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Acute toxicity, biochemical and histological of fenitrothion and thiobencarb on fish Nile tilapia (*Oreochromis niloticus*)

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Abstract. *Fouad MR, El-Aswad AF, Aly MI. 2022. Acute toxicity, biochemical and histological of fenitrothion and thiobencarb on fish Nile tilapia (*Oreochromis niloticus*). Nusantara Bioscience 14: 217-226.* The results show that the tested fenitrothion and thiobencarb are highly toxic to fish. However, fenitrothion is more toxic (1.6 times) on *Oreochromis niloticus* (Linnaeus, 1758) than thiobencarb. The determined 96-h LC_{50} values using a static bioassay system to Nile tilapia fingerlings (8-10 g) were 0.20 and 0.32 mg L^{-1} for fenitrothion and thiobencarb, respectively. The mortality rate of fish exposed to ½ 96-h LC_{50} of fenitrothion (0.10 mg/L) and thiobencarb (0.16 mg/L) for four days demonstrated was 20% mortality rate. Fish showed tremors, lethargy, decreased movement, and increased respiratory rhythm. The total activity of AChE in control was 5.61 ±0.03; it was significantly reduced to 4.92 ±0.03 in fenitrothion treatment and 1.13 ±0.02 in thiobencarb treatment. Fenitrothion decreased the specific activity from 0.83 ±0.01 for the control to 0.68 ±0.01, whereas thiobencarb reduced the specific activity to 0.22 ±0.01. Generally, thiobencarb inhibited AChE activity much more than fenitrothion; it produced 80% inhibition, while fenitrothion produced 12.5% inhibition. It showed a significant increase in liver GST and SOD activity of Nile tilapia exposed to the tested pesticides compared to the control. There were no histological alterations in the tissues of the control individuals. It was found that the herbicide thiobencarb affected the gills, kidneys, and liver of Nile tilapia more than the insecticide fenitrothion.

Keywords*:* Biochemical, fish, histological, Nile tilapia, pesticides, toxicity

INTRODUCTION

Over the world, fish is an important human food due to its protein content. About 25,000 different known species of fish, including 15,000 marine species and 10,000 freshwater species (Nelson 1994). The annual capture (direct and from fish farms) was about 149 million tons (FAO 2012). Egypt is considered the second largest producer of tilapia in the world, where Nile tilapia accounts for about 80% of fish production (Tahoun et al. 2008). Fish is a source of important nutrients essential for human health (Stanley et al. 2016; Aboagye et al. 2020). Fish farming is practiced on farmlands as a component of sustainable agriculture in many tropical countries. Thus, to supplement the risk assessment, it is crucial to gather information on the impact of pollutants on fish (Peebua et al. 2007). Fish serve as a bioindicator of water quality due to two key characteristics: their availability in a larger range of habitats and their stronger reactivity to contaminants (Stanley et al. 2016).

Once a pesticide is applied to a field, it travels through the watershed and may have negative consequences far away from the application site (Akan et al. 2014). Even though pesticides are diluted in rivers and streams, various mechanisms cause the pesticide to become concentrated and harmful (Stanley et al. 2016). Through soil erosion, the pesticides may reach the rivers (de Melo Plese et al. 2005). However, surface runoff due to rainfall is the major mechanism for pesticide transport into water bodies. It was reported that pesticide exposure to aquatic biota was achieved in three ways; direct absorption of polluted food, water absorption through gills, and integument. Each of the three routes varies with pesticide type, organism, and environmental conditions. The absorption through the gut and gills are the two important pesticide routes. The relative importance of absorption via water or food depends on the exposure, dose level, duration, and the individual organism (Stanley et al. 2016). The absorption of pesticides by an aquatic organism occurs through partitioning. The organisms living in the water (fish) must combat the water pollutants, especially the break-down products of pesticides, since they live in that water medium. Fish is exposed to contaminants by direct absorption through the skin and dermal contact (Akan et al. 2014). Fish is affected by pesticides, due to acute mortality or by sublethal effects. Commonly, acute mortality is expressed in LC_{50}, typically done for 96 hours. Behavioral changes such as excitation, avoidance, respiration, and feeding can be due to pesticide toxicity. Chemicals can also cause affect reproduction, blood cells, enzymes, and hormones (Akan et al. 2014).

Pesticide contamination is common in the agricultural sector. It can cause damage to human and beneficial organisms and eventually lead to aquatic environment pollution; thus, it becomes hazardous to aquatic life (El-Murr et al. 2015). Sub-lethal effects of pesticides could be studied in different beneficial organisms such as birds, fish, earthworms, and bees with a highlight to identify the biochemical responses that may be useful to monitor sublethal levels of exposure in the field. The biochemical responses may be detected by measuring enzyme activities in different tissues. In general, the enzyme activities measurement may be useful for exposure assessment and sub-lethal effects of chemicals on the non-target organisms, causing structural and functional changes in liver tissues (Dahamna et al. 2004). Pesticides at low levels may produce various biochemical changes, some of which could not necessarily lead to observable symptoms (Araoud et al. 2012). Environmental pollution due to extensive usage of pesticides without proper management has affected the survival potential of fish as some of these toxic chemicals in the environment may persist for long periods (Gill et al. 1988), producing many physiological, biochemical, and histological changes in freshwater organisms, particularly of the fish. Therefore, histology is a useful tool to determine the pollution degree, particularly for sublethal and chronic effects. Histological examination of fish tissues, especially their liver, proved to be an extraordinarily sensitive tool to detect detrimental effects in fish induced by organic contaminants since the fish liver is a major storage site and pesticide biotransformation and excretion (Cengiz and Unlu 2006).

