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The influence of di -ammonium phosphate (DAP) on serum glutamate pyruvate transaminase of fresh water edible fish *wallago attu*

Indu Kumar* and V.M.S. Sriwastwa
Deptt. of Zoology, K.N.Govt.P.G.College Gyanpur, S.R.N Bhadohi- , India
***e-mail:** kumar.indu6@gmail.com

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Abstract: The present paper deals with the toxic effect of fertilizer di-ammonium phosphate on the SGPT levels of fish *Wallago attu*. Due to the toxic effect of fertilizer di-ammonium phosphate the enzyme level were higly disturbed. The effect of fertilizer di-ammonium phosphate was observed as SGPT levels of fish *Wallago attu* at six different concentrations for 24 to 144 hr of exposure. At all the concentrations and exposures, the enzyme levels have fallen below control at the terminal hr. In 0.42 and 0.45 g L^{-1} stress of DAP the SGPT increased at initial hours of exposure and decreased in the end but in all other concentrations it decreased below the control.

Key words: Di-ammonium phosphate, SGPT, *Wallago attu,* Serum

Introduction

Aquatic organism can survive in very low concentrations of the pollutants. But when these concentrations increased abnormally, they become fatal to the sensitive organisms like fishes (Awasthi et al., 2008; Bhatnagar et al., 1962). For each species the tolerance limits for each pollutant are different. The toxicity levels of the pollutatns are affected by several environmental factors such as termperature, oxygen content, pH, hardness of water, chloride *etc*. Some of the pollutants eliminate certain indigenous species while the more resistant ones which can survive, replace them (Bartsch and Ingram, 1966). Organic matter in the water deoxygenates the water and increases the suspended solids, ammonia and sulphides. The lethal conentration for 50% animals of the commonly used fertiilzer Di-ammoonium phosphate (DAP) was determined for fishes *Wallago attu* by follwing the linear graphical methods. Different concentrations were determined for 24, 48, 72, 96, 120 and 144 hr for the above fish. The effect of LC_{50} of individual fertilizer was seen as serum glutamate pyruvate transaminase (SGPT) levels of fish *Wallago attu* at different concentration of fertilizer DAP. The fishes of uniform weight and length were taken for the experiment (Dash, 1978; Iqbal et al., 1992).

Increasing population, industrialization and agricultural production has resulted in increasing the number of freshwater systems which are being impaired by the contaminants present in wastewater releases (Toro et al., 2001; Santore et al., 2001). Micronutrient interact with toxic metals at several points in body, absorption and excretion of toxic metals, transport of metals in body, binding to target proteins, metabolism and sequestration of toxic metals, and finally in secondary mechanisms of toxicity such as oxidative stress (Payle, 1992; Tao, 2001; Singh et al., 2008 a & b).

The aim of present study was performed to evaluate effect on metabolic indicator (SGPT) by differnt concentration of DAP to a freshwater fish, *Wallago attu*. This parameter can be used as a indicator / tool in monitoring of water polluton in lake, pond and rivers etc.

Materials and Methods

Live fresh water fishes were obtained from river Gomati in suburbs of Sultanpur with the help of fisherman. Fishes were transported to the laboratory in natural waters, in wide mouthed large plastic containers, avoiding injuries and stresses of all kinds, as for as possible. The fishes were then treated with 2% $KMnO_4$ to remove external infections like protozoa, bacteria, fungi, arthropods and monogenetic treamatodes *etc*. Before the experiment fishes were acclimated in glass aquaria for seven days. The fishes were starved for a period of 24 hr before the tests. Ten specimen of fish were taken for each concentration of the fertilizer DAP 0.35, 0.42, 0.45, 0.65, 0.75 and 0.90 g L^{-1} and were kept in these concentrations of DAP for 24, 48, 72, 96, 126 and 144 hr. During the experiment water quality was tasted by the method of APHA, 2005.

Selection of concentration of test solution and duration of test: The test soluations were selected by taking the highest concentration of the fertilizer when 50% of the test fishes died within 24 hr. The oxygen content of water in test solution was adequately maintained. Then progressively lower concentrations were used to find the concentration at which 50% fishes survived up to 144 hr, the maximum time of test in these studies. The longest duration of test was kept of 144 hr at the lowest concentration used, then increasing concentrations at which 50% fishes survived for 120, 96, 72, 48 and 24 hr were used.

Preparation of fish for blood sampling: Control and experimental fish were taken out of the aquaria with the help of hand net at the required intervals. The caudal end of fish was washed with distilled water and blotted dry with the help of clean towel.

Table - 1: The influence of di -ammonium phosphate (DAP) on serum glutamate pyruvate transaminase (SGPT) of fresh water edible fish *wallago attu*

