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EFFECT OF MALACHITE GREEN FUNGICIDE ON PHYSIOLOGICAL AND BIOCHEMICAL PROFILES IN NILE TILAPIA (*OREOCHROMIS NILOTICUS)*

Adel M. E. Shalaby[1*]; Ahmed A. AbdEl-Rahman[1] Maaly A. Mohammed[2] and Amany A. Ghareb[1]

[1] *Fish Physiology Department*
[2] *Ecological Department, Central Laboratory for Aquaculture Research, Abbassa, Abo- Hammad, Sharkia, Egypt.*
** Corresponding author: Adel. M. Shalaby Tel: 01148342902*
E- Mail address: adele_shalaby@yahoo.com

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Abstract

The 24, 48, 72 and 96-h LC_{50} for Nile tilapia "*Oreochromis niloticus"* exposed to malachite green (MG) were determined and found to be 1.38, 0.90, 0.80 and 0.76 ppm respectively.

Nile tilapia "*O. niloticus*" (40-50 g/fish) was assigned to three group with three replicates each. The first group was kept as control group. The second and third ones were exposed to sublethal concentration (1/8 and ¼ 96-hr LC_{50}) of malachite green.

The haematological and biochemical changes induced in Nile tilapia (*O. niloticus*) exposed to these sublethal concentration of malachite green (0.095 and 0.19 mg/l respectively) were carried out under labotratory condition for 30 days.

All treatments produced significant fall of erythrocyte count (RBCs), haemoglobin content (Hb) and packed cell volume (PCV). However, blood indices (MCV and MCH) were increased significantly in fish exposed to herbicide. While, the MCHC was decreased significantly in fish exposed to high dose of malachite green.

The plasma glucose content was significant increased mean while, the plasma total protein was significant reduced in fish exposed to all treatments of malachite green. Also, the total lipids was decreased significantly in the exposed fish with malachite green .The activity of aspartate

aminotransferes (AST) and alanine amino transferase (ALT) were also significantly increased in fish exposed to subleathal dose of the tested fungicide.

Key words: Nile tilapia, malachite green, haematology, Biochemistry changes.

INTRODUCTION

Fish are persistently bathed in potential pathogens, including bacteria, fungi and parasites. Among them, parasitic diseases in fish are most frequently caused by protozoa. Most protozoan infections are comparatively easy to control using common fishery chemicals, such as copper sulfate, malachite green, formalin or potassium permanganate, methylene blue, brilliant green, parasite green, and trichlorforn (Reardon and Harrell, 1990)

Malachite green (MG) is widely used in aquaculture as a fungicide and in food, health, textile and other industries for one or the other purposes. It controls fungal attacks, protozoan infections and some other diseases caused by helminthes on a wide variety of fish and other aquatic organisms. The toxicity of this dye increases with exposure time, temperature and concentration. It has been reported to cause carcinogenesis, mutagenesis, chromosomal fractures, teratogenecity and respiratory toxicity. Histopathological effects of MG include multi-organ tissue injury (Srivastava *et al.,* 2004b)

Malachite green is a triphenylmethane dye, widely used as a strong antifungal, antibacterial, antiparasitical agent in the aquaculture industry and as a very common multipurpose dyestuff in various dye industries. However, malachite green is not approved for use in commercial fishes in many countries, due to its high toxicity (Zhai *et al*., 2007). Exposure to malachite green may cause the physiological and biochemical indexes' imbalance, such as the serum calcium, protein levels and total cholesterol level (Srivastava *et al*., 1995). It may cause lesion of organs, such as the sensory organ, respiratory organ and

pituitary gland (Kumar *et al*., 2007). For its high toxicity, many techniques have been developed to treat malachite green residual in the environment. However, this fungicide has been widely used in aquaculture to control fungal attacks, protozoan infections and some other diseases (Mei –Jie *et al*., 2012).

Malachite green lead to closing of taste pore, disintegration of taste hairs and cellular components of taste buds and decline in glycoprotein moieties, which is followed by great reduction in the ability of cat fish, *Mystus vittatus* (Kumar *et al.*, 2007). For the Nile tilapia, *"O. niloticus"*, opercular ventilation rate decreased with increase in the concentrations of malachite green (Omoregie *et al*., 1998). The studies showed that exposures to subacute and sublethal concentrations of malachite green inhibite the activity of gonadotropic cells in the pituitary gland which resulted in degenerative changes in the gonads of Heteropneustes fossilis (Srivastava *et al*., 1998). Malachite green also affects hematological parameters: decreases in haematocrit values and anaemic responses have been reported in rainbow trout and *Clarias gariepinus* (Musa and Omoregie, 1999). It had also immunosuppressive effect on rainbow trout (Yonar and Yonar, 2010).

Therefore this study was assigned to determine the lethal concentration of malachite green and to investigate the effects of sub lethal concentration of this substance on survival, biochemical and hematology parameters in the Nile tilapia fingerlings.

