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HAEMATOTOXIC AND BIOCHEMICAL HAZARDS INDUCED BY LONG-TERM EXPOSURE OF FEMALE NILE TILAPIA (*Oreochromis niloticus*) TO CARBOFURAN

Mohamed A. Al-Zahaby[2*], M.S.A. Ayyat[1],

G.E. Abd El-Rahman[1] and A.M.E Shalaby[2]

1. Dept. of Animal Production, Fac. Agric., Zagazig Univ., Egypt
2. Fish Physiol. Dept., Cent. Lab. for Aquacult. Res., Abbassa, Sharkia, Egypt

ABSTRACT

The present study was carried out to measure the influences of sublethal toxicity of carbofuran (carbamate), which is one of the most commonly used nematicides, on the reproductive performance of *Oreochromis niloticus.* The 96 hr LC_{50} of the carbofuran was determined for the adult Nile tilapia and it was 3.04 µg/ml. The present experimental assay was evaluated on the 60^{th} day of exposure to 1/50, 1/25 and 1/10 LC_{50} of the pesticide in comparison with control fish group. The induced hazard effects were obvious in blood parameters as well as blood chemistry. The insignificant decrease of the erythrocytes count (RBCs), haemoglobin content (Hb) and hematocrit value (Hct) were dose depended. The plasma glucose level of the treated fish was significantly increased (hyperglycaemia) in response to dose dependent of carbofuran exposure. Nonetheless, the total plasma lipid (highly significant) and total plasma protein (significantly lowered) under the effect of intoxication with the pesticide. The plasma Aspartate amino-transferase (AST) and Alanine amino-transferase (ALT) enzymes activities were elevated significantly in fish exposed to carbofuran. Also the plasma urea (highly significant) and creatinine (insignificant) were increased in toxicated fish in comparison with those of control specimens. The blood levels of estradiol-β17 in the toxicated fish were highly

*Corresponding author: Mohamed A. Al-Zahaby , Tel.: +20102343903
E-mail address: alzahaby85@yahoo.com

significant decreased with the different doses of carbofuran, causing reproduction dysfunction, indicated by the obvious decrease of gonado somatic index as well absolute and relative fecundity of the stressed females.

Keywords: Nile tilapia, *oreochromis niloticus*, pesticides, carbofuran, haematotoxic effect, biochemical hazards.

INTRODUCTION

Problems associated with pesticide hazards to man and the environment are not confined to the developing countries. Developed nations have already suffered these problems, and still facing some problems in certain locations. For many reasons, the severity of pesticide hazards is much pronounced in third world countries (Mansour, 2004).

The widespread use of pesticides and their presence in waters used for fish production are of particular concern. Many pesticides are complex organic molecules which are very persistent in the environment, and are passed on through the food chain. Thus, carnivorous fish can accumulate high levels of pesticides from their prey (Redding and Midlen, 1990).

Egypt has one major source of water supply, the River Nile, which supplies over 95% of annual water needs. Egypt possesses approximately 50000 km of irrigation and drainage canals, of which approximately 3532 km are of a suitable size for aquaculture. The potential for some form of aquaculture in these waters is considerable according to Jauncey and Stewart (1987). Irrigation canals are the recipients of the residual loads of the wide range of chemicals used in crop production, and often exhibit high levels of pesticides and fertilizer (Redding and Midlen, 1990). The expansion and optimization of agricultural and industrial production have lead to augmented use of pesticides in agriculture which resulted in high contamination of aquatic environments. Contamination of water by pesticides is mainly due to intensive agriculture combined with surface runoff and subsurface drainage, usually within a few weeks after application (Nouri *et al.*, 2000). These are polluted as a result of discharging agricultural drains daily, with about 30000 tons of the pesticides used for protection of different crops annually (El-Sebae and Soliman, 1982). Carbofuran (Fura-Z) which is a carbamate nematicide is used for control of soil-dwelling and foliarfeeding insects especially nematodes in vegetables, rice, cotton, maize, potatoes, peanuts, soya beans and others (BCPC, 2004).

