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Comparative Studies on the Effects of Herbicide Atrazine on Freshwater Fish *Oreochromis niloticus* and *Chrysichthyes auratus* at Assiut, Egypt

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In recent years, aquatic herbicides are widely used for controlling undesirable weeds (Maroni and Bersani 1994). The use of herbicides to control aquatic weeds has applied in fish management where they used in aquatic habitats especially rice fields and some fish farms (Wu et al 1980). Atrazine (2-chlor-4-ethylamino-6-isopropyl-amino-S-triazine) is considered the main aquatic herbicide used (Brusick 1994). It enters the aquatic system through run-off from agricultural fields or directly through careless application. It has been found display in different sources: up to 3.5 µg/L in rainwater, up to 1.25 µg/L in surface waters, more than 0.5 µg/L in ground water, exceeding of 0.1 µg/L for drinking water in Germany while in USA was 88.4 µg/L, and up to 69.4 µg/L in surface water. Moreover, the high concentrations normally occur in the environment for a short period after accidents (Fischer-Scherl et al 1991). Third world countries have the problem of adverse effects of these chemical compounds in ecosystem on fish production (Parkinson and Agius 1988). The application of herbicides has hazard effects on the fish beside the change of available plankton food (Mason 1991). However, Aaronson (1980) concluded that a fish kill in the ponds were mainly due to atrazine, where the use of 0.01, 10 and 1000 µg/L atrazine produced characteristic amplitude and frequency changes in rainbow trout.

Hematological tests and analysis of serum constituents have proved to be useful in the detection of the stress and metabolic disturbances (Aldrin et al 1982). Atrazine effects could be evaluated through hematological tests since RBCs, hemoglobin and haematocrit were significantly affected by the exposure of fish to atrazine (Prasad et al 1991). Egaas et al (1993) declared the significant effects of atrazine on serum components. Being the most vital enzyme in the most tissues of animals acetylcholinesterase (AChE) lend itself to be the most sensitive enzyme to hazard effects. The inhibition of AChE activity is a useful indicator of fish exposure to atrazine (Chaturvedi, 1993). Moreover, atrazine plays an important role in osmoregulation since it control urinary excretion and stress on the gills and kidney (Catenacci et al 1993).

There are little data on atrazine effects on Nile fish although its widely application in our locality. Therefore, the objective of this study was to achieve atrazine effects on hematology, selected serum components and AChE of Nile fish, Oreochromis niloticus and Chrysichthyes auratus,

MATERIALS AND METHODS

Samples of Oreochromis niloticus and Chrysichthyes auratus were obtained from River Nile and transported immediately to the laboratory. Fish were reared in aerated glass aquarium (100 L capacity) and acclimatized for two wk in dechlorinated tap water (pH 6.95, DO 5.5 mg/L, 150 mg/L as CaCo3) before being used in the experiment. Fish were fed with a commercial pellets supplied by Alexandria company at a rate of 3% of wet weight twice daily, feces and

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unused food were aspirated regularly. The water temperature was maintained at 21.77 ± 1.88°C. At the first day of study the fish were weighed and measured the standard and total length. Tilapia had weights of 38.46 ± 2.70 g and total length of 12.62 ± 1.35 cm and Chrysichthyes auratus had weights of 25.85 ± 1.47 g and total length of 12.21 ± 1.37 cm. Fish were divided randomly into three groups of 30 each for each species. The first group was kept as control (normal water), the second and third groups were exposed to 3 and 6 mg/L atrazine for four wk respectively. Five fish samples from both the experimental and control groups were sacrificed at 0, 14 and 28 d. The brains were excised and kept frozen at -20°C in individual vials until analysis. Toxicoclinical signs as well as changes in fish behavior in all groups were observed and recorded.

