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Biomarker Studies of Potential Hazards of chlorpyrifos to Nile Tilapia, *Oreochromis Niloticus*

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ABSTRACT

The introduction of organophosphorus insecticide into the aquatic ecosystem will adversely affect many non-target organisms including fish. Fish is an important organism of any aquatic system and so is one of the major sources of protein for human beings in Egypt. This study investigated the impact of acute and chronic exposure of chlorpyrifos on freshwater fish, Oreochromis niloticus. Results indicated that the exposure to 0.3and0.8 mg/l of chlorpyrifos for 24h induced significant decreasing in some investigated metabolites as total lipid, AChE, T3, Na^+ and Cl^-.While, cholesterol, cortisol, T4 andK^+ revealed marked elevation during the acute period when compared to control value whereas total protein was fluctuated during acute exposure. Furthermore, the chronic exposure of Tilapia to 0.5 mg/l of chlorpyrifos showed significant increasing in total lipid, cholesterol, total protein, cortisol, T4 and K^+. There was marked reduction in AChE, T3, Na^+ and Cl^-. The depuration process indicated that Nile Tilapia is trying to restore their metabolic parameters, thyroids and electrolyte levels but still did not reach the control one.

Key word: Chlorpyrifos, Insecticide, Toxicity, Biomarkers, *Oreochromis niloticus*

Introduction

Fish is an important part of any aquatic system as non-target organisms, and so is one of the major sources of protein for human beings in Egypt. Pollution of aquatic environment by pesticide, especially organophosphorus insecticide (OP) brings changes in the metabolic and enzyme activities and thereby changing the biochemical constituents of aquatic organisms.

Chlorpyrifos is an effective organophosphate (OP) insecticide used heavily throughout the world for agriculture and domestic purposes. LORSBAN is a trade name for agricultural-use products of chlorpyrifos (US EPA, 2009). The main target of OP pesticides is acetylcholinesterase (AChE), which hydrolyses acetylcholine (ACh) in cholinergic synapses and at neuromuscular junctions this results in the accumulation of ACh in the synapses which in turn induces hyperactivity in cholinergic pathways (Palma *et al.*, 2009). Inhibition of AChE leads to increased secretions, sensory and behavioral disturbances, respiratory depression, hepatic dysfunction, immunological abnormalities, embryo toxicity, genotoxicity changes and finally death. It is classified by WHO as toxicity Class II. Therefore, chlorpyrifos is very toxic to fish and also it can have significant effects on aquatic community structure(Zhong *et al.*, 2012). Chlorpyrifos' persistence, that when it has been used as a soil termiticide, summertime levels in indoor air did not decline over a period of 7 years after initial application (Zhong *et al.*, 2012). Degradation of chlorpyrifos is significantly slower in seawater (49.4 days at 10 ^oC) than it is in fresh water (18.7 days at 20 ^oC). Chlorpyrifos is an endocrine disruptor as it reduces serum levels of cortisol and thyroid hormone thyroxin (T4), induces alterations in thyroid and adrenal glands and differentially affects levels of thyroid-stimulating hormones in Nile tilapia fish (Bondarenko *et al*., 2004; Meriel, 2013 & Ghisari and Bonefeld, 2006).

Depletion in serum and total lipids may be a secondary effect to the hyperglycemia as a result of pesticide toxicity since, hyperglycemia enhance and activate the rate of lipids decomposition (Bentzene *et al*., 1996).Increasing of cholesterol may be due to deprivation of insulin levels which increasing the breakdown of lipids into free fatty acids and glycerol that migrate via blood to the liver and enhanced hepatic synthesis of cholesterol(Demael *et al*., 1990). Also, elevation of cholesterol may be related to levels of catecholamine that increases after pesticide exposure (Janz *et al*., 1991).

Kidney plays a vital role in the maintenance of anorganism's internal environment, being the key to theregulation of extracellular fluid volume andcomposition as well as acid–base balance. It is also atarget of toxic chemicals, which can disrupt its functions, and cause temporary or permanent derangement of homeostasis (Na^+ and K^+). Several authors recorded histopatho logical changes in the kidney of fresh water fish

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that exposed to organophosphate insecticides diazinonmonocrotophos, dimethoate and elsan, respectively (Banerjeeand Bhattacharya, 1996; Bhattacharya, 1993 & Miller, 2002).

Investigations into the effects of insecticides and metals on fish have a diagnostic significance in evaluating the adverse effects of these toxicants to human health. Most studies on the effects of environmental pollutants are confined to reporting biochemical and physiological changes after either insecticide treatment or metal exposure, and very little attention has been paid to compare the effects of these toxicants on biochemical parameters of fish, especially acute and chronic effect of chlorpyrifos on Nile Tilapia. Therefore, the primary aim of this study was to compare the inducing effects metabolites (total lipid, total protein, cholesterol, AChE and cortisol), thyroid function (ThyroxinT3 and TriiodothyronineT4) and ions (Na^+, Cl^- and K^+) levels of commercially valuable freshwater fish, *Nile Tilapia.*

Materials and Methods

Sample collection:

