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The analysis of Haematological investigation on fresh water teleost and study the endosulfan on Indian cat fish

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Abstract

The Present paper deals with the result of water quality parameters showed there were no significant difference ($P > 0.05$) between the test media and the control. Abnormal behaviour such as imbalance position, secretion of mucus, erratic swimming, respiratory distress flashing and lethargy were observed before death during the exposure period. The exposure leads to a decrease in the levels of erythrocytes, leucocytes, haemoglobin, total protein and an increase in alkaline phosphatase, total bilirubin and plasma glucose when compared with the control. The implications of the findings in relation to the exposed fish and the aquatic environment are discussed. Stinging catfish *Heteropneustes fossilis* exposed to completely different concentrations of associate organochlorine chemical endosulfan below static conditions, discovered statistically vital increase in sterol contents of liver, brain and gill tissues even at rock bottom concentration (0.0010 mg/l). Fish additionally evoked behavioural changes thanks to chemical toxicity. symptom gave the impression to ensue to fret induced metabolic alterations caused by intoxication.

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Keywords: Behavioral alt sensitivity erations, *Heteropneustes fossilis*, *toxicants*.

Introduction

With rapid global economic development and need for more food production, pollution from agricultural practices through pesticides remain a major threat to aquatic ecosystem. Highly effective pesticides are used indiscriminately, which result in aquatic pollution on entering the aquatic environment and brings multiple changes in organism by altering growth rate, nutritive value, and behavioural pattern of the fish

Endosulfan (6, 7, 8, 9, 10-hexachloro-15, 5, 9, 9a-hexahydro-6, 9-methano- 2, 4, 3-benzodioxathiepine-3-oxide) is an organochlorine compound introduced in the 1950's with a blood spectrum effects against insects and ants in agriculture and allied sectors. Kay (2006) reported that acute toxicity exposure to endosulfan produces symptoms like hyperactivity, convulsions, staggering, difficulty in breathing, nausea and vomiting, diarrhea and unconsciousness.

Widespread application of various pesticides has aggravated the problem of pollution to aquatic environment. Silva and Gammon (2009) noted that runoff of pesticides like endosulfan from agricultural field killed fish in various parts of the world. Repeated exposure to sub lethal dose of some pesticides can cause physiological and behavioural changes in fish and reduces their population such as its abandonment of nests and broods decreased immunity to disease and increased failure to avoid predators. Erickson *et al.* (2008) Organochlorine pesticides are still used for crop protection in many countries while not considering their cyanogenic effects on aquatic life. These pesticides reach in water bodies principally through run-off from agricultural fields and have an effect on the lifetime of non-target organisms like fishes, that are a lot of vulnerable than invertebrates. Endosulfan could be a non-systemic pesticide and is usually used for agricultural functions, biological science and agriculture. Sterol is a crucial element of nice significance in living systems. It holds a key and central position within the metabolism of the many closely connected biologically necessary compounds (Shell, 1961), precursor of steroid hormones (Lehninger, 1975) [3] and a crucial constituent of cell wall

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wherever it modulates their runniness, fragility and consequently the membrane operate gift paper embodies observations on cyanogenic effects of endosulfan on sterol contents of liver, brain and gill tissues of fresh stinging catfish *Heteropneustes fossilis*, a vigorous and hardy fish of economic importance and features a nice demand owing to its healthful price. Pollutants like pesticides are glorious to change the behavioural pattern, growth, replica and resistance to diseases of aquatic organism, touching on a spread of organic chemistry and physiological mechanism.

Experimental design

Fishes were weighed, measured and classified randomly into 8 groups (10 fish/tank) according to dose of lead, tomato paste in terms of lycopene, vitamin E and their combinations (Table 1). The diets (maize and soy bean, 15 g/kg fish) were pelleted after addition of vitamin E and tomato paste doses for the treated groups and the

addition of suitable amounts of molasses and water. The diets were dried at room temperature and stored in small bags for fish feeding.