Atrazine (2-chlor-4-ethyl-amino-6-isopropyl-amino-s-triazine) as powder ingredient (96% purity) was obtained from Plant Protection Dept., Faculty of agriculture, Assiut University. It was dissolved in distilled water to a saturated mixture, filtered and added constantly to aquarium at rate of 3 and 6 mg atrazine/L water according to Pluta (1989). The test water was replaced daily along with the required amount of stock solution to prevent deterioration of water quality and to replenish atrazine levels,

Blood samples were taken from the caudal vein of live fish per each group at 0, 14 and 28 d. A portion (1 mL) was mixed well in a clean dry vials contained EDTA anticoagulant (1.5 mg/mL) according to McKnight (1966) to evaluate the RBCs, hemoglobin and haematocrit. The RBCs were counted by haemocytometer, hemoglobin and haematocrit were measured according to methods of Stoskopf (1993). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Coles (1980). The remaining blood was transferred to plastic vials and left to clot for 3 hr at 4°C prior to centrifugation at 3000 rpm to obtain serum. The serum was subsequently decanted into glass vials and stored at -20°C until analysis.

Serum glucose, cholesterol, urea nitrogen, total protein, albumin concentrations (globulin was obtained by difference) using assay kits supplied by Diamond Diagnostic, Egypt (Cairo) and serum triglycerides and total lipids concentration were determined using assay kits of BioMerieus, France. Blood serum sodium (Na) and serum potassium (K) were determined by Flame photometer (Corning 400). Serum chloride (Cl) was estimated by chloride analyzer model 925 USA. AChE activity was determined by measuring the rate of hydrolysis of acetylthiocholine iodide in 0.1 M sodium phosphate buffer (pH 8.0) according to the method of Ellman et al (1961).

Data were analyzed using general linear model (GLM) procedures of SAS (1987) for personal computers. Effects of treatments on serum constituents were analyzed on individual fish basis by factorial analyses of variance. Treatment, period and treatment X period interactions were determined with the variables; treatment effects were also examined within time periods by one - way ANOVA (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The present study dealt with the alterations in the physiological effects of atrazine herbicide on two of the most important Nile fish (Oreochromis niloticus and Chrysichthyes auratus). We investigated the changes of hematological, some selected serum components and AChE.

Fish exposed to atrazine in concentrations of 3 and 6 mg/L showed some clinical signs such as rapid respiration and increased rate of gill cover movements, Slow-down of reflexes and swimming movements and reduction in the feeding activities were also observable signs in exposed fish. Before death, fish floated at the surface of water gasping for more oxygen, very rapid movements in various directions. Other fish showed jerky movement with its head down and tail upwards. These signs were more pronounced in Chrysichthyes auratus than tilapia fish. About 25% of the treated fish had abdominal swelling (ascites) in the two species. This abnormal behavior could be attributed to the effect of atrazine on CNS and cardiovascular

system (Antychowicz et al 1979), and may be due to lowering of the oxygen content of the water owing to presence of the herbicide (Olli Ojala, 1966). The LC_{50} of atrazine was 9.37 and 6.37 mg/L for Oreochromis niloticus and Chrysichthyes auratus. These obtained results were less than the obtained value by Neskovic et al 1993 (18.8 mg/L) on carp (Cyprinus carpio). This difference may be due to the difference in water temperature since the present results obtained under about 22°C but Neskovic's results obtained at about 15°C. Also, the difference in LC_{50} may be due to the fish species difference. Moreover, the difference in LC_{50} for Oreochromis niloticus and Chrysichthyes auratus which were in the same conditions and exposed to the same atrazine levels may be due to fish species. The difference in LC_{50} could be also explained by the lipid content which was 22.87 and 11.28% (as dry matter) for Chrysichthyes auratus and Oreochromis niloticus (Hussein 1995). The increase of lipid content of the fish may be the reason of mortality due to stress action. This finding confirm that of Pluta (1989) who studied the atrazine effect on Cyprinus carpio and Salmo gairdneri at level of 3 and 6 mg/L atrazine.

