Toxicological Effects of Abamectin and Chlorpyrifos on Some Biochemical and Histopatological Parameters in Tilapia Nilotica Fish

Loutfy, N.*, M. S. Almatari*, L. A. Reda*, A. A. Dessouki and Y. M. Ahmed***
* Plant Protection Department, Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt.
** Pathology department , Faculty of veterinary medicine, Suez Canal University, 41522 Ismailia, Egypt.

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Abstract: Pesticides are generally toxic to many non-target invertebrate species such as fish. The present work was carried out to evaluate the toxicity effects of Chlorpyrifos and Abamectin against tilapia nilotica fish. Also, the effect of sublethal concentrations (LC_{10} and LC_{25}) of tested pesticide on the activities of some biochemical parameters of fish after exposure period of 21 days was investigated. The effects of tested pesticides on the histopathology of gills and liver of fish were studied as well. The 96 hr LC_{50} value of Chlorphos and Abamectin was found to be 0.05 and 0.02 mg/L respectively. The results showed that the serum AchE activity decreased in all treatments for both Abamectin and Chlorpyrifos pesticides. The activity of AST declined in fish exposed to Abamectin sublethal concentrations, whereas an increase in ALT activity was observed in case of Chlorpyrifos treatments (LC_{25} and LC_{10}). Serum glucose level was greatly increased with percentage of 29.14 % and 32.39% in case of treatment with LC_{10} and LC_{25} of Chlorpyrifos, and 128.45% and 215.37 % in case of treatment with the same of Abamectin respectively. While urea level decreased in all the treatments for tested sublethel concentrations pesticides. In case of Abamectin pesticide total lipid was elevated by 10.01% and 101.04% in comparison to control, and a reduction in the total lipid content was reported in Chlorpyrifos treated fish. Both of the tested pesticides had marked effects on the histology of the liver and gills of fishes.

Keywords: Pesticides toxicity, Chlorpyrifos; Abamectin; biochemical parameters; histopathology; *Tilapia niloticus.*

INTRODUCTION

Pesticides have tremendously benefited mankind by increasing food and fiber production, and by controlling the vectors of serious diseases and weeds of crops. Even so, the use of pesticides has caused considerable negative effects for the environment and human. In most studies the proportion of pesticides applied reaching the target pest has been found to be less than 0.3%, so 99.7% went 'somewhere else' in the environment (Pimentel, 1995). Therefore the use of pesticides may lead to the contamination of surface waters by drift, runoff, drainage and leaching. The pollution of water by pesticides is a topic of considerable environmental interest. However, these chemicals may reach other ecological compartments as lakes and rivers through rains and wind, affecting many other organisms away from the primary target. Pesticides can cause major damage to aquatic life, with fish kills as the most visible exponent. Determination of sub-lethal impacts on non-target organisms are among the environmental parameters for assessing consequences of pesticides activities (Lois Levitan 1997).

Abamectin, a mixture of 22, 23–dihydroavermectin B1a and B1b, is produced by an actionmycete, *Streptomyces avermectilis*. Abamectin is a highly effective and generally well tolerated microfilaricide that may soon become an essential component of many public health initiatives to interrupt transmission of lymphatic filarial infection in an effort to eliminate Lymphatic Filariasis globally (Brown *et al.,* 2000). Moreover, Ivermectin has a high acute and chronic toxicity for several taxonomic groups (Halley *et al.,* 1989). They have been shown to have effects on reproduction, biological function and survival of non-target aquatic and terrestrial organisms (Halley *et al.,* 1993; Moore *et al.,* 1993; Steel and Wardhaugh 2002; Wislocki *et al.,* 1989). It has been proved that Abamectin is highly toxic to fish and extremely toxic to aquatic invertebrates (US EPA, 1990). However, there are very few reports on the toxicity of Abamectin to aquatic animals, especially fish (US EPA, 1990).

Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyrinidyl)-phosphorothioate] is a broad-spectrum organophosphorous insecticide used in agriculture and more recently in residential areas (Barrett *et al.,* 2000). Evaluation of biochemical effects on contaminants in fish is a powerful tool in anticipating the adverse impacts of these material on the aquatic ecosystem. Despite the high toxicity of Abamectin to fish, no information is available regarding its chronic toxicity to fish.

Very few studies were found in the literature concerning Abamectin effects on tilapa fish and few data exists for Chlorpyrifos toxicity effects on Nile tilapia fish, an economically very important species around the world. Therefore, the aim of this study was to investigate the toxicity and latent effect of sublethel concentrations of the tested pesticides on certain's biochemical parameters in tilapia fish as non target organism.

MATERIALS AND METHODS

Fish:

Nile tilapia fish is the main cultured species in Egypt. 400 Nile tilapia fish (*Oreochromis niloticus*) with an average weight of 50±10 g and average length of 13-15 cm were used in this study. Tilapia fish were obtained from the Fish Research Center located at Suez Canal University, Ismailia, Egypt. Fish reared in glass aquaria each of 60 liters capacity provided with a good air supply and dechlorinated water and kept in natural

Corresponding author: maglamag@yahoo.com

illuminating system under normal prevailing photoperiod conditions. Water temperature was maintained at about 25±3 °C. Fish were fed on commercial pellets twice a day and acclimatized under laboratory conditions for 4 days before the experiment.