Preparation of tomato paste to adjust the lycopene dose

Tomatoes used for the experiment were obtained from the local market. Fresh peeled, deseeded tomatoes were pulped well to a smooth consistency in a warring blender. The lycopene content in tomato paste was estimated spectrophotometrically according to the methods of Ranganna (1976) and Choudhari and Ananthanarayan (2007). The lycopene concentration in the tomato paste was 30.028 mg/100 g. Based on the review of Xianquan *et al.* (2005), such concentration could not be affected by current conditions of diet preparation and storage of a short time (37°C for 4 weeks). In addition to lycopene, tomato paste composition include water, proteins, carbohydrates, fibres, calcium, potassium, zinc, copper, manganese, iron, vitamin C, vitamin E, b-carotenoids and other phytonutrie.

Table 1: The fish groups exposed to lead (7mg/l) and lycopene (9mg/kg body weight) and vitamin E (50 mg/kg body weight) and their

Treatments	C	VE	LYC	LYC+VE	Pb	Pb+VE	Pb+LYC	Pb+LYC+VE
Lead (mg/L)	0	0	0	0	7	7	7	7
Vitamin E (mg/kg)	0	50	0	50	0	50	0	50
Lycopene (mg/kg)	0	0	9	9	0	0	9	9

Combinations.

C= control, VE= vitamin E, LYC= lycopene and Pb= lead dos

Stock solution (1,000 ppm) of lead as lead nitrate Pb (NO₃)₂ was prepared and stored in clean glass bottles and diluted to concentration of 7 mg/l. Such low sublethal lead concentration (1/4 of 96 h LC₅₀) was chosen according to levels monitored by Adeyemo *et al.*, (2007). Lead doses were prepared and added constantly to the aquarium for 4 weeks. The test water was replaced daily with the required amount of stock solution to prevent deterioration of water quality and replenish cadmium levels. Tomato paste was added to the diet in concentration of 30 g/kg BW (9 mg lycopene/kg BW). Dose response of lycopene was described previously by Rodriguez *et al.* (2004). Also, vitamin E (α -tocopherol) was supplemented in 50 mg/kg BW. Such vitamin E concentration was chosen according to levels monitored by Ortun˜o *et al.* (2001). It is worthy to mention that vitamin E (α tocopherol) in tomato paste was estimated to be 38.67 \pm 2.29 mg/100 g tomato paste dry weight with no effect by industrial processing (Capanoglu *et al.*

dechlorinated water throughout the experiment were- temperature 19-21 0C; pH seven.0-7.4; dissolved atomic number 8 seven.2-7.6 mg/l; total pH 108-115 mg/l; and total hardness 118-128 mg/l. Their liver, brain and gill were compound out, washed and unbroken in zero.7% cold isosmotic solution. 2.5% homogenates were ready in glacial ethanoic acid and sterol level was calculable following technique of Rosenthol *et al.* (1957), exploitation Bausch and Lomb Spectronic - twenty photometer at 560 nm.

Consequence Analysis

The effects of endosulfan toxicity on sterol levels in liver, brain and gill tissues are summarized in Table a pair of. At rock bottom concentration of zero.0010 mg/l the sterol levels in liver, brain and gill multiplied incessantly upto seventy two hours of chemical exposure and so shrunken when ninety six hours however remained still on top of their various management levels. At 0.0015 mg/l, 0.0020 mg/l and zero.0025 mg/l chemical concentration the sterol levels in liver and brain rose continuously until the terminal hours of exposure.

Treatment of Problem

They were properly fed and so starved for twenty-four hours before the experiment. Fish from constant cluster unbroken below traditional conditions, were used as management. Live *Heteropneustes fossilis* obtained from watercourse Gomti at Lucknow were transported and acclimatized to the laboratory atmosphere, as delineated earlier. solely healthy wanting and active fishes of weight vary 100-150 grams were chosen for the experiment. The recommendations created by Doudoroff *et al.* (1951) and APHA (1992) were followed for the static bioassay tests. The chemical concentrations, chosen on the premise of 80-100% survival of fishes (Table I). Fish were exposed to completely different concentrations of chemical from twenty four to ninety six hours relying upon their survival at the actual concentration. Fish were taken out frequently when each twenty four hours interval from the experimental in addition as from management cluster and washed with H₂O. The chemical science characteristics of