The present study was performed on fresh waterfish, Nile Tilapia, weighting 87.3±3g and 15.6±0.6cm in length. It was purchased from Islamic company-animal productions, El-Qanater, Qalubia Governorate, Egypt and transported to the laboratory in a plastic ice-box containing dechlorinated and oxygenated water to keep the fish population alive. Nile Tilapia were reared in aerated glass aquarium (50x50x50cm, 100L capacity) and acclimatized for two weeks under the condition of laboratory in dechlorinated tap water. The physico-Chemical characteristics of the test water used for this bioassay were measured according to American Public Health Association (APHA, 2005) and recorded as follow. pH: 7.5 ± 0.06, temperature: 27 ± 0.5 ºC, hardness 97.8± 2.7 mg/l as $CaCO_3$, Na^+: 25.72 mg/l, K^+: 6.49 ± 0.25 mg/l, Mg^{+2}: 13.5 ± 0.3 mg/l, F^-: 0.458 ± 0.01 mg/l, Cl^-: 36.88 ± 0.7 mg/l, NO_3^-:2.41± 0.16 mg/l, SO_4^{-2}:38.00 ± 0.8 mg/l, dissolved oxygen:6.4 ± 0.24 and alkalinity 2.43 ± 0.18 meq. Fish were fed once daily with a commercial diet (25% protein; fish meal, soya bean, bran, corn yellow and oil) at a rate of 2% of the body weight (Sprague, 1969).Aquarium were siphoning once daily before a feeding day to remove any faeces and unused food from the previous day. Following the two weeks of acclimation then fish were transferred randomly into the glass aquaria to test the pesticide.

Chemical Materials:

Chlorpyrifos is a chlorinated organophosphorus insecticide, acaricide, and nematicide widely used in agriculture and non-agricultural settings. Chlorpyrifos was produced by Misr for Agricultural Development Company, Cairo, Egypt. Chlorpyrifos is a white crystalline solid with a melting point of 41.5-42.5ºC. This compound is relatively stable to hydrolysis in neutral pH and acidic aqueous solutions. However, stability decreases with increasing pH. Chlorpyrifos has a half-life of 16 days at pH 9. Chlorpyrifos was persistent in the water columns of some aqueous systems with relatively long hydrological residence times.
Common name: Chlorpyrifos 500EC
IUPAC name: O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl3, 5, 6- Phosphorothioate
Commercial name: Lorsban, Dursban, Suscon Green, Empire, Equity
Molecular formula: $C_9H_{11}C_{l3}NO_3PS$
Molecular weight: 350.6

Metabolites and Degradates:

The major degradates of chlorpyrifos in the environment under most conditions is trichloro-2-pyridinol (TCP). TCP appears to be more persistent than chlorpyrifos (substantial amounts remain 365 days post-application) and it exhibits similar toxicity as chlorpyrifos to fish and invertebrate species. Chlorpyrifos may also oxidize to its active metabolite chlorpyrifos-oxon, a more toxic compound than chlorpyrifos (US EPA, 2009).

Chlorpyrifos and chlorpyrifos oxon, Figure 1, inhibit acetylcholinesterase by reacting with the active site to form a stable dialkylphosphorylated enzyme that cannot hydrolyze acetylcholine. Chlorpyrifos oxon, the active metabolic intermediate of chlorpyrifos, is much more potent than chlorpyrifos in inhibiting acetylcholinesterase (ATSDR, 1999 & FAO/WHO, 1999).

Determination of (LC_{50} (Median Lethal Concentration)) of chlorpyrifos for Nile Tilapia:

Nile Tilapia were starved 24hrs before beginning of the experiment. Nine concentrations of chlorpyrifos (0.3, 0.6, 0.9, 1.0, 1.2, 1.5, 1.8, 2.1 and 3.4mg/l) were prepared in nine equal sized test aquaria, in addition to one test aquarium for the control. Ten fish individuals were transferred for each individual chloropyrifos

containing aquaria. The fish were exposed to the prepared test solutions for 96 hours. The fish dead were watched and removed per day till the end of the fourth day. By the end of the fourth day, the mortality percentage was then calculated according to the profit analysis method (Finney, 1971). The experiment was repeated triplicate and the average of LC_{50} value for chlorpyrifos was recorded as 1.5mg/l, Figure 2. The mortality percentage of Nile Tilapia exposed to different concentrations of chlorpyrifos showed a linear equation (12.667X-22.222) with R^2= .9542.

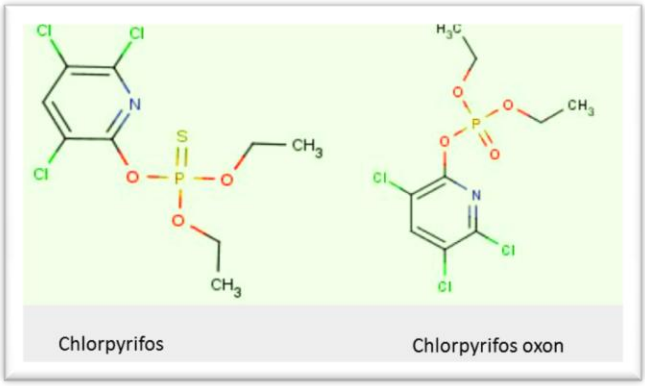

Fig. 1: Chemical structure of chlorpyrifos and their oxon metabolites.

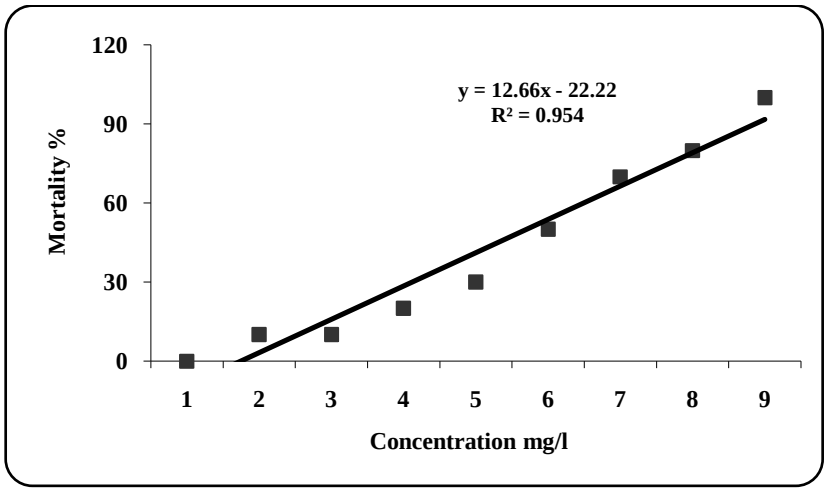

Fig. 2: Determination of $LC_{50}$of Nile Tilapia exposed to chlorpyrifos.