MATERIALS AND METHODS

Experimental materials

Tested pesticides

Fenitrothion

UPAC name: O, O-dimethyl O-4-nitro-m-tolyl phosphorothioate, Chemical formula: $C_9H_{12}NO_5PS$, Solubility: Water 0.038 g/L, Pesticide type: Insecticide, miticide, Group: Organophosphate, Production Company: Shandong Chuangying Chemical Co., Available formulations: EC 50%; ULV 100%; WP 25, 40, 50%; G 5%; D 2, 2.5, 3, 5%, Usage: For controlling chewing and sucking insects on rice, orchard fruits, vegetables, cereals, cotton, and forest.

Thiobencarb

UPAC name: S-4-chlorobenzyl diethyl thiocarbamate, Chemical formula: $C_{12}H_{16}ClNOS$, Solubility: Water 0.030 g/L, Pesticide type: Herbicide, Group: Thiocarbamate, Production Company: Shandong SanYoung Industry Co., Ltd, Available formulations: EC 50%; G 10%, Usage: It is herbicide for weed control for pre and early post-emergence in rice paddy fields and other situations.

Fish

Nile tilapia was used (*Oreochromis niloticus* Linnaeus, 1758), a common type in Egypt, particularly in rice fields. Tilapia of both genders weighing 8-10 g and measuring 6-8 cm total length were obtained from a Department of Animal Production, Faculty of Agriculture, Alexandria University, Egypt, without pesticide exposure. Fish were acclimated to laboratory conditions for 10 days in the tank (50 L) containing dechlorinated water with aeration and a natural photoperiod (12 h light/12 h dark) before the experiments. During acclimation and pesticide exposure, commercial fish pellets were fed once a day at a proportion of 1% of the total weight of fish in each tank (Peebua et al. 2007).

Experiments

Toxicity of tested pesticides on fish by static bioassay technique

A series of concentrations (0.1, 1, 2.5, 5, 10, 25, 50, and 100 mg L^{-1}) of fenitrothion and thiobencarb were added to the dechlorinated water. The pesticide solutions (2 L for each) were added into 4 L plastic containers, 3 replicates per concentration, and the control was tested. Five Nile tilapia fishes (*O. niloticus*) were exposed per replicate. Under aeration conditions and a natural photoperiod (12 h light/12 h dark), commercial fish pellets were fed daily for 4 days. The mortality percentages were recorded daily, and the LC_{50} values were calculated by LdP line software (Saka 2010).

Side-effects of tested pesticides on biochemical indicators

The side effects of fenitrothion and thiobencarb were studied on the AChE as a target of action of many pesticides, GST, which plays a key role in cellular detoxification, and SOD which prevent oxidative stress. First, the enzyme activities were tested in vivo; Nile tilapia fish (*O. niloticus*) was exposed to concentrations that achieved LC_{50} of fenitrothion and thiobencarb for 4 days, and the fish was taken to extract the enzymes.

Acetylcholinesterase (AChE) assay

AChE activity was determined by colorimetric method according to the procedure of Ellman et al. (1961) using acetylthiocholine iodide (ATChI) as a substrate. The definite weight of fish brain tissue was homogenized in 0.1M potassium phosphate buffer (pH 7.0) using a glass/Teflon homogenizer with ice. Determined protein concentrations by the Lowry et al. (1951) method.

Superoxide Dismutase (SOD) assay

The SOD activity was determined in the liver tissue of fish. The enzyme activity was determined according to Nishikimi et al. (1972). The assay depends on the ability of an enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye.

Glutathione S-transferees (GST) assay

Total GST activity (cytosolic and microsomal) was determined according to Habig et al. (1974) by measuring the conjugation of 1-Chloro-2,4-dinitrobenzene (CDNB) as substrate with reduced glutathione. An increasing absorbance accompanies the conjugation at 340 nm.

Side-effects of tested pesticides on histological characteristics

The live exposed fish to concentrations of fenitrothion and thiobencarb equivalent to their LC_{50} for 4 days was taken the histological examination. Moreover, to study the changes in the histology of these organs, different organs of fish, such as the liver, kidney, and gills, were isolated from the control and the exposed fish. First, the physiological saline solution (0.75% NaCl) was used to rinse and clean the tissues. Next, they were fixed in aqueous bouin's fluid. Then, they were dehydrated through gradual ethanol (70-100%). Finally, they were xylene-cleared and embedded in paraffin wax. Five microns thick paraffin sections were prepared, stained with Ehrlich hematoxylin/eosin (H&E) dyes, dissolved in 70% alcohol, and mounted in Canada balsam (Humason 1972). The slides were examined under a microscope. The possible changes in the tissues of fish exposed to tested pesticides were observed, and photo monographs were taken from the Olympus Microscope (El-Murr et al. 2015).

Statistical analysis

The Ldp line software performed experimental fish data presented as LC_{50} statistical analysis. In addition, a probit analysis developed by Chi (1997) was employed to assess the acute toxicity of pesticides to fish Nile tilapia.

RESULTS AND DISCUSSION

Toxicity of tested pesticides on fish by static bioassay technique

The acute toxicity as mortality percentages of fenitrothion and thiobencarb (50% EC commercial grade formulation for each) using a static bioassay system to Nile tilapia (*O. niloticus*) was tested. While using only the active ingredient in the tests is insufficient (Benli and Özkul 2010). The operational formulations of the herbicide are more toxic than the technical grade chemical (Jiraungkoorskul et al. 2002). Therefore, in this investigation, the results were given regarding the herbicide's commercial formulation, not the active ingredient used, because farmers only use the commercial formulation for agricultural uses.