MATERIALS AND METHODS

Fish Culture Management:

Healthy fish of Nile tilapia, *Oreochromis niloticus* weighing 40-55 g/ fish were collected from the ponds of Central Laboratory for Aquaculture Research at Abbassa, Abo-Hammad, Sharkia, Egypt. Fish were acclimated in an indoor tank for 2 weeks to laboratory conditions.

Acclimated fish were exposed to different concentration of malachite green and mortality were observed for 24, 48, 72 and 96- h. A static renewable bioassay method according to (Spraggue, 1973) and the median lethal probity analysis according to (Litchfield and Wileoxon, 1949) was adopted for the determination of 24, 48, 72 and 96-hr LC_{50}. A control group was maintained dechlorinated tap water. The 24, 48, 72 and 96 hr LC_{50} of malachite green for *O. niloticus* was 1.38, 0.90, 0.80 and 0.76 ppm respectively. A stock solution of malachite green was prepared by dissolving 0.38 g of annular grad of malachite green in100 ml of distilled water and the diluted with water to obtain the desired concentration (1/8 and 1/4 96 hr LC_{50} (0.095 and 0.190 ppm respectively) for this experiment.

The fish were distributed randomly in 120-liter glass aquaria, at a rate of 10 fish / aquarium that containing aerated tap water. These aquaria were divided into three groups with three replicates each per group. The first group was free of malachite green and maintained as a control. The second groups and third group were exposed to 0.095 and 0.190 ppm of malachite green. (Equivalent to 1/8 and 1/4 96 – h LC_{50}) respectively. Each aquarium was supplied with compressed air via air-stones from air pumps. The well-aerated water was provided from a storage fiberglass tank. The temperature was adjusted at 27±1 ^{o}C by means of thermostats.

Malachite green was obtained from El- Nasr chemical company (Egypt) and prepared in aquatic solution to provide the required concentrations.

Table (1): Showed experimental groups and their notation.

S. No.	Groups	Nation
1	Control (Malachite green free water)	C
2	Malachite green (0.095 ppm)	1/8 96 – h LC_{50}
3	Malachite green (0.190 ppm)	1/4 96 – h LC_{50}

Fish were fed frequently a diet containing 30% crude protein (CP) at a rate of 3% of live body weight twice daily for 30 days. Siphoning three quarters aquariums was done every day for waste removal and replacing it by an equal volume of water containing the same concentration of Malachite green. Dead fish were removed and recorded daily.

Physiological Analyses:

At the end of the experiment, samples of blood were taken from three fish specimens from each aquarium.

Fish were not fed for 24 h before sampling and were anaesthetized with buffered MS222 (50 mg /L) and blood samples were taken from caudal vein of an anaesthetized fish by sterile syringe using EDTA solution as anticoagulant. These blood samples were used for determining erythrocyte count according to (Dacie and Lewis 1984) and hemoglobin content according to (Van Kampen, 1961). Heamatocrit value (Hct) was calculated according to the formulae mentioned by Britton (1963).

Plasma was obtained by centrifugation of blood at 3000 rpm for 15 min and nonhaemolyzed plasma was stored in deep freezer for further biochemical analyses. After decapitation of fish, samples of liver and muscle were taken and frozen for further biochemical analyses. Plasma glucose was determined, using glucose kits supplied by Boehring Mannheium kit, according to Trinder (1969). Total protein content was

determined colorimetrically according to Henry (1964). Total lipids contents were determined colorimetrically according to Joseph *et al.* (1972). Activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel (1957.

Statistical Analysis:

The obtained data were subjected to analysis of variance according to Snedecor and Cochran (1982). Differences between means were done at the 5% probability level, using Duncan' s new multiple range test (Duncan, 1955).

RESULTS

Behavioral changes:

Upon addition of higher (lethal doses) concentration of the tested herbicides, fish showed initial disturbed swimming movements, rapid opercular movement and surfacing behavior indicative of avoidance response. This was followed by blackening of the whole body, unusual lethargy and tendency of the fish to settle at the bottom motionless with slow opercular movements. Lower levels of sublethal pesticide did not produce any obvious changes in fish behavior for the entire duration of the experiment.

Toxicity:

The median lethal concentration (LC_{50}) values and mortality percentage of malachite green on *Orechromius niloticus* after 24, 48, 72 and 96- hr of exposure were 1.38, 0.90, 0.80 and 0.76ppm, respectively in table (1A, B and 2 A, B).

Table (1 A & B): LC_{50} of malachite green for *Orechromis niloticus* after 24 and 48 hr of exposure, respectively.

