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PHYSICAL FINGER PRINT AND BIOCHEMICAL STUDIES ON THE INSECTICIDE "LUFENURON" AGAINST THE NILE TILAPIA, *Oreochromis niloticus* AND ITS RESIDUE LEVELS IN TISSUES

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ABSTRACT

Physical finger prints of Lufenuron (active ingredient and its formulation Match 5% EC) were studied. Lufenuron was a colorless crystals with melting point 170°C and 99% as purity. Also Match 50% EC comply the FAO/WHO specification of EC where it was slightly acidic with pH 4.88, flash point over 75°C, refractive index 1.489, density about 0.932 gm/cm^3 and it passed cold and hot cold test for 1 hr at 0°C and hot storage at 54°C for 3 days without any degradation or sedimentation. The physicochemical properties of spray solutions (soft, hard and tap water) showed that a good emulsion stability and decrease the surface tension of water from 72 dyne/cm to 37 dyne\cm which gave high degree of wettability on the treated surface.

The adverse effects due to exposure of the Nile tilapia, *Oreochromis niloticus* to sublethal concentration (1/10 LC_{50}) of Lufenuron insecticide (i.e. technical and formulation form) for 21 days, then allowed to a recovery for seven days, were investigated through some biochemical parameters and tissues residues determinations. The results showed a significant increase of serum glucose levels, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, in addition to creatinine levels, while the total serum protein and total serum lipid showed a significant decrease. The residual analysis exhibited that the concentration of Lufenuron residues in the liver were 0.506 and 1.505 ppm and in fish muscles were 0.281 and 0.417 ppm after 21 days of

treatments as active ingredient and formulation form, respectively. However higher concentration of residues were detected in the fish gills (3.139 and 1.726 ppm) after one day of treatment. Furthermore, the higher persistent rate of the tested insecticide "Lufenuron" in the water was detected after 24 hours of application and concentration losses of tested insecticide were increased by time.

Key words: Nile tilapia – Insecticide – Lufenuron - Tissues residue - Biochemical parameters - Physical properties - Physical print.

INTRODUCTION

The toxicity of pesticides refers to how poisonous it is. Some pesticides are extremely toxic, whereas others are relatively of low toxicity. So, the capacity of pesticides to harm fish and aquatic animals is largely a function of its toxicity, exposure time, concentration and persistence in the environment (Louis, 1996). The misuse of pesticides in agriculture can cause environmental problems especially to aquatic system by altering the quality of water and affecting the physiology and biochemistry of non-target organisms such as fish (Shakoori *et al.,* 1996). The basic mechanism of action for most pesticides is proved to be an alteration in the transfer of a signal along a nerve fiber and across the synapse from one nerve to another or from nerve to a muscle fiber. The signal is transferred across the synapse to the next nerve cell by the release of neurotransmitters such as acetylcholine (Ach) (Katherine, 2003). The biochemical processes represent the most sensitive and relatively early events of pollutant damage. Thus, it is important that pollutant effects be determined and interpreted in biochemical terms, to delineate mechanisms of pollutant action, and possibly ways to mitigate adverse effects (Begum, 2004).

The objectives of the present work were to study the physical finger print of the Lufenuron and its spray solutions (soft, hard and tap water) and evaluate some physiological, biochemical responses and also the residues in some organs of the Nile tilapia, *Oreochromis niloticus,* in relation to 1/10 LC_{50} (0.006 ppm and 0.013 ppm) sublethal exposure to the insecticide Lufenuron as an active ingredient and formulation form, respectively.

MATERIALS AND METHODS

Lunfenuron is an insecticide of benzyle urea family and it was manufactured by Syngenta and its formulation type is Emulsifiable Concentrate (Match 5% EC). It is insect growth regulator for controlling Lepidoptera and Coleoptra larvae on cotton with 40 ml/100 Liter of water.

1. The physical properties and physical print:

The physical properties of the Lufenuron 5% EC and their spray solutions were carried out according to (FAO and WHO specifications 2006) and (CIPAC Methods, Volume F 1995), Flash point (MT 12), Acidity/or alkalinity (MT 31), pH (MT 75), Density and Specific Gravity (MT 3), Foam Test (MT 47), Emulsion stability (MT 36.1) Conductivity (MT32) and storage test at 0°C and 54°C (MT 39 and 46.3.3).

Also viscosity, Surface Tension and Refractive Index were carried out according to American Society of Testing and Materials (ASTM) 1989, 2001 and 2005.

2- Determination of 96-h LC_{50}:

The 96-hour medium lethal concentration (LC_{50}) was determined according to the method of Behreus and Karbeur (1953). 66 fish were distributed in eleven aquaria with 100 liter capacity (six fish for each aquarium). The aquaria were supplied with dechlorinated tap water and kept at constant aeration, temperature, pH and feeding. Ten concentrations of active ingredient and formulation form of Lufenuron were distributed in the aquaria 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1 and 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.11, 0.12, 0.13 and 0.14 mg/l, respectively. The aquarium number 11 was kept as a control. All fish were observed for 96 hours to record the number of dead and active fish in each aquarium. The 96-hr LC_{50} was calculated according to the following equation:

$$LC_{50} = \text{highest dose} - \sum ab/n$$

Where:

a: is a constant factor between two successive concentrations.

b: is the mean of dead fish in two successive groups.

n: is the number of fish in each group (6 fish).