***Toxicity test*:**
Fish were exposed to series of concentrations of the tested pesticides Chlorpyrifos and Abamectin to determine medium lethal concentration (LC_{50}). Each concentration was replicated 2 times each contain 8 fish. Control treatment represented in similar aquaria that contain only clean dechlorinated tap water. Water was changed each 96 hr to avoid increase of ammonia level in water in order not to cause inhibition of the acetylcholinesterase (AchE) (Kumar, 1984). Dead fish was also removed from the aquaria as soon as confirmed to avoid changes in the water conditions. Mortality was recorded each 24 hr for 4 days (96 hr) continuously for both treatments and control. Results were then subjected to Ld-P line analysis or probit analysis (Finney, 1971).

***Subchronic toxicity test*:**
This study was designed to examine the effect of two sublethal concentrations of tested pesticides on Nile tilapia fish. Fish were divided to 2 groups, exposed to LC_{25} and LC_{10} for each pesticides for 21 days. Control was the fifth group, where fish was maintained in clean dechlorinated tap water. Two replicates were used per concentration of Chlorpyrifos and Abamectin, with 8 fish in each aquarium. At the end of the exposure interval, samples were taken for the biochemical assessments.

***Sampling*:**
At the end of subchronic exposure experiment, fish were taken from the aquaria and were stunned by gently striking their heads on the table. Blood samples were collected by direct puncture of the heart using needle without anticoagulant and collected in eppendorf tubes. Serum was obtained by centrifugation (6000 rpm for 20 min at 4 °C) and stored in the deep freeze for further studies. Fish were immediately dissected after blood was obtained, then gill and liver were removed and kept in 10% Formalin solution until histological examination was conducted on.

***Biochemical analysis*:**
All determinations were done by using commercial kits and U.S.A. Spectrophotometer, AchE was determined according to Ellman *et al.*, (1961).

Serum AST and ALT were measured according to the methods of (Murry, R. 1984). Glucose was determined according to the methods of (Young, DS. 1995 and Young, DS. 2001). Total lipid and urea level were measured using commercial kits according to the methods by (Zollner and Kirsch 1962).

***Histological Examination*:**
The influence of the tested pesticides on the histopathology of liver and gills was investigated. At the end of this experiment (21 days), specimens from liver and gills were collected and fixed in 10% neutral buffered formalin for histopathological examination. After fixation the specimens were dehydrated in graded alchol, embedded in paraffin. Five microns sections were obtained and stained with routine Hematoxylin and Eosin stain (H&E) as described by (Bancroft, and Stevens, 1990)

RESULTS AND DISCUSSION

Toxicity of tested pesticides

Results in Table (1) shows the values of $LC_{10, 25, 50, 75, 90}$ for Abamectin and Chlorpyrifos. The table also shows the line equation and the slops for both pesticides. Figure (1) represents the toxicity lines for both pesticides when tested on Nile tilapia for 96 hr. The values of LC_{50} observed in this study for Abamectin and Chlorpyrifos were 0.018 and 0.052 ppm respectively, and this values represent high toxicity to tilapia fish. It can be seen that Abamectin was more toxic than Chlorpyrifos to tilapia fish. This results are in harmony with many of the previous studies as can be seen in the following discussion.

No data was found dealing with toxicity of Abamectin to Tilapia fish. Therefore we used some other studies which report the toxicity of Abamectin to another species of fishes to give an overview about Abamectin toxicity to aquatic species in general. Wislocki *et al.* (1989) found that LC_{50} (96 hr) of Abamectin to different organism was 3.2 ppb, 9.6 ppb, 15 ppb, 24 ppb, 42 ppb, 1.6 ppb, 0.02 ppb, 340 ppb, 153 ppb, 3.2 ppb and 0.34 ppb to rainbow trout, bluegill sunfish, sheep head minnow, channel catfish, carp ,pink shrimp, mysid shrimp, eastern oysters, blue crab, *oncorhynehus mykissand Daphnia magna* respectively. Jencic *et al.* (2006) found that LC_{50} for of Abamectin to rainbow trout was 3.2 ppb, and the 58 hr LC_{50} was 1.5 µg/L. Tisler and Erzen (2006) reporter that 96 hr LC_{50} of Abamectin to Zebrafish was 30.3 µg/L (EC 10), LC_{50} was 55.1 µg/L (EC 50).

The present results are in harmony of those obtained by EXTOXNET (1990) reported that 96 hr LC_{50} for Chlorpyrifos was 0.009 mg/L in mature rainbow trout, 0.098 mg/L in lake trout, 0.806 mg/L in goldfish, 0.01 mg/L in bluegill, and 0.331 mg/L in fathead minnow. Grant and Briggs (1998) reported that the LC_{50} of Abmectin to *Carcinus maenas* was 957ppb. Also Chindah *et al.* (2004) found that LC_{50} of Chlorpyrifos on *Tilapia guineensis* was 0.002 mg/L. According to Chandrasekera and pathiratne (2006) LC_{50} of Chlorpyrifos for fry, fingerline and sub-adult for Nile tilapia were 0.53 (0.31-072), 0.75 (0.44-1.07), 3.86 (2.83-5.12) µg/L respectively.

Table (1): Toxicity of Abamectin and Chlorpyrifos (mg/l) to Nile tilapia fish after 96 hr.