	A				B			
Concentration Of malachite green	**No. of Dead fish**	**a**	**b**	**ab**	**No. of Dead fish**	**a**	**b**	**ab**
0.6	0	0	0.2	0	0	1	0.2	0.2
0.8	0	0.5	0.2	0.1	2	3	0.2	0.6
1	1	1	0.2	0.2	4	5	0.2	1
1.2	1	1.5	0.2	0.3	6	6	0.2	1.2
1.4	2	3.5	0.2	0.7	6	6	0.2	1.2
1.6	5				6			
Sum				1.3				4.2

LC_{50} = biggest dose - $\frac{(a \times b)}{n}$

a= sum of dead fish in two consequent groups. b= difference between used doses

n= number of fish used in each group which 6 fish specimens

A . 24 hr- LC_{50} of malachite green = 1.6- 1.3/6 =1.6-0.216=1.38 ppm

B. 48 hr- LC_{50} of malachite green = 1.6- 4.2/6 =1.6- 0.7= 0.9 ppm

Table (2 A & B): LC_{50} of malachite green for *Orechromis niloticus* after 72 and 96 hr of exposure respectively.

	A				B			
Concentration Of malachite green	**No. of Dead fish**	**a**	**b**	**ab**	**No. of Dead fish**	**a**	**b**	**ab**
0.6	0	2	0.2	0.4	0	2.5	0.2	0.5
0.8	4	4.5	0.2	0.9	5	5	0.2	1
1	5	5.5	0.2	1.1	5	5.5	0.2	1.1
1.2	6	6	0.2	1.2	6	6	0.2	1.2
1.4	6	6	0.2	1.2	6	6	0.2	1.2
1.6	6				6			
Sum				4.8				5

Lc50 = biggest dose - $\frac{a \times b}{n}$

a= sum of dead fish in two consequent groups b= difference between used doses

n= number of fish used in each group which 6 fish specimens

A- 72 hr -LC_{50} of malachite green = 1.6- 4.8/6 =1.6- 0.8= 0.8. ppm

B-96hr- LC_{50} of malachite green = 1.6- 5/6 =1.6- 0.83= 0. 76 ppm

Haematological parameters:

Data of the erythrocyte count (RBC**s**), haemoglobin content (Hb) and haematocrit % (Hct) obtained from fish exposed to sublethal dose of were given in table (3). It showed that higher level (1/8 and 1/4 LC_{50}) of malachite green induced a reduction in all blood parameters examined, and were significantly different from the control ones. Also the erythrocyte count was decreased significantly (1.715 ± 0.018 and, 1.53 ± 0.039 million mm3) in fish exposed to 1/8 and ¼ LC_{50} of malachite green, respectively when compared to the control ones (2.345 ± 0.044). The haemoglobin content was decreased significantly in fish exposed to1/8 and ¼ LC_{50} of malachite green, also haematocrit value was significantly decreased in two dose of malachite green.

Table (3): The erythrocyte count (C/mm^3), haemoglobin content (g /100 ml) and haematocrit value (%) of the *O. niloticus* after exposure to sublethal dose of malachite green

Item	**Erythrocyte Count (C/mm^3)**	**Haemoglobine Content (g/100ml)**	**Haematocrit value %**
Control	2.354 ± 0.044	7.568 [a] ± 0.107	24.71 ± 0.487
1/8 LC_{50}	1.715** ± 0.018	6.33*** ± 0.058	22.00** ± 0.534
1/4 LC_{50}	1.53*** ± 0.039	6.614*** ± 0.143	17.84*** ± 0.294

Data are represented as mean ± S.E n=6 * Significant at $p < 0.05$
** Significant at $p < 0.01$ *** Significant at $p < 0.001$

The blood indices calculated from the mean values of blood parameters for the aftermentioned treatments are given in (Table 4). Data shows that the MCV increased significantly in fish exposed to sublethal doses of malachite green. Also, the MCH was decreased significantly to (36.92 ± 4.13 and 130.45 ± 3.16) in fish exposed to 1/8 and ¼ LC_{50} of malachite green , respectively compared with the control. While, the MCHC concentration was decreased significantly (28.5 ± 0.45) *in O. niloticus* after exposed to low level of malachite green in table 4.

Table (4): Changes in mean cell volume (MCV), mean celll haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in the blood of Nile tilapia (*O.niloticus*) exposed sublethal dose of malachite green.