Toxicity tests showed that 96-hr LC_{50} values of the active ingredient and formulation form of the Lufrnuron for the Nile tilapia

Oreochromis niloticus were 0.063 and 0.126 ppm of the active ingredient and formulation form, respectively.

3- Animals and experimental design:

Fish *Oreochromis niloticus* weight 85.8±2.8 g and length 13.5:1:1.5 cm. were obtained from the fish farm of the Central Laboratory for Aquaculture Research (CLAR) Abbasa, Sharkia Governorate, Egypt. They were acclimatized on the laboratory conditions for two weeks and fed with commercial balance diet once/ day (2% of body weight). The physicochemical characteristics of the tap water were: pH 7.28±0.153, temperature 25±1°C, total hardness 229.56±3.39 mg/l and alkalinity 3.0l±0.4 mg/l as $CaC0_3$, dissolved oxygen 7.24±0.04 mg/l. The insecticide Lufenuron was supplied by Syngenta Agro. Egypt for agricultural pesticides. Fish were exposed to 0.006 ppm of active ingredient and 0.013 ppm of formulation form of Lufenuron.

Fish were divided into three groups. The first group (35 fish) was kept in fresh untreated water and used as a control. The second and third groups (35 fish each) were exposed to sublethal concentrations of 0.006 and 0.013 ppm of the active ingredient and formulation of Lufenuron, respectively. A sample of six fish from each group was removed successively after 1, 3, 5, 7, 14 and 21 days of exposure and after recovery period of seven days.

4-Blood sampling and biochemical analysis:

Blood samples were collected from caudal vein. Sera were taken in dry clean vials and immediately kept in deep freezer at -20°c for later biochemical analysis.

The serum glucose concentration was determined by using the method of Trinder, (1969). The total serum protein was determined according to the method of Henry (1964). The total serum lipid concentration was determined calorimetrically by sulphophospho-vanillin reaction according to Schmit (1964). The serum creatinine was measured by kinetic method described by Henry (1974). The serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities were determined calorimetrically according to, Reitman and Frankel (1957).

4-1: Fish tissue and water sampling:

Fish samples (0.5-30 g) were taken to determine the residues concentration for tested pesticide in gills, liver and muscles that were immediately removed from the sacrificed fish in each treatment.

Water samples (100ml) were taken to evaluate the persistence of tested pesticide in water.

5-Residues analysis

Determination of pesticide residues in water and fish tissues.

5-1-Extraction and clean up of tested pesticide:

Extraction and clean up of Lufenuron residues from water and fish tissues were carried out according to pesticide analytical manual (Tamlin, 1997).

5-2- Determination of Lufenuron residues by HPLC:

The obtained samples were cleaned up and then dissolved in 1 ml methanol, HPLC grade and determined using HPLC instrument with the following condition: a) DV detector, b) C18 column, c) mobile phase was 90% methanol 10% acetonitryl, d) flow rate was 1 ml/min and detection line was 0.005 µg/kg. Duplicate injection (2 µl) of calibration solution and each sample was injected and integrated areas for each peak were recorded and standard peak under ideal condition for Lufenuron.

6- Statistical analysis:

Student's (t) test was used to analyze the Statistical significance between the control and pesticide - treated fish (Gad and Weil, 1989).

RESULTS AND DISCUSSION

The physical properties (finger print) of Lufenuron active ingredient and its formulation (Match 5% EC) were measured and illustrated in table (1). The Lufenuron active ingredient was 99% purity, melting point 170°C and acidic in nature. The WHO, FAO and CIPAC described the specification which the Emulsifiable Concentrates must be comply with. Its flash point should not be lower than 22.8°C, no solid or oily separation when it cold storage at 0°C for 1 hr, its acidity or alkalinity being not more than 0.1-0.3%. Also the EC should be chemically and physically stable after storage at 54°C for 3 days FAO and WHO specifications 1970, 1983.

The sample was clear, transparent, pale yellow in color, has characteristic odor and free from suspended matter and sediments which complying with the FAO and WHO specifications 2006. It was slightly acidic by 0.0098% as H_2SO_4 where its pH value was 4.88 and 1.489 was its refractive index at 25°C. The density and specific gravity were 0.931 and 0.932 gm/cm^3, respectively. The rotational

Table (1): Physical properties (Finger Print) of Lufenuron (Match) 5% EC.

Test	Instruments - Reference	Results
Flash point	*Fisher Tag - CIPAC (MT 12)*	Over 75°C
Acidity	*Titration - CIPAC (MT 31)*	9.8×10^{-3} as H_2SO_4
pH	*pH meter - CIPAC (MT 75)*	4.88
Storage test	*Cold storage at 0 °C – CIPAC (MT 39)*	No sedimentation
	Heat storage at 54 °C – CIPAC (MT 46.1.3)	No sedimentation
Viscosity	*Brookfield Viscometer - ASTM (D 2196-05)*	2.834 cp
Surface tension	*Surface Tensiometer - ASTM (D 1331-89)*	32 dyne/cm
Refractive Index	*Refractometer - ASTM (D 1218–02)*	1.489
Density	*Pyknometer - CIPAC (MT 3)*	0.931 gm/cm^3
Specific gravity	*Hydrometer - CIPAC (MT 3)*	0.932 gm/cm^3

Table (2): Physical properties of Lufenuron (Match) 5% EC spray solutions.