Pesticides	LC_{10}	LC_{25}	LC_{50}	LC_{75}	LC_{90}	Line equation	Slope
Abamectin	0.008	0.012	0.018	0.027	0.038	Y= 11.8+ 3.9X	1.62±1.439
Chlorpyrifos	0.0081	0.02	0.052	0.137	0.33	Y = 7.1 + 1.6X	1.593 ±0.58

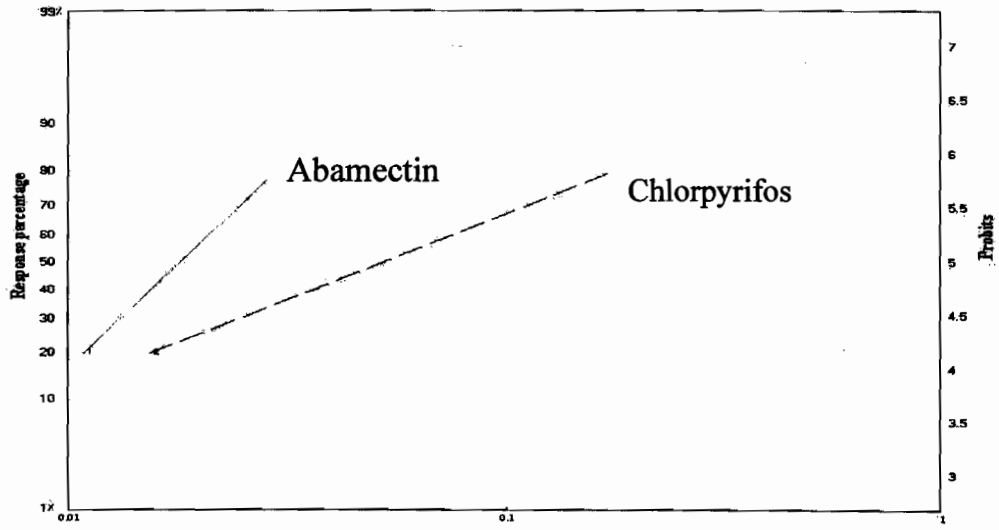

Figure (1): Toxicity lines for Abamectin and Chlorpyrifos against Nile tilapia fish after 96 hr.

Effect of tested pesticides on AchE, AST and ALT:

In view of the scarcity of data on Abamectin chronic toxicity to Nile Tilabia fish, subchronic effects of LC_{10} and LC_{25} of Abamectin and Chlorpyrifos as well were studied in this part after exposure period of 21 days. Table (2) shows the effect of tested pesticides on acetylcholinesterase (AchE), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes in serum of Nile tilapia fish.

AchE activity has measured as an important indicator especially for organophosphorus pesticides. As can be seen in table (2), the serum AchE activity decreased in all treatments for both Abamectin and Chlorpyrifos pesticides. The inhibition rates were 20% at LC_{10} level and 60% at LC_{25} level for Abamectin, while it were 20 and 80 % for Chlorpyrifos at the same level of concentrations. This results means that AchE activity is inhibited strongly by Chlorpyrifos than that by Abamectin and such inhibition was concentration-dependent and this result are in line with Chandrasekara and Pathiratne (2007) who reported that the inhibition of AChE activity in the fish brain tissue was dependent on the concentration of the pesticide. Acetylcholinesrerase is an enzyme that regulates cholinergic signaling by hydrolyzing the transmitter acetylcholinesterase at centeral and peripheral synapses in the vertebrate nervous system (Bayley *et al.* (1995). The inhibition of AChE enzyme leads to the accumulation of ACh at synaptic junction hence, the altered locomotor behavior of fish could be due to the accumulation of ACh, which interrupted coordination between the nervous and muscular junctions. Abamectin which has potent acaricidal, insecticidal and anthelmintic properties in animals, acts by stimulating the pre-synaptic release of the inhibitory neurotransmitter, ã-aminobutyric acid (GABA), binding to the post-synaptic receptors (i.e. GABA agonist) and thus disturbing Cl passage through the end plate resulting in the inhibitory post-synaptic potentials and eventual paralysis (Corbitt *et al.*, 1992; Martin *et al.*, 2002). However, the effects of Abamectin are not restricted to signal transmission disruption. In fish the avermectins can also pass the blood/ brain barrier and could cause toxic effects (Høy *et al.*1990)

The alteration in serum levels of (ALT) may be indicative of internal organs damage especially in liver (Kaneko *et al.*, 1997). Our results are in good harmony with those reported by Gamal et *al.*, (2008) who found that AchE activity decreased when exposed *Culex pipiens* and *Anopheles multicolor* to Bio-Insecticides (Spinotoram and Vertemic).

Also, Thangnipon *et al.*, (1995) reported a progressive decrease in brain AChE activity as the concentration of Monocrotophos increased in the Nile Tilapia fish after 96 hr exposure to acute levels. Similarly Chandrasekera and Pathiratne (2006) found an inhibition of brain AchE activity in juvenile tilapia exposed to Chlorpyrifos and Carbosulfan. In all groups of fish exposed to Chlorpyrifos, the AChE inhibition in the brain tissue was dependent on the concentration of the pesticide. Same trend of result reported by Rao *et al.*, (2008) for AChE activity of brain, gill and muscle of tilapia (*Oreochromis mossambicus*). The enzyme was inhibited by 67%, 77% and 73% respectively on day-30 of exposure to organophosphorus insecticide. Also Rao (2008) showed significant inhibition of AChE activity at 6 hr (>40%) and reached 90% at 24 hr when exposed tilapia fish to 24 hr LC_{50} (43.52 µg/ L) of Chlorpyrifos.

Also high inhibition levels of AChE activity observed in tilapia fish after exposure to Carbaryl and Chlorpyrifos insecticides (Liwag *et al.*, 2009).