Item	MCV	' MCH	MCHC
Control	105.79 ± 3.47	33.52 ± 0.984	31.58 ± 0.67
1/8 LC_{50}	120.26* ± 4.13	36.92** ± 0.562	36.31*** ± 0.51
1/4 LC_{50}	130.45*** ± 3.16	43.39*** ± 1.08	28.5** 28.5± 0.45

Data are represented as mean ± S.E n=6 * Significant at $p < 0.05$
** Significant at $p < 0.01$ *** Significant at $p < 0.001$

Biochemical changes: The toxic effects on biochemical parameters demonstrated in table (5-6) :The plasma glucose concentration of control group was 48.94 ± 5.43 (mg %). Data presented in table (5) indicated that treatment of *O. niloticus* with sublethal dose (1/8 and 1/4 $LC_{50)\ of}$ malachite green induced highly significant increase in glucose concentration to (73.67 ± 7.01 and 92.97 ± 4.51 (mg/L), respectively as compared to control group. Similarly, the plasma total protein was decreased significantly to (3.164 ± 0.133 and 2.97 ± 0.33 g/100ml) in fish exposed two doses of malachite green when compared to the control fish group (3.744 ± 0.093 g/100ml) in table 5. On other hand, the mean value of plasma total lipids of the Nile tilapia exposed to malachite green for 30 are shown in table 5. It can be observed that the mean value of total lipid in fish exposed to high dose (0 .019 mg/l) of malachite green fungicides showed a significant decreased to (12.12 ± 0.99; P< 0.01) when compared the control fish group (13.19 ± 0.28 g/L).

Table (5): Changes in glucose, total protein and total lipids concentrations in plasma of Nile tilapia (*O.niloticus*) exposed to sublethal dose of malachite green.

Item	Glucose (mg/L)	Total protein (g/100ml)	Total lipid (g/L)
Control	48.94 ± 5.43	3.744 ± 0.093	13.91 ± 0.28
1/8 LC_{50}	73.67* ± 7.01	3.164*** ± 0.133	13.25 ± 0.298
1/4 LC_{50}	92.97** ± 4.51	2.974*** ± 0.33	12.12*** ± 0.99

Data are represented as mean ± S.E n=6 * Significant at $p < 0.05$
** Significant at $p < 0.01$ *** Significant at $p < 0.001$

Bioassay of plasma AST activity in blood of *O. niloticus* revealed a remarkable highest (72.30 ± 3.874 and 86.266 ± 3.33 IU/l) at two sublethal dose (1/8 and 1/4 C_{50}) of malachite green groups, respectively versus control group (58.46 ± 2.46 IU/l) in table 6.

The average level of ALT activity in plasma of *O. niloticus* of the control group was 4.12 ± 0.845, IU (Table 6). As shown in table (6) the ALT levels were significantly increased significantly fish exposed to all treatment of malachite green (5.72 ± 0.452 and 11.11 ± 0.847, respectively).

Table (6): Changes in aspartate aminotransferase activity (AST)_and alanine aminotransferase (ALT) activity (IU/L) in plasma of Nile tilapia (*O.niloticus*) exposed to Cd with or without EDTA.

Item	**AST**	**ALT**
Control	58.46 ± 2.46	4.12 ± 0.845
1/8 LC50	72.30** ± 3.874	5.72 ± 0.452
1/4 LC50	86.266** ± 3.33	11.11* ± 0.847

Data are represented as mean ± S.E n=6 * Significant at $p < 0.05$
** Significant at $p < 0.01$ *** Significant at $p < 0.001$

DISCUSSION

Clinical signs shown in this experiment have been demonstrated to be sensitive indicator of physiological stress in fish subjected to sublethal concentration of pollution (Davis, 1973). Thomas and Rice (1975) and Cruz *et al.* (1988) suggested that increased opercular movement may be caused by decrease efficiency in oxygen uptake or transport.

96-hour LC_{50} is useful measure of relative acute lethal toxicity to organisms under certain experimental conditions. However, these values do not represent safe concentration in natural habitats. The safe level of a compound is derived by multiplying the 96- hours LC_{50} with an application factor of 0.1 to 0.01 for the less persistent organophosphate pesticides (Koesomadinata, 1980).Toxicity of malachite green to aquatic animals depends on the concentration and exposure time . Exposure time is very important. Static test has been performed to determine the acute toxicity of malachite green to the fingerlings of Nile tilapia. The results showed that LC_{50} values were 1.38, 0.9, 0.8 and 0.76 mg/L for 24, 48, 72 and 96 hr, respectively. The results suggested that malachite green is highly toxic to the teleost fish *Orechromis niloticus*, and the toxicity increases with increase in the concentration of the toxicant These data are in agreement with of those (Srivastava *et al*., 2004a) showed that LC_{50} values of malacihite green for fingerlings *Cyprinus carpio* were 2.65, 1.95, 1.60 and 1.30 mg/L for 24, 48, 72 and 96 h, respectively. It indicated that the longer the exposure time is, the smaller the LC50 values. Similarly, El-Neweshy and Abou Srag (2011) found that the toxicity (96 – h LC50) of malachite green for *O. niloticus* was 0.76 mg/l. Also, Hanan (2001) determined 96-hrs LC_{50} as 0.075 mg/L for Nile tilapia fingerlings. While, Srivastava *et al.* (2004) reported that acute toxicity (96-hr LC_{50}) of malachite green was 0.238 for Channel catfish (*Ictarulus punctatus*).