Test	Instruments - Reference	Result		
		Soft water	Hard water	Tape water
Foaming	*Manual - CIPAC (MT 47)*	0	0	0
Emulsion stability	*Manual - CIPAC (MT 36.1)*	Stable	Stable	Stable
pH	*pH meter - CIPAC (MT 75)*	5.22	5.26	7.49
Viscosity	*Brookfield Viscometer - ASTM (D 2196-05)*	2.272 cp.	2.284 cp.	2.28 cp.
Surface tension	*Surface Tensiometer - ASTM (D 1331-89)*	31.25 dyne/cm	36.5 dyne/cm	33.75 dyne/cm
Conductivity	*Conductivity and Salinity meter CIPAC (MT 32)*	123.9 µs	482 µs	260 µs
Salinity		0.1 ‰	0.2 ‰	0.1 ‰
TDS		60 mg/L	223 mg/L	120 mg/L
Density	*Pyknometer - CIPAC (MT 3.1)*	0.9915 gm/cm^3	0.9906 gm/cm^3	0.9921 gm/cm^3
Specific gravity	*Hydrometer - CIPAC (MT 3.2)*	0.9924 gm/cm^3	0.993 gm/cm^3	0.967 gm/cm^3

viscosity of sample was 2.834 cp and the surface tension was 32 dyne/cm where its flash point was over 75°C. At cold storage test at 0°C for 1 hr, no crystals or sedimentation appeared where for heat storage at 54°C for 3 days, it was chemically and physically stable .

The physical properties of Lufenuron (Match 5% EC) spray solutions table (2), were measured at concentration 5 ml/100 ml waters (soft, tap and hard). The spray solutions appeared no foams and they are stable in emulsion stability test without any oily or creamy separation. The conductivity, salinity and TDS of spray solution were increased generally from soft to tape till hard water. The conductivities were 123.9, 260 and 482 µs, the salinities were 0.1, 0.1 and 0.2‰ and the TDS's were 60, 120 and 223 mg/l, respectively for soft, tap and hard water. The soft water spray solution was more acidic than hard water where their pH values were 5.22 and 5.26 while the tap water spray solution was slightly alkaline and its pH value was 7.49. The densities of the soft, hard and tap water spray solutions were 0.995, 0.9906 and 0.992 gm/cm^3, respectively where the specific gravities were also 0.9924, 0.9930 and 0.9670 gm/cm^3, respectively. The surface tension of spray solutions was changed according to the type of water. They were 36.5, 31.3 and 33.75 dyne/cm for hard, soft and tap water, respectively. The formulation EC decrease the surface tension of water alone from 73 dyne/cm to obtained results which indicated that the high degree of wettability of spray solutions on the treated surfaces. The rotational viscosity was not affected by changing the type of water where it was in the range 2.272–2.282 cp.

Toxicity tests of the present study indicated that the 96-hr LC_{50} values were 0.063 and 0.126 ppm for the active ingredient and formulation form of the insecticide Lufenuron respectively, in *Oreochromis niloticus*. Tomlin (1997) reported that 96-hr LC50 for rainbow trout is > 73, carp > 63, bluegill sunfish> 29 and catfish> 45 mg/l for this insecticide. Therefore the technical Lufenuron was more toxic than the formulation of Lufenuron (5% EC). This may attributed to hypoexic conditions was occurred where fish try to meet these conditions by increasing its ventilation rate. Thereby, drawing more amount of pesticides containing water through the gills. This implies that more toxicant is being brought into contact with gill lamella which causes more and more damage to gill epithelia. This results in a steer decline in oxygen absorption capacity of the gills. This suggestion confirmed by increasing accumulation of technical

Lufenuron residues in the gills rather than the gills of fish expulsed to formulation of Lufenuron (5% EC) as shown in our results.

The serum glucose concentration of the control tilapia (Table 3) was within the same range for other fishes (Marie *et al.,* 1990). The results revealed hyperglycemia in the tilapia exposed to 0.006 & 0.013 ppm sublethal concentrations of active ingredient and formulation of Lufenuron, respectively. The source of such hyperglycemia seems to be the liver glycogenolysis, resulting from the increased plasma catecholamine and corticosteroid hormones according to Mazeaud *et al.* (1977) and Pickering (1981), as well as amino acids through the activation of gluconeogenesis process (Abo-He gab and Hanke, 1984). Consequently, changes are produced in the glucose level of blood serum. Moreover, according to Gupta (1974) the hyperglycemia induced by malathion might be explained by the inhibition of cholinesterase at neuron effectors sites in the adrenal medulla, leading to hypersecretion of adrenaline which stimulates the breakdown of glycogen to glucose. Ghazaly (1995) observed a significant increase in serum glucose of *Clarias garieplenus* following exposure to sublethal concentrations of lindane, malathion and sevin. Marie *et al.* (1998) reported an increase in serum glucose level to the common carp, *Cyprinus carpio,* after 21 days of. exposure of an organophosphorus insecticide "profenfos".