Pesticide loss, originating from areas where agricultural pesticides are mixed or handled, and where sprayers are parked or washed down, can be responsible for 20-70% of the total pesticide loading in surface waters in arable catchments (Mason *et al.*, 1999 and Carter 2000). This can affect water quality, and subsequent water treatment costs, as well as ecosystem health.

Fish exposed daily to the synthetic chemical compounds even in small doses, can disrupt the development of its reproductive, nervous and immune systems by mimicking and/or blocking the action of hormones and then alter the biochemical, hematological and histopathological characteristics of fishes (Begum and Vijayaraghavam, 1996). Some of these pesticides are known to accumulate in plant and animal tissues to very high level, posing a potential toxic hazard to the organisms themselves, or organisms higher in the food chain including humans, which may consume them (Mansour, 2004).

The objective of the present studies was to investigate the haematological and biochemical changes which take place in the toxicated *Oreochromis niloticus*. The study also throw light on the amino acid metabolism through the measurements of transaminases as well as urea and creatinine in the blood plasma of the studied fish. These studies may be of great help to understand the healthy status of the fish under the effect of the studied pesticide, carbofuran.

MATERIALS AND METHODS

Apparently healthy Nile tilapia, *Oreochromis niloticus*, specimens with an average body weight of 55 - 65 g obtained from Abbassa fish farm, Abbassa, Abou-Hammad, Sharkia were acclimated in laboratory conditions for two weeks prior the experiment. The 96 hour half lethal concentration (96hr LC_{50}) of the carbofuran (carbamate) nematicide (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) was determined according to Bebreus and Karbeur (1953) as 3.04 µg/ml, however the applied field dose in the rice fields is about 14.28 µg/ml.

Four groups of the studied fish were holded for two monthes (from the beginning of April to the end of June) to evaluate the effects of different sublethal concentrations of carbofuran on the reproductive performance of *Oreochromis niloticus*. Each group consisted of 27 fish was divided into three replicates, six fish (6 females). Each group maintained in glass aquaria of 100 liter capacity each supplied with dechlorinated aerated tap water at a temperature of 26°±2 C, pH 7.2±0.2 and dissolved oxygen 5.5 - 0.5 mg/l. The first group was kept as a control but the other groups were exposed to the 1/50 LC_{50}, 1 /25 LC_{50}, and 1/10 LC_{50} (0.061, 0.122 and 0.304 µg/ml, respectively) of the carbofuran, in the order mentioned. The feeding rate was holded at the percent of 3% of the body weight twice daily.

The 96 hours LC_{50} of Fura-Z (carbofuran) was done according to Behreus and Karbeur (1953) and shown in Table 1.

In the applied field dose of carbofuran in the rice fields is about 1.428 g/100 l which equal to 14.28 mg/l, the applied three concentration in the present experiment were: 1/50 LC_{50}= 0.061 mg/l, 1/25 LC_{50} = 0.122 mg/l and 1/10 LC_{50} =0.304 mg/l.

Table 1. Successive concentrations and number of dead and live fishes after 96 hours of exposure to carbofuran (Fura Z) to determine its 96- LC_{50}

Group	Conc. mg	No of dead fishes	(A) Mean of dead fish in the two successive groups	(B) Difference between the two successive concentrations	A X B
Group I	2.50	3	--	--	--
Group II	3.00	5	4.0	0.5	2.00
Group III	3.500	4	4.5	0.5	2.25
Group IV	4.00	5	4.5	0.5	2.25
Group V	4.500	5	5.0	0.5	2.50
Group VI	5.00	6	5.5	0.5	2.75
					∑ of AB=11.75

LC_{50} = Higher dose - (∑ AB / № of specimen in each group).

LC_{50} = 5.00 – (11.75/6) = 3.04 mg/l.