The RBCs number, hemoglobin concentration and haematocrit percentage of Oreochromis nitoticus and Chrysichthyes auratus which exposed to 3 and 6 mg/L atrazine were recorded in Table 1. It is evident that the exposure of the fish to 3 and 6 mg/L atrazine resulted in significant ($p<0.01$) decrease of RBCs, hemoglobin and haematocrit as compared with the control group in both species. Also, there were significant ($P<0.01$) changes of MCV, MCH and MCHC for the two species (Table 1). However, it is obvious that Chrysichthyes auratus much more affected from atrazine exposure than tilapia which represented in significant changes in hematological parameters. The obtained results of RBCs count, hemoglobin content and haematocrit in control tilapia were register by Mohmed (1979). The significant ($P<0.01$) decrease in RBCs, hemoglobin and haematocrit in exposed fish to atrazine may be attributed to the lowering of the oxygen content of the water. It was reported that the oxygen tension in the water is decreased in presence of herbicide (Olli Ojala 1966). It is well known that reduced oxygen tension lead to anoxia and mortality in fish. Also, Prasad et al (1991) showed that significant changes in hematological constituents such as RBCs, Hb, PCV, MCV, MCH and MCHC of Tilapia mossambica exposed to atrazine. They explained this effect by respiratory stress in the fish. Otherwise, Guthmann (1989) investigated that hematological parameters for rainbow trout exposed to atrazine decreased significantly in the total number of erythrocytes and haematocrit. This can be explained by the morphological alterations in renal interstitium. This is in agreement with these findings of Horn and Hanke (1980) who found a decline in the number of erythrocytes after exposure of Cyprinus carpio to 0.1 mg/L atrazine. Moreover, the significant decrease ($P<0.01$) of RBCs, Hb and PCV in both concentrations for the two fish species may indicate the toxic effect of atrazine on spleen, liver, and anterior kidney, this suggestion is supported by Rehwoldt (1978). However, there was correlation between the red blood cells count, packed cell volume and hemoglobin content in fishes as shown in Table 1. Therefore erythrocytic counts, packed cell volume and hemoglobin can be useful in the evaluation of stress in fishes. Otherwise, Houston and Dewilde (1972) showed that the PCV value could be used as a general index of hematological status in most fishes.

Serum glucose level (Table 1) remained unchanged over the course of the experiment in control groups for the two species. Comparable, treatment means of serum glucose concentration were recorded at 14 and 28 d of the experimental period (Table 1). It increased in exposed fish to 3.0 mg/L atrazine only at 14 d while at 28 d it was significantly reduced ($P<0.05$). While, the exposure to 6 mg/L atrazine resulted in significant decrease ($P<0.05$) in serum glucose at 14 and 28 d for the two species. Moreover, at 14 d the level of 6 mg/L atrazine led to 20.39 and 41.52% less glucose in the serum than the control while at 28 d the reduction in glucose level was 19.69 and 45.34% for Oreochromis niloticus and Chrysichthyes auratus respectively.

Although analysis of serum constituents have proved useful in the detection and diagnosis of metabolic disturbances with the aim of securing optimal production (Aldrin et al 1982) the studies of atrazine effects on serum metabolites are lacking, where only brief informations

about metabolism of atrazine in fish were available. In the present trial. the increase of serum glucose at low level (3 mg/L) atrazine and short period (14 d) can be attributed to stress effect such as rapid respiration of the fish and float at the surface of water gasping for more oxygen. These stress factors led to disturbances in respiratory and circulatory systems of exposed fish and increased the glucose level in this case. On the other hand, the exposure of the fish to higher level (6 mg/L) for 14 and 28 d resulted in significant decrease ($P<0.05$) in serum glucose. This decrease could be attributed to the toxic effect of atrazine on the liver (Braunbeck et al 1992). Reduction in food intake of the treated fish could be also considered a cause of glucose level reduction as the diet is the main source of energy