Acute exposure of chlorpyrifos for Nile Tilapia:

Fish were randomly divided three groups; each group consists of ten fish. The first group was exposed to control water (chlorpyrifos = <0.1µg/l), while the other two groups of fish were exposed to test water prepared with 0.3 and0.8mg/l chlorpyrifos for 24 hours.

Chronic exposure of chlorpyrifos for Nile Tilapia:

Fish were collected and divided randomly into eight groups. The first four groups represent the control and the other four groups were toxicant groups were exposed to one third LC_{50} (0.5mg/l) for 7, 14, 21 and 28 days. The number of fish for each group was ten fish.

Fish were sampled in all previous mentioned intervals for both acute and chronic periods. Blood was obtained directly from puncture of the heart of each fish, and collected in heparinized tube and centrifuged at 5000 rpm for 10 minutes to obtain plasma then, plasma were separated into Eppendorf tubes and stored at -40C^0 till all assay were carried out.

Stress related biomarkers:

Plasma total protein was determined colorimetrically based on Biuret method (Gornall, 1949) by using centronic Gmb H Kit-Germany. Plasma total cholesterol: Serum cholesterol was determined as CHOD-PAP method (enzymatic colorimetric test) according to Allain *et al.*, (1974).Plasma total lipid: Total lipid was

estimated colorimetrically by sulfo-phospho-vanillin mixture using Diamond Kit-Egypt according to Zollner and Kirsch(1962).Plasma cortisol concentration was determined according to the method of Foster and Dunn(1974) using RIA (radioimmunoassay) kit supplied by Diagnostic Products Corporation (D.P.C.), Los Anglos, USA. The activity of plasma cholinesterase was determined kinetically according to the method of Ellman *et al.,* (1961)using kit Boehringer Mannheim, Germany.

Thyroid function tests:

Thyroxine (T4) determination was carried out in plasma according to the method adapted by Britton *et al*., (1975)by Coat-A- count technique for (T4).Triiodothyronine (T3) assay using radioimmunoassay (RIA) kits of DPC, Los Anglos, U.S.A.

Homeostasis analysis:

Determination of sodium concentration: Colorimetric determination of sodium concentration in plasma was carried out according to method of Maruna *et al.,* (1958) using kits obtained from Bio-Analytics (U.S.A.). Determination of chloride concentration: Colorimetric determination of chloride in plasma was measured by the method adopted by Skeggs and Hochstrasset (1964),using kits purchased from Bio-Merieux, France. Determination of potassium concentration: Colorimetric determination of potassium concentration in plasma was carried out according to method of Sunderman and Sunderman (1958), using kits obtained from Bio-Analytics (U.S.A.).

Depuration:

Fish samples exposed to chronic chlorpyrifos for 28 day were subjected to clean water (free chlorpyrifos) for 28 days.

Statistical analysis:

The statistical analysis was estimated according to the method of Snedecor and Cochran(1980); ANOVA: One Way Complete Randomized (WRC) was applied to analyze the data using Costat statistical program, ver. 6.400, USA.

Results and Discussion

Insecticides can cause serious impairment to physiological and health status of fish. Therefore, biochemical tests are routine laboratory tests useful in recognizing acute or chronic toxicity of insecticides (Banaee *et al*., 2008&Al-Kahtani, 2011) and can be a practical tool to diagnose toxicity effects in target organs and to determine the physiological status in fish. Blood biochemistry test gives indicates what is happening in the body of fish exposed to insecticides. Chlorpyrifos forms the active ingredient in DURSBAN TM and LORSBAN TM insecticides (Kienle *et al*., 2009). Acute toxicity tests with lorsban on different fish species, at different life stages and under different environmental conditions were studies (Karen *et al.*, 1998 & Karen, *et al.*, 2001).

Metabolic parameters:

Table (1) showed the acute exposure of Nile Tilapia to 0.3 and 0.8 mg/L chlorpyrifos for 24 hrs caused highly significant ($P<0.05$) decrease of total lipid content in comparison with the control samples. The total lipid decreased from 12.1±0.22(in non-exposed samples) to 6.07±0.06; this refers to 50% of the total lipid was lost after 24hrs exposure to 0.8 mg/L chlorpyrifos,Figure3.

On the other hand, after chronic exposure (0.5 mg/l) the results showed marked increment in total lipid after 7, 14 and 21 days of exposure then the effect was reversed and deduced significant reduction ($P<0.05$) at 28 days,Table (1). The percentage of increase was 221.9, 83.6 and 19.8 after 7, 14 and 21 days, respectively, while the percentage was decrease to 16.5after 28 days, Figure 4. These indicate that chlorpyrifos inhibit total lipid at the end of experiment 28 days, Table (1) and Figure 4.

Such irregular changes in the total lipid content were in agree with (Kotb, 2000) during the exposure of Nile Tilapia to anilofos and cinmethylin for long term exposure. Also, El-Amin (2002)suggested the same result after both acute and chronic exposure of *Cyprinus carpio* to dithiopyridine. Sweilum (2006) recorded an irregular change in serum lipid after chronic exposure of Nile Tilapia to malathion and dimethoate.