The total serum protein of the Nile tilapia, *Oreochromis niloticus* exposed to either active ingredient and formulation form concentrations of Lufenuron was significantly decreased (Table 3), after 1,3,7,14 & 21 days of exposure. The decrease in total protein level may be due to augmented proteolysis. The reduction in total protein may be related to the action of chemicals on nucleic acid. A chemical could affect the protein content of a tissue by inhibiting RNA synthesis, by inhibiting protein synthesis due to anaerobiosis resulting from Lufenuron toxicity (Davi, 1981 and Seshagiri Rao *et al.,* 1983). The minimum values were recorded on the 5th day after Lufenuron application. These results are in accordance with Sharma (1999) in freshwater catfish, *Clarias batrachus* exposed to sublethal concentrations of carbaryl, a carbamate pesticide for 15 days and Tilak *et al.* (2005) in the freshwater fishes, *Carla cattle, Label rosita* and *Cyprinus regale* exposed to sublethal concentrations of an organophosphate pesticide, chlorpyrifos. Lipids are important metabolites for the locomotory and reproductive activities of fish. The

Table (3): Changes of serum total proteins, total lipids and glucose levels of the tilapia nilotica (*Oreochromis niloticus*) exposed to 0.006 and 0.013 ppm of (active ingredient and formulation form) of Lufenuron, respectively. (mean±S.E.)

Time	T. Protein g/dl			T. lipid g/l			Glucose mg/dl		
	Control	0.006 ppm	0.013 ppm	Control	0.006 ppm	0.013 ppm	Control	0.006 ppm	0.013 ppm
1st day	5.5605 ±0.2537 A	2.2850 ±0.2478 B	2.2261 ±0.0865 B	4.554 ±0.1216 B	1..9100 ±0.1954 B	3.7687 ±0.5361 A	25.057 ±0.8559 A	27.275 ±0.5574 A	25.917 ±1.2381 A
3rd day	5.4715 ±0.2213 A	1.3890 ±0.0762 C	2.4692 ±0.0821 B	4.3258 ±0.2590 A	1.8980 ±0.0570 B	2.0868 ±0.0638 B	24.947 ±0.4236 B	29.767 ±1.0415 A	3.100 ±1.4018 A
5th day	5.3301 ±0.3137 A	1.0609 ±0.0947 B	1.2660 ±0.1583 B	4.9014 ±0.1187 A	0.6276 ±0.0206 C	1.1185 ±0.0067 B	25.125 ±0.7230 B	35.054 ±2.2713 A	40.15 ±1.2350 A
7th day	5.2615 ±0.4513 A	2.2643 ±0.0193 B	2.0334 ±0.0015 B	4.1902 ±0.2064 A	2.5752 ±0.2861 B	2.1089 ±0.2317 B	23.998 ±0.3256 B	26.485 ±0.1302 B	39.690 ±2.2040 A
14th day	4.7613 ±0.2752 A	1.9089 ±0.2725 B	1.8609 ±0.1875 B	4.4368 ±0.3014 A	1.8000 ±0.3703 B	2.6054 ±0.3333 B	24.879 ±0.2581 B	29.191 ±1.4073 A	28.016 ±0.2751 AB
21st day	7.5510 ±0.1241 A	1.3217 ±0.0112 C	2.0427 ±0.1360 B	3.9910 ±0.0994 A	1.3750 ±0.0448 B	2.8400 ±0.5426 B	25.062 ±0.5426 B	26.806 ±1.2586 AB	31.346 ±1.8427 A
28th day (recovery)	4.9812 ±0.2581 A	3.1801 ±0.2871 B	3.1307 ±0.0976 B	4.1937 ±0.3641 A	2.9455 ±0.3647 B	3.0243 ±0.0604 B	25.1830 ±0.5454 A	24.6180 ±0.9370 A	26.1160 ±1.4650 A

- Mean with the same letter for each parameter are not significantly different

Table (4): Changes of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities and creatinine levels of the tilapia nilotica (*Oreochromis niloticus*) exposed to 0.006 and 0.013 ppm of active ingredient and formulation form of Lufenuron, respectively. (mean±S.E.)

