Journal of

Fisheries and Aquatic Science

ISSN 1816-4927

Academic Journals Inc.

www.academicjournals.com

Journal of Fisheries and Aquatic Science 4 (4): 169-177, 2009
ISSN 1816-4927

Evaluation of Diazinon Toxicity on Nile Tilapia Fish (*O. niloticus*)

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Abstract: Diazinon was used in the laboratorial study to investigate its biochemical effect on tilapia as it is the most popular fish in Egypt. Two hundred and twenty appeared 40±2 g adult male Nile tilapia were reared in glass aquaria of 60 L capacity, provided with a good air supply and dechlorinated tap water, Fish were maintained under suitable condition for the fish growth. Results of the study are summarized as follow: (1) The bioassay test revealed that the LC_{50} for tilapia after 96 h of exposure was 2.8 ppm, (2) Fish was very excited after being exposed to lethal concentrations of diazinon (5, 10, 200 ppm) for 96 h. Meanwhile, fish exposed to sublethal concentrations of diazinon for 30 days didn't cause mortality to fish and (3) Exposing fish to 0.28 and 1.87 ppm for 30 days caused the following changes: (A) A reduction in total protein content in muscles up to 13.69 and 21.5% for 0.28 and 1.87 ppm, respectively, (B) A reduction in total protein content in blood serum up to 22.23 and 24.32% for 0.28 and 1.87 ppm, respectively and (c) 52, 27 and 6.8 kDa proteins were not scanned in the treated or the recovered samples in both treatments, a slight reduction in the 33.55, 31.72, 24.31 and 20.8 kDa proteins in both treatments, (4) Exposing the treated fish to 7 days of recovery in un poisoned water caused the following changes: (A) A recovery in total protein content in muscles up to 95.59 and 90.58% for 0.28 and 1.87 ppm, respectively, (B) A recovery in total protein content in blood serum up to 89.36 and 95.14% for 0.28 and 1.87 ppm, respectively and (C) 52.27 and 6.8 kDa proteins were still not scanned after recovery of both treatments. A slight increase in the rest of affected proteins after recovery of both treatments was recorded. Therefore, it can be emphasized for good environmental administration of the water bodies to save human health and environment from the dangerous pesticides.

Key words: Nile tilapia, diazinon, bioassay, muscular protein

INTRODUCTION

The intensive agriculture regime in Egypt was designed to employ the land at its maximum uses. This partially covers the needed food by people live in this over populated country. By this strategy, the use of intensive pesticides in controlling pasts and diseases became common (Abdelhamid, 2003, 2005).

Subsequently, the food chain including fish production was affected negatively due to the toxicity of the pesticides uses (Abdel Fattah, 1992; Tawfic *et al*., 2002).

The present investigation was carried out in order to study the physiological and biochemical changes in quality and quantity of protein of Tilapia fish due to its exposure to Diazinon pesticide found in the waterways and lacks.

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MATERIALS AND METHODS

Toxicity tests and some biochemical determinations were performed on tilapia fish (*O. niloticus*) at the Laboratory of Plant Protection. Faculty of Agriculture, Suez Canal University, Ismailia, Egypt- in season 2006. The tested compound (Diazinon) was a product of ADWIA Company, its concentration was 60% and it was brought from the Agricultural Requirements and Equipments market. The tested compound was reported by Kamrin (1997).

Tested Fish

Two hundred and twenty appeared uniform 40±2 g adult male Nile tilapia, *O. niloticus* were used in the experiment. They were provided from, Al-Tal Alkber fish farm, Ismaila, Egypt. Fish were transported live in tanks supplied with air pumps to the laboratory where they were reared in glass aquaria of 60 L capacity, provided with a good air supply and dechlorinated tap water. Fish were fed on commercial pellets contain 30% protein twice a day for 15 days. Feeding rate was made by 4% of fish weight (NRC, 1993). Fish was maintained in natural illuminating system under normal prevailing photoperiod conditions. Water temperature was maintained at about 25 ±1°C and water pH 7.7±0.5 which is suitable for the fish growth (Brown and Gratzek, 1979).