The reduction in total lipid may be due to a direct effect of the utilization of body fat as energy supply to meet the increasing physiological demands. Fayed *et al*. (2001) attributed the serum total lipid reduction in fish

to increase secretion of catecholamines as a result of pollutant stress, which enhanced metabolic rate and in turn reduced metabolic reserves. Also, Bentzene *et al*. (2008) attributed the depletion in serum and total lipids may be a secondary effect to the hyperglycemia as a result of pesticide toxicity since, hyperglycemia enhance and activate the rate of lipids decomposition.

Table 1: Levels (mean±S.D) of some metabolic parameters in Nile Tilapia after acute and chronic exposure to chlorpyrifos.

Parameter	Total lipid (g/l)	Cholesterol (mg/l)	Total protein (g/l)	AChE (µg/l)
Acute exposure to 0.3 and 0.8 mg/l chlorpyrifos				
Control	12.1 ^{a} ±0.22	138.14 ^{c} ±0.52	2.60 ^{b} ±0.02	163.81 ^{a} ±2.10
0.3 mg/l	6.71^{b} ±0.10	195.44 ^{b} ±1.48	2.31 ^{c} ±0.03	128.29 ^{b} ±4.95
0.8 mg/l	6.07^{c} ±0.06	266.69 ^{a} ±2.58	2.76 ^{a} ±0.04	115.76 ^{c} ±3.08
Chronic exposure to 0.5 mg/l chlorpyrifos				
Control	12.10 ^{d} ±0.22	138.10^{e} ±0.52	2.60^{d} ±0.02	163.81^{a} ±2.10
7d.	28.95^{a} ±0.25	294.72^{a} ±0.79	5.53^{b} ±0.06	124.73^{b} ±1.32
14d.	22.21^{b} ±0.47	283.19^{b} ±1.67	6.902^{a} ±0.07	116.58^{c} ±2.54
21d.	14.50^{c} ±0.30	193.32^{c} ±1.30	3.59^{c} ±0.51	96.82^{d} ±1.88
28d.	10.10^{e} ±0.07	176.67^{d} ±2.85	2.29^{d} ±0.03	87.94^{e} ±1.22

Data are represented as mean ± S.D of 10 individuals, Variation between different single letters (a, b, c, d and e) in each component is significant, while variation between similar single letters in each component is not significantly different.

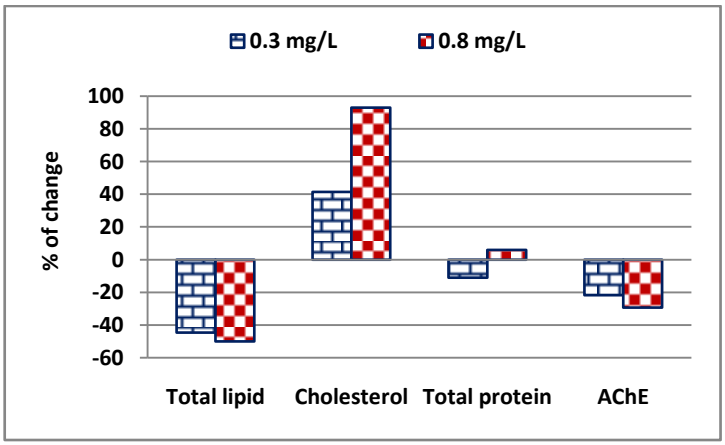

Fig. 3: Percentage of change of total lipid, cholesterol, total proteins and AChE after 24hexposure to 0.3 and 0.8 mg/l chlorpyrifoscompared to the control group.

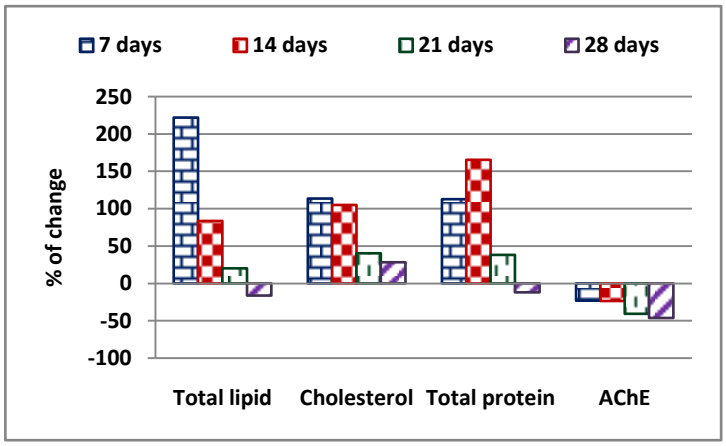

Fig. 4: Percentage of change of total lipid, cholesterol, total proteins and AChE after chronic exposure to 0.5 mg/l chlorpyrifos compared to the control group.

On the other hand, significant increase of serum total lipids after 14 and 21 days of chronic exposure may be a result of partial or full inactivation of insulin as responses to pesticide toxicity in blood and the pancreatic

tissues. It promotes fat storage by activation of lipoprotein lipase and supplying breakdown product of glucose, α- glycerophosphate which provides the glycerol portion of newly forming triglyceridesFayed *et al*. (2001).

Table (1) and Figure 3clarified that cholesterol concentration increased significantly ($p<0.05$) with percentage 41.5% and 93.1% after exposure to 0.3 and 0.8 mg/l of chlorpyrifos, respectively. Also, the chronic exposure showed significant ($p<0.05$) increase with respect to control sample with percentage 113.4%, 105%, 40% and 27.9% after 7, 14, 21 and 28 days, respectively as shown in Table (1) and Figure 4. So that, we concluded that cholesterol showed highly significant ($P<0.05$) increase during acute and chronic exposures. This results are in agreement with the findings of Öner *et al*. (2008) who found that cholesterol concentrations in the serum of metal-exposed Nile Tilapia generally increased when compared to that of the control value; due to liver and kidney failure causing the release of cholesterol into the blood. Also, Yousef *et al*. (2003) reported that changes in blood cholesterol levels are related to changes caused by pesticides in the permeability of hepatic cells and that accumulation of pesticides in the liver disrupt lipid metabolism and increase serum cholesterol levels.