Time	AST u/l			ALT u/l			Creatinine mg/dl		
	Control	0.006 ppm	0.013 ppm	Control	0.006 ppm	0.013 ppm	Control	0.006 ppm	0.013 ppm
1st day	10.954 ±0.5002 B	16.896 ±0.8544 A	17.377 ±1.3007 A	4.6951 ±0.0756 B	7.0104 ±1.0377 A	5.7734 ±0.0243 AB	0.138 ±0.0024 A	0.164 ±0.0059 A	0.151 ±0.0202 A
3rd day	11.158 ±0.4521 B	19.976 ±1.5472 A	18.625 ±0.3803 A	4.4251 ±0.0921 C	14.088 ±0.1904 A	11.773 ±0.1645 B	0.181 ±0.0104 B	0.1897 ±0.0136 A	0.1755 ±0.0169 AB
5th day	11.253 ±0.4421 C	21.471 ±1.2027 B	28.354 ±1.2544 A	4.1125 ±0.0771 B	15.209 ±0.9605 A	15.540 ±1.0261 A	0.1095 ±0.0121 C	0.2676 ±00.41 B	0.3129 ±0.0143 A
7th day	9.898 ±0.3982 C	21.203 ±0.945 B	28.096 ±0.9825 A	4.2251 ±0.0621 C	15.497 ±0.2861 B	2.1089 ±0.2317 B	23.998 ±0.3256 B	26.485 ±0.1302 B	39.690 ±2.2040 A
14th day	10.259 ±0.4301 C	20.005 ±0.8600 B	24.285 ±1.0026 A	4.3681 ±0.0625 B	10.880 ±0.2764 A	10.366 ±0.9285 A	0.144 ±0.0122 B	0.3222 ±0.0109 A	0.3262 ±0.0163 A
21st day	10.116 ±0.3987 B	18.465 ±0.5250 A	19.339 ±0.6386 A	3.9861 ±0.1025 B	10.546 ±0.4619 A	9.6158 ±1.2605 B	0.152 ±0.1102 B	0.4371 ±0.1338 A	0.3411 ±0.019 AB
28th day (recovery)	9.876 ±0.2984 C	13.948 ±0.6702 B	18.726 ±0.5947 A	5.0131 ±0.1102 B	10.655 ±0.4807 A	9.6771 ±1.2694 A	0.1291 ±0.1055 A	0.1838 ±0.0507 A	0.151 ±0.0065 A

- Mean with the same letter for each parameter are not significantly different

total serum lipid concentration of the control tilapia (Table 3) was within the same range for other freshwater fish (Abo-Hegab *et al.*, 1990 and Mousa, 1996). Exposure of the tilapia to 0.006 & 0.013 ppm of active ingredient and formulation of the Lufenuron, respectively revealed a significant decrease in total serum lipid after 1, 3, 5, 14 & 21 days of exposure. The minimum values were recorded on the 5th day of Lufenuron exposure. The results are in agreement with those reported by Singh and Singh (1980) for catfish.

The decrease in serum total lipid may be due to lipolysis as a result of releasing of stress hormones (i.e., adrenalin and cortisol) and/or as result of decreasing of biosynthesis of lipid in liver. However, our results indicated to the higher level of Lufenuron residues as a technical or formulation was occurred in the liver of exposed fish, this probably due to the liver is principle detoxifying sits of Lufenuron toxicity. Therefore, these results reflected a significant decrease in serum total proteins and lipids or Lufenuron–exposed fish.

Transamination represents interplay between carbohydrate, protein and. fat metabolism, an activity which can serve the changing demands of the organisms. Exposure of *Oreochromis niloticus* to 0.006 & 0.013 ppm of active ingredient and formulation of Lufenuron, respectively showed significant increases in transaminase, aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Table 4) after 1, 3, 5, 7, 14 & 21 days, with maximal values of such enzyme activities on the 5^{th} day and 7^{th} day of active ingredient and formulation of the insecticide Lufenuron, respectively. The increase in the activities of AST and ALT enzymes might be due to release of such enzymes from liver tissue damage to blood stream. These results confirmed by increasing of Lufenuron residues in hepatic tissues, and this in turn, induced hepatoxicity in exposed fish (Verma *et al.*, 1981; Sharma, 1999). Poleksic and Karan (1999) demonstrated an increase in the activities of (AST) and (ALT) enzymes was observed in serum of *Cyprinus carpio* after 14 days after exposure to sublethal doses of the herbicide, trifluralin. Also, Tilak *et al.* (2005) reported a significant increase in the activity of AST and ALT following exposure to sublethal concentrations of an organophosphate pesticide, "chlorpyrifos" in tissues of freshwater fish, *Catla catla, Labeo rohita* and *Cirrhinus mrigala.* In addition, Venkateswara (2006) detected an increase in serum AST and ALT activities of the tilapia, *Oreochromis*

mossambicus, after 30 days of exposure to sublethal dose of an organophosphorus insecticide "RPR-II".

Serum creatinine can be used as rough index of the glomerular filtration rate (Hemanz and Coulson, 1967). High values of creatinine indicate to nephrotoxicity of kidney (Maxine and Benjamin, 1985). Table (4) shows that exposure of *Oreochromis niloticus* to 0.006 & 0.013 ppm of active ingredient and formulation of Lufenuron, respectively, revealed a significant increase in creatinine after 5,7 & 14 days of exposure. The maximum values were on the 21th day of exposure. Moussa *et al.* (1997) noticed an elevation in creatinine level of *Clarias lazera* after exposure to two derivatives of chinnaberry plant *Melia azearach* for 4 weeks. Jyothi and Narayan (2000) detected an increase in urea, uric acid and creatinine in the catfish, *Clarias batrachus* after exposure to sublethal concentration of two pesticides, carbaryl, a carbamate and phorate, an organophosphorus insecticide for 7 days. There was a significant recovery in the above biochemical parameters during a recovery period of (7 days). There was a significant recovery in the above biochemical parameters using recovery period.