Toxicity Studies

This study was designed to examine the influence of both acute and chronic toxicity of diazinon on biochemical parameters of tilapia *O. niloticus.*

Acute Exposure

Approximately 150 healthy fish (10 fish/aquarium, 3 replicates/treatment) at equal size and length were divided in groups in aquaria and then fish was starved for 2 days before the test was performed. Fish were exposed to series of concentrations of the tasted pesticide (diazinon) to determine 50% lethal concentration (LC_{50}); 5, 10, 50 and 200 mg L^{-1} for 96 h. Control fish was maintained in similar aquaria that contain only clean dechlorinated tap water. Feeding was stopped within the exposure time. And behavioral changes and clinical signs were recorded. Dead fish was removed from the aquaria as soon as possible to avoid polluting the contained water causing changes in the aquaria's maintained conditions. Mortality was recorded each 24 h. Results were then subjected to probit analysis (Finney, 1971) to produce regression lines and to determine LC_{50} and line slopes.

Chronic Exposure

Sixty fish were exposed to the LC_{10} and $LC_{33.5}$ of diazinon (30 fish for each) for 30 days which rarely, if ever, occurs under natural conditions, (Scholz *et al.*, 2000). Fish were fed on 30% protein pellets, the pellets were brought from the Fish Research Center at Suez Canal University. Water was changed each 96 h to avoid the increase of ammonia level in water. At the end of the exposure interval, samples were randomly taken from the aquaria and then fish were removed from the treated aquaria to be transferred, for recovery, in other similar aquaria for 7 days, provided with clean dechlorinated tap water. Also, control fish were kept for a similar period in similar conditions. At the end of this recovery interval, new samples were taken from the aquaria and biochemical assessment was conducted on them.

Blood Sampling

Blood samples were taken from the tested fish to be biochemically analyzed. Fish were anaesthetized in ethyl 3-aminobenzoat methanesulphat (MS 222) by dissolving 0.02 g of

MS 222 L^{-1} of water, then dipping the fish in it for 30 sec. Blood plasma was obtained by direct puncture of the heart with a heparinized injection, as heparin is a good antithrombin anticoagulant recommended for most fresh water fish (Smit and Hattingh, 1980); the sample was then collected in eppendorf tubes. On the other hand, Blood sampling of serum was obtained by direct puncture of the heart with an injection without using any antithrombin anticoagulant.

Biochemical Studies

Total protein was measured in fish blood serum and muscles by using a total protein kit (Henry, 1964). Proteins changes due to the lethal and sublethal concentrations of diazinon in the isolated muscles were identified using Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis (SDS/PAGE) by the method of Laemmili (1970).

Statistic Analysis

Data were statistically analyzed to test the significant differences ($p<0.05$) using SAS (1998).

RESULTS AND DISCUSSION

The Bioassay and Toxicity Studies

Results of the present study showed that LC_{50} of diazinon for 96 h was 2.8 mg L^{-1}. LC_{50}, MLC50 and the slope of diazinon are shown in Table 1.

Results reported by US EPA (1995) show close results with rainbow trout, while other fishes showed higher results; LC_{50} for Sheepshead minnows juveniles was 1400 μg L^{-1} (Goodman *et al*., 1979). Killifish 48 h LC_{50} was 4400 ppb for diazinon and 220 ppb for the diazoxon (Tsuda *et al*., 1997). Ninety 6 h LC_{50} for common carp juveniles was 26.7 mg L^{-1} of basudin 600 EW (preparation which is 16.0 mg L of diazinon), (Svoboda *et al*., 2001) and 839 μg L^{-1} for congeneric rainbow trout (*Oncorhynchus mykiss*), 2620 μg L^{-1} for *Oncorhynchus clarki* (Novartis, 1997). 770 ppb for Brook trout (*Salvelinus fon tinalis*), 7800 ppb Fathead minnow (*Pimephales promelas*), 460 ppb for bluegill sunfish (*Lepomis macrochirus*) (Allison and Hermanutz, 1977).

There has been different opinions reported about diazinon toxicity; Scholz *et al*. (2000) suggested that diazinon concentrations in nature aquatic ecosystems are more commonly less than 10 μg L^{-1}; these concentrations, lasting for only a few days, are unlikely to kill fish outright. This difference of results and conclusions can possibly be related to differences of conditions on which each experiment was processed; such as containing water parameters, physiological differences between fish species and sexes since that salt water fish are more susceptible than freshwater fish to pollution (Kamrin, 1997). Also, differences in feeding types and habitats and pond capacity can be sources of differentiations. Moreover, differential sensitivity of various species to diazinon is largely due to the transformation rates within those species as suggested by Fujii and Asaka (1982). Differences in diazinon toxicity among various spices of fish could strongly be related to metabolic balances in the liver and with the features of the target enzyme as suggested by Keizer *et al*. (1995).