Regarding protein level, Table (1), was fluctuated after acute and chronic exposure of Tilapia to chlorpyrifos since; protein content showed significant reducing ($P<0.05$)with respect to control sample after 0.3 mg/l for 24hrs and 28 days during chronic exposure to 0.5 mg/l to the same pesticide. Furthermore, the levels were reversed and revealed marked elevation($P<0.05$) with respect to control after acute exposure to 0.8 mg/l andafter 7, 14 and21 days during chronic exposure Table (1).The changes induced in the total protein may be due to pesticide effects on spleen, liver and anterior kidney since the decrease in serum total protein may be due to a direct enhancement use for muscle energy which led to degradation of proteins and tissue breakdown as well as due to inhibition of protein synthesis (Fouda, 2004). On the other hand, the reversed increase ($P<0.05$) of protein after chronic exposure may be due to increase in liver synthesis, disturbances in liver function or the immune response to pesticide which lead to an increase in the formation of protein-insecticide complex (El-Sayed, and Saad, 2008). This investigation in agreement with previous studies have shown that metals and pesticides can cause either increase or decrease in levels of serum protein, cortisol, glucose, cholesterol, ions, and in the activities of serum enzymes depending on the toxicant type, species of fish, water quality, and length of exposure(Vaglio, and Landriscina, 1999; Monteiro *et al*., 2005 &Jee *et al*., 2005).

Concerning to the acetylcholinesterase (AChE), the exposure of Tilapia to both concentrations of chlorpyrifos (0.3 and 0.8mg/l) for 24hrs during acute treatment and also during the chronic exposure (0.5 mg/l) to chlorpyrifos showed highly significant reduction ($P<0.05$) of AChE levels during the whole exposure periods with respect to control sample Table (1). The percentage of reduction of AChE was 21.7% and 29.3% after acute exposure to 0.3 and 0.8 mg/l, Fig. (3), respectively. While the percentage of reduction of AChE was 23.25%, 23.86%, 40.9% and 46.3% after 7, 14, 21 and 28 of chronic exposure to 0.5 mg/l chlorpyrifos, Figure4.

These results reveal an inhibition during the acute and chronic exposure. This is one of the main actions of chlorpyrifos in fish, as with other organophosphates, is to inhibit the AChE through phosphorylation. This enzyme breaks down the neurotransmitteracetylcholine which activates cholinergicneurons, the nerve cells that control signalsin the peripheral nervous system, brain, andspinal cord. If acetylcholine is not inactivated immediately after it has done its job, the neurons become over-stimulated leading to increased secretions, sensory and behavioral disturbances, in coordination, respiratory depression, and finally death(Colborn, 2006). Recovery depends on manufacturing moreAChE. This effect of chlorpyrifos is said to be caused by the metabolite, chlorpyrifos oxon, rather than the parent compound (Slotkin, 2004).

Thyroid function:

It is obvious from Table (2) that serum cortisol levels in treated Tilapia showed a significant increasing ($P<0.05$) during acute and chronic exposure to chlorpyrifos with respect to control. For cortisol, the percentage of increase was 90.5% and 157.0% after acute exposure to 0.3 and 0.8 mg/l chlorpyrifos, respectively, Figure 5.Chronic exposure, Figure 6, to 0.5 mg/lchlorpyrifos showed that the percentage of increase was 220.6%, 447.6%, 296.8% and 239.7% after 7, 14, 21 and 28 days, respectively. The elevation of cortisol as a result of organophosphorus exposuredue to serum cortisol levels are widely used as a primary response to stressors caused by organophosphorus. Stress is a response to every situation which threatening homeostasis and result in activation of hypothalamic pituitary-adrenal (HPA) axis and sympathetic autonomic nervous system which consequently lead to hyperglycemia (Mechanick, 2006). The hypothalamo-pituitary-interrenal (HPI) axis of fish is activated to produce cortisol and other corticosteroid hormones for the maintenance of disturbed homeostasis (Gagnon *et al*.,2006).

Table (2) showed that chlorpyrifos inhibited T3 after acute and chronic exposure. The reduction of T3 was significant ($p<0.05$) during acute and chronic treatment. The percentage of reduction was 9.6% and 48% after acute exposure to 0.3 and 0.8 mg/l, while after chronic exposure to 0.5 mg/l the percentage of reduction was 22.6, 33.7, 46.7 and 60.2% after 7, 14, 21 and 28 days, respectively, Figures 5 and 6. Also, Table (2) showed that T4 levels in treated Tilapia showed a significant increasing ($P<0.05$) during acute and chronic exposure to chlorpyrifos with respect to control. The percentage of increase T4, Figure 5, was 24.6% and 186%, while chronic exposure, Figure 6, to 0.5 mg/l chlorpyrifos showed percentage of increase was 147.5%, 82.5%, 40.7% and 14.1% for T4 after 7, 14, 21 and 28 days, respectively.These results reveal that chlorpyrifos decreased

serum T3 level but accelerated T4; these results are in the same line with Sinha *et al*., (1991)they found that endosulfan provoked a significant increase in T4 and a decrease in T3.

Table 2: Levels (mean±S.D) of thyroid function of Nile Tilapia after acute and chronic exposure to chlorpyrifos.