Serum total protein and globulin tended to decrease significantly ($P<0.05$) with the increase of atrazine level and exposure time in tilapia, while total protein and globulin decreased significantly ($P<0.01$) in Chrysichthyes auratus (Table 1). Moreover, Chrysichthyes auratus suffered with atrazine exposure since at 6 mg/L total protein and globulin decreased by about 59% versus about 26% in tilapia after 28 d. The decrease of total protein was mainly due to globulin (Table 1) this means that atrazine has toxic effects on immune system in these fishes. Although serum albumin did not differ significantly ($P>0.05$) in tilapia groups and it was significantly decreased ($P<0.01$) in Chrysichthyes auratus groups (Table 1). These changes in total protein, albumin and globulin as affected by atrazine exposure can be explained by different theories: Santa Maria et al (1986) showed that atrazine exposure led to increase of protein amount in the urine. Moreover, atrazine has harmful effects on spleen. liver and anterior kidney (Jackim et al 1970 and Rehwoldt 1978). Otherwise, Kettle et al (1987) have shown that atrazine has an inhibitory effect on macrophyte communities as photosynthesis inhibitor. This led to a decline of the invertebrate fauna which is essential as prey for fish. The decline of the invertebrate fauna as essential prey for fish negatively affected the diet of fish. Moreover. the exposure of Cyprinus carpio to 1.5, 3.0 and 6.0 mg/L atrazine for 14 d resulted in biochemical changes such as alkaline phosphatase, glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase activities (Neskovic et al 1993).

The overall mean of serum cholesterol concentration was 206.27 ± 39.53 and 848.70 ± 42.33 mg/dL in control group of Oreochromis niloticus and Chrysichthyes auratus. Serum cholesterol decreased significantly ($P<0.01$) in exposed group of Chrysichthyes auratus while this difference was not significantly in exposed tilapia to the same atrazine levels, At 6 mg/L atrazine the decrease percentage in serum cholesterol of Chrysichthyes auratus was about three times more than tilapia at the same level.

Triglycerides and total lipids concentrations obtained in the present study are presented in Table 1. Both triglycerides and total lipids were significantly decreased ($P<001$) with the increase of atrazine levels in Chrysichthyes auratus. The pattern of change during the course of the experiment in both parameters was differ where the decrease in case of tilapia was less significant ($P<0.05$, Table 1). The decrease in triglycerides concentration in exposed fish to atrazine compared with control fish could be due to decreased glucose availability in exposed fish (Table 1). Glucose is essential for triglycerides synthesis because it forms alpha glycerophosphate which is the specific precursor of glycerol with which fatty acids are esterified for triglycerides formation (Bergman 1983). In addition glucose furnishes NADPH which is required as a reducing agent in the synthesis of long chain fatty acids. In general, the decrease in serum levels of both glucose and triglycerides indicate that the exposed fish to atrazine were in worse condition compared with control fish. The liver plays a key role in metabolism of triglycerides and cholesterol (Barnhart 1969) otherwise atrazine caused fatty degeneration and fatty infilteration changes in the liver of rainbow trout at level of 10, 20, 40 and 160 µg/L for 5 wk and also in zebra fish at 0.1, 1.0 and 10 mg/L for 3 mon.

From Table 1 it is cleared that urea nitrogen was significantly increased ($P<0.01$) in case of Chrysichthyes auratus. This increase was related to atrazine level and exposure time. While, in case of tilapia the differences were not significantly differ ($P>0.05$). The significant increase of urea nitrogen in exposed Chrysichthyes auratus to atrazine may be due to necrosis of

endothelial cells and renal hemopoietic tissue. This opinion supported by Fischer-Scherl et al (1991) using 1.4 and 2.8 mg/L atrazine in rainbow trout. Also, Wotton and Freem (1982) reported that the herbicides led experimentally to degeneration of kidneys in fish. Gunkel and Streit (1980) indicated that atrazine was accumulated via the gills and blood during its exposure phase. This accumulation of atrazine in the gills caused their dysfunction and resulted in kidney stress which lead to increase of urea in the blood.