Table 1: Toxicity of diazinon on Nile Tiapia

LC_{10}	LC_{25}	$LC_{33.5}$	LC_{50}	LC_{90}	SLOOPW	MCL_{50}
(mg L^{-1})						
0.28	0.7	1.87	2.8	2.52	8.14	6.88×10^{3}

MLC50: Molar LC50 = LC50/Molecular weight of compound

Behavior Response and Clinical Signs of Diazinon on Nile Tilapia

Acute Exposure

After exposing fish to lethal concentrations of diazinon (200, 50, 10 and 5 mg L^{-1}), fish were notably excited. They swam around erratically and very rapidly and tried to jump out of the container. They surfaced gulping air. Their eyes were surrounded with black circles. In latter stages of exposure, the exposed excited fish laid on their sides on the bottom of the container, making very slight movement. Fish died within less than an hour from the exposure time when concentrations were equal to/or higher than 10 mg L^{-1}. By the time fish died, they were all turned black. The dead fish which were exposed to 2.8 mg L^{-1}, showed approximately similar clinical signs; they showed rapid movements at the first few hours of the exposure, then they swam less rapidly. Later, they laid on their sides on the bottom of the container till death, fish died within 96 h. Observed clinical signs caused by acute exposure are shown in Table 2.

Chronic Exposure

When fish was exposed to sublethal concentrations of Diazinon, LC_{10} and $LC_{33.5}$ (0.28 and 1.87 mg L^{-1}, respectively), fast movements of operculum and mouth was observed during the first 7 days. Later, fish skin turned black and the operculum moved normally. At the end of the exposure period, dark circles were noticed surrounding the eyes. No abnormal swimming activity was observed. No mortality occurred in the containers. Recovered fish showed lighter skin color, the black circles around the eyes were recovered. This agrees with the determinations reported by (Vaid and Mishra, 1999) that fish show a remarkable recovery against their toxicity when the source of toxicity is vanished. Rath and Misra (1981) reported that the degree of recovery follows an inverse relationship with the time of exposure, however, observed clinical signs caused by chronic exposure are shown in Table 3. The changes in skin colour in fish is in apart controlled by the sympathetic nervous system, which is probably affected by the exposure to Diazinon which is a nerve poisonous that affects the nervous system as the a main target to its activity. Color is most likely affected and regulated by a Melanophore Stimulating Hormone (MSH) as well when fish is stressed.

The observed fast movements of the operculum can be related to the cytochromoxidase which is responsible of the respiration system, most likely, when fish were stressed by the exposure, the enzyme was affected and resulted in reducing the efficiency of the extracting the oxygen from the water

Table 2: Clinical signs of acute toxicity

	Time (h)		
Signs	25	48	72/96
Skin color	Gray	Black	Black
Operculum movement	Opens and closes very fast	Opens and closes very fast	Opens and closes very slow
Eyes	Surrounded by black cricles	Surrounded by black cricles	Surrounded by dark cricles
Mouth	Opens and closes rapidly and gulps air	Opens and closes less rapidly	Normal
Food appetite	Non	Very bad appetite	Non
Activity	Rapid movements	Slight movements	Almost stays still

Table 3: Clinical signs of chronic toxicity

	Time (day)			
Signs	7	14	30	Recovery
Skin colour	White	Black	White	White
Operculum	Opens and closes slightly faster	Opens and closes normally	Opens and closes slightly faster	Normal
Evey	Normal	Normal	Surrounded by dark circles	Normal
Mouth	Often gulps air	Normal	Normal	Normal
Food appetite	Normal	Normal	Normal	Normal
Activity	Normal swim	Normal swim	Normal swim	Normal

causing those fast movements of both, the mouth and the operculum of the fish in trying to come over the shortage of oxygen. Eventually when fish is exhausted by those fast movements and the shortage of oxygen, the movements are slowed gradually. Fish lost the appetite of food when they were treated with Diazinon for a chronic exposure; this is probably because the energy of the fish is almost totally directed toward resisting the pollutant effect on the biochemical functions of its body. The stored energy is then used instead of consuming food.