Parameter	Cortisol (µg/l)	T3 (µg/l)	T4 (ng/l)
Acute exposure to 0.3 and 0.8 mg/l chlorpyrifos			
Control	0.63^{c}±0.04	183.26^{a}±1.42	0.263^{c}±0.01
0.3mg/l	1.20^{b}±0.03	165.62^{b}±2.26	0.328^{b}±0.01
0.8mg/l	1.62^{a}±0.03	95.43^{c}±1.94	0.753^{a}±0.01
Chronic exposure to 0.5 mg/l chlorpyrifos			
Control	0.63^{e}±0.04	183.26^{a}±1.42	0.26^{e}±0.01
7d.	2.02^{d}±0.03	141.78^{b}±0.88	0.65^{a}±0.01
14d.	3.45^{a}±0.13	121.56^{c}±1.45	0.48^{b}±0.01
21d.	2.50^{b}±0.13	97.75^{d}±1.61	0.37^{c}±0.02
28d.	2.14^{c}±0.04	73.00^{e}±1.52	0.30^{d}±0.01

Data are represented as mean ± S.D of 10 individuals, Variation between different single letters (a, b, c, d and e) in each component is significant, while variation between similar single letters in each component is not significantly different.

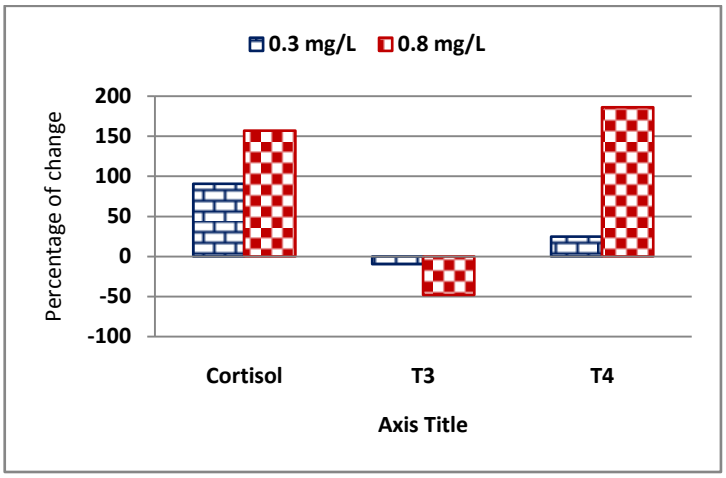

Fig. 5: Percentage of change of Cortisol, T3 and T4 after24h exposure to 0.3 and 0.8 mg/lchlorpyrifos compared to the control group.

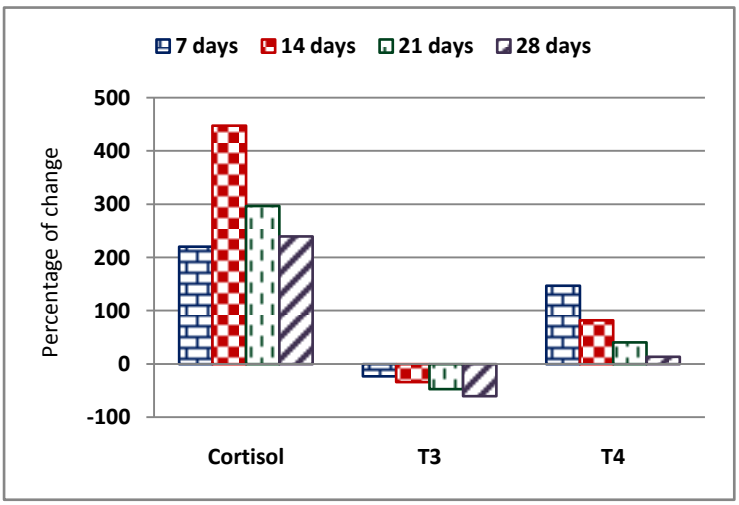

Fig. 6: Percentage of change of Cortisol, T3 and T4 after chronic exposure to 0.5 mg/l chlorpyrifos compared to control group.

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Electrolyte levels:

The variations osmotic and ionic regulation, and the base–acid balance in an attempt to resist and adapt to pesticide stress (Chyuan *et al.*, 2006). Exposure to acute (0.3 and 0.8 mg/L) and chronic (0.5 mg/L) concentrations of chloropyrifos resulted in significantly ($p<0.05$) reduction ofNa^+ and Cl^- levels, while K^+ levels showed significant increased ($p<0.05$), Table (3)&Figures 7 and 8. The percentage of decrease of Na^+ was 3.27% and 9.64% after exposure to 0.3 mg/L and 0.8 mg/L of chlorpyrifos, while after exposure to 0.5 mg/L of chloropyrifos; the percentage of decrease was 3.70%, 7.16%, 10.80% and 19.00% after 7, 14, 21 and 28 days. The percentage of decrease of Cl^- was 30.80% and 10.40% after exposure to 0.3 mg/L and 0.8 mg/L of chlorpyrifos, while after exposure to 0.5 mg/L of chloropyrifos; the percentage of decrease was 3.80%, 8.60%, 12.77% and 19.60% after 7, 14, 21 and 28 days.

Table 3: Levels of some electrolyte levels on Nile Tilapia after acute and chronic exposure to chlorpyrifos.