The importance of haematology in diagnosis of fish diseases and for the assessment of the effect of pollution has been widely accepted. Changes in haematology of fishes in response to stress agents are indicators of stress. Erythrocyte count, haemoglobin and haematocrit values in O. *niloticus* exposed to malachite green were lower than the control resulting in hypochromic anemia. This type of anemia could attributed to the effect of malachite green on the erythrocytes as a result of prooxidative effect (Vutukuru *et al*., 2005) and tubular nephrosis that leading to drop in erythropoietin production and attenuation of erythropoiesis ((Horiguchi *et al.*, 1994). These data are in agreement with El-Neweshy and Abou Srag (2011), they reported significant decreased in RBC, Hct and MCHC in Nile tilapia after exposure to malachite green at the 2nd and 4th weeks. This may be attributed to depletion of interstitial hemopoietic tissue in posterior kidney and white pulp depletion in spleen. This explanation was supported by El-Boushy (1994) and Robert (2001). These results are partially agreed with Musa and Omoregie (1999) who reported RBCs and haematocrit reduction. Also, the decrease in RBCs count may be attributed to haematopathology or acute haemolytic crisis that results in sever anemia in most vertebrates including fish species exposed to different environmental pollutants (khangarot and Tripathi, 1991) or may be the decrease in the RBCs may be attributed to reduction of growth and other food utilization parameters which results in sever aneamia (James and Sampath, 1999). Shalaby *et al.* (2007) found that the RBCs, Hb and Hct were decreased significantly in Nile tilapia poisoned bybutataf herpacide. The reduction of erythrocyte count (RBCs), haemoglobin count (Hb) and haematocrit value (Hct) levels can be attributed possibly to progressive damage to the kidney by the herbicide and for lysis of red blood cells.

The calculated blood indices MCV, MCH and MCHC have a particular importance in anemia diagnosis in most animals (Coles, 1986). The perturbations in these blood indices (increase MCV, MCH and

decreased MCHC) may be attributed to a defense against malachite green toxicity through the stimulation of erythropiosis or may be related to the decrease in RBCs, Hb and Hct due to the exaggerated disturbances that occurred in both metabolic and hemopoietic activities of fish exposed to sublethal concentration of pollutants (Mousa, 1999). These data were in agreement with Srivastava *et al.* (2009) who study the effects of four days therapeutic bath of malachite green and formalin (F) on catfish(Heteropneustes fossilis),0.25 ml F/litre water revealed that the total RBC count, Hb, Hct, MCV and MCHC values showed a significant decrease after four days bath.

The blood glucose measurements are known to be sensitive indicator of environmental stress in fish . From these results, it is clear that the malachite green as shown by the elevated blood glucose level affected as stresses on fish. The elevated blood glucose level in our study could be attributed to the generalized stress response leading to an increase in the pituitary internal activity with an increase in the secretion of corticosteroid Yildiz and Pulatsu (1999) stated that, Nile tilapia exposed to varying concentrations (0.1, 0.5 and 1.0 mg/litre) of a mixture of formalin, malachite green and methylene blue (FMC) for 1, 10 and 60 min. treatment of fish with FMC elicited marked elevations of plasma glucose. Endosulfan pesticide induced hyperglycemia was observed in common carp, *Cyprinus carpio* exposed to sublethal dose of endosulfan for 21 days (Chandrasekar and Jayabalan, 1993). These increases may be due to decrease in serum insulin required to control the glucose level either through a reduction in the rate of its production or the effectiveness of the available insulin. This could be attributed to alteration in the rate and degree of digestion, absorption and utilization of glucose due to the exposure to the pesticide, i.e impaired carbohydrate due to exposure to the pesticides.

The quantitative determination of the total protein in serum and liver reflects the liver capacity of protein synthesis and denotes the osmolaity of the blood and the renal impairments. So, it is of a valuable factor in the diagnosis of toxicity in fish. In the present study the total plasma protein was decreased significantly and the effect was dose dependent. This decrease might have been attributed to several pathological processes including plasma dissolution, renal damage and protein elimination in the urine, a decrease in liver protein synthesis, alteration in hepatic blood flow and/ or hemorrhage into the peritoneal cavity and intestine (Salah El-Deen *et al.,* 1996). These results agree with those of Mousa (2004) who found a significant decrease in plasma protein in the Nile tilapia after toxication with machete herbicide. Also the decrease in protein content of dimethoate intoxicated fish indicated the physiological adaptability of the fish to compensate for pesticide stress. To overcome the stress the animals require high energy. This energy demand might have led to the stimulation of protein catabolism (Begum and Vijayaraghavan, 1995). The decrease in serum and tissue protein may occurred due to the increase of protein breakdown as a result of stress stimulated corticosteroid hormones to supply amino acids for gluconeogenesis to provide glucose to compensate for the increase in energy demands under stressful conditions.

