Biochemical Effects of Certain Pesticides on Common Carp (*Cyprinus Carpio L.*)

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ABSTRACT

Effects of sublethal concentrations of herbicide; glyphosate (4 ppm) and fungicide; $CuSO_4$ (8 ppm), on the activity of certain enzymes of common carp after 96 hours of exposure were investigated. The data showed that $CuSO_4$ caused significantly inhibition in the activity of blood δALAD, while glyphosate did not change the enzyme activity. Also, brain AChE activities were significantly inhibited by the tested pesticides, but the differences between the treated groups and control on serum AChE were not significant. Both pesticides non-significantly inhibited brain and serum BuChE activities. Glyphosate and CuSO4 caused significantly increase in liver; AST and ACP activities, while ALT activity was no significantly changed. On the other hand, an oxidative stress with both pesticides was found by increasing the activity of liver GST and decreasing SOD activity. The activity of C-ase was significantly increased by glyphosate, but decreased by $CuSO_4$ compared to the control. The level of LPO was significantly increased in all carp organs; i.e brain, gill, muscle, kidney and liver treated with glyphosate. The decrease of LPO level in gill and liver treated with $CuSO_4$ was not significant.

ABBREVIATIONS: CuSO4: copper sulfate; δALAD: delta amino levulinic acid dehydratase; AChE: acetyl cholinesterase; BuChE: buteryl cholinesterase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ACP: acid phosphatase; GST: glutathione - S – transferase; SOD: superoxide dismutase; C-ase: catalase; LPO: lipid peroxidation.

INTRODUCTION

Pesticides are substances designed to kill, rep+el, or mitigate pests. They include a number of chemical and biological agents commonly used to control a broad range of pests. Extensive uses of these pesticides cause serious problems on non-target organisms **(Parker and. Goldstein, 2000)**. Also, use pesticides in pest control programmers seem to produce many physiological and biochemical changes in freshwater organisms by influencing the activities of several enzymes **(Heath, 1995)**. Several authors investigated the biochemical effects of different pesticides on fish and reported changes in some fish enzymes activities; cholinesterase **(Ferrari *et al.*, 2004 and Rao, 2004)**, glutathione – S – transferase, transaminases, phosphatases, **(Orue and Uner 2002, El-Gendy *et al.*, 1990a and Bayoumi *et al.*, 1996)**.

Reactive oxygen species (ROS) include the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and lipid peroxides. They are known to cause oxidation of membrane phospholipids, lipid peroxidation, and protein damage leading to cellular dysfunction and eventually to disease. ROS are continuously produced in living cells naturally as a result of leakage of electrons on the electron transport chain in mitochondria. In addition, they can be produced in cells by various enzymatic mechanisms, auto-oxidation of small molecules, or in response to xenobiotics and exogenous environmental exposures. Cells have well-developed antioxidant systems to protect themselves from ROS. These include low molecular-weight antioxidants like ascorbic acid and glutathione; and antioxidant enzymes such as catalase, glutathione peroxidases and superoxide dismutases (SODs) **(Halliwell and Gutteridge, 1989)**. Many environmental and industrial agents such metals and pesticides will provoke an oxidative stress response, overwhelm antioxidant defense systems and result in oxidative damage to tissues. The toxicities usually involve tissue damage in kidney, liver or central nervous system **(Sies, 1991)**. Many pesticides causing oxidative stress by affecting on the antioxidant enzymes activity and the lipid peroxidation level in the different organs of fish, **(Sharma *et al.*, 2005, Orue *et al.*, 2004, Zhang *et al.*, 2004, Yarsan *et al.*, 2002, Orue and Uner , 2002)**.

The present study was carried out to investigate the effects of the sublethal concentrations of glyphosate and copper sulphate on some enzymes activities in different organs of (*Cyprinus carpio L*) after 96 hours of exposure. Various parameters of the pesticides oxidative stress were also determined in fish organs.

MATERIALS AND METHODS

Chemicals

Formulated organophosphorus herbicide, glyphosate 48 % EC (Monsanto Company, USA) and fungicide, copper sulfate ($CuSO_4$, chemical pure grade) were used in this study. All other chemicals were of highest purity grade commercially available.