Parameter	Na^+ (meq/l)	K^+(meq/l)	Cl^-(meq/l)
Acute exposure to 0.3 and 0.8 mg/l chlorpyrifos			
Control	$128.01^{a}\pm1.61$	$5.16^{c}\pm0.04$	$147.91^{a}\pm0.64$
0.3mg/l	$123.83^{b}\pm1.01$	$5.54^{b}\pm0.11$	$132.56^{b}\pm0.72$
0.8mg/l	$115.67^{c}\pm0.76$	$7.55^{a}\pm0.08$	$102.37^{c}\pm0.29$
Chronic exposure to 0.5 mg/l chlorpyrifos			
Control	$128.01^{a}\pm1.61$	$5.16^{e}\pm0.04$	$147.91^{a}\pm0.64$
7d.	$123.22^{b}\pm0.16$	$6.74^{d}\pm0.11$	$142.30^{b}\pm0.76$
14d.	$118.83^{c}\pm0.32$	$6.96^{c}\pm0.02$	$135.16^{c}\pm0.50$
21d.	$114.14^{d}\pm0.33$	$7.13^{b}\pm0.02$	$129.02^{d}\pm0.88$
28d.	$103.70^{e}\pm0.41$	$7.71^{a}\pm0.17$	$118.85^{e}\pm0.73$

Data are represented as mean ± S.D of 10 individuals, Variation between different single letters (a, b, c, d and e) in each component is significant, while variation between similar single letters in each component is not significantly different.

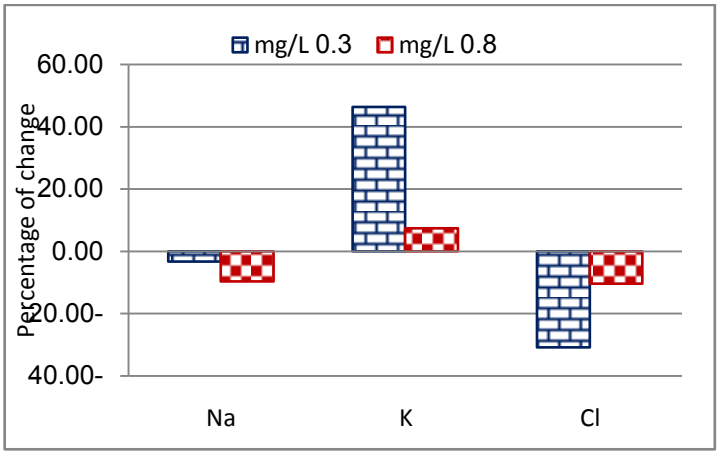

Fig. 7: Percentage of change of Na^+, K^+ and Cl^- after 24h exposure to 0.3 and 0.8 mg/l chlorpyrifos compared to the control group.

The reduction in Na^+ and Cl^- concentrations after pesticide exposure may be considered as a result of electrolyte balance disturbance due to the toxicity of pollutant. There must occur an impairment of sodium pumping system in gills and/or an increase of bronchial sodium permeability and subsequent net sodium loss is possible (Abo-Hegab *et al.*, 1990).

The percentage of increase of K^+ was 46.30% and 7.40% after exposure to 0.3 mg/L and 0.8 mg/L of chlorpyrifos, while after exposure to 0.5 mg/L of chloropyrifos; the percentage of increase was 30.6%, 34.9%, 38.2% and 49.4% after 7, 14, 21 and 28 days, Figure 8. The elevation of potassium level (hyperkalemia) may be due to redistribution of electrolytes between intercellular or extracellular compartments and /or impairment of renal function. Moreover might be due to impairment of their electrolyte influx at gills or shock of treated fish. The damage of erythrocyte membranes or cell membranes and subsequent leakage of K^+ into plasma or as compensatory mechanism for decreasing of Na^+(Walmsley, and White, 1994).

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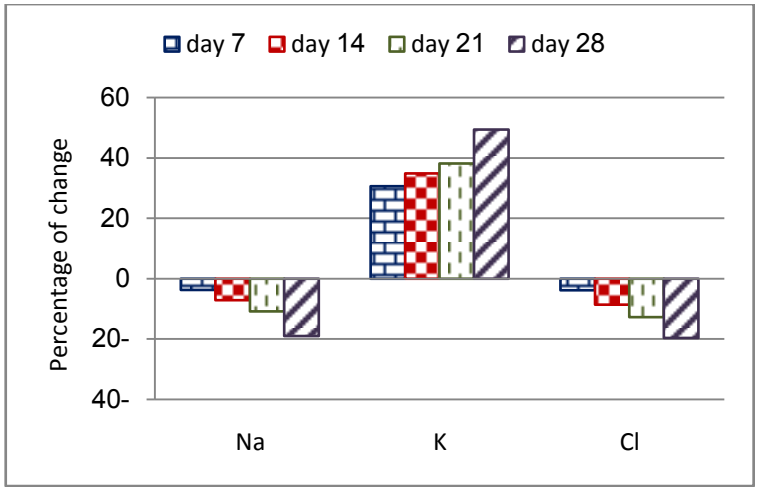

Fig 8: Percentage of change of Na^+, K^+ and Cl^- after chronic exposure to 0.5 mg/l chlorpyrifos compared to control group.

Depuration:

Fish samples treated with water containing chlorpyrifos for 28 days were transferred to clean water for depuration for 28 days. The chronic exposure to chlorpyrifos for 28 days showed a significant decreased ($p<0.05$) of total lipid, total protein AChE, T3, Na^+ and Cl^-, while showed a significant increased ($p<0.05$) for cholesterol, cortisol, T4 and K^+ with respect to control fish.

Table 4: Levels of metabolic parameters, thyroid function and some electrolyte levels on Nile Tilapia chronic exposure to chlorpyrifos for 28 day and depuration with free water.

