
17. Patil, V. and Vikram Ghole (2010) : Comparative toxicological studies of distillery effluent treatment such as UASB reactor followed by on oxidizer unit using cyprinus carpio fish : Environmental Technology : 31 (2) : 185-191.
18. Raju K., Rannu, K., Manickan, T.S. (1997): Use of distillery and sugar industry waste in agriculture. In proceedings of the sixth National Symposium of Enviroment, Tamil Nadu Agricultural University, Coimbatore, India : 286 – 290.
19. Rama Krishnan, M., Elumali M., Jayakumar, R. and Balsubramanian, M.P. (1999): Influence of distillery effluent on feeding energetic of freshwater fish in relation to water temperature. *Cytobios* : 98 : 1-15-87.
20. Ramakritinan, C.M., A.K. Kumaraguru and M.P. Balasubramanian (2005): Impact of distillery effluent a carbohydrate metabolism of freshwater

fish, *Cyprinus carpio : Ecotoxicology*. 14:693-707.

21. Srivastava, S.K., Singh D., Prakash, S. and Ansari, K.K. (2007): Effect of sublethal concentration of distillery effluent on the hematological and biochemical parameters of *Clarius batraschus*. *Eco*. *Env. & Cons*, 13 (3) : 511-514.
22. Shukla, Anuradha and J.P.Shukla (2011): Distillery effluent Inducted alterations in the nucleic acid and protein during testicular cycle of *Colisa fasciatus*, (Bl.& Schn), a tropical freshwater perch. *Int.J.Pha.Bio.Sci*.:3(1).
23. Zsigmond Jeney, E. Tellervo Valtonen, Galina jeney and E Lamari Jokinen (2002): Effect of pulp and paper mill effluant (BKME) on physicological parameters of roach (*Rutilus rutilus*) infected by the digenean Rhipidocotyle fennica: *Folia Parasitologica*: 49:103-108.