The maximum value of the residue concentrations of Lufenuron as active ingredient and formulation (Table 5) in gills of *Oreochromis niloticus* was detected on the 1st day of exposure, and then started to decline gradually from the 3rd day till recovery at 28th day. But the residues concentration of Lufenuron as an active ingredient and formulation (Table 5) in liver increased from the 1st day of exposure and reached its maximum values on the 21st day then declined on the recovery period at 28th day of treatment. Lufenuron as active ingredient and formulation (Table 5) in muscle of *Oreochromis niloticus* was not detected until the 7th day of treatment, but increased from 14th day and reached its maximum value on the 21st day. It started to decline from the 28th recovery day. The maximum residue limit (MRL) was 0.271 mglkg body weight of Lufenuron.

This 'limit is considered to be high and therefore, people should be aware when eating Nile tilapia either reared in Lufenuron treated ponds or from fish ponds adjacent to Lufenuron treated agricultural drainage. Similar results were reported by Sacher (1978) in the blue gill sunfish, rainbow trout, large mouth bass and channel catfish exposed to glyphosate; and by Purdum (1980) in four fish species (rainbow trout; Channel catfish; large mouth bass and carp) after

Table (5): Concentrations residues in gills, liver and muscles (mg/kg body weight) of the tilapia nilotica (*Oreochromis niloticus*) exposed to 0.006 & 0.013 ppm of (active ingredient and formulation form) of Lufenuron, respectively.

Pesticides / Time day	Active ingredient			Formulation Form		
	Gills	Liver	Muscle	Gills	Liver	Muscle
1st day	4.9596 ±17.98	0.244 ±8.84	ND	3.2550 ±1.36	0.1449 ±0.617	ND
3rd day	2.547 ±9.236	0.2819 ±9.494	ND	1.700 ±16.19	0.1901 ±0.798	ND
5th day	2.320 ±8.4141	0.3044 ±11.031	ND	1.457 ±0.6140	0.2451 ±0.1120	ND
7th day	1.919 ±0.8103	0.5103 ±0.5279	ND	1.5741 ±0.176	0.4506 ±16.34	ND
14th day	1.1556 ±0.486	0.5721 ±0.240	0.2305 ±0.9680	0.7635 ±0.176	0.588 ±0.249	0.272 ±0.115
21st day	0.8836 ±0.371	0.7723 ±0.324	0.2711 ±1.1380	0.8198 ±0.349	0.9189 ±13.602	0.4523 ±0.189
28th day (recovery)	0.1638 ±0.145	0.119 ±0.499	0.0918 ±3.330	0.3634 ±5.547	0.10 ±3.6269	0.1063 ±0.451

Each value represents the mean±STD
ND: Not detected under the limit of detection 5 ng.

Table (6): Persistence rate of two different forms (active ingredient and formulation form) of Lufenuron in water at different periods.

Pesticides / Time day	Lufenuron	
	Active ingredient	Formulation Form
1st day	1.9707 ±4.8467	1.3501 ±4.5513
3rd day	1.6364 ±4.0256	1.1237 ±3.2562
5th day	1.3766 ±3.3863	0.9577 ±2.3559
7th day	0.9617 ±2.3659	0.4636 ±1.1403
14th day	0.3649 ±0.8976	ND
21st day	ND	ND

Each value represents the mean±STD
ND: Not detected under the limit of detection 5 ng.

exposure to glyphosate for 14 days. Mousa (1996) found that the residue of glyphosate in muscles of *Oreochromis aureus* increased on the 1st day and reached its maximum value on the 7th day of experimental period, and then it declined on the 14th day till the end of exposure time.

The results in Table (6) exhibited that the Lufenuron was less persistent in water as an active ingredient and formulation were recorded at 14th day and 7th day, respectively.

REFERENCES

Abo-Hegab, S. and Hanke, W. (1984). The significance of cortisol for Osmoregulation in carp *(Cyprinus carpio)* and tilapia *(Sarotherodon mossambicus)*. Gen. Comp. Endocrinol., 54: 409-417.

Abo-Hegab, S.; Marie, M.A.S. and Kandil, A. (1990).Changes in Plasma Lipid and total protein of grass carp *(Ctenopharyngodon idella)* during environmental pollutant toxicity. Bull. Zool. Soc. Egypt. 39: 211-222.

American Society of Testing and Materials (ASTM) D 1218–02. Standard Test Method for Refractive Index and Refractive Dispersion of Hydrocarbon Liquids.

American Society of Testing and Materials (ASTM) D 1331–89 (Reapproved 2001). Standard Test Method for Surface and Interfacial Tension of Solution of Surface-Active Agents.

American Society of Testing and Materials (ASTM) D 2196–05. Standard Test Method for Rheological Properties of Non-Newtonian Materials by Rotational (Brookfield type) Viscometer.

Begum, G. (2004). Carbofuran insecticide induced biochemical Alterations in liver and muscle tissues of the fish, *Clarias batrachus* (Linn) and recovery response. Aquatic Toxicol. 66: 83-92.