Table 2: Present survival of fishes in endosulfan activity

Pesticide Conc. mg/l	Time of exposure in Hours				
	24	48	72	96	120
Control	100%	100%	100%	100%	100%
0.0011	100%	90%	80%	70%	50%
0.0014	100%	90%	80%	40%	20%
0.0020	100%	80%	50%	10%	5%
0.0024	70%	40%	10%	5%	00%

In gills the cholesterol contents enlarged upto 48 hours of toxicant exposure at 0.0015 mg/l concentration and then declined. However, with increasing concentration of pesticide, cholesterol levels of gills enlarged during initial exposure period but later decreased. Interestingly the maximal rise in cholesterol level during the experiment was observed in gills (36.09%), followed by brain

(29.65%) and liver (27.07%) at 0.0015mg/l concentration. Elevated cholesterol contents in liver, brain and gills due to toxic effect of endosulfan were statistically significant ($P<0.01$) as compared to their respective control levels. Endosulfan also produced behavioral changes in *H. fossilis* such as convulsions which were more frequent during the terminal hours of experiment, rapid air gulping, copious mucus secretion, loss of balance and sensitivity to touch and sound.

Statistical analysis

The basic statistics, means, standard errors and ranges of the measured parameters were estimated. The patterns of variation due to lead, lycopene and vitamin E doses and their combinations were studied by three- and four-way analysis of variance using the SPSS package (SPSS 1998) at the 0.05 significance level. Levene's test of equality of error variance of the dependent variables was applied, with rejection of the null hypothesis for raw, log-transformed and SQRT-transformed data. So, the homogeneity of variance was assumed for raw data. The pattern of variations was also recorded by one-way analysis of variance, revealing significant difference due to lead, lycopene and vitamin E ($P<0.0001$); The Tukey-HSD test was considered for multiple comparisons.

Results and Discussion:

The toxicants on entering into fish body cause deleterious effects on functional activity of endocrine system and metabolism leading to physiological, pathological and biochemical disorders (Bais and Arasta, 1995; Karuppasamy 2002; David *et al.*, 2003). Certain pathological informations along with physiological and biochemical data may provide accurate and early indication of toxicity of organochlorine pesticide (Krishnagopal *et al.* 1988). Arasta *et al.*, 1999, Pandey *et al.*, 2000; Endosulfan had significantly enlarged cholesterol levels of liver, brain and gill tissues of *H. fossilis* at all concentrations in these experiments. It was further observed that the rate of enlarge in cholesterol was higher in the initial period of pesticide exposure to fishes but following prolonged exposure the same was much lower. However, the level remained above the control level. Lipids provide an essential, readily available energy source for fish, of which cholesterol is of major importance because of its relationship to many physiologically active steroids and hormones (Tiez, 1970; Evans, 1998). Alterations in cholesterol level may be an indirect result of toxicant effect on metabolic enzymes (Mayer *et al.*, 1992; Padmini *et al.*, 2004). Marked biochemical alterations in blood and tissues of freshwater cat fish *H. fossilis* have been reported by Singh and Srivastava (1998) and Chandra (2008) following formothion and malathion exposure. Biochemical changes are primarily due to shift in the respiratory metabolism caused by pesticide in the ambient environment and utilization of organic reserves yielding excess energy to compensate the stress. Toxic stress to fish *H. fossilis* was quite evident in these experiments showing abnormal behavioral changes. Rao *et al.* (1981) reported reduced oxygen consumption in fish *M. aculeatum* treated with endosulfan. Exposure to sublethal concentration of thiodon lead to 40% decline in oxygen consumption rate in *Mystus vittatus* (Reddy and Gonathy, 1977). Varied respiratory response may occur in fishes (Thosar and Lonker, 2004) depending upon exposure period of toxicant, stress and the intensity of damage caused to gills. Further dose dependent behavioral changes in fish *H. fossilis* clearly indicated stages of stress response, the alarm reaction, the state of resistance and the stage of exhaustion in present