Mortality percentages of fish exposed to concentrations of 0.1, 1, 5, 10, 25, and 50 mg L^{-1} were recorded at 24, 48, 72, and 96 h. The control mortality was zero during the experiment. Regarding fenitrothion, the lowest tested concentration (0.1 mg L^{-1}) caused 13.33, 20.00, 33.33, and 53.33 % mortality. Consequently, the concentration of 5 mg L^{-1} caused 33.33, 40.00, 53.33, and 80.00 % mortality at 24, 48, 72, and 96 h, respectively. The highest tested concentration (50 mg L^{-1}) of fenitrothion caused 100% mortality at 24 h; the concentration of 25 mg L^{-1} gave 100% mortality after 72 hours of exposure. Concerning thiobencarb, the mortality percentages were (20.00, 26.67, 33.33, and 40.00 %) for 0.1 mg L^{-1}, (40.00, 53.33, 60.00, and 73.33 %) for 5 mg L^{-1} at an exposure time of (24, 48, 72, and 96 h), respectively (Table 1). The concentrations of 25 and 50 mg L^{-1} gave 53.33 and 86.67 %, then 100% at 24 and 72 h, respectively. The results indicated that all tested concentrations' effects on fish increased mortality, with the increasing concentration of the pesticides and exposure time. It was observed that the mortality rate of tested pesticide exposure ranged from 15-55% in acute doses, which began high and decreased gradually. This observation also supported the mortality rate of thiobencarb exposure ranged from 25-30%, which began high and decreased gradually (Eissa et al. 2015).

The calculated 96-h LC_{50} value (95% confidence limits) of fenitrothion, using a static bioassay system to Nile tilapia (*O. niloticus*) fingerlings, was 0.20 mg L^{-1} (0.04-0.22). LC_{50} at 24 h was found to be 4.07 mg L^{-1} (3.28. 5.01) (Table 2). Similarly, the calculated 96-h LC_{50} value of thiobencarb was 0.32 µg /mL (0.19. 0.49). LC_{50} at 24 h was found to be 5.63 mg L^{-1} (4.23. 7.48). The selected species are as recommended by the standard methods (OECD 1993). The slope value was ≤ 1 at all probability levels. The Chi-Square values ranged from 13.36 to 85.99 for fenitrothion and from 22.63 to 61.92 for thiobencarb. The p-values were ≤ 0.01 for fenitrothion and thiobencarb at different time intervals. Moreover, based on the LC_{50} values, the toxicity of fenitrothion at 96 h was 20.7, 14.1, and 6.5 times more than its toxicity at 24, 48, and 72 h. Also, thiobencarb toxicity at 96 h was 17.7, 7.4, and 3.5 times more than its toxicity at 24, 48, and 72 h, respectively. Therefore, from comparing the effect of two pesticides according to 96-h LC_{50} values, fenitrothion was 1.6 times more toxic than thiobencarb. Additionally, from comparing according to the relative toxicity, at 48 and 72 h, thiobencarb has relative toxicity = 100, with fenitrothion having relative toxicity of (85.82 and 85.57) compared to thiobencarb. In contrast, at 24 and 96 h, fenitrothion has relative toxicity = 100, while thiobencarb has relative toxicity of 72.17 and 61.44 compared to fenitrothion. The results show that the tested pesticides are highly toxic to fish. However, fenitrothion is additional deadly on *O. niloticus* than thiobencarb. This result agreed with those obtained by Benli and Özkul (2010), who stated that fenitrothion is highly toxic to Nile tilapia.

The results demonstrated the calculated 96-h LC_{50} value of fenitrothion, using a static bioassay system to Nile tilapia (*O. niloticus*) of body weight (8-10 g) was 0.20 mg L^{-1}. Despite being the same species, the difference in LC_{50} was referred to as the difference in body weight (Eissa et al. 2015). The results showed that fenitrothion is highly toxic to Nile tilapia. However, it was reported that fenitrothion is considered moderately toxic to fish (Benli and Özkul 2010). Still, it is less toxic to most other species, such as 48-h LC_{50} values for carp (*Cyprinus carpio*) 8.2 mg L^{-1}, eel (*Anguilla anguilla*) 3.2 mg L^{-1}, and 96-h LC_{50} values compared with guppy (*P. reticulata*), peppered corydoras (*Corydoras paleatus*), medaka (*Oryzias latipes*) and mullet (*M. cephalus*) 3.21, 3.51, 2.1 and 2.6 mg L^{-1}, respectively (Sarikaya et al. 2007). Also, the experiments showed that the LC_{50} at 96 h of fenitrothion was 0.2 mg L^{-1} in *A. anguilla* (Ferrando et al. 1991) and 1.64 mg L^{-1} for top mouth gudgeon (Solomo et al. 2000). Experimentally, the LC_{50} of thiobencarb in this study on *O. niloticus* (8-10 g of body weight) was found to be 0.32 mg L^{-1} for 96 h, which conformed by Eissa et al. (2015), which was 0.40 mg L^{-1} while; was less than that recorded by Abbas et al. (2007), and Abumourad et al. (2010) which was 0.72 mg L^{-1} on Nile tilapia of body weight (15-20 g). Thiobencarb, at very low doses, is widely used to eradicate the larvae of mosquitoes and control argulus disease and milkfish during pond preparation, which can be toxic to aquatic organisms

(Abbas et al. 2007). It should be emphasized that sublethal concentrations are as significant as acute concentrations to understand the earlier toxicity in non-target species (Benli and Özkul 2010). There is a global interest concerning the problems of a polluted ecosystem, which comprises hazards to fish health and human health (Ulrich et al. 2004).