Animals

The common carp (*Cyprinus carpio l.*), 110.5 ± 7.45 g weight and 16.3 ± 1.2 cm length was supplied from Saft Khalid Farm in Etay Al-Barood, Behera Governorate. The fish were acclimatized in glass aquaria with dechlorinated tap water and were fed daily with commercial diet for two weeks before the experiment.

Treatment of animals:

The healthy (*Cyprinus carpio l.*) was fasted 24 hours before treatment and during the exposure. Fish were divided into three groups; one group served as control, while the remaining two groups were exposed to the sublethal concentrations; 4 and 8 ppm which represented 2/3 LC_{50} of glyphosate and $CuSO_4$, respectively, as we previously determined **(El-Gendy *et al.*, 1990a)**. Nine fish were used for each treatment. After the exposure period (96 hours), blood samples of treated and untreated fish were withdrawn from the caudal vein in two tubes, the first containing the anticoagulant (EDTA) and the second without anticoagulant for serum. Then brain, liver, kidney, gill and muscle were removed rapidly, washed with physiological saline solution and prepared for biochemical investigations.

Enzymes Assay:

Tissues from control and treated groups were homogenized in 10 volumes (w/v) of physiological saline solution. The homogenates were centrifuged at 8,000 xg for 30 min at 4 ° C. The supernatants were used for measurements of each enzyme activity. The activity of δALAD in blood was determined and calculated by the method of **Burch and Siegel (1971)**. The activities of AChE and BuChE in brain and serum were determined according to **Ellman *et al.* (1961)**. Activities of the transaminases, ALT and AST were assayed according to the **Reitman and Frankel method (1957)** using a commercially available kit. ACP activity was measured according to **Bessey *et al.* (1946)**. GST activity was determined spectrophotmetrically by the method of **Vessey and Boyer (1984)**. C-ase activity was estimated by the

method of **Beers and Sizer (1952)**. SOD activity was measured by the adrenochrome method of **Misra and Fridovich (1972) and Hien *et al.* (1974)**.

Lipid peroxidation (LPO):

LPO in all organs of fish; i.e brain, gill, liver, kidney and muscle was determined according to **Placer *et al.* (1966)** and **Nair and Tuner (1984)** using thiobarbituric acid (TBA). The LPO levels expressed as n moles malondialdhyde (MDA).

The protein was determined by the method of **Lowry *et al.* (1951)** using bovine serum albumin as a standard.

Statistical analysis:

The data analyzed using analysis of variance technique (ANOVA) and probability of 0.5 or less was considered significant. Statistical analysis was done with Costat Program **(Version 2, CoHort Software, 1986)**.

RESULTS AND DISCUSSION

δALAD activities:

δALAD is the sulphydryl enzyme responsible for the concentration of the two molecules of delta amino levulinic acid (ALA) to form the monopyrolle porphobilinogen needed for hemoglobin synthesis.

Table (1) illustrated the effect of glyphosate and $CuSO_4$ on red blood cell δALAD activity. Data showed that $CuSO_4$ caused significant inhibition in the enzyme activity to 74.8 % of control, while glyphosate caused no significant inhibition compared to the control. Copper sulphate showed mild interaction and inhibition of δALAD activity (*in vitro*) in wild and domestic quail **(El-Gendy *et al.*, 1990b)**. Inhibition of red blood cell ALAD activity became accepted as a standard bioassay to detect the acute and chronic metal exposure especially, lead. **Stone, *et al.* (1977)** demonstrated that the activity of the red blood cells δALAD in Japanese quail is a very sensitive indicator of lead exposure. **El-Gendy *et al.* (1988)** reported a correlation between δALAD activity and lead level in blood of occupational lead exposure. In our study the δALAD activity was inhibited by CuSO4 exposure so, it can be depending on ALAD activity as sensitive biomarker and accurate for the copper effect as well as lead toxic effect.