Transaminases AST (GOT) and ALT (GPT) are liver-specific enzymes, which considered to be sensitive indicators of hepatocellular damage and within limits can provide a quantitative assessment of the degree of damage sustained by the liver (Peng *et al.*, 2007). Tilak *et al.*,(2005) reported a significant increase in (AST) and (ALT) enzyme activities exposed to sublethal concentrations of Chlorpyrifos an organophosphate pesticide in tissues of freshwater fish, *Catla catla, Labeo rohita* and *Cirrhinus mrigala.* Increasing or decreasing in AST and ALT activity levels are suggested to reflect tissue damage in liver and gill (Oluah 1999; Venkateswara 2006 b).

It has been mentioned that in many biological systems enzymes reaching the blood stream after cell necrosis of certain organs, may be used to indicate the tissue damage. Also Table (2) shows the activity of serum transaminases (AST and ALT) of Nile tilapia fish exposed to LC_{10} and LC_{25} of Abamectin and Chlorpyrifos for 21 days. The activity of AST in control fish was 78.9 ± 0.87 U/L. Exposure to the

LC_{10} and LC_{25} of Abamectin has caused an inhibition in the activity of AST by ratio of 15.15 and 32.6% respectively. Whereas exposure to the LC_{10} and LC_{25} of Chlorpyrifos has caused an elevation of 30.77 % and 44.55% respectively.

Marked elevation was noticed in the activity of ALT enzyme when fish exposed for sublethal concentrations of both pesticides. In case of Chlorpyrifos, the ALT activity increasing percentages were many times higher (194.11% and 327.52%) than the increasing percentage when fish exposed to sublethal concentration of Abamectin.

Elevation of AST, a cytosolic enzyme of the hepatocytes, reflects the increase of plasma membrane permeability resulting from the damage of hepatocytes and is used to detect liver damage. In case of ALT enzyme, our results were disagreed with Ewies *et al.* (1995) and Abd El-Wahab *et al.* (2002) who found that Abamectin caused a decrease in ALT activities in rats.

According to Walton and Cowey (1982), Pequin and Serfaty (1963) transamiases are responsible for detoxification processes, metabolism and biosynthesis of energetic macromolecules for different essential function and they are catalyze the intercessions of the amino acids. The enzymes AST and ALT are known to act as a link between carbohydrate and protein metabolism (Murugesan *et al.*, 1999). And the increase in the activities of these enzymes activities might be due to a decrease in metabolic activity sites and to tissue damage (Verma *et al.*, 1981 and Sharma, 1999).

From the previous results it could be concluded that the changes in transaminases activity may be due to the liver damage in tilapia fish when exposed to Chlorpyrifos and our histopathological results are confirming this openion.

Oure results concerning the effect of Abamectin on AST and ALT are disagree with Eissa and Zidan (2009) who reported an elevation in AST activity and a reduction in ALT when exposued Albino rats to Abamectin and *Bacillus thuringiensis*.

Klaassen and Eaton, (1991) and Tilak *et al.* (2005) reported a significant increase in AST and ALT enzyme activities exposed to sublethal concentrations of Chlorpyrifos in tissues of freshwater fish, *Catla catla, Labeo rohita* and *Cirrhinus mrigala.* Venkateswara (2006) also reported an increase in serum AST and ALT enzyme activities of the tilapia, *Oreochromis mossambicus*, after 30 days of exposure to sublethal dose of an organophosphorus insecticide.

Effect of tested pesticides on serum glucose, urea and total lipid content:

Changes in blood glucose level have been suggested as useful general indicator of environmental stress in fish (Nemcsock and Bores 1982). Table (3) shows the results obtained for serum glucose level after 21 days of exposure to sublethal concentrations of tested pesticides. The results revealed that the exposure for LC_{10} and LC_{25} concentrations of tested pesticides produced an elevation in serum glucose level, with percentage of to 29.14 % and 32.39% in case of Chlorpyrifos, and 128.45% and 215.37 % in case of Abamectin. The elevation of glucose level was many times higher in case of Abamectin, giving an impression that Abamectin is more toxic than Chlorpyrifos to tilapia fish and this result support the toxicity data (LC_{50s}) that reported in this study.

Generally the alterations in carbohydrate metabolism in fish under pesticides stress are depending on exposure time, class of pesticides and concentration used. Glucose increase is a general response of fish to acute pollutant effects, including organophosphates (Sancho *et al.*, 1997). According to Subramamian *et al.*, (1989) the increase in the blood glucose level after exposure to pesticides, may be due to decreased levels of insulin which intern may be due to inhibited synthesis and/or reduced release of insulin from the pancreatic β cells. Glycogenolysis in case of precenseal of pollutants may be the reason of increase in blood glucose. The source of such hyperglycemia seems to be liver and muscle glycogen, as well as amino acids through the activation of glueoneogenesis (Abo Hegab *et al.*, 1990).

The results seem to reconcile well with those reported by several authors like Ramesah and Saravanan (2008) who reported an increasing in plasma glucose level in fish treated with Chlorpyrifos. Adedeji (2010) who found that plasma glucose concentration in the experimental group was significantly higher than that of the control group in African catfish when exposed to Diazinon. And Bdint *et al.*, (1995) also found that the blood glucose levels in Carp were highest after the 6 hr treatment with organophosphorus Methidathion.

Table (2): Effect of Abamectin and Chlorpyrifos on serum AchE, AST and ALT activity after 21 days of exposure.