At the end of the experiment, the total body length of females only to the nearest 0.1 cm of each fish was recorded then blood samples were taken from the caudal vein of non anaesthetized female fish by sterile syringe, 0.5 ml of the blood containing EDTA as an anticoagulant was used for erythrocyte count (Dacie and Lewis 1984), haemoglobin content (Hb; Vankampen 1961) and haematocrit value (Hct; Britton, 1963). The derived indices of the red blood cells which are the mean cell volume (MCV), mean cell hemoglobin content (MCH), mean cell hemoglobin concentration (MCHC) and the Colour Index (CI) presents the ratio between the haemoglobin content and the number of red blood cells in a specific amount of blood are calculated according to (Houston, 1990).

MCV = Hct X 10 / RBCs x10^6......μ^3

MCH = Hb X10^7/ RBCs X 10^6....Pg

MCHC = Hb / Hct X 100 ....%

CI = Hb / RBCs in million

Plasma was obtained by centrifugation of blood at 3000 rpm for 15 mm and non-haemolyzed plasma was stored in deep freezer for further analyses. The gonads and liver of all fish specimens were remove wet, weighed then the gonado-somatic index (GSI) and hepato-somatic index (HSI) were calculated by assessing the gonad and liver weights as a percentage of the total body weight. Ripening egg numbers in the ovaries of the female were counted to determine the absolute (total) and relative fecundity, the number of eggs per gram of total fish weight (Munkittrich and Dixon, 1988).

Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were determined according to the method of Reitman and Frankel (1957). The urea and glucose were estimated according to the method of Trinder (1969), while total protein and creatinine were determined according to Henry (1964) and total lipids according to Schmit (1964). All of these parameters were measured using specific reagent kits purchased from Diamond Diagnostic Company. Estradiol-β17 was determined by the radioimmune assay method according to Xing *et al.* (1983) using kit Immunchem provided by Diagnostic products corporation, Los Angeles.

The data were statistically analyzed by Statical package SPSS version 15, in which data was subjected to one way ANOVA

(Sokal and Rohif, 1981). To determine the significance of the differences between each treatment and the control group Duncan multiple range tests was used comparisons were made at the 5% probability level.

RESULTS

Behavioral Responses

In respect to the mobility of exposed Nile tilapia (*Oreochromis niloticus*), it was noticed that female fish are excited firstly as they exposed to the pesticide. The unexposed fish group (control group) did not suffer some impact when they were placed into the aquatic medium. Conversely the exposed fish suffered a shock at the beginning and showed respiratory distress manifestations, represented by rapid and wide opening of mouth, hyperexcitability, erratic movements and active swimming. Severe convulsive reflexes, static equilibrium and hanging horizontally in the water were observed especially with the high dose of exposure (1/10 LC_{50}) when compared with the control fish group. Afterwards, in the second week of exposure, fish showed exhaustion appearance, sluggish movements and less general activity. After three weeks the experimental fish stayed motionless on the aquarium bottom, although in the following days they survived. The dead fish specimens were replaced from the acclimated stock fishes in the first week of the begging only.

The hepato somatic index (HSI) was affected insignificantly with exposed to the sub-lethal concentrations of carbofuran in female fish. The HIS decreased by 16.12, 16.06 and 43.57% in fish exposed to 1/50, 1/25 and 1/10 LC_{50} of pesticide, respectively, compared with the values of control group (Table 2).

Gonado Somatic Index (GSI) was more significantly decreased by 42.45, 58.60 and 74.65 % in females toxicated with 1/50, 1/25 and 1/10 LC_{50} of the examined pesticide, respectively (Table 2). Similarly, absolute fecundity was also decreased with highly significant pattern by 32.44, 53.37 and 70.73 % in females stressed the tested doses of carbofuran, respectively compared to the control fish group. Consequently, relative fecundity was also lowered more significantly with a percentage of 26.01, 49.67 and 69.56%, respectively.