Serum sodium and potassium concentrations tended to decrease significantly ($P<0.01$) with the increase of atrazine levels and exposure time in the two fish species (Table 1). Also, serum chloride concentrations were reduced significantly ($P<0.01$) in the exposed Chrysichthyes auratus than the control while, the decrease in serum chloride of tilapia was less ($p<0.05$) than Chrysichthyes auratus at the same time and the same atrazine level (Table 1). These obtained results were in agreement with the findings of Fischer-Scherl et al 1991 and Catenacci et al 1993 who mentioned that atrazine plays an important role in osmoregulation since it controlled urinary excretion and stress on the gills and kidney. Also, Ferreira (1981) proved significant correlations between benzocaine hydrochloride (BH) concentrations and blood plasma sodium, potassium and chloride. However, Kolberg et al (1974) reported that 2,4D may affect the permeability of the plasma membrane of fish.

AChE activity obtained in the present study (Table 1) was 2232.00 ± 121.89 and 2454.00 ± 118.88 IU in the brain and 119.70 ± 0.62 and 471.90 ± 58.15 IU in the serum of Oreochromis niloticus and Chrysichthyes auratus. There was a significant difference between the serum AChE activity as well as brain AChE in the treated groups in comparison to the control one. The inhibition degree of enzyme activity increased significantly ($P<0.01$) with the increase of atrazine level and exposure time. It is obvious that a dose -dependent decrease in AChE activity after exposure to 3 and 6 mg/L atrazine. Inhibition of AChE activity of fish may cause it to exhibit a number of symptoms, These symptoms include a reduction in the ability of the fish to tolerate reduced oxygen tension, a slow-down of its reflexes and swimming movements and a reduction in the feeding activities of the fish. So, inhibition of AChE activity is generally regarded as a useful indicator of exposing the fish to hazard compounds. There was lacking in the literature on atrazine effect on AChE except Chaturvedi (1993) who showed that serum AChE activity was inhibited by ca. 50% in 2.5 and 5 mg atrazine/kg for mice. Otherwise, using other pollutants on tilapia, El-Elaimy et al. (1990) recorded that the inhibition in AChE activity in brain, liver and gills of Tilapia nilotica was followed up after exposure of fish to chloropyrifos or lannate. On other hand, the obtained results on inhibition of AChE activity are in agreement with findings of Nemcso et al (1990) using other chemicals and various fish species.

The role of the herbicide on inhibition of AChE may be caused by their ability to inhibit AChE in various parts of the nervous system and thereby disrupt nervous transmission at that location (O'Brien et al 1974). Also, the inhibition of AChE may be caused by accumulation of AChE at cholinoceptive sites and thus are potentially capable of producing effects equivalent to continuous stimulation of cholinergic fibers throughout the central and peripheral nervous system.

In conclusion, the present study suggested the urgent need to the natural and non-pollutant substances to eradicate the undesirable weeds for protecting fish and man. On the basis of these findings atrazine can not be recommended for aquatic use in total water application. The use of atrazine may be the reason for the decrease of Chrysichthyes auratus population in Assiut, Egypt since this fish is very sensitive to this substance.

Table (1): Means of hematological and selected serum components for Oreochromis niloticus and Chrysichthyes auratus after the exposure to atrazine