El-Khateib and Afifi (1993) suggested that the onset of death due to the exposure with Diazinon is related to the nerve hemorrhagic gills and the lamellar effusion which cause reduction of the respiratory efficiency resulting in acute death. The entrance of the Diazinon was most likely by gills since toxicants enter the blood stream through the gills or/and gastrointestinal tract as reported by Doving (1991). There are many agreements with the result of this study; Beauvais *et al.* (2000) reported significant changes in swimming speed and distance in larval rainbow trout (*Oncorhynchus mykiss*) after been exposed to diazinon. They suggested that there was a correlation between physiological and behavioral changes in fish as well as in mammals. In addition, Moore and Waring (1998) suggested dangerous effect of Diazinon on the population of salmonids resulted from the effect of diazinon on the olfactory system of mature male Atlantic salmon (*Salmo salar*), which in sequence led to losing the ability of recognizing the prostagaglandin F sub (2 alpa) and ovulated female urine and both are known to have important roles in synchronizing reproductive physiology and behavior in salmonids as well as in other fish. El-Khateib and Afifi (1993) observed the same signs on Tilapia niloticus when it was exposed to Diazinon.

On contrast with the results reported in this study and in the previously mentioned studies, Scholz *et al.* (2000) reported that diazinon had no effect on swimming behavior or visually guided food capture on chinook salmon (Oncorhynchus tshawytscha) when treated with 0.1, 1.0 and 10.0 μg L^{-1}. But in a partial agreement, they reported that Diazinon significantly inhibited olfactory-mediate alarm response at concentration as low as 1.0 μg L^{-1} in addition to a homing behavior that was impaired at 10.0 μg L^{-1}. Parallel clinical signs were observed in fish after the exposure to other pesticides.

Effect of *in vivo* Sublethal Concentration of Diazinon on Total Protein Content in Blood and Muscles

Total protein content in both muscles and blood serum of the tested fish were significantly ($p<0.05$) reduced after been exposed to both of the tested sublethal concentrations of Diazinon (LC_{10} and $LC_{33.5}$) for chronic exposures of 30 days compared with the control. Total protein in muscles and blood serum was significantly raised after fish were recovered for 7 days. Compared to the control group, total protein content in serum was reduced by 22.3% for LC_{10} and 24.3% for $LC_{33.5}$. Both were increased in 7 days recovery to 10.64 and 4.86%, respectively. The same period caused reduction in muscle's total protein content by 13.7% for LC_{10} and 21.9% for $LC_{33.5}$. Both were increased in 7 days recovery to 4.41 and 9.42%, respectively. Results are shown in Table 4 and 5.

Table 4: Effect of sublethal concentrations of diazinon on total protein content in blood serum

		30 days		Recovery	
Concentration	Control (g dL^{-1})	g dL^{-1}	Change (%)	g dL^{-1}	Change (%)
0.28 mg L^{-1}	11.56±0.26^{a}	8.99±0.27^{b}	-22.23	10.33±0.13^{a}	-10.64
1.87 mg L^{-1}	11.10±0.57^{a}	8.40±0.43^{b}	-24.32	10.56±0.20^{a}	-4.86

Similar letter(s) indicate to non significant differences ($p \geq 0.05$), Different letter(s) indicate to significant differences ($p \leq 0.05$)

Table 5: Effect of sublethal concentrations of diazinon on protein content in muscles

		30 days		Recovery	
Concentration	Contrrol (mg g^{-1})	mg g^{-1}	Change (%)	mg g^{-1}	Change (%)
0.28 mg L^{-1}	11.57±0.32^{a}	9.985±0.07^{c}	-13.69	11.06±0.17^{a}	-4.41
1.87 mg L^{-1}	12.00±0.38^{a}	9.420±0.33^{c}	-21.50	10.87±0.34^{b}	-9.42

Similar letter(s) indicate to non significant differences ($p \geq 0.05$), Different letter(s) indicate to significant differences ($p \leq 0.05$)

Table 6: Relative molecular weights and bands intensity of the SDS/PAGE separated proteins of muscles of tilapia treated with 1/10 LC_{50} of Diazinon

	Intensity				
Band No.	M.W.	St.	CON.	REC.	TRE.
1	214.00	14112			
2	210.00		60902	59968	50268
3	120.98		33966	31432	30176
4	118.00	12838			
5	111.35		27195	27352	26164
6	99.76		23310	23480	28220
7	92.40	12740		26656	26724
8	70.79		30969	26240	23902
9	64.23		19684	18792	17658
10	61.16			19808	34612
11	58.15		27121	26128	25758
12	52.20	11368	19980		
13	47.12		68561	74880	92884
14	44.91		29341	28928	27604
15	43.00		27158	25472	25158
16	39.77		54760	52928	50792
17	36.70		33300	36288	44200
18	35.00	10528			
19	33.55		23606	23488	23382
20	31.72		23095	23020	23090
21	28.90	8862			
22	27.00		20868		
23	26.00		14726	20416	24548
24	25.00			11616	12614
25	24.31		18279	18268	18228
26	20.80	7840	37444	37388	37410
27	18.00		18093	18088	18002
28	6.80	12096	12506		
Band No.		8	21	21	21