Blood Components

The given data in Table 2 for the effect of carbofuran on the erythrocyte counts (RBCs) of

Oreochromis niloticus revealed insignificant dose dependent decrease with reference to the value of control group by 1.26, 5.18 and 7.86 % for fish exposed to 1/50, 1/25 and 1/10 LC_{50} of carbofuran, respectively. Likewise, the hemoglobin content (Hb) of toxicated fish was also lowered insignificantly by 9.18, 16.14 and 29.35 %, respectively than that of the controls to reach its lowest value with 1/10 LC_{50} of the pesticide. Concerning the haematocrit value (Hct), it showed a similar trend and insignificant decreased in the treated specimens comparable to the control ones in percentage of 13.11, 19.67 and 22.95 % under the effect of 1/50, 1/25 and 1/10 LC_{50} of carbofuran, respectively.

Table 2. The hepato- gonado somatic index (HIS - GSI), absolute fecundity (AbF), relative fecundity (ReF) erythrocyte count (RBCs), hemoglobin content (Hb) hematocrit value (Ht), color index (CI), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) in the blood of female Nile tilapia exposed the sub-lethal concentrations of carbofuran

Items	Control group	0.061 mg/l (1/50 LC50)	%	0.122 mg/l (1/25 LC50)	%	0.304 mg/l (1/10 LC50)	%	Significance
HSI	1.501±0.089	1.259 ±0.088	-16.1	1.260±0.260	-16.1	0.847± 0.072	-43.6	NS
GSI	2.773^{a} ±0.437	1.596^{b} ±0.180	-42.4	1.148^{bc} ±0.076	-58.6	0.703^{c} ± 0.042	-74.6	**
AbF egg	849.155^{d} ±11.52	573.672^{c} ±19.79	-32.4	395.962^{b} ±58.49	-53.4	248.541^{a} ±45.17	-70.7	***
ReF egg/g	14.228^{c} ±0.828	10.5272^{b} ±1.114	-26.0	7.161^{a} ±0.628	-49.7	4.331^{a} ±0.681	-69.6	**
RBCs (10^{6}/ml)	1.273±0.047	1.257±0.072	-1.3	1.207±0.030	-5.2	1.173±0.057	-7.9	NS
Hb g/dl	7.529±0.937	6.838±0.966	-9.2	6.314±0.538	-16.1	5.319±1.438	-29.4	NS
Hct %	20.333±3.756	17.667±1.667	-13.1	16.333±0.882	-19.7	15.667±1.202	-22.9	NS
CI ratio	5.984±0.981	5.405±0.495	-9.7	5.241±0.559	-12.4	4.514±1.118	-24.6	NS
MCH Pg	59.844±9.808	54.052±4.945	-9.7	52.410±5.588	-12.4	45.138±11.176	-24.6	NS
MCHC %	37.953±2.641	40.061±8.232	5.6	38.530±1.266	1.5	33.104±6.409	-12.8	NS
MCV μ^{3}	162.260±36.10	142.140±17.70	-12.4	135.450±10.41	-16.5	133.590±7.19	-17.7	NS

Means within each row with the deferent letter are significantly different (P<0.05).

The tested sublethal concentrations of carbofuran also induced insignificant decreased in all blood derived indices. The mean cell haemoglobin (MCH) and the colour index (CI) of Nile tilapia females decline from the control level with 9.68, 12.42 and 24.57% in fish toxicated with 1/50, 1/25 and 1/10 LC_{50} of carbofuran, respectively. However, mean cell haemoglobin concentration (MCHC) was increased insignificantly by 5.55 and 1.52%, respectively in 1/50, 1/25 LC_{50} groups, while the group 1/10 LC_{50} decreased with 12.78% in reference to the values of control group. Concerning with the mean cell volume (MCV) of Nile tilapia erythrocytes lowered under the effect of the same sublethal concentrations of pesticide by 12.40, 16.52 and 17.67% from value of control fish group.