Side-effects of tested pesticides on biochemical indicators

Effects of tested pesticides on AChE activity

Measurement of acetylcholinesterase activity is routinely used as a biomarker of exposure to different groups of contaminants, such as organophosphate and carbamate insecticides. Previous experiments carried out in this study showed that 0.20 and 0.32 mg/L for fenitrothion and thiobencarb were the LC_{50}-96 h in Nile tilapia (*O. niloticus*). Based on these results, constant sublethal exposure of Nile tilapia fish to ½ LC_{50}-96 h (0.10 and 0.16 mg/L) of fenitrothion and thiobencarb for 96 h resulted in the inhibition of the AChE activity in the brain tissue. The mortality rate of fish exposed to ½ 96-h LC_{50} of two pesticides for 4-days demonstrated about (20%) mortality rate, the dead fish were avoided, and live fish were examined. No mortality occurred in control during the experiment, but fish in treatments showed signs of tremors, lethargy, decreased movement, and increased respiratory rhythm. As reported in Table 3, total and specific brain AChE activity were significantly reduced. The total activity of AChE in un-exposed control was 5.61 ±0.03; it was significantly reduced to 4.92 ±0.03 in fenitrothion treatment and significantly reduced to 1.13 ±0.02 in thiobencarb treatment. On the other hand, fenitrothion decreased the specific activity from 0.83 ±0.01 for the control to 0.68 ±0.01, while thiobencarb reduced the specific activity to 0.22 ±0.01. In general, thiobencarb inhibited AChE activity much more than fenitrothion; it produced 80% inhibition, whereas fenitrothion produced 12.5% inhibition. Similar findings have been described by Sancho et al. (1998). They reported 57% inhibition of AChE activity at 96 h exposure to 0.04 mg L^{-1} and 0.02 mg L^{-1} of fenitrothion produced only a 51% reduction in AChE at 96 h, too. A 20% decline in AChE activity in fish was used as evidence of exposure to OPs (Zinkl et al. 1991), those obtained in fish that have encountered fenitrothion concentration of O. 4 mg/L (Solomo et al. 2000). Fenitrothion insecticides induced significant inhibitory effects on the AChE activity of *A. anguilla*, ranging from > 40% inhibition at a sublethal concentration of 0.02 mg L^{-1} to > 60% inhibition at a sublethal concentration of 0.04 mg L^{-1} (Sancho et al. 1997). A few fish studies indicate a greater decrease in AChE activity with exposure to higher concentrations of OP insecticides such as fenitrothion (Morgan et al. 1990).

Table 1. Toxicity of fenitrothion and thiobencarb on fish (Mortality% ±SE) by static bioassay technique

Conc. (mg L^{-1})	24h	48h	72h	96h
		Fenitrothion		
0.1	13.33 ±6.67	20.00 ±11.55	33.33 ±6.67	53.33 ±6.67
1	20.00 ±11.55	26.67 ±13.33	40.00 ±11.55	73.33 ±6.67
2.5	26.67 ±6.67	33.33 ±6.67	46.67 ±6.67	73.33 ±6.67
5	33.33 ±6.67	40.00 ±0.00	53.33 ±6.67	80.00 ±0.00
10	66.67 ±6.67	73.33 ±6.67	73.33 ±6.67	86.67 ±6.67
25	93.33 ±6.67	93.33 ±6.67	100.00 ±0.00	100.00 ±0.00
50	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00
		Thiobencarb		
0.1	20.00 ±0.00	26.67 ±6.67	33.33 ±6.67	40.00 ±0.00
1	26.67 ±6.67	33.33 ±6.67	40.00 ±11.55	66.67 ±6.67
2.5	33.33 ±6.67	46.67 ±6.67	46.67 ±6.67	66.67 ±6.67
5	40.00 ±0.00	53.33 ±6.67	60.00 ±0.00	73.33 ±6.67
10	46.67 ±13.33	53.33 ±17.64	73.33 ±6.67	93.33 ±6.67
25	53.33 ±6.67	73.33 ±6.67	100.00 ±0.00	100.00 ±0.00
50	86.67 ±13.33	93.33 ±6.67	100.00 ±0.00	100.00 ±0.00

Table 2. Toxicity indices and their parameters for fenitrothion and thiobencarb on fish by static bioassay technique

Pesticide	Time (day)	LC_{50} (mg L^{-1})^{a}	Confidence limits at 95%	Slope^{b}	$\chi^{2\ c}$	P	Relative toxicity^{d}
Fenitrothion	24	4.07	3.28-5.01	1.19 ±0.07	85.99	0.001	100
	48	2.76	2.15-3.52	1.03 ±0.01	72.20	0.001	85.82
	72	1.28	0.86-1.82	0.72 ±0.06	45.27	0.001	88.57
	96	0.20	0.04-0.22	0.57 ±0.01	13.36	0.010	100
Thiobencarb	24	5.63	4.23-7.48	0.77 ±0.04	61.92	0.001	72.17
	48	2.37	1.71-3.22	0.77 ±0.01	45.07	0.001	100
	72	1.13	0.80-1.54	0.86 ±0.01	58.31	0.016	100
	96	0.32	0.19-0.49	0.80 ±0.01	22.63	0.001	61.44

Note: a: Concentration causing 50% mortality of the fish. Results of LC_{50} are expressed by the mean of three replicates ± standard error (SE). b: Slope of the concentration-mortality regression line ± SE. c: Chi-square value. d: Relative toxicity = (LC_{50} level for the most effective/LC_{50} level for the other pesticide) × 100

Table 3. In-vivo AChE activity in the brain of the Nile tilapia exposed to ½ LC50 for 96 h of fenitrothion and thiobencarb individually

Parameters	Control	Fentrothion	Thiobencarb
Total AChE activity	5.61[a] ±0.03	4.92[b] ±0.03	1.13[c] ±0.02
Specific activity	0.83 ±0.01	0.68 ±0.01	0.22 ±0.01
Inhibition %	-	12.35 ±0.96	79.93 ±0.51

Note: Total activity (OD 412/min g tissue), Specific activity (OD 412/min mg protein). I%, Inhibition % ((activity of control - activity of treatment)/activity of control) * 100. The means that do not share a letter differ greatly. Data significantly different from the control at P < 0.05

Effects of tested pesticides on GST and SOD activities

Results showed a significant increase in liver GST activity of treated fish compared to the control (Table 4). On 96 h, it showed a significant increase of GST activity as Unit/g tissue (OD_{340}/min g tissue) from control (155.63 ± at 2.82 to 2107.45 ±20.86 for fenitrothion and 3475.67 ±25.24 for thiobencarb. GST activity in the liver, gills, and muscle of fish *Labeo rohita* was significantly increased by endosulfan and chlorpyrifos compared to the control (Naz et al. 2019). Our results also show induction in GST-specific activity at 96 h exposure; the specific activity of GST for fish exposed to thiobencarb and fenitrothion individually was more than 30 times and 10 times that of the control (Table 4). Therefore, it seems likely that GST-dependent detoxification of fenitrothion might have also contributed to low acute toxicity. The acute toxicity level and liver esterases' susceptibility to fenitrothion were inversely related (Solomon et al. 2000).