Treatment	AchE activity (mg protein/ min)		AST activity (U/L)		ALT activity (U/L)	
	Mean +S.D	% Change	Mean +S.D	% Change	Mean +S.D	% Change
Control	0.005 ± 0.0001	-	78.9 ± 0.87	-	8.32 ± 0.32	-
Abamectin (LC_{10})	0.004 ± 2.85	(-) 20	66.95 ± 9.45	(-) 15.15	10.64 ± 2.38	(+) 27.88
Abamectin (LC_{25})	0.002 ± 2.84	(-) 60	53.18 ± 7.64	(-) 32.60	11.23 ± 3.58	(+) 34.98
Chlorpyrifos (LC_{10})	0.004 ± 0.0008	(-) 20	103.18 ± 0.83	(+) 30.77	24.47 ± 2.04	(+) 194.11
Chlorpyrifos (LC_{25})	0.001 ± 0.0001	(-) 80	114.05 ± 2.55	(+) 44.55	35.57 ± 1.83	(+) 327.52

Percentage change = (Ttreatment – Control/ Control) X 100, (+): Increase (-): Decrease

In conterary Eissa and Zidan (2009) found that glucose level decreased in Albino rats when exposed to Abamectin and *Bacillus thuringiensis*.

Urea is the main waste product of protein breakdown. It formed in the liver from carbon dioxide and ammonia, passes into the extracellular fluid, and is excreted almost entirely by the kidneys. The measurement of urea is an important in diagnosing kidney damage. Table (3) represents the changes in serum urea level in Nile tilapia fish exposed to LC_{10} and LC_{25} of Abamectin and Chlorpyrifos. The results indicated that urea level decreased in all the treatments for tested pesticides.

When fish exposed to LC_{10} and LC_{25} of Abamectin, urea level deceased by 13.12% and 44.23% respectively in comparison to control group. While in case of Chlorpyrifos, urea level decreased by 31.83% and 70.21% when exposed to LC_{10} and LC_{25} respectively. The results are in agreements with Mona *et al.,* (2010) who observed a significant decrease in the urea level in cat fish when exposed to Malathion (4.5 mg/L) for 96 hr. Such effect may be resulting from kidney damage due to exposure to insecticides.

The increased level of urea in all treatments suggests the stepped up conversion of toxic amonia to less toxic urea thus it can be inferred that pesticides used in this study interacts with the biochemical sequences of nitrogen metabolism and the fish tries to adapt to the toxic stress condition by converting the toxic amonia to less toxic urea (Bahuguna and Bahuguna, 1987). Our results are similar to the results of Gaafar *et al.,* (2010), who reported a significant increase in urea level when Nile tilapia fish exposed to Edifenphos.

Table (3) shows the effect of tested pesticides on total lipid content. Results indicated that exposure to sublethal concentration of Abamectin for 21 days has caused an elevation of 10.01% and 101.04% in comparison to control for LC_{10} and LC_{25} respectively. In contrary, exposure to both sublethal concentrations of Chlorpyrifos has caused a reduction in the total lipid content. The decreasing percentage were (33.87%) and (59.28%) when the fish treated with LC_{10} and LC_{25} respectively. Also from the above result, it was observed that either the increasing (in case of Abamectin) or the decreasing (in case of Chlorpyrifos), the response was correlated to concentration. According to Ghosh and Chatterjee (1989) the observed reduction in total lipids content in serum upon exposure to Chlorpyrifos could be due to several mechanisms. These could include: 1) Formation of lipoproteins which are utilized for repair of damaged cell and tissue organelles. 2) Direct utilization by cells for energy requirements. 3) Increased lypolyses.

Our results for total lipid content are in good agreement with Vadhva and Hasan (1986) who found dose-related increase in the levels of total lipids in the fish exposed to Dichlorvos. While the results of Chlorpyrifos concerning to the decreasing of total lipid content are similar to the results of Sastry and Dasgupta (1991) when exposed *Channa panucatatus* to sublethel concentration (1ppm) of Monocrotophos. The total lipid content of the muscle decreased significantly after 30 and 60 days. Agrahari1 *et al.,* (2006) found that the total lipid content of all the selected tissues in a freshwater fish *Channa punctatus* (Bloch) increased significantly after 96 hr of exposure to Monocrotophos and to the sublethal concentrations for 30 days.

***Effect of tested pesticides on liver Histopathology*:**

In this part we investigated the effect of sublethal concentrations (LC_{10} and LC_{25}) of tested pesticides on the histological profile of livers and gills of Tilapia fish at the end of the exposure period (21 days). The results shows that in comparison to the control, the liver exposed to LC_{10} of Abamectin showed mild to moderate focal areas of vacuolar degeneration, congestion of blood vessels and minute focal areas of fatty change compared with control (Fig 2&3). While Fish liver exposed to LC_{25} of Abamectin showed diffuse vacuolar degeneration, moderate to severe areas of accumulated fat, in addition mild to moderate areas of necrosis (Fig. 4&5). Our results agreed with that of Vlasta *et al.,* (2006) who observed single cell necrosis in the liver of rainbow trout after exposure to Abamectin, and mononuclear foci were found in some fishes. In the study of Athanassopoulou et al. (2002) the main histopathological changes after administration of ivermectin to sea bass (*Dicentrarchus labrax*) were observed in gills, intestinal tissue and in smaller fish also in kidneys. The lesions that reported in this study were observed in rats treated with Abamectin (Eissa and Zidan 2009).