The obtained data of the plasma glucose recorded for the treated groups was more significantly increased by 10.71, 13.29 and 17.77% in 1/50, 1/25 and 1/10 LC_{50} of pesticide groups, respectively, when compared with the control group (Table 3). The plasma total protein decrease significantly by 13.28, 26.48 and 42.59 %, respectively, in 1/50, 1/25 and 1/10 LC_{50} groups than the value of the control fish. However, the lipid concentrations decrease with 21.57, 34.43 and 41.60%, respectively.

Regarding with the transaminases, it was found that aspartate amino-transferase (AST) and alanine amino-transferase (ALT) activities were more significantly dose dependent increased with the increase of carbofuran concentration. Their percentage of decrease from their control levels were 3.90, 9.25 and 11.29 % for AST and 5.67, 9.25 and 17.25% for ALT, respectively after exposure to 1/50, 1/25 and 1/10 LC_{50} of carbofuran. In the same way, the plasma urea and creatinine levels, in the present investigation significantly increased in female *O. niloticus* exposed to the same sublethal concentrations of pesticide. Urea was more significant increased by 17.61, 23.14 and 37.25% but creatinine level was insignificant raised by 12.74, 18.11 and 30.69% in comparison with their control rank (Table 3). Finally, the level estradiol-β 17 in plasma of Nile tilapia was a more significant lowered, under the effect of the tested concentrations of pesticide, with percentage of 9.01, 22.42 and 60.41 %, respectively commencing with the control group.

Table 3. Some blood components; glucose, protein and lipid plasma content as well as AST, ALT, urea, creatinine and estradiol β17 levels in plasma of female Nile tilapia, *Oreochromis niloticus* exposed the sub-lethal concentrations of carbofuran

Items	Control group	0.061 mg/l (1/50 LC50)	%	0.122 mg/l (1/25 LC50)	%	0.304 mg/l (1/10 LC50)	%	Significance
Glucose mg/dl	$37.160^{b} \pm 0.796$	$41.140^{a} \pm 1.041$	10.7	$42.100^{a} \pm 0.999$	13.3	$43.763^{a} \pm 0.842$	17.8	**
Protein g/dl	$2.644^{a} \pm 0.261$	$2.293^{a} \pm 0.205$	-13.3	$1.944^{ab} \pm 0.081$	-26.5	$1.518^{b} \pm 0.270$	-42.6	*
Lipids g/l	$5.428^{a} \pm 0.336$	$4.257^{b} \pm 0.151$	-21.6	$3.559^{c} \pm 0.146$	-34.4	$3.170^{c} \pm 0.081$	-41.6	***
AST Iµ/l	$98.32^{b} \pm 1.006$	$102.150^{b} \pm 1.494$	3.9	$107.333^{a} \pm 1.499$	9.2	$109.420^{a} \pm 1.403$	11.3	**
ALT Iu/l	$16.677^{c} \pm 0.385$	$17.623^{bc} \pm 0.273$	5.7	$18.220^{ab} \pm 0.436$	9.3	$19.553^{a} \pm 0.549$	17.2	**
Urea mg/dl	$10.603^{c} \pm 0.212$	$12.470^{b} \pm 0.288$	17.6	$13.057^{b} \pm 0.237$	23.1	$14.553^{a} \pm 0.190$	37.3	***
Creatinine mg/dl	1.75 ± 0.156	1.973 ± 0.087	12.7	2.067 ± 0.291	18.1	2.287 ± 0.158	30.7	NS
Estradiol β17 ng/l	$4999.76^{a} \pm 107.45$	$4549.47^{b} \pm 176.39$	-9.0	$3878.650^{c} \pm 105.06$	-22.4	$1979.640^{d} \pm 41.34$	-60.4	***

Means within each row with the deferent letter are significantly different (P<0.05).