On the other hand, Superoxide Dismutase (SOD) plays a very important role in the process of scavenging reactive oxygen species (ROS) (Livingstone 2001). The results showed that the activity of SOD in Nile tilapia liver exposed to fenitrothion and thiobencarb (Table 4) significantly increased in the two individual exposure pesticides. Fenitrothion promoted SOD activity after a 96 h exposure period from 43.10 ±0.71 in the control to 135.00 ±2.18. Also, thiobencarb significantly increased the SOD activity to 106.80 ±2.89. In addition, the specific activity of SOD was 3.81 ±0.12 in the control.

In contrast, it was 4.50 ±0.16 and 7.51 ±0.39 in the treatment of fenitrothion and thiobencarb, respectively, was revealed a significant increase in SOD in the treatment of fenitrothion-exposed fish compared with the control; this induction may be attributed to the high production of superoxide anion radical after fenitrothion exposure (Zeid and Khalil 2014). SOD is one of the most important defense mechanisms against the toxic effects of oxygen metabolism. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, maintaining low steady-state concentrations of ROS and alleviating their toxic effects (Oruc et al. 2004). Furthermore, the dismutation of the superoxide anion radical is catalyzed by SOD to H_2O and H_2O_2, which is detoxified (Monteiro et al. 2006). Considering this, pro-oxidant conditions elicited by pesticides could trigger increases in the activity of this antioxidant enzyme as an adaptive response (Oruc and Usta 2007). In addition, it has been found that SOD as an antioxidant maintains the enzyme balance in cells and protects them from oxidative damage to the tissues (hepatopancreas, muscle) to fish after chronic exposure to the herbicide prometryne (Stará et al. 2014).

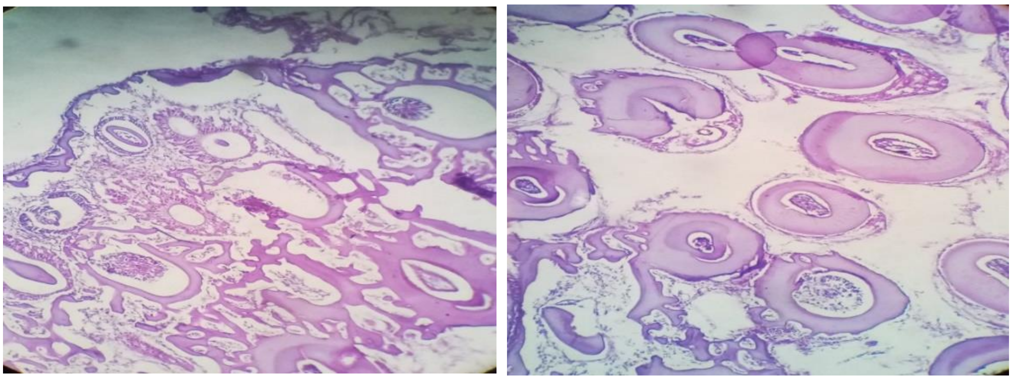
Side-effects of tested pesticides on histological characteristics

Water pollution induces pathological changes in fish. Therefore, histology is a valuable method for assessing pollution degree, particularly for sublethal and chronic impacts, as an indicator of pollutant exposure (Cengiz and Unlu 2006). Tissue damage brought about by water-borne pollutants can be easily observed because the fish gills come into immediate contact with the environment (Cengiz and Unlu 2006). No morphological changes were observed in fish exposed to 1.0 and 1.6 mg L^{-1} concentrations of fenitrothion and thiobencarb; the fish were unchanged, similar to the control group. However, respiratory distress was observed, one of the early symptoms of pesticide poisoning. Control individuals did not show any histological changes in the tissues examined by the light microscope.

Table 4. In-vivo GST and SOD activities in the liver of the Nile tilapia exposed to ½ LC_{50} for 96 h of fenitrothion and thiobencarb individually

Parameters	Control	Fentrothion	Thiobencarb
GST-Activity	155.63[c] ±2.82	2107.45[b] ±20.86	3475.67[a] ±25.24
GST-Specific activity	0.36 ±0.02	3.51 ±0.27	11.88 ±0.65
SOD-Activity	43.10[c] ±0.71	135.00[a] ±2.18	106.80[b] ±2.86
SOD-Specific activity	3.81 ±0.12	4.50 ±0.16	7.51 ±0.39

Note: GST-Activity U/g tissue (OD_{340}/min g tissue), GST-Specific activity (OD_{340}/min mg protein). SOD-Activity U/g tissue (OD_{560}/min g tissue), SOD-Specific activity (OD_{560}/min mg protein). Data significantly different from the control at P < 0.05



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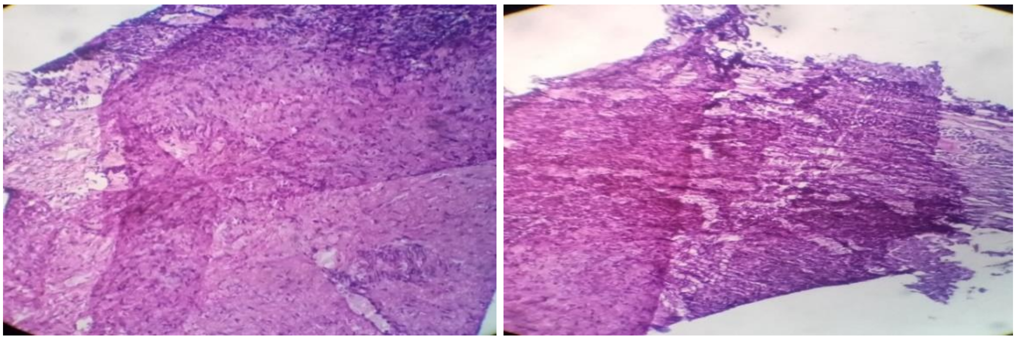