Treatment (mg/L)	Control			3.0		6.0	
Time (day)	0	14	28	14	28	14	28
Species (1)	***Oreochromis niloticus***						
a) Haematology:							
1- RBCs (No)	1980000	1420000	1310000	650000b	631000c	760000a	610000d
2- Haemoglobin (g/L)	6.0	7.0	6.8	3.2c	3.1b	4.5a	4.0b
3- Haematocrit (%)	20.0	22	21	11a	11a	11a	10b
4- MCV (fl)	101.0	154.9	160.3	169.2c	174.3d	144.7a	163.9b
5- MCH (pg)	30.3	49.3	51.9	49.2a	49.1a	59.2c	65.6b
6- MCHC (%)	30.0	31.8	32.4	29.1b	28.2c	40.9a	40.0d
b) Serum components:							
1- Glucose (mg/dL)	52.33	52.18	47.37	63.99	43.29b	41.54c	38.04d
2- T.protein (g/100 mL)	3.40	3.73	3.41	2.72b	2.44d	2.90a	2.48c
3- Albumin (g/100 mL)	0.67	0.71	0.73	0.67a	0.70	0.73	0.50b
4- Globulin (g/100 mL)	2.74	2.56	2.68	2.05b	1.74d	2.17a	1.98c
5- A/G ratio	0.24	0.45	0.27	0.33	0.40	0.33	0.25b
6- Cholesterol (mg/dL)	161.34	221.73	235.74	176.3c	178.7b	191.3d	182.6a
7- Triglycerides (mg/100 mL)	209	323.76	437.76	72.96b	142.9a	20.52d	37.24c
8- T.lipids (g/L)	22.44	19.95	22.07	13.30d	17.83a	17.33b	13.64c
9- Urea (mg/100 mL)	8.41	11.71	11.11	10.31a	8.25c	11.90	7.77d
10-Sodium (mmol/L)	162.40	148.40	165.20	140.0a	134.0c	134.4b	128.6d
11-Potassium (mmol/L)	5.8	5.3	5.9	5.0a	4.8b	4.8b	4.2c
12-Chloride (mmol/L)	105	105	111	91a	91a	93a	90b
c) AChE							
1- Brain (IU)	2130	2199	2367	1991a	1471b	1214c	431.2d
2- Serum (IU)	119.50	119.20	120.40	55.96a	53.54a	50.01b	43.23c
Species (2)	**Chrysichthyes auratus**						
a) Haematology:							
1- RBCs (No)	1390000	1460000	1380000	940000b	820000c	720000d	600000e
2- Haemoglobin (g/L)	6.0	6.5	6.0	5.0a	4.4b	3.18c	2.84d
3- Haematocrit (%)	30.5	35	31	25b	22c	20d	18e
4- MCV (fL)	219.4	239.7	224.6	265.9a	268.3b	277.8c	300.0d
5- MCH (pg)	43.2	44.5	43.5	53.2b	53.7c	44.2d	47.3e
6- MCHC (%)	19.7	18.6	19.4	20.0b	20.0c	15.9d	15.8e
b) Serum components:							
1- Glucose (mg/dL)	51.60	52.40	51.32	64.20	32.79c	30.64d	28.05e
2- T.protein (g/100 mL)	3.89	3.85	3.42	3.34b	2.66c	1.83d	1.37e
3- Albumin (g/100 mL)	0.97	0.76	0.80	0.72b	0.57d	0.60c	0.30e
4- Globulin (g/100 mL)	2.92	3.09	2.62	2.62a	2.09b	1.23c	1.07d
5- A/G ratio	0.33	0.24	0.31	0.27c	0.27c	0.49	0.28b
6- Cholesterol (mg/dL)	835.26	814.72	896.13	517.4c	639.1b	280.9e	314.0d
7- Triglycerides (mg/100 mL)	1637.80	1583.68	1272.08	140.6c	140.0d	144.8b	136.48e
8- T.lipids (g/L)	27.83	26.98	27.0	23.13b	18.86c	7.91d	6.92e
9- Urea (mg/100 mL)	8.88	7.30	7.86	8.73c	11.58b	9.6e	14.20d
10-Sodium (mmol/L)	134.40	134.40	135.20	128.8a	128.8a	122.1b	118.6c
11-Potassium (mmol/L)	4.8	4.8	4.9	4.6a	4.6a	4.0b	3.8c
12-Chloride (mmol/L)	120	120	115	102a	91c	95b	90d
c) AChE							
1- Brain (IU)	2542.5	2319	2501	1364b	1019c	1019d	621.3e
2- Serum (IU)	456.50	423	536.2	420.1c	451.7a	220.8d	170.0e

Values are least-sqare means and standard error of five fish/tratment
Means with the same letters are not significant

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