Livers treated with LC_{10} Chloropyrifos showed moderate diffuse vacuolar degeneration and congestion of blood vessels (Fig. 6). While fish treated with LC_{25} Chloropyrifos showed diffuse vacuolar degeneration, severe fat degeneration were also observed and mild to moderate areas of necrosis along with hyperplasia of hepatopancrease (Fig 7 and 8). These changes may be attributed to direct toxic effect of Chloropyrifos on hepatocytes since the hepatopancreas is the site of detoxification of all types of toxins and chemicals (Robert, 2001). These results came in agreement with (Tilak *et al.,* 2005) who observed same changes in liver parenchyma.

***Effect of tested pesticides on gill Histopathology*:**

Gills are important not only for gaseous exchange but also for osmoregulation and excretion of toxic waste products (Robert, 2001), thus any harm in the gills leads to impairment of such vital functions revealing respiratory distress, impaired osmoregulation and retention of toxic wastes .

Gills of control group showed normal histological appearance (Fig 9).While fish gills exposed to LC_{10} of Abamectin showed mild congestion of blood vessels, mild vacuolation and hyperplasia of the epithelial lining the secondary lamellae (Fig. 10). In fish gills exposed to LC_{25}, showed moderate congestion of blood vessels, mild vacuolation and hyperplasia of secondary lamellar epithelium along with leukocytic infiltration mainly lymphocytes (Figs.11).

Gills treated with LC_{10} of Chloropyrifos showed mild congestion of blood vessels, mild histological alterations represented by vacuolar degeneration, moderate mononuclear cell infiltration with loss of

secondary lamellae (fig 12). While fish treated with LC_{25} showed moderate to severe congestion of blood vessels, vacuolation of lamellar epithelial cells and massive leukocytic infiltration mainly lymphocytes along with proliferation and fussion of secondary lamellae (fig 13). These results came in agreement with those of (De Silva and Samayawardhena, 2002, Rao *et al.*, 2003 and Gaafar *et al.*, 2010). The epithelial proliferation of secondary gill lamellae resulted as a response of the malpighian cells to chemical irritation, as they migrate distally resulting in an accumulation of cells at the edge of the secondary lamella, progression of this migration leads to lamellar fusion (Robert, 2001). This may be attributed to that Chloropyrifos has a direct effect on gill filaments as cytotoxic and irritating substance which resulted in proliferation and fusion of secondary lamellae.