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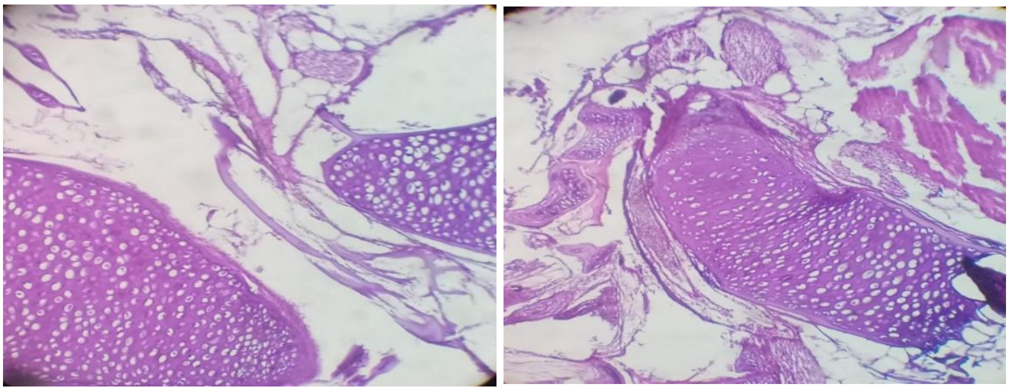


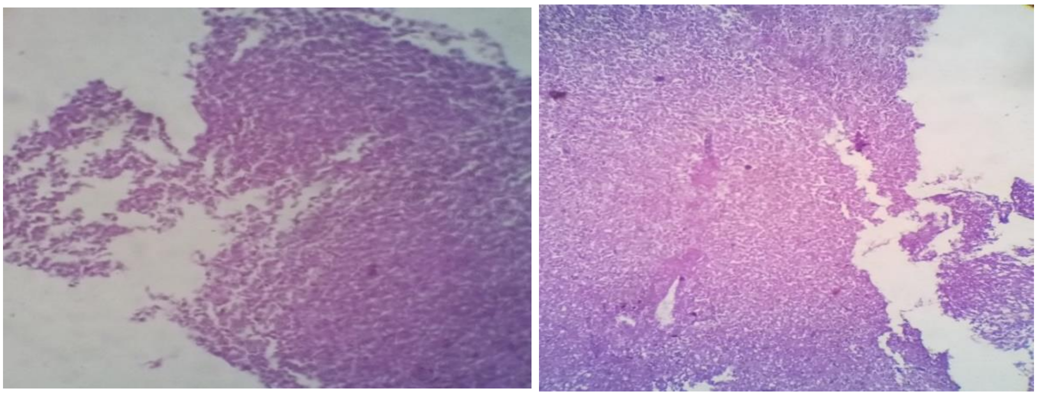
Thiobencarb

Figure 1. Histological appearance of the gills tissue of Nile tilapia exposed to ½ LC_{50} 96-h for fenitrothion (0.1 mg/L) and thiobencarb (0.16 mg/L) compared to control (H and E stained, X40, X100). The control showed bronchial arch, gills lamellae, epithelial layer, and normal cells. Fenitrothion treatment showed hyperplasia and necrosis. Thiobencarb treatment showed bronchial arch, gills lamellae, epithelial lifting, necrosis, and desquamations

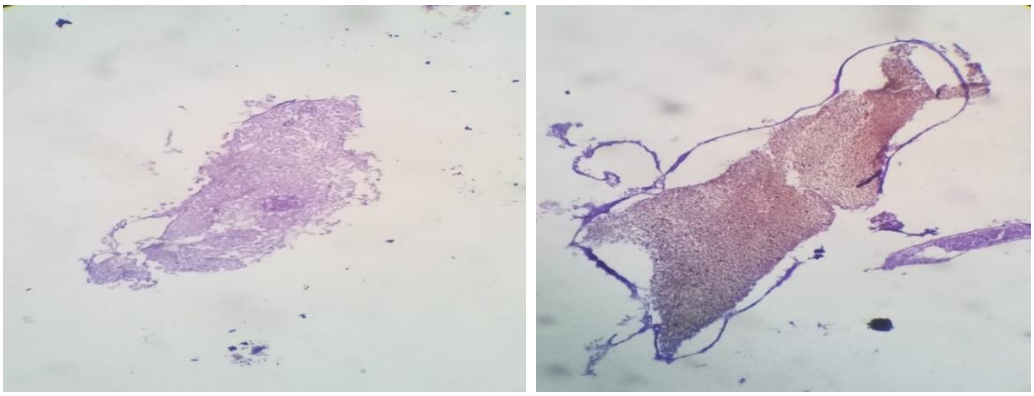
Histological examination of the gills of the control group showed no microscopical abnormalities. The gills were observed to be made up of double rows of filaments, which arise perpendicularly to the lamellae. A squamous epithelium lined the lamellae; below that epithelium were lamellar blood sinuses. Between the lamellae, the filament is lined by a thick stratified epithelium (Figure 1-the Control). The gills of fish subjected to fenitrothion; showed hyperplasia, necrosis, cell lysis, and hyperemia (Figure 1-Fenitrothion). The gills subjected to thiobencarb showed articulated the bronchial arch, which carries gills lamellae, epithelial lifting, necrosis in the fusion of some lamellae, and desquamations (Figure 1-Thiobencarb). Similar to our findings, thickening of the lamellar epithelium (fusion) was also reported after glyphosate exposure in Nile tilapia (Jiraungkoorskul et al. 2003). The first target organ of pollutants was the gills because of their large interface area between the external and internal fish environment, vital functions such as gas exchange and ion osmoregulation; particularly, the gills are sensitive to adverse environmental conditions (Abbas et al. 2007).