M.W.: Molecular weight (kDa), St.: Standard marker, CON.: Control fish, TRE.: Treated fish, REC.: Recovered fish

A similar result was reported by Luskova *et al.* (2002) evidences a significant decrease in protein concentration in blood serum of carp (*Cyprinus carpio* L.). Khalaf-Allah (1999) indicated that total protein was lower in fish exposed to 1/10 LC_{50} of diazinon compared to the non-exposed ones even though they were vaccinated before exposure to diazinon. Danasoury *et al.* (1997) demonstrated a decrease in muscle protein in acute and chronic exposure, they reported that in recovery condition the decrease was continued but with tendency to recovery. Sakr and Gabr (1992) reported that in some cases, diazinon exposure to fish caused changes in muscles.

Effect of Sublethal Concentration of Diazinon on Muscles' Protein Banding Patterns

Analyzing the pictured stained SDS/PAGE gel by gel documentation system (GDS) indicated the presence of 21 bands in the control sample and 21 bands in the 1/10 LC_{50} treated and recovered fish Table 6. While 20 bands only were scanned in the 1/3 LC_{50} treated and recovered sample as shown in Table 7. Bands of 92.4, 61.16 and 25.00 kDa were induced in the 1/10 LC_{50} treated and recovered fish and were not scanned in the control sample. While in the 1/3 LC_{50} treatment, only bands of 92.4 and 25.00 kDa were induced in the treated and recovered samples and were not scanned in the control sample. Meanwhile, 99.76 and 26 kDa proteins were also induced in the treated and recovered fish, but they were scanned in thin bands in the control lanes.

The 52, 27 and 6.8 kDa proteins were not scanned in the treated and recovered samples in both treatments, which indicate that these proteins were probably highly affected by the stress caused by the treatment, which led to its high reduction. Also, 33.55, 31.72, 24.31 and 20.8 kDa proteins showed a slight reduction in both treatments. The rest of bands were all remarkably thinner in the treated

Table 7: Relative molecular weights and bands intensity of the SDS / PAGE separated proteins of muscles of tilapia treated with 1/3 LC_{50} of Diazinon

	Intensity				
Band No.	M.W.	St.	CON.	REC.	TRE.
1	214.00	15223			
2	210.00		78696	63396	48924
3	120.40		34868	32588	27216
4	118.00	14576			
5	111.99		29160	29024	25500
6	99.76		26460	26888	28880
7	92.40	14256		21924	29440
8	70.66		35064	28440	25164
9	64.40		23472	22284	20908
10	61.16				
11	58.60		29052	28368	24372
12	52.20	13424	20628		
13	47.33		64872	80748	86016
14	45.00		32004	30492	29196
15	43.00		29304	27108	22248
16	39.08		59868	56268	53092
17	36.70		36360	38268	21276
18	35.00	12048			
19	33.46		22824	22168	21972
20	31.73		22320	22280	22240
21	28.90	9664			
22	27.00		19584		
23	26.00		15300	21832	27676
24	25.00			12816	10872
25	24.31		21034	21031	21028
26	20.61	8848	33796	33732	33732
27	18.00		18316	18306	16012
28	6.80	17248	12708		
Band No.		8	21	20	20

M.W.: Molecular weight (kDa), St.: Standard marker, CON.: Control fish, TRE.: Treated fish, REC.: Recovered fish

sample and the recovered sample in both treatments, indicating to a reduction in the protein of these bands. Protein bands of the treated fish were obviously thinner than those in the recovered fish, which indicated that the proteins were highly reduced most likely due to the Diazinon treatment and that they were induced in the recovered fish after the poison was removed. The bands were reduced to nearly normal in the recovered fish in both treatments. The Induction of these proteins was most likely caused by the treatment which simulated the accumulation of existing proteins or synthesis of new proteins (Singh *et al*., 2002). The results presented in this research revealed that diazinon at concentration ranged from 13.7-21.5% significantly reduced the muscular protein in Nile tilapia fish.

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