DISCUSSION

Most information about the effect of pesticides on aquatic animals has been commonly obtained from mortality studies. Very little about the damage of different internal organs and consequently physiological and biochemical disruptive effects of these poisons is known, particularly about the presently tested carbofuran. It is necessary to predict the potential harmfulness of this chemical to the aquatic environment and Nile tilapia in particular.

In the present investigations, abnormal behavioral response (loss of equilibrium, hanging vertically in the water, rapid gill movement, erratic swimming and staying motionless on the aquarium bottom) of *Oreochromis niloticus* stressed especially with high concentrations of carbofuran was evident. This have been also recorded in *Poecilia reticulata* (Viran *et al.,* 2003), *Heteropneustes fossilis* (Saha and Kaviraj, 2003) and *Cyprinus carpio* (Calta and Ural, 2004) exposed to sublethal concentrations of pyrethroid pesticides. These responses are usually related to impaired respiration and feeding behaviour of fish which are badly affected by pollutants (Sprague, 1971). Later on, Dutta and Arends (2003) declared that fish behavior is greatly influenced by the accumulation of the acetylcholine following the decrease of the brain acetylcholinesterase activity induced by the pesticide effect.

Because the fish is in direct contact with its environment, so every unfavorable change in the water should be reflected on the fish hematological parameters, other wards, blood is a mirror to what occur inside fish body and can help to assess the health condition of fish (Shehata and Shehata, 1994). As indicated by Rizkalla *et al.* (1999) pollutants produce relatively destructive changes in blood characteristics of fish, thus it provides good information about the level of organ damage in the fish.

In the present investigation, the hazard effects induced by carbofuran (Fura-Z) in *Oreochromis niloticus* were obvious in blood parameters as well as blood chemistry. The concentration of RBCs count, Hb and Hct was insignificantly decreased in the fish groups exposed to sub lethal concentrations of carbofuran compared to the control groups are in accordance with results recorded by Glusczak *et al.* (2006) in *Leporinus obtusidens* exposed to Roundup and those of Kumar *et al.* (2010) in *Oreochromis mossambicus* long term exposed to sublethal concentrations of endosulfan. Ahmed *et al.* (1992) found that ammonia resulting in irregular pattern of changes in the blood parameters of *Oreochromis niloticus*. Nonetheless, Round-up (Herbicide) not exerted any differences in the blood parameters of *Prochilodus lineatus* (Modesto and Martinez, 2010).

The differently response of hematological parameters in fish towards different chemical stressors are however, non-specific to a wide range of substances. Some of these changes may be resulted in the activation of protective mechanisms (Cazenave *et al.*, 2005). Another explanation is that given by Elahee and Bhagwant (2007), where they pointed out that the effects of environmental toxicants on hematological characteristics of fish vary according to the target fish species.

Glucose which is one of the most important sources of energy has been studied as an indicator of stress caused by pollutants (Manush *et al.,* 2005). In the present investigations, plasma glucose content of *Oreochromis niloticus* was more significantly ($P<0.05$) dose dependent increased in response to sublethal concentrations of carbofuran exposure. The same results have been also recorded in gilthead exposed to Malathion (Rosety-Rodriguez *et al.,* 2005). This indicates the quantum of stress forced on fish during the acute toxicity of pesticide and physiological attempts of fish to overcome it, as was assumed by Kumar *et al.* (2010). Almeida *et al.* (2001) stated that, the chemical stress may elevates the secretion of catecholamine which in turn enhances glycogen break down (glycogenolysis) in *O. niloticus* liver and consequently raise blood glucose level causing hyperglycemia. Hyperglycaemia, indicating the disruption of carbohydrate metabolism, was also observed in *Mystus vittatus* exposed to dichlorvos and carbofuran (Dalela *et al.*, 1981) and *Clarias batrachus* exposed to endosulfan (Venkateshwarlu *et al.*, 1990). Hyperglycaemia (evidenced in the present study as increased plasma glucose levels) is viewed as a physiological response of the fish to meet the critical needs for energy under toxic stress.