Lipids, because of their rapid metabolic transformation, are considered transient body materials, but they represent the major source of stored chemical energy and their presence or absence reflects the physiological capacity of fish (Schreck and Moyle, 1990). The influence of stress on lipid metabolism in fishes was studied by several authors (El- Nagar *et al.,* 2001). In the present study *O. niloticus* exposed to sublethal concentration of saturn or glyphosate herbicide showed a gradual decrease in their serum total lipids and cholesterol. These changes may be of value for energy production to succumb the increasing demand of energy in Nile tilapia on exposure to saturn and glypohosat.

This assumption is supported by that of El-Nager *et al.* (2001) and Shalaby (2004).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes are frequently used to diagnose the sublethal damage to certain organs specially the liver (Benedeczky *et al.,* 1984). In the present study showed that the fish plasma AST and ALT enzyme activities were decreased at the 30^{th} day of exposure of malachite green. These results were attributed to the hepato-cellular damage and inhibition of enzymes synthesis as a result of toxic effect of the herbicide (Mousa, 2004). It could be concluded that malachite green are found to be highly toxic to Nile tilapia nilotica (*O. nilotucus*) that reflected hematological as well as biochemical findings in fish.

REFERENCE

Begum, G. and S. Vijayaraghavan. 1995. In Vivo toxicity of dimethoate on protein and transaminases in the liver tissue of fresh water fish *Clarias batrachus* (Linn). Bulletin of Environ. Contam . Toxicol., 54: 370- 375.

Benedeczky, I.; B. Biro and Z.S. Scaff. 1984. Effect of 2, 4-D containing herbicide (Diconirt) on ultrastructure of carp liver cells. Biol. Szeged., 30: 107-115.

Britton, C.J. 1963. "Disorders of the Blood" 9th ed. I. A. Churchill, Ld. London

Chandrasekar, S .and N. Jayabalan. 1993. Hematological responses of the common carp, *Cyprinus carpio* L. Exposed to the pesticide endosulfan. Asian. Fish.Sci., 6 (3): 331-340

Coles, E.H. 1986. Veterinary Clinical Pathology .W. B. Saunders, Philadelphia. pp. 10- 42.

Cruz, E.R.; M.C. De La Cruz and N.A. Sunaz. 1988. Hematological and histopatholofical changes in *Oreochromis mossambicus* after exposure to molluscicides aquatin and Brestan., p.99-110 in R. S. V. Pullin , T. Bhukaswan, K. Tonguthi andJ. L. Maclean(eds). The second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, 623p. Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines.

Dacie, J.V. and S.M .Lewis. 1984. Practical Haematology. Churchill Living Stone. London.

Davis, J.C. 1973. Sublethal effects bleached kraft puple mill effluent on respiration and circulation in sockeye salmon (*Oncorhyncus nerka*). J. Fish. Res. Board. Can., 30: 369 – 377.

Duncan, D.B. 1955. Multiple range and multiple (F) test. Biometrics, 11: 1- 42.

El-Boushy, M.A. 1994. The effect of molluscicides polluted on the blood picture and serum biochemical parameters in Clarias lazera. M.V.Sc. Thesis Faculty of Veterinary Medecine, Suez-Canal University.

El- Nagar, G.O.; M. Salah El-Deen and H.H. Abbas. 2001. Effect of copper and lead toxicity on some physiological characteristics of blue tilapia *Oreochromis aureus* and African cat fish (*Clarias gariepinus*). J. Egypt. Ger. Soc. Zool., 34 (A): 225 - 244.

El-Neweshy, M.S and M.A. Abou Srag. 2011. Chronic malachite green toxicity in Nile tilapia: Pathological and hematological studies with special reference to quantitative histopathological assessment Researcher., 3(4):55-64.

Hanan, A. 2001. Determination of the Toxic and Therapeutic Levels of some Water-Borne Chemotherapeutics Administered to Cultured

Fresh Water Fishes in Egypt; Assuit University center for environmental studies.; Thesis (M.Sc).

Henry, R.J. 1964. Colorimetric determination of total protein. In: Clinical Chemistry. Harper and Row Publ., New York, pp 181.

Horiguchi, H.; H. Ternishi; K. Niiya; K. Aoshima; T. Katoh; N. Sakurayawa and M. Kasuy. 1994. Hypoproducton of erythropoietin contributes to anemia in chronic cadmium intoxication.Clinical study on Itaiitai disease in Japan. Arch. Toxciol., 68: 632-636.

James, R. and K. Sampath. 1999. Effect of the ion- exchanging agent, Zeolite, on reduction of cadmium toxicity: an experimental study on growth and elemental uptake in *Heteropneustes fossilis* (Bloch). J. Aqua. Trop., 14 (1): 65- 74.

Joseph, A.; M. Knight; S. Anderson; M. James and H. Rawie. 1972. Chemical basis of the sulfophospho-vanillin reaction for estimating total serum lipid. Clin. Chem., 18 (3): 198-201.