Fertilizer (gL^{-1})	Exposure time					
	24	48	72	96	120	144
Control	3.42 + 0.08 (3.34 – 3.50)	3.42 + 0.08 (3.34 – 3.50)	3.42 + 0.08 (3.34 – 3.50)	3.42 + 0.08 (3.34 – 3.50)	3.42 + 0.08 (3.34 – 3.50)	3.42 + 0.08 (3.34 – 3.50)
0.35	3.11 + 0.05 (3.06 – 3.16)	2.94 + 0.04 (2.90 – 2.98)	2.87 + 0.02 (2.85 – 2.89)	2.65 + 0.07 (2.58 – 2.72)	2.39 + 0.06 (2.33 – 2.45)	2.11 + 0.04 (2.07 –2.15)
0.42	3.61 + 0.06 (3.55 – 3.67)	3.90 + 0.09 (3.81 – 3.99)	4.09 + 0.05 (4.04 – 4.14)	3.76 + 0.04 (3.72 – 3.80)	3.19 + 0.03 (3.16 –3.22)	
0.45	3.76 + 0.04 (3.72 – 3.80)	3.88 + 0.06 (3.82 – 3.94)	3.24 + 0.07 (3.17 – 3.31)	3.14 + 0.03 (3.11 – 3.17)		
0.65	2.93 + 0.06 (2.87 – 2.99)	2.57 + 0.05 (2.52 – 2.62)	2.27 + 0.03 (2.24 – 2.30)			
0.75	3.25 + 0.05 (3.20 – 3.30)	3.32 + 0.07 (3.25 – 3.39)				
0.90	1.98 + 0.03 (1.95 – 2.01					

SGPT µM pyruvate formed per ml per hour mean ± S.D.

Collection of blood: Blood was collected from caudal vessels by shaving off the caudal end. For SGPT blood was collected in clean dry test tube and allowed to clot at 10°C. soon after clothing the contents of test tube were contrifuged at 2,000 rpm for 15 min and serum was transferred to another dry clean test tube and stored in refrigerator at 0°C. The biochemical estimation were done within 24 hr of blood collection.

Biochemical estimation: Standard analytical chemicals doubly distilled water, freshly prepared reagents and freshly separated serum stored in refrigerator were used. The serum glutamate pyruvate transaminase (SGPT) of blood was estimated by the method Reitman and Frankel (1957).For the estimation of this enzyme in serum 0.9 ml DL-alanine solution (20 ml) were mixed to make the substrate. The substrate was taken in two separate clean dry test tubes, one for 'Test' and other for 'Control', 0.2 ml serum was added to test and incubated at 37°C for 30 min. 1.0 ml of 2, 4-di-nitrophenyl hydrazine solution was added in each test tubes, 0.2 ml serum was then added and mixed. Optical density was determined at 505 nm against water blank. Blank and standard were also prepared as given in the method. Sodium pyruvate was used in standard and volume of serum was replaced by water. SGPT level calculated as the mole pyruvate formed/ml/hour in serum. Optical density was determined with the help of SPEKOL spectrophotometer.

Results

The result obtained on SGPT level of fish *Wallago attu* exposed for 24 to 144 hr to six different concentration of DAP have been summarized in Table 1. Due to the effect of fertilizer DAP enzyme levels were highly disturbed. There was maximum increase in enzyme level in the initial exposure periods which declined with the increasing time intervals and was maximum at the end when 50% fished died. Maximum elevation was after 72 hours interval at 0.42 g L^{-1} concentration while minimum was within 24 hours at the concentration 0.90 g L^{-1}. However in the longer exposures enzyme level had fallen even below control. At 0.35 g L^{-1} concentration after 24, 48, 72, 96, 120 and 144 hr of exposures enzyme levels increased 9.07, 14.04, 16.09, 22.52, 30.12 and 38.31% respectively below control. At 0.42 g L^{-1} concentration after 24, 48, 72 and 96 hr of exposures SGPT level increased 5.55, 14.03, 19.59 and 9.94% respectively above control but it decreased 6.73% below control at the end of 120 hr. At 0.45 g L^{-1} concentration the activity increased 9.94 and 13.45% of 24 and 48 hr of exposures respectively above control then fell 5.27 and 8.19% below control after 72 and 96 hr. At 0.65 g L^{-1} concentration after 24, 48 and 72 hr of exposures SGPT level decreased 14.33, 24.86 and 33.63% respectively below control. At 0.75 g L^{-1} concentration the fishes survived for 48 hr only and the levels decreased 4.98 and 2.93% below control after 24 and 48 hr of exposures respectively. At 0.90 g L^{-1} concentration the fishes died in 24 hr only. The enzyme activity was greatly inhibited and a decrease of 42.11% below control has occurred.

Discussion

The biochemical estimation of SGPT of fish *Wallago attu* was higyly disturbed by the toxic effect of fertilizer DAP. The maximum SGPT levels in 19.59% above control was observed at the concentration 0.42 g L^{-1} concentration after 72 hr of exposure while minimum SGPT levels in 42.11% below control at the concentration 0.90 g L^{-1} after 24 hr of exposures. This dose proved lethal. Phosphate fertilizers on soil and stored phosphorus contaminated river water which resulted harmful environmental effects specially the aquatic animals (Lata et al., 2008; Naqvi and singh, 1992; Gladusbko, 1979; Sathi et al., 1986; Campbell, 1978), excess phosphate ingestion resulted in increase of unspecific phosphates in rats (Tondon and Gupta, 1980). Biochemical basis of toxicity and

its mechanism was discussed in details in relation to humans (Ballantyne *et al*.,1983). Mechanistic explanation of toxic phenomenon are critically important as they account for the origin of toxicity, at prevention of toxicity by chemical or biological means and to provide a rational basis for the use of animal data to anticipate the consequences of human exposure to a chemical. It has been clearly established the water of river Gomati is highly contaminated at measures sampling stations which may affect the aquatic fauna specially the fishes. This was correlated with biochemical estimation of SGPT levels in common food fish *Wallago attu* . The cause of pollution need to be worked out in detail. In the future along with mechanisitc approach of toxicity of fertilizer DAP to fishes, it is also necessary to survey the presence of different chemical in the river Gomati (Redhaiah et al., 1987; Trivedi et al., 1990).

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