Behreus, A.S. and Karbeur, L. (1953). Determination of LC50. Arch. Exp. Path. Pharm., 28: 177-183.

Devi, A. P. (1981). Studies of the toxicity of endosulfan to some fresh water fish with special reference to cortain physiological changes induced in *Channa punctata* (Bloch). Ph.D. Thesis, Nagarjuna University, Nagarjuna Nagar, South India.

Dobrat, W. and Martijn, A. (1995). CIPAC hand book, Volumes F, J, K and L. Collaborative International Pesticides Analytical Council Limited..

FAO and WHO, Pesticide Specifications, 1970 and 1983.

FAO/WHO (2006). Joint Meeting on Pesticide Specifications (JMPS), Manual on Development and Use of FAO and WHO Specifications for Pesticides, 2nd edition, 125–127.

Gad, S.C. and Weil, C.S. (1989). Statistics for toxicologists in principles and methods of toxicology. Hayes, AW. (Ed) (2nd.ed. Raven press. Ltd., New York, pp.435-483.

Ghazaly, K.S. (1995). A comparison of hematological changes in *Clarias lazera* induced by three different pesticides at sublethal Levels. J. Egypt. Ger. Soc. Zool., 16(A): 253-268.

Gupta, P. K. (1974). Malathion-induced biochemical changes in rats. Acta. Pharmacol. Toxicol. 35: 191-194.

Hemandz, T. and Coulson, R.A (1967). Amino acid excretion in the Alligator. Comp. Biochem. Physiol., 23:775-784.

Henry, R.J. (1964).Clinical chemistry, Harper & Row publishers, New york.181 pp.

Henry, R.J. (1974). Clinical chemistry, principles and techniques, 2nd Edition, Harper and Row. 525 pp.

Hesser, E.F. (1960). Methods for routine fish hematology. Prog. Fish. Cult., 22: 164-170.

Jyothi, B. and Narayan, G. (2000). Pesticide induced alterations of nonProtein nitrogenous constituents in the serum of a freshwater Catfish, *Clarias batrachus* (Linn.). Indian J. Exp. Biol. Pet., 38(19): 1058 – 61.

Katherine, S. (2003). Toxicology mechanisms of trace metal metabolism and cellular toxicity, chemical interactions and risk assessment. Division of Environ. Epidemiol. and Toxicol.

Louis, A (1996). Pesticides and aquatic animals: A guide to reducing impacts on aquatic system. Aquatic Toxicol., 420-013.

Marei, M.A.S.; Haggag, A; Mohamed and Badawi, A.A (1998). Physiological and biochemical responses of the common carp, *Cyprinus carpio,* to an organophosphorus insecticide "profenfos". Egypt. J. Zool., 31: 279-302.

Marei, M.A.S.; Abo-Hegab, S. and Hanke, W. (1990). Regulation of Calcium ion concentration in stenohaline carp *(Cyprinus carpio)*

and euryhaline tilapia, *Sarotherodon mossambicus*. Bull. Zool. Soc. Egypt. 39: 301-318.

Maxine, M. and Benjamin, B.S. (1985). Outline of veterinary pathology. 3rd edition, Colorado state university. Printed in India at Rekha Printers PVT. LTD., New Delhi

Mazeaud, M.M.; Mazeaud, F. and Donaldson, E.M. (1977). Primary and Secondary effects of stress in fish, some new data with a general Review. Trans. Am. Fish. Soc., 106:201-207.

Mousa, M.AA (1996). Effect of the herbicide "glyphosate" on some Biological aspects of tilapia species. M.Sc. Thesis, Fac. Sci., Zagazig Univ. (Banha Branch).

Moussa, F.L.; Adhem, K.H. G; Hashem, H.O. and Abd-El-Hamed, N. (1997). Some physiological and histological alterations in *Clarias lazera* Induced by Melia azedarach derivatives. J. Enviro. Biol. 18(2): 149-165.

Pickering, A.D. (1981). Stress and compensation in teleostean fishes. Response to social and physical factors. In:" Stress and Fish" (Pickering, A.D., Ed.) Pp.295-322.Academic press, New York, London.

Poleksic, V. and Karan, V. (1999). Effect of trifluralin on carp: Biochemical and histological evaluation. Ecotoxicol. Environ. Saf. Jun.; 43(2): 213-21.

Purdum, W.R. (1980). Estimation of bioconcentration factors (BCF) for Glyphosate in selected aquatic species with the pharmacokinetic Model BIOF AC. Mansanto Report MSL- 1116.

Reitman, S. and Frankel, S.A. (1957). J. Clin. Path., 28: 56.

Sacher, R.M. (1978). Safety of round up in aquatic environment. Proe. Europ. Weed Research Soc. 5th symposium on aquatic weeds. Amsterdam, pp. 315-322.

Schmit, J.M. (1964). Determination of total (lipids Kit) thesis, Lyon. Shakoori, A.R., Mughal, A.L. and Iqbal, M.J. (1996). Effect of sublethal Doses of fenvalerate (A synthetic pyrethroids) administration Continuously for four weeks on the blood, liver and muscles of Freshwater fish, *Ctenopharyngodon Idella*. Bull. Environ. Contam. Toxical., 57: 487-494.