Parameter	Total lipid (g/l)	Cholesterol (mg/l)	Total protein (g/l)	AChE (µg/l)	Cortisol (µg/l)
Control	12.10^{a}±0.22	138.10^{b} ±0.52	2.60^{a} ±0.02	163.81^{a} ±2.10	0.63^{c} ±0.04
Subjected to chlorpyrifos 28d.	10.10^{c} ±0.07	176.67^{a} ±2.85	2.29^{b} ±0.03	87.94^{b} ±1.22	2.14^{a} ±0.04
Depuration after 28d.	11.50^{b}±0.17	138.38^{b}±4.02	2.32^{b} ±0.30	162.27^{a}±1.90	0.83^{b}±0.09
Parameter	T3 (µg/l)	T4 (ng/l)	Na^+ (meq/l)	K^+(meq/l)	Cl^-(meq/l)
Control	183.26^{a} ±1.42	0.26^{b} ±0.01	128.01^{a} ±1.61	5.16^{b} ±0.04	147.91^{a} ±0.64
Subjected to chlorpyrifos 28d.	73.00^{c} ±1.52	0.30^{a} ±0.01	103.70^{c} ±0.41	7.71^{a} ±0.17	118.85^{b} ±0.73
Depuration after 28d.	150.16^{b}±2.94	0.26^{b}±0.01	124.01^{b}±2.13	5.07^{b}±0.07	147.10^{a}±1.07

The depuration process, Table (4) and Figure 9,using clean water resulted in total lipid, total protein (non-significant), AChE, T3, Na^+ and Cl^- showed a significantincreased ($p<0.05$) with respect to samples treated with chlorpyrifos for 28 days and but still did not reach the control value. The percentage of increase was 13.9%, 0.4%, 84.5%, 105.7%, 19.6%, and 23.8% respectively. While cholesterol, cortisol, T4 and K^+ after depuration showed significant decreased ($p<0.05$) with respect to treated fish (chlorpyrifos for 28 days) but still did not reach the control value. The percentage of decrease was 21.7%, 61.2%, 13.3% and 34.2%, respectively. This result indicated that the fish is trying to restore the natural environment but still did not reach their metabolic, thyroid and electrolyte level, indicating that chlorpyrifos is not rapidly excreted or metabolized.

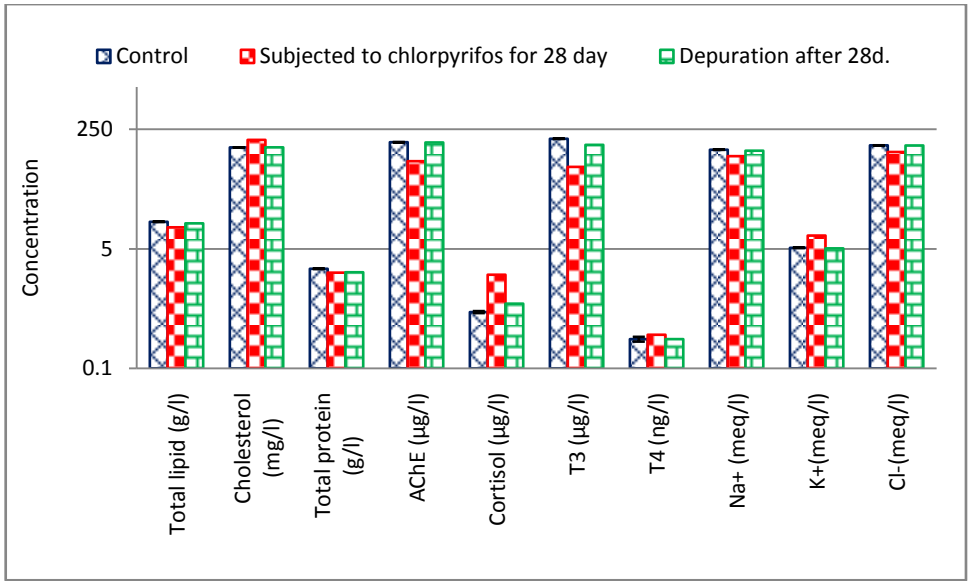

Fig. 9: Levels of metabolic parameters, thyroid functions and electrolyte level for control, subjected to chlorpyrifos and depuration after 28 days for Nile Tilapia.

Conclusion:

The relative stress, thyroid and homeostasis biomarkers exposed to chlorpyrifos can be used as rapid and sensitive biomonitoring indicators of hazardous effects on an aquatic non-target organism and ultimately whole of the ecosystem. So, human population is at high risk by consuming these toxicated fish.This study also may contribute to an understanding aboutthe stress of fish physiology exposed to chlorpyrifos in theaquatic environment of agricultural settings.

The LC_{50} value of chlorpyrifos were determined using Nile Tilapia,to be 1.5 mg/L for 96 hours reveals that chlorpyrifos was effect on aquatic organism and can be rated to be highly toxic to fish samples. The results clarified that the exposure to 0.3 and 0.8 mg/l of chlorpyrifos for 24h induced significant decreasing in some investigated metabolites as total lipid, AChE, T3, Na^+ and Cl^-. While, cholesterol, cortisol, T4 and K^+ revealed marked elevation during the acute period when compared to control value whereas total protein was fluctuated during acute exposure. The chronic exposure of Tilapia to 0.5 mg/l of chlorpyrifos showed significant increasing in total lipid, cholesterol, total protein, cortisol, T4 and K^+. There was marked reduction in AChE, T3, Na^+ and Cl^-. Depuration when placed in uncontaminated water wasn't also rapid, indicated that Tilapia tries to return to the control one, but still fish was affected.

Recommendations:

Blood biochemical parameters of fish can provide the information that facilitates the evaluation of potential toxic hazard resulting from exposure to different levels of toxic compounds depicts the effects of insecticides on the survival chance.

Applying the right amount of pesticide is especially important to achieve good control, avoid non-target toxicity, eliminate unnecessary expense, and comply with the legal requirements.

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