The plasma total lipid of *Oreochromis niloticus* was high significantly lowered under the effect of carbofuran intoxication. However, Singh and Singh (1980) found that in Indian cat fish, *Heterpneustes fossilis*, exposed to malathion and endrin, the plasma lipid was significantly increased. This abnormal alteration in plasma fats may be due to the induced imbalance between fat production and utilization (Sevgiler *et al.*, 2004). Pesticides induced lipid peroxidation has already been described in various fish species (Glusczak *et al.*, 2007 and Modesto and Martinez, 2010). Plasma lipid peroxidation was significantly increased in *Oreochromis mossambicus* exposed to high dose of endosulfan causing a dose dependent increase in blood glucose and decrease plasma lipid as well as fish immunity (Kumar *et al.*, 2010).

The significant decrease of plasma total protein in *Oreochromis niloticus* under the effect of sublethal concentrations of carbofuran is in accordance with that happened in *Clarias gariepinus* toxicated with phenolic compounds (Assem *et al.*, 1992). *Oreochromis niloticus* inhabited the effluents of raw and treated toxic industrial wastes (El Deeb *et al.,* 1990) and/or sub-lethal concentrations of cyanide effluent (Adekunle *et al.*, 2007) also suffering from a marked decrease of total plasma protein content. However, plasma total protein was not altered in African cat fish, *Mystus vittatus*, toxicated with thiotox and carbofuran (Dalela *et al.*, 1981). Blood plasma protein fairly adjusts the biochemical system, specifically reflects the condition of the fish under influence of internal and external stressors (Shalaby *et al.,* 2006 and Hadi *et al.*, 2009). Jacobes *et al.* (1990) proposed that, the decline of plasma total protein may be due to the high degree of haemodilution under the stress of pesticides. Hence in fish exposed to stressors (pesticide), the gills become spongy and permeable to water and ions, this often resulting in osmoregulatory imbalances (Mazeoud *et al.*, 1977). Pesticides may enhance the intracellular generation of reactive oxygen species (ROS) (Tellez-Banuelos *et al.*, 2009). Ahmad *et al.* (2000) claimed that, due to the high reactivity of these free radicals (ROS), it induces oxidative harm to biological systems and may damage lipids, and proteins as referred by Hermes (2004). When the animal's defenses are insufficient to neutralize these free radicals, oxidative damage may occur, and one of the most serious damages is the membrane lipid peroxidation (Scandalios, 2005). Total plasma protein showed general significant decrease after exposure to carbofuran may be due to liver damage where most of plasma protein synthesis usually occurs in the liver, this result is in accordance with that of Gaafar *et al.* (2010).

Concerning plasma transaminases (AST and ALT) in the studied fish, they were more significantly ($P < 0.05$) dosed dependent increased under the effect of sublethal concentration of carbofuran as compared with the value of control specimens. These findings are in close agreement with those of Begum (2004) who investigated the effect of carbofuran on *Clarias batrachus*. Verma *et al.* (1981) recorded more obvious dose dependent increase of (AST) than (ALT) in *Mystus vittatus* exposed to carbofuran. Transaminases (AST and ALT) are considered to be very important in assessing the state of the liver and some other organs (Daabees *et al.*, 1992). Urea level in the blood of Nile tilapia also showed highly significant dose dependent increase following the exposure to different tested concentrations of carbofuran. Creatinen also increased under the effect of carbofuran, but with statistically insignificant pattern. These findings are in close accordance with those given by Gaafar *et al.* (2010) in Nile tilapia stressed by edifenphos due to harmful effect of pesticide on kidney tissue.