Khangarot, B.S and D.M. Tripathi. 1991. Changes in humoral and cell-mediated immune responses and in skin and respiratory surfaces of cat fish *Saccobranchus fossillis*, following copper exposure. Ecot. Envir. Safety., 22 (3): 291- 308.

Koesoemadinata, S. 1980. Pesticides as a major constraint to integrated agriculture aquaculture farming system, p. 45-51. In R.S.V. Pullin and Z.H Shehadeh (eds.) Integrated agriculture-aquaculture farming system. ICLARM Conference Proceedings 4. International center for living Aquatic Resources Management, Manila, Philippines and Southeast Asian Regional Center for Graduate Study and Research in Agriculture , Los Banos, Philippines.

Kumar S. A.; A.K. Maurya and J.P.N Singh. 2007. Acute toxicity of malachite green to sensory organs of a catfish *Mystus vittatus*. J. Exper. Zool. India, 10: 21-25.

Litchfield, J. T and F. Wileoxon. 1949. A simplified method for evaluatin dose- effect experiments. J. Pharmacol. . Exp. Ther., 96: 59-113

Mei-Jie, L.; L. An-Na; X. Xiang-Rong; W. Shan; I. Sha; A. Xu-Xia and L. Hua-Bin. 2012. Degradation and Removal of Malachite Green in Environment. International Journal of Environment and Bioenergy, 2 (1): 1-18.

Mousa, M.A. 1999. Biological and physiological studies on the effect of the gramoxon and stomp herbicides on Nile tilapia *(Oreochromis niloticus*). Fact. Sci. Zool. Dep. Cairo. Univ.,200p (Ph.D. Thesis)

Mousa, M.A. 2004. Toxicological studies on the effect of machete herbicide on some fish species. Egypt. J. Appl. Sci., 19 (5): 1-11

Musa, S.O. and E. Omoregie. 1999. Haematological changes in the mudfish, Clarias gariepinus (Burchell) exposed to malachite green. Journal of aquatic sciences. 14: 37–42.

Omoregie, E.; P.C. Ofojekwu.; J.C. Anosike and A.O. Adeleye. 1998. Acute toxicity of malachite green to the *Nile tilapia*, *Oreochromis niloticus* (L.). J. Aquat. Trop. 13 (4): 33–237.

Reardon, I.S. and R.M. Harrell. 1990. Acute toxicity of formalin and copper sulfate to striped bass fingerings held in varying salinities. Aquaculture, 87: 255–270.

Reitman, S. and S. Frankel. 1957. Colorimetric determination of glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. Clin. Pathol., 28: 53-56.

Roberts, R.J. 2001. Fish pathology Third edition. Harcourt publishers limited 2001.

Salah El-Deen, M.A. and W.A. Rogers. 1996. Changes in total protein and transaminases activity of grass carp exposed to diquat. J. Aquat. Animal Health, 5: 280-286.

Schreck, C.B. and P.B. Moyle. 1990. Methods of fish biology. Am. Fish. Soc. Bathesda, Maryland.

Shalaby, A.M. 2004. The opposing effect of ascorbic acid (Vitamin C) on ochratoxin toxicity in Nile tilapia (*Oreochromis niloticus*). p.209-221 in R. Bolivar.; G. Mair and K. Fitzsimmons (eds). The 6 th International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 60, 623p. Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines.

Shalaby, A.M.; M.A. Mousa.; H.A. Tag-Eldain. 2007. Toxicological effect of butataf herbicide on some physiological aspects and THE reproductive performance of Nile TILAPIA Oreochromis niloticus., Egypt Aqyatic Biology & Fish. 11 (2): 145-163.

Snedecor, G. W. and W. G. Cochran. 1982. Statistical Methods. 6*th* edition. Iowa State Univ. Press., Amer., IA, USA, pp 593.

Spraggue, J.B. 1973. The ABCs of pollutant bioassay using fish In Biological methods for Assessment of water quality. ASTM 528. Amer. Testing. Material., pp.6-30.

Srivastava, S.; A. Gupta and P.K. Srivastava. 2004a. Acute toxicity of malachite green to fingerlings of common carp, *Cyprinus carpio*. *Biol. Memoirs*, 30: 120-121.

Srivastava, S.; A. Gupta; S. Chaturvedi; A. Abhinavand and R. Singh. 2009. Effects of short-term therapeutic bath of malachite green

and formalin on certain hematological parameters of afreshwater catfish, (*Heteropneustes fossilis*) (Bloch). Journal of Experimental Zoology, India. 12 (1): 79-83.

Srivastava, S.J.; N.D. Singh; R. Sinha and A.K. Srivastava. 1998. Acute and chronic toxicity of malachite green: Microscopic changes in the pituitary gonadotropic cells and gonads in a freshwater catfish, *Heteropneustes fossilis* (Bloch). Biol. Sci., 68: 253-256.