experiment. It appeared that fishes exposed to lowest concentration of pesticide did not reach upto the state of exhaustion, rather they were able to accommodate and adapt the stress. However, fishes exposed to higher concentration of endosulfan could not resist the stress for longer duration and died. Nath (2003) also reported abnormal behavior in *Clarias batrachus*, *Heteropneustes fossilis* and *Labeo rohita* of wetland pond of Bihar, contaminated with endosulfan. Matsumura (1980) reported that susceptibility and survival time in fishes are directly related to body size, weight and age in ambient environment. Deshmukh and Sonawane (2007) reported significant changes in blood corpuscles number and attributed as an adaptation to meet stressful condition in the fish *Channa gachua* following long term exposure to endosulfan. Chandra *et al.* (2007) reported significant enlarge in Glutamic oxalacetic and pyruvic transaminase in brain and liver of *C. batrachus* due to toxicity of malathion and dimicron pesticides which indicated impaired carbohydrate and protein metabolism.

Table 3: Cholesterol levels of non-identical tissues of *H. fossilis* following endosulf toxicity

Pesticide Conc. mg/l	Cholesterol mg/100mg. Fress weight			
	Mean \pm Std. deviation Time of exposure in hours			
	24	48	72	96
	Liver –Control -2.29 \pm 0.12			
0.0011	2.62 \pm 0.13	2.80 \pm 0.17	2.85 \pm 0.30	2.60 \pm 0.30
0.0014	2.80 \pm 0.16	2.98 \pm 0.15	3.14 \pm 0.32	
0.0020	3.04 \pm 0.42	3.10 \pm 0.24		
0.0024	3.00 \pm 0.25			
	Brain–Control -2.80 \pm 0.08			
0.0011	3.27 \pm 0.17	3.46 \pm 0.24	3.70 \pm 0.23	3.40 \pm 0.42
0.0014	3.40 \pm 0.38	3.66 \pm 0.30	3.98 \pm 0.31	
0.0020	3.60 \pm 0.28	3.84 \pm 0.42		
0.0024	3.37 \pm 0.38			
	Gills–Control -1.40 \pm 0.28			
0.0011	1.28 \pm 0.13	1.35 \pm 0.08	1.40 \pm 0.06	1.20 \pm 0.12
0.0014	1.39 \pm 0.08	1.69 \pm 0.12	1.46 \pm 0.28	
0.0020	1.48 \pm 0.19	1.60 \pm 0.21		
0.0024	1.22 \pm 0.25			

Aldolase, a relevant gluconeogenic enzyme which plays an essential role in mobilization of energy to tolerate additional stress, was also reported to escalate significantly in liver, brain and gill of *C. batrachus*, following pesticide exposure. Such biochemical changes clearly indicated utilization of organic reserves yielding excess energy to cope up the toxic stress of environment at their level best. Since cholesterol plays a relevant role in body metabolism and being associated with almost every organ of body, was the foremost biochemical component of tissues revealing significant alterations in present observations. The percentage elevation of cholesterol were noted to be higher during early period of exposure to toxicant than prolonged one, which suggested that the fish tried its best to accommodate with the situation and doing so body metabolism became rapid to fulfil energy requirement, utilizing organic reserves, yielding excess energy to compensate the toxic stress. The mobilization of energy substances as secondary response and reduced capacity to tolerate additional stress have been described as a part of integrated response in fish. Enlarged glucose contents in brain of *H. fossilis* (Shrivastava *et al.*, 2002) and changes in liver cholesterol have been observed following exposure of fish to various pollutants. Gills being the primary sites of absorption but having poor drug metabolizing

capacity, indicated maximum rise (36.09%) in cholesterol level. Endosulfan toxicity have also been reported to cause degeneration, necrosis, hyperplasia and hypertrophy in gills, liver and muscle tissues (Kumar *et al.*, 2000) ^[5]. These observations further support the decreasing cholesterol levels in liver, brain and gill tissues of *H. fossilis* following prolonged exposure and increasing doses to pesticide when fish became exhausted. Thus it can be concluded that even very low concentration of endosulfan pesticide significantly altered cholesterol level of fish *H. fossilis* and behavioral changes are the early symptoms to gauge the potential toxicity of a toxicant.

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