Seshagiri, R.K.; Srinivasa, M.K.; Dhanumjaya, N.M.; Steeramula, C.C. and Swami, K.S. (1983). Changes in nitrogen metabolism in tissue of fish *Sarotherodon mossambicus* exposed to binthicarb. Bull. Environ. Contam. Toxicol., 30: 473

Sharma B. (1999). Effect of carbaryl on some biochemical constituents of The blood and liver of *Clarias batrachus,* a freshwater teleost. 1. Toxicol. Sci. Aug., 24(3): 157-64.

Singh, H. arid Singh, T.P. (1980). Effects of two pesticides on testicular Uptake, gonadotrophic potency, lipid and cholesterol contents of Testis, liver and blood serum during spawning phase in *(Heteropneustes jossilis) (Bloch).* Endokrinologic (Ind.), 76: 288298

Tilak, K.S.; Veeraiah, K. and Rao, D.K. (2005). Biochemical changes Induced by chlorpyrifos, an organophosphate compound in sublethal concentrations to the freshwater fish *Catlacatla, Labeo rohita* and *Cirrhinus mrigala.* J .Environ. Biol. Jun; 26(2): 341-7.

Tomlin, C.D.S. (1997). The pesticide manual. Eleventh ed.p.753

Trinder, P. (1969). Ann. Clin. Biochem. 6: 24.

Venkateswara, R.J. (2006). Sublethal effects of an organophosphorus InseCticide (RPR-II) on biochemical parameters of tilapia, *Oreochromis mossambicus.* Comp. Biochem. Physiol. Toxicol. Pharmcol. Aug, 143(4): 492-8.

Verma, S.R.; Rani, S. and Dalela, R.C. (1981). Isolated and combined Effect of pesticides on serum transaminase in *(Mustus vittatus).* Toxicol. Lett., 9: 67-71.

دراسة البصمة الطبيعية والبيوكيميائية للمبيد الحشري"لوفينيورون" على سمك البلطي النيلي وكذلك مستويات متبقيات المبيد في الأنسجه

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تم دراسة البصمة الطبيعية للمادة الفعالة ومستحضر مبيد الماتش ٥٪ EC وقد وجد أن المادة الفعالة ليوفيرون عديمة اللون ذات درجة نقاوه ٩٩٪ ودرجة إنصهار ١٧٠°م، وكذلك فان خواص مستحضر الماتش ٥٪ EC تتفق من مواصفات منظمتـي الأغذيـة والزراعة والصحة العالمية حيث انه يميل الى الحموضـة حيـث كـان تركيـز الأس الهيدروجيني له ٤,٨٨ ودرجة الوميض أكبر من ٧٥°م، معامـل الإنكـسار ١,٤٨٩ ، وكذلك الكثافة ٠,٩٣٢ جرام/سم^3 ولقد نجح المستحضر في إجتياز إختبار التخزين الحار عند درجة ٥٤°م لمدة ٣ أيام وكذلك التخزين البارد عند درجة الصفر المئوي لمدة ساعة بدون أن يحدث أي ترسيب أو فصل أو تدهور في الخـواص الكيميائيـة أو الفيزيقيـة للمستحضر.

ولقد وجد أن الإستحلاب لمحاليل الرش في الماء اليسر والماء العسر وماء الصنبور ثابتة لم يحدث فيها أي فصل وكذلك تأثرت هذه المحاليل بإنخفاض ملحوظ فـي التـوتر السطحي من ٧٣ الى ٣٢ داين/سم مما يؤدي الى زيادة البلل للسطح المعامـل بمحلـول الرش بدرجة كبيرة مما يزيد من كفاءة المستحضر.

تم تعريض أسماك البلطي النيلي لتركيزين أقل من التركيز النصف مميـت وهمـا ٠,٠٠٦ و ٠,٠١٣ جزء في المليون وذلك من المادة الفعالة كذلك صورة المبيد المجهزة لمبيد لوفينيورون وتركت في هذين لمده ٢١ يوماً. وقد كـشفت اختبـارات الـسمية إن التركيز النصفي المميت للنصف خلال ٩٦ ساعة (LC_{50}) في سمك البلطي النيلي كـان ٠,٠٦٣ ، ٠,١٢٦ جزء في المليون على الترتيب. وأظهرت النتائج زيادة ملحوظة فـي تركيز كل من الجلوكوز في سيرم الدم بينما أظهر تركيز البروتين الكلي والدهن الكلـي في سيرم الدم انخفاضا واضحا. وعلى الجانب الآخر أظهر تركيز كل من الكريـاتينين ونشاط كل من الإنزيمات الناقلة لمجموعة الأمين (AST و ALT) زيادة واضحة دليلا على حدوث خلل في الكلى والكبد.

ولقد أظهرت النتائج تراكم كل من المادة الفعالة في كلا المعاملتين (سـواء بالمـادة الفعالة أو الصورة المجهزة) من المبيد الحشري "ليوفينورون" في الأعضاء المختلفة على النحو التالي الخياشيم > الكبد> العضلات على الترتيب.