Srivastava, S.J.; N.D. Singh.; A.K. Srivastava and R. Sinha. 1995. Acute toxicity of malachite green and its effects on certain blood parameters of a catfish, *Heteropneustes fossilis*. Aquat Toxicol., 31: 241-247.

Srivastava, S.; S. Ranjana and D. Roy. 2004b. Review, Toxicological effects of malachite green. Aquatic Toxicology 66: 319–329

Trinder, P. 1969. Determination of glucose concentration in the blood. Ann. Clin. Biochem., 6:24.

Thomas, R.E. and S.D. Rice. 1975. Increased opercular rates of pink salmon (*Onchorhynchus garbuscha*) fry after exposure to the water-soluble fraction of prudhoe Boy Crude oil. J . Fish. Res. Board. Can., 32: 2221-2224.

Van Kampen, E.J. 1961. Determination of haemoglobin. Clin. Chem. Acta, 5: 719-720.

Vutukuru, S.; C. Suma; K. Madhavi; S. Juveria; J. Pauleena; J. Raoand and Y. Anjaneyulu. 2005. Studies on the Development of Potential Biomarkers for Rapid Assessment of Copper Toxicity to Freshwater Fish using Esomusdanricus as Model. Int. J. Environ. Res. Public Health, 2 (1): 63-73

Yildiz, H. and S. Pulatsu. 1999. Evaluation of the secondary stress response in healthy Nile tilapia (Oreochromisniloticus) after

treatment with a mixture of formalin, malachite green and methyleneblue.Aquacult. Res.,30 (5): 379-383

Yonar, M and S. Yonar. 2010. Changes in selected immunological parameters and antioxidant status of rainbow trout exposed to malachite green (Oncorhynchus mykiss, Walbaum, 1792). Pesticide Biochemistry and Physiology, 97: 19–23.

Zhai, Y.X.; Y.Y. Guo; X. Geng; C. Zhang and J. S. Ning. 2007. Advances in studies on metabolic mechanism and bio-toxicity of malachite green. J. Ocean Univ. China, 37: 27-32.

التاثير السام لمبيد ملاكيت جرين على النواحى الفسيولوجية والبيوكميائية لاسماك البلطى النيلى

عادل محمد عيسى شلبى[1]، أحمد عبد الله عبد الرحمن على [1]،
معالى عبدالرحمن محمد[2]، امانى عبد العزيز غريب[1]

[1]قسم بحوث التفريخ وفسيولجيا الأسماك.
[2]قسم بحوث البيئة وبيولوجيا الأسماك.
المعمل المركزى لبحوث الثروة السمكية- العباسة- الشرقية- مصر.

الملخص العربى

أجريت هذه الدراسة للوقوف على تأثير التركيزات الغير المميتة للملاكيت جرين والمستخدم لمقاومه الفطريات على بعض النواحى الفسيولوجية والبيوكميائية لاسماك البلطى النيلى. فبعد تعين الجرعة القاتلة لنصف أسماك البالغة خلال ٢٤، ٤٨، ٧٢، ٩٦ ساعة وكانت ١.٣٨، ٠.٩، ٠، ٠.٨، ٠.٧٦ جزء فى المليون، على التوالى. وتم اختبار ٨/١، ٤/١ الجرعة الميتة خلال ٩٦ ساعة منها لدراسة تأثيرها على الحالة الصحية للاسماك . ولقد أظهرت الأسماك المعاملة بالمبيد اضطرابا فى سلوكها بما تأديه من حركات غير متزنة و كانت النتائج المتحصل عليها كالآتى : المبيد بتركيزاته المختلفة تسبب فى النقصان الغير معنوي لعدد كرات الدم الحمراء (RBCs) ومحتوى الهيموجلوبين (Hb) ونسب الهيماتوكريت فى الدم (Hct) ومتوسطات حجم كرات الدم الحمراء (MCV) وكم الهيموجلوبين بها (MCH) كذا تركيز الهيموجلوبين فى الدم (MCHC) بالإضافة إلى معامل لون الدم (CI). كما أن المبيد بتركيزاته المختلفة تسبب فى نقصان احصائى متباين المعنوية فى الدهون والبروتينات الكلية فى دم الأسماك الواقعة تحت تأثيره و هذا بخلاف ما حدث للجليكوز فى الدم كذا الإنزيمات الناقلة لمجموعة الأمين (AST، ALT) والتى لوحظ زيادتها بمعنوية عالية فى نفس هذه الأسماك مقارنتا مع أفراد المجموعة الضابطة. لذلك نوصى بعدم استخدامة فى البيئة المصرية والبحث عن بدائل اخرى اكثر اماناوسلامة منة للحفاظ على الثروة القومية والصحة العامة للمواطنين.