Reproductive success sharply affected through inhibition of spawning or through toxicity of fish (Sprague, 1971). The fecundity as well gonado-somatic index (GSI) were obviously lowered in females of *Oreochromis niloticus* stressed with long term exposure of sublethal concentrations of carbofuran. Similarly, Estradiol-β17, secreted by the ovarian follicles stimulates the egg shell production (Senthilkumaran *et al.*, 1999), more significantly decreased in the present investigations. These findings are in harmony with those of Senthilkumar *et al.* (2001), they noticed that pesticides obviously decreased estradiol- β 17 causing dysfunction of reproduction activity of the stressed fishes. Poorni (2001) claimed that pesticides may produce adverse effects on fish reproduction directly by germ cell destruction or indirectly by structural similarity to endogenous hormones and consequently interrupt reproduction via increase or decrease of steroid hormone level. The present study confirms those of El-Gawahir (2008) and Oruc (2010) in *O. niloticus* stressed with NP and chlorpyrifos, respectively, they stated that, the decreasing effect of plasma estradiol is significantly correlated with the fecundity and GSI decline.

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الأخطار الناتجة عن تعرض إناث السمك البلطى لجرعات تحت قاتلة من مبيد الكاربوفيوان ولمدة طويلة على بعض النواحى الكيميائية والفسيولوجية

محمـــد الأحمـــدى الـــذهبى[٢] – محمد صـــلاح الدين عياط[١]
جمال الدين على عبدالرحمن[١] – عادل محمد عيسى شلبى[٢]

١– قسم الإنتاج الحيوانى – كلية الزراعة – جامعة الزقازيق
٢– قسم فسيولوجى الأسماك– المعمل المركزى لبحوث الثروة السمكية– العباسة– الشرقية– مصر

أجريت هذه الدراسة للوقوف على تأثير التركيزات الغير المميتة لمبيـد الكربوفيـوران (فيورازد) وهو من الكربمات التى تستخدم لمقاومـه النيمـاتودا علـى بعـض النـواحى الفسيولوجية والبيوكميائية التى لها ارتباط وثيق بالأداء التكاثرى فى إناث البلطى النيلـى. فبعد تعين الجرعة القاتلة لنصف أسماك البالغة خلال ٩٦ ساعة والتى وجـد أنهـا كانـت ٣،٠٤ جزء فى المليون، تم اختبار ١/٥٠ ، ١/٢٥ ، ١/١٠ منها لدراسة تأثيرهـا علـى الإناث أثناء دورتها التكاثرية . ولقد أظهرت الأسماك المعاملة بالمبيد اضطرابا فى سـلوكها بما تأديه من حركات غير متزنة و كانت النتائج المتحصل عليها كالآتى : المبيد بتركيزاتـه المختلفة تسبب فى النقصان الغير معنوي لعدد كرات الـدم الحمـراء (RBCs) ومحتـوى الهيموجلوبين (Hb) ونسب الهيماتوكريت فى الدم (Hct) ومتوسطات حجم كـرات الـدم الحمراء (MCV) وكم الهيموجلوبين بها (MCH) كذا تركيز الهيموجلـوبين فـى الـدم (MCHC) بالإضافة إلى معامل لون الدم (CI). كما أن المبيد بتركيزاته المختلفة تسـبب فى نقصان احصائى متباين المعنوية فى الدهون والبروتينات الكلية فى دم الأسماك الواقعـة تحت تأثيره و كذا هرمون الاستراديول فى الإناث وكان هذا النقصان يزداد بزيـادة تركيـز المبيد. هذا بخلاف ما حدث للجليكوز واليوريا والكرياتين فى الدم كـذا الإنزيمـات الناقلـة لمجموعة الأمين (AST , ALT) والتى لوحظ زيادتها بمعنوية عاليـة فـى نفـس هـذه الأسماك مقارنتا مع أفراد المجموعة الضابطة. وهذا جميعه أدى إلى إحداث ضرر كبير فـى كبد ومناسل الأسماك مما كان له الأثر البالغ على نقصان معامل الكبد والمناسل (المبايض) وكذا الخصوبة المطلق والنسبية فى عند إناث البلطى النيلى.