AChE and BuChE activities

The activities of AChE and BuChE in brain and serum of carp *(Cyprinus carpio L)* are summarized in Table (1). It was found that the brain AChE activities were significantly inhibited by glyphosate and $CuSO_4$. On the other hand, there were no significant differences between the treated groups and control of serum AChE activity. In addition, there were no significant changes in the brain BuChE by the two pesticides, but glyphosate caused significant inhibition in the serum BuChE activity. Many investigators reported that organophosphate inhibit the cholinesterase activity in carp (**Ferrarri *et al.*, 2004 and Das and Mukherjee, 2000**). Moreover, **Osten *et al.* (2005)** reported that the commercial glyphosate, chlorpyrifos and carbofuran formulations caused AChE inhibition of brain mosquito fish in a single pesticides exposure or a mixture of them. On the other hand, treatment of carp with $CuSO_4$ (5 ppm) for 96 hours led to 50 % decrease in the serum AChE followed by a transient increase over the control level after two weeks (**Szabo *et al.*, 1992**). The inhibition of these enzymes disturbs the normal nervous function and finally results in the death of animals.

Detoxifying enzymes in liver

Transaminases and phosphatases are important in the biological processes. They are responsible for detoxification processes, metabolism and biosynthesis of energetic macromolecules for different essential function. Any interference with these activities out of the normal range should lead to biochemical impairment and lesions of the tissues and cellular functions (**Latner, 1978**).

Transaminases:

Transaminases are important and critical enzymes in the biological processes. They play a role in amino acid catabolism and biosynthesis. Data presented in Table (2) showed that AST activities significantly increased with glyphosate and $CuSO_4$ treatment. While ALT activities not significantly changed in both treatments compared to the control. The results clearly indicated that $CuSO_4$ had more toxic effects on the transaminases of carp. These results are matched with those of **Aly (2000)** who reported that 4 ppm of pyrazophos caused a significant increase in the activities of liver AST and ALT of *Cyprinus carpio L.* Also, **Jiraungkoorskul *et al.* (2003)** reported that a structural damage could be correlated to the significant increase in AST and ALT activities in Nile Tilapia at the second and third months of exposure to glyphosate sublethal concentrations

(5 and 15 ppm). Several studies reported that $CuSO_4$ caused tissue damage and stress effects in carp **(Nemcsok and Boross 1982; Asztalos *et al.*, 1990; Karam *et al.*, 1998)**. In addition, $CuSO_4$ caused serious disturbances in energy uptake and secretion possesses for three fish species, i.e. common carp, silver carp and European wells by increasing the serum transaminases activities **(Rojik *et al.*, 1983)**. The disruption of transaminases from the normal value denotes biochemical impairment and lesions of tissue and cellular function because they are involved in the detoxification processes and metabolism **(Tordlor and Van Heemstra-Leqin, 1980)**.

Acid phosphatase

The effects of glyphosate and $CuSO_4$ on liver acid phosphatase of carp are shown in Table (2). The results showed that the activity increased significantly by both pesticides compared to the control. There were significant differences between glyphosate and $CuSO_4$ on the enzyme activity. **El-Gendy *et al.* (1990a)** reported that LC_{50} and 1/2 LC_{50} of glyphosate and $CuSO_4$ increased the activity of liver carp. **Das and Mukherjee (2000)** found that the activity of carp ACP was affected by the sublethal concentrations (1.12 and 0.22 ppm) of quinalphos. **Aly (2000)** reported that up to 18 ppm of pyrazophos caused no significant increase in the carp liver ACP activity. The changes in the ACP activity may be related to the biotransformation and elimination of the tested pesticide **(Hans, *et al.*, 1993)**. It is known that the detoxification of the toxic materials that enter the body occurs mainly in liver. The toxic effect occur by pesticides could affect the liver cells and consequently its function, responding to the release of many enzymes in fish body **(Hinderer and Menzer, 1976)**.

Antioxidant enzymes:

The hepatic oxidative stress of glyphosate and $CuSO_4$ on fish estimated by the antioxidant enzymatic defenses; GST, catalase and SOD, and LPO are summarized in Table (2) and Fig. (1).

GST activity

Glutathione transferases are ubiquitous group of detoxification enzymes involved in the metabolism of pesticides and other toxins. Glutathione transferases have direct antioxidant activity and are involved in the metabolism of dopamine. The effects of glyphosate and $CuSO_4$ on (*Cyprinus carpio L*) liver GST activity were summarizes in Table (2). The present results showed that liver GST activity was not significantly increased in the treated animals with glyphosate and $CuSO_4$. Slight changes in liver GST activity of carp occurred by the fungicide pyrazophos

(Aly, 2000). Gill GST activity of mosquito was not altered by treatment with formulated glyphosate and chlorpyrifos, but carbofuran was significantly inhibited the enzyme activity **(Osten *et al.*, 2005)**.