In addition, histological investigations of fish organs, especially the liver, repeatedly proved to be an extraordinarily sensitive tool to reveal both adaptive processes and detrimental effects in fish induced by organic pollutants. Since the fish liver is regarded as a major site of storage, biotransformation, and excretion of pesticides and since the gut is the first organ to come into contact with foodborne contaminants, histological changes of these organs were chosen as criteria for the sublethal action of two pesticides. The liver of the treated fish showed hepatic lesion, necrosis, and pycnotic nuclei for insecticide fenitrothion. Side effects on fish by herbicide thiobencarb were congestion, degeneration of many hepatocytes, nuclease, hepatic lesions, vacuole pyknotic nuclei, necrosis degeneration, and dilatation of sinusoids in Figure 2.

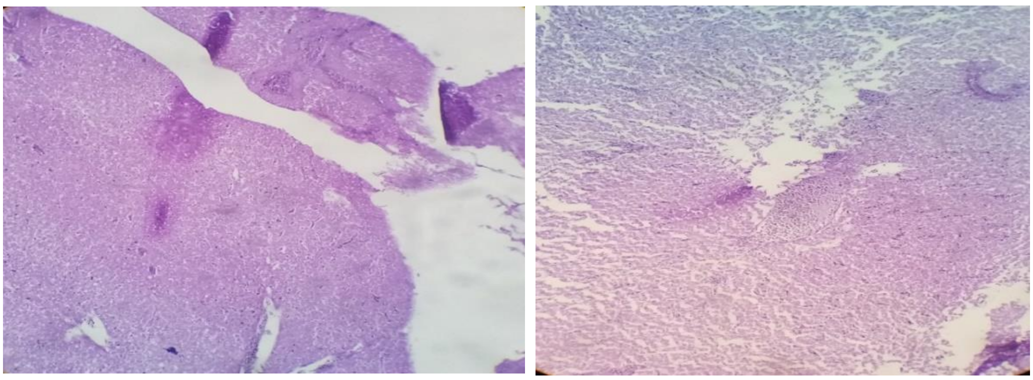
Previous studies investigating the side effects of pesticides have also shown similar alterations. For example, hypertrophy, vacuolization, and degeneration of hepatocytes, widespread nuclear pycnosis, focal necrosis have been reported in the *Puntius conchonius* exposed to dimethoate and carbaryl (Gill et al. 1988). The same alterations (hypertrophy, vacuolization, nuclear pycnosis, karyolysis, and fatty degeneration of hepatocytes) have also been recorded by Gill et al. (1990). In addition, they investigated the effects of aldicarb, phosphamidon, and endosulfan on the liver.



Control



Fenitrothion



Thiobencarb

Figure 2. Histological appearance of the liver tissue of Nile tilapia exposed to ½ LC_{50} 96-h for fenitrothion (0.1 mg/L) and thiobencarb (0.16 mg/L) compared to control (H and E stained, X40, X100). The control showed normal architecture of the liver tissue showing a continuous mass of polygonal cells called hepatocytes, distinct nuclei, sinusoid vessels, and blood vessels. Fenitrothion treatment showed hepatic lesion, necrosis, pycnotic of nuclei (left), and necrosis, pycnotic of nuclei (right). Thiobencarb treatment showed congestion, degeneration of many hepatocytes, nuclease (left), hepatic lesions, vacuole, pycnotic nuclei, necrosis degeneration, many hepatocytes degeneration, and dilatation of sinusoids (right)

In another research study, Cengiz et al. (2001) found hepatic lesions, including hypertrophy, degeneration, sinusoid enlargement, hemorrhage, pycnosis position of nuclei, vacuolization of the cell cytoplasm, infiltration of mononuclear lymphocyte. Also, Cengiz and Unlu (2006) found hepatic lesions in the liver tissues of fish exposed to deltamethrin pyrethroid, characterized by hypertrophy of hepatocytes, circulatory disturbances, and a significant increase of kupffer cells, focal necrosis, fatty degeneration, narrowing of sinusoids and nuclear pycnosis. In addition, roundup concentration corresponded to the 96-h LC50 value for adult fish tilapia, induced histopathological alterations in the liver, nuclear pyknosis, and vacuolation of hepatocytes (Jiraungkoorskul et al. 2002).

The fish kidney comprises three distinct systems: hematopoietic, endocrine, and excretory. Lesions developed in the kidney may involve one or all three tissue systems. Thus it is essential to examine the changes that may occur in different kidney cell types (Abbas et al. 2007). It can be seen in Figure 3 the effect of tested pesticides on the histological kidney examination. The effect of fenitrothion on the posterior kidney of Nile tilapia shows Malpighian corpuscles (bowman's capsules and glomerulus), epithelial hypertrophy of bowman capsules, elongation of bowman capsules and broader of B-C, necrosis, and atrophy in the glomerulus, necrosis in epithelial cells of B-C for fenitrothion.