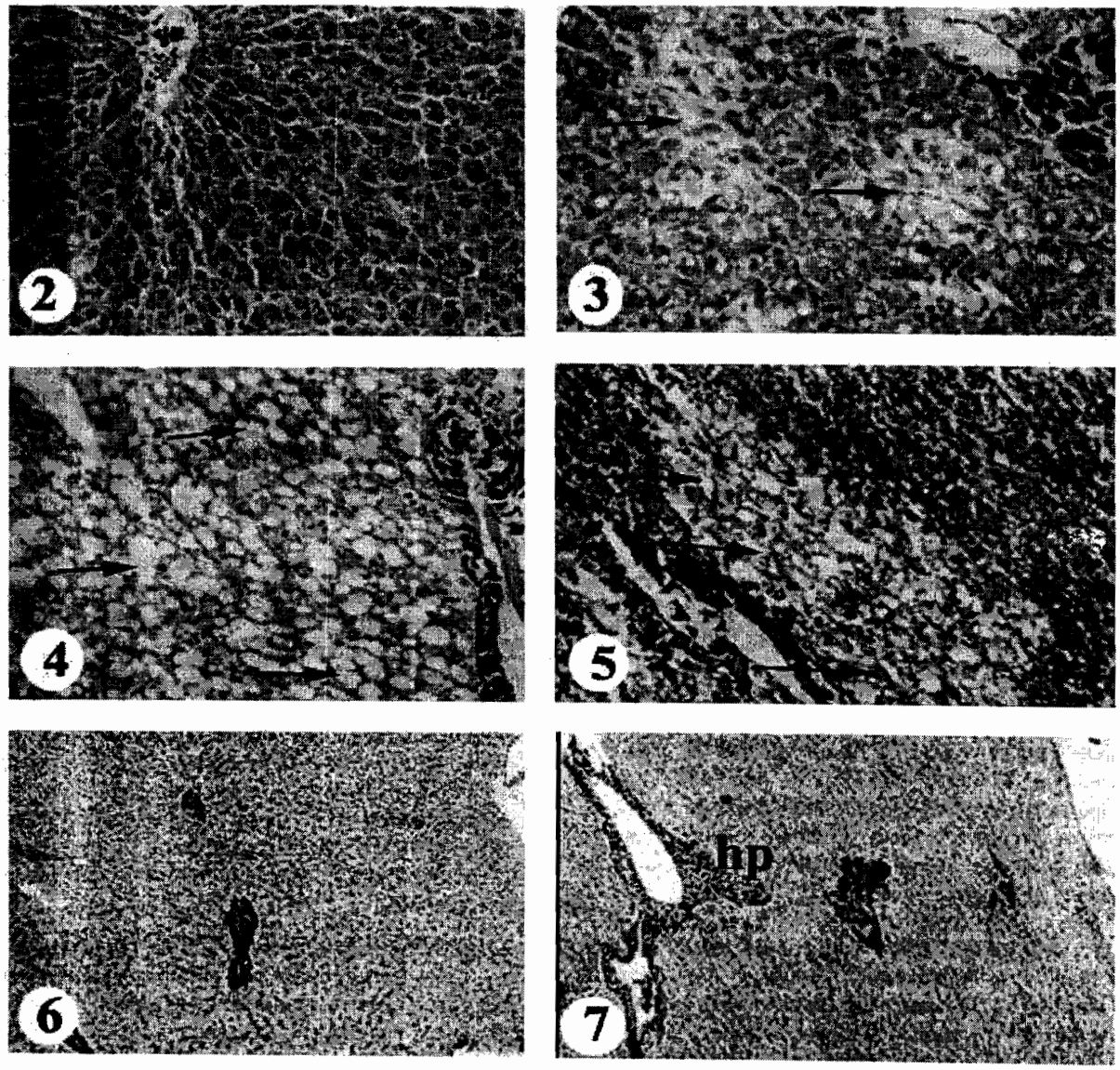

Fig (2): liver, control group showing normal histological appearance and normal hepatopancrease. H&E. X 400.
Fig (3): liver treated with LC_{10} of Abamactin showing focal areas of minute fatty degeneration of hepatocytes (arrows). H&E. X 400.
Fig (4): liver treated with LC_{25} of Abamactin showing fatty change of the hepatocytes (arrows). H&E. X 400
Fig (5): liver treated with LC_{25} of Abamactin showing focal area of hepatocytic necrosis (arrows) , atrophy and degeneration of hepatopancrease H&E. X 400
Fig (6): liver treated with LC_{10} of Chloropyrifos, showing diffuse mild vacuolar degeneration of hepatocytes. H&E. X 200.
Fig (7): Liver treated with LC_{25} Chloropyrifos , showing vacuolar degeneration of hepatocytes and hyperplasia of hepatopancreas (hp) . H&E. X 200.

Table (3): Effect of Abamectin and Chlorpyrifos on blood glucose, urea level and total lipid content after 21 days of exposure.

Treatment	Glucose level (mmol/L)		Urea level (mg/DL)		Total lipid (mg/dL)	
	Mean +S.D	% Change	Mean +S.D	% Change	Mean +S.D	% Change
Control	48.6 ± 1.12	-	19.67 ± 0.44	-	359.24 ± 0.34	-
Abamectin (LC_{10})	108.46 ± 1.08	(+) 128.45	17.09 ± 7.10	(-) 13.12	395.19 ± 51.17	(+) 10.01
Abamectin (LC_{25})	135.27 ± 3.26	(+) 215.37	10.97 ± 7.20	(-) 44.23	722.2 ± 32.35	(+)101.04
Chlorpyrifos (LC_{10})	62.76 ± 12.14	(+) 29.14	13.41 ± 1	(-) 31.83	237.06 ± 20.80	(-) 33.87
Chlorpyrifos (LC_{25})	64.34 ± 5.90	(+) 32.39	5.86 ± 1.75	(-) 70.21	146.3 ± 114.38	(-) 59.27