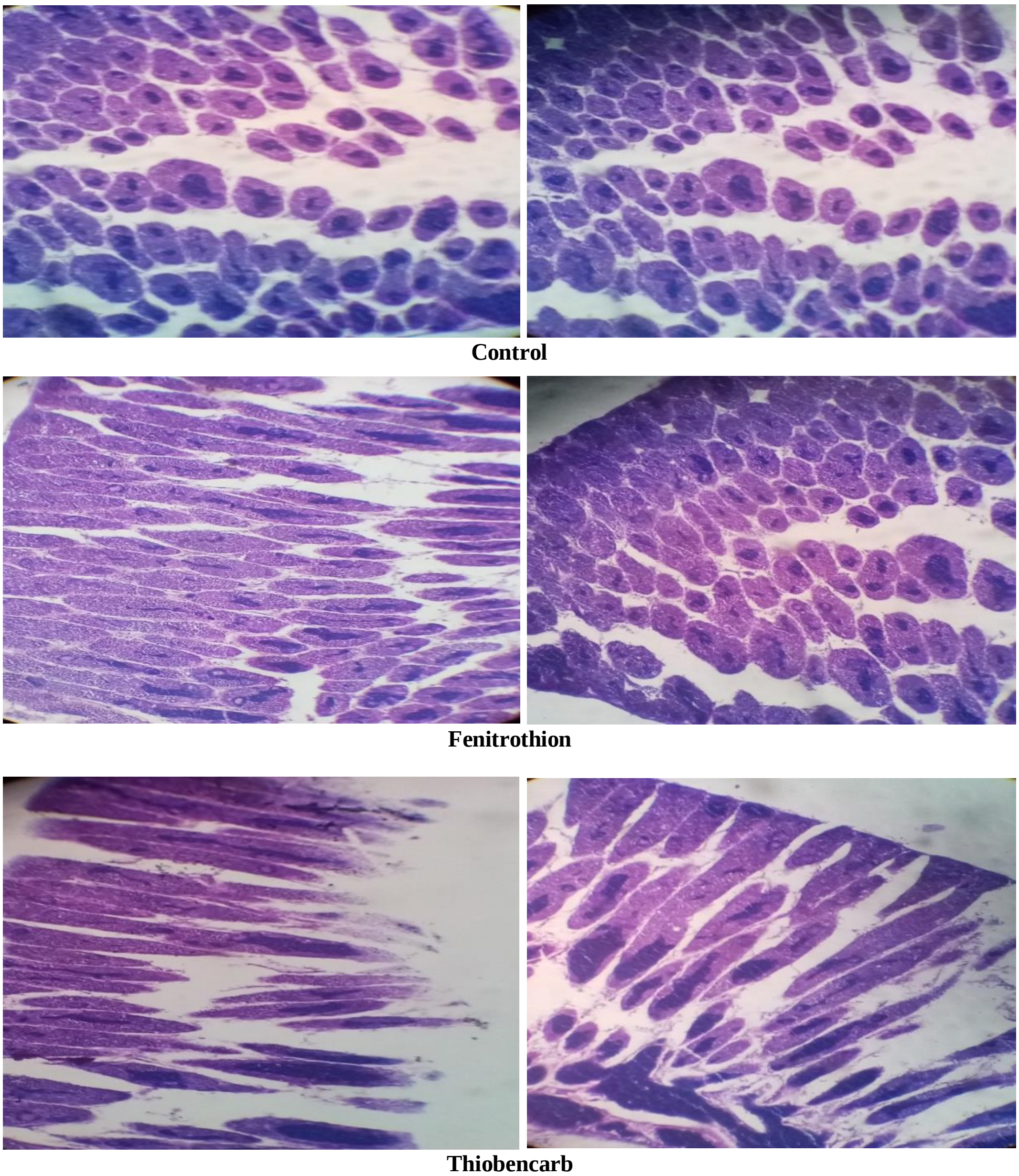


Figure 3. Histological appearance of the kidney tissue of Nile tilapia exposed to ½ LC_{50} 96-h for fenitrothion (0.1 mg/L) and thiobencarb (0.16 mg/L) compared to control (H and E stained, X40, X100). The control showed normal histological structure and normal cells. Fenitrothion treatment showed Malpighian corpuscles (bowman's capsules and glomerulus), epithelial hypertrophy of bowman capsules, elongation of bowman capsules and broader of B-C, necrosis, and atrophy in the glomerulus necrosis in epithelial cells of B-C. Thiobencarb treatment showed broader B-Cs, elongation of B-Cs, necrosis, and atrophy of glomeruli (blood capillaries)

Also, the effects of thiobencarb were epithelial hypertrophy of bowman capsules with broader B-Cs, elongation of B-Cs, necrosis, and atrophy of glomeruli (blood capillaries). In a similar; effect of the herbicide glyphosate on mosquito fish, Gambusia affinis; showed in kidney tissue, necrosis, and degeneration of epithelial cells of tubules, increasing the size of Bowman's capsule, congestion and atrophy, and disappearance of the glomerulus, separation of tubules, increasing of size and congestion of blood vessels, bleeding between tubules and bilirubin pigment diffusing around blood vessels (Al-Kawaz 2019). Histological changes in the kidney associated with pesticides in fish have been studied by many authors (Mostakim et al. 2015).

It was recorded that herbicide thiobencarb affects gills, kidneys, and liver more than insecticide fenitrothion. These results may be attributed to the lipophilic nature of the herbicides; this observation was supported by El-Sayd and Radwan (2004). Furthermore, the highest concentration of thiobencarb was in the liver, while the lowest was found in the fish brain (Abumourad et al. 2010). Our results are also compatible with the results obtained by Abbas et al. (2007) and Eissa et al. (2015), who studied the side effects of Nile tilapia *O. niloticus* exposed to thiobencarb. In addition, the results of fenitrothion are in agreement with the results of other studies that investigated the effects of sublethal fenitrothion on Nile tilapia; it showed histopathological alterations in the gills, liver, and kidney (Benli and Özkul 2010), the effect of organophosphorus insecticides malathion and fenitrothion on fish tilapia (Ohaida and Akrawee 2010), the effect of fipronil on Nile tilapia (El-Murr et al. 2015), and histological effects of deltamethrin on tissues of gills, liver, and kidney of Nile tilapia (Yildirim et al. 2006), mosquitofish (Cengiz and Unlu 2006). In general, our study shows a relation between the type of the pesticide and biochemical changes, as well as the severity of expression of the histological alteration in the Nile tilapia tissues. Due to humans' high consumption of Nile tilapia and considering the pesticides used in agriculture, the possible toxic effects of these pesticides in fish tissues for commercial interest have become a great concern. These side effects may be tissue alteration, detected by the histological examination. Overall, such experiments could be successfully used in research and applied in monitoring programs to monitor the side effects of pesticides on fish. Furthermore, this research supports earlier findings that highlight the value of scientific inquiry in the natural world (Zaki et al. 2018; Zaki et al. 2019; Saber et al. 2020; Saber et al. 2021).

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