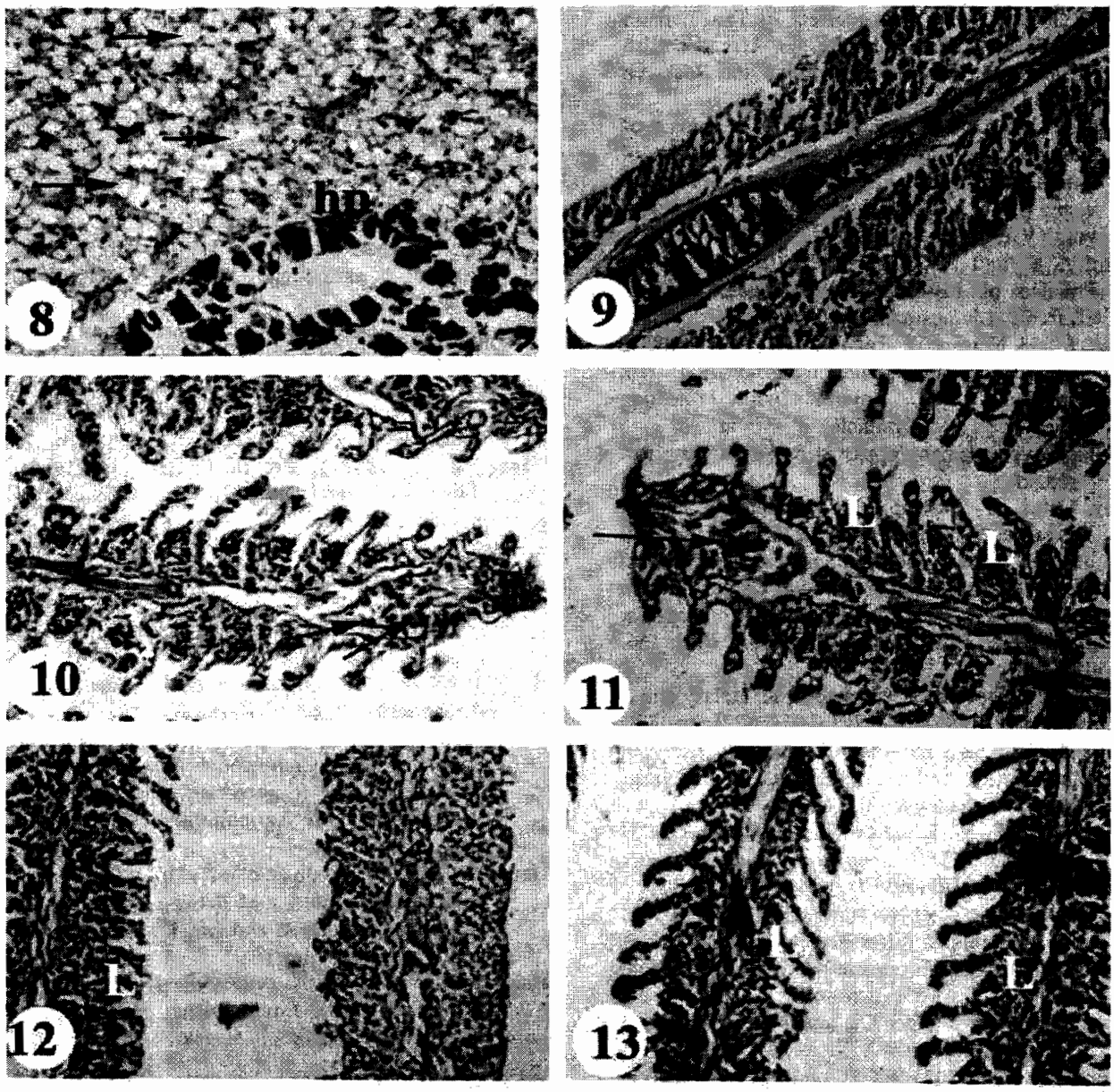

Fig (8): Liver treated with LC $_{25}$ of chloropyrifos, showing fat degeneration of hepatocytes and degeneration of hepatopancreas (hp). H&E. X 400.

Fig (9): gills, control group showing normal histological appearance. H&E. X 400

Fig (10): gills treated with LC $_{10}$ of Abamactin showing congestion of blood vessels (arrows), mild vacuolation of gill epithelium. H&E. X 400.

Fig (11): gills treated with LC $_{25}$ of Abamactin showing congestion (arrow) and mononuclear cell infiltrations (L). H&E. X 40

Fig (12): gills treated with LC $_{10}$ of Chloropyrifos showing congestion of blood and mononuclear cell infiltration (L) with loss of secondary lamellae. H&E. X 400

Fig (13): gills treated with LC $_{25}$ of Chloropyrifos showing congestion of blood vessels and massive lymphocytic infiltration (L). H&E. X 400.

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تأثير مبيد ألابامكتين ومبيد الكلوربيريفوس على بعض المقاييس البيوكيميائية والتشريحية في اسماك البلطي النيلي

نجلاء محمد لطفي*- محمد صالح محمد المطرى*- ليلى علي رضا- امينة الد سوقي*- يسرى محمد أحمد *

* قسم وقاية النبات- كلية الزراعة- جامعة قناة السويس- الاسماعيلية- مصر

** قسم الباثولوجى-كلية الطب البيطرى- جامعة قناة السويس- الاسماعيلية- مصر

تعتبرالمبيدات من اهم الملوثات للعديد من اللأنواع غير المستهدفة من اللافقاريات مثل الأسماك. تهدف الدراسة الحالية إلى تقديرالتأثيرات السامة لمبيدى الكلوربيريفوس والابامكتين ضد اسماك البلطي النيلي وكذلك دراسة تاثير التركيزات تحت المميتة (LC_{10} و LC_{25}) لهذة المبيدات المختبرة على بعض المقاييس البيوكيميائية بعد فترة من التعرض لمدة ٢١ يوما. كما تم ايضا دراسة تاثيرهذة التركيزات التحت مميتة للمبيدات المختبرة على هيستوباثولوجيا الخياشيم والكبد في الاسماك.

وجد ان قيم ال LC_{50} لمبيدي الكلوربيريفوس والابامكتين كانت ٠,٠٥ و ٠,٠٢ ملغم / لتر على التوالي. وأظهرت النتائج أن نشاط انزيم الاسيتايل كولين استريز انخفض في كل المعاملات للمبيدات المستخدمة.

ايضا انخفض نشاط انزيم ال AST في حالة الأسماك المعرضة للأبامكتين في حين لوحظ زيادة في نشاط هذا الانزيم (AST) عند المعاملة بمبيد الكلوربيريفوس. كما ارتفع نشاط نشاط انزيمALT عند المعاملة بكلا المبيدين. وزاد بشكل كبير مستوى السكر في السيرم حيث وصلت الزيادة الى ٢٩,١٤ ٪ و٢,٣٩ ٪ في حالة الكلوربيريفوس عند المعاملة ب LC_{10}، LC_{25}على التوالى و في حالة ألابامكتين ١٢٨,٤٥ ٪ و٢١٥,٣٧ ٪ عند المعاملة بنفس التركيزات التحت مميتة على التوالى . وقد انخفض مستوى اليوريا في جميع المعاملات من لكلا المبيدين المستخدمين. بالنسة لمحتوي الدهون الكلية فقد ارتفع فى حالة المعاملة بتركيزات LC_{10}، LC_{25} لمبيد الابامكتين بنسبة 10،٠١ ٪ و ١٠١,٠٤ ٪ على التوالى بالمقارنة مع الكنترول بينما انخفض محتوى الدهون الكلية عند معاملة الاسماك بنفس التركيزات لمبيد الكلوربيريفوس.اظهرت الدراسة ايضا مدى تاثر الانسجة لكلا من الخياشيم والكبد نتيجة للمعاملة بكلا المبيدين تاثرا سلبيا .