Catalase activity:

C-ase, which eliminates H_2O_2 from environment, is a part of the cellular defense system against ROS. A significant increase was found in the enzyme activity in treated group with glyphosate (4 ppm). In contrast, a significant decrease in liver C-ase activity was produced by a concentration (8 ppm) of $CuSO_4$.

SOD activity:

Superoxide dismutase is an enzyme, which catalyzes the dismutation of superoxide free radical ions ($O_2.^- + O_2.^- + 2H^+ \rightarrow O_2 + H_2O_2$).

Glyphosate and $CuSO_4$ caused a decrease in total SOD activity, but $CuSO_4$ was more toxic to SOD than glyphosate. Oxidative stress describes a condition in which cellular antioxidant defense or insufficient to keep the level of ROS below a toxic threshold. This may by either due to excessive production of ROS, loss of antioxidant defenses or both **(McCord and Fridovich, 1969)**. Many authors reported that pesticides induce oxidative stress in carp by affecting the antioxidant enzymatic defenses **(Hai *et al.*, 1997; Poovala *et al.*, 1998; Yarsan *et al.*, 2002)**. **Orue *et al.* (2004)** reported that the herbicide; 2,4dichlorophenoxy acetic acid (2,4 D) and the insecticide; azinophos methyl induce oxidative stress in bolti (*O. niloticus*) and carp (*Cyprinus carpio L*) fish by increasing the activity of SOD in fish gill and GST activity in kidney.

Lipid peroxidation (LPO) levels

LPO can be defined as the oxidative deterioration of lipids containing any number of carbon-carbon double bonds. The relatively high content of fat in fish tissues especially of polyunsaturated fatty acids is a well-known fact. Lipid hydro peroxides are non-radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol itself. Their formations occur in enzymatic or non-enzymatic reactions involving activation of the ROS, which are responsible for toxic effects in the body via various tissue damages. Increase of LPO level pointed to the phenomenon connected with degeneration processes of the dystrophic muscle, entailing increase in the free radicals **(Matcovics *et al.*, 1980)**.

Figure (1) illustrated the effects of glyphosate and $CuSO_4$ on LPO in the different organs of (*Cyprinus carpio L*). It is clear that glyphosate significantly increased the level of LPO in all carp organs compared to the control. The value of LPO in muscle and kidney were also significantly increased with the $CuSO_4$. In contrast, there were no significant decrease in

LPO of gill and liver treated with $CuSO_4$, and no significant increased in brain compared to the control. Many studies reported that pesticides cause increase in the LPO in the different tissues of carp **(Li *et al.*, 2004; Sayeed *et al.*, 2003; Poovala *et al.*, 1998)**. Our data also, coincided with **El-Gendy *et al.* (1990a)** who reported that LPO level increased in liver of carp by increasing the concentrations of glyphosate and fenvalerate.

Our results showed that glyphosate and CuSO4 affects the cholinesterases, δALAD and liver detoxifying enzymes. They also induce changes that characteristic of "oxidative stress". Finally, it can be concluded that fish exposure to these pesticides cause a marked alterations in many enzymatic functions in the different organs. Accordingly, care must be taken into account to avoid fish exposure in particular to pesticides.

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Table 1: *In vivo* effect of glyphosate and $CuSO_4$ on δALAD, AChE and BuChE activities of Cyprinus *carpio L.*

Treatment	Specific activity (Mean ± SE)				
	δALAD	AChE		BuChE	
	RBC's	Brain	Serum	Brain	Serum
Control	207.7±1.6^{b}	30.70±0.9^{a}	1.96±0.40^{a}	0.042±0.03^{ab}	0.124±0.003^{b}
Glyphosate	198.4±6.8^{b}	24.85±0.5^{b}	1.86±0.02^{a}	0.35±0.02^{a}	0.096±0.008^{a}
$CuSO_4$	155.3±7.4^{a}	27.75±0.7^{c}	1.84±0.04^{a}	0.046±0.01^{b}	0.11±0.006^{ab}

Means have the same letter is considered non-significant different, $p < 0.05$.

Activity of δ ALAD in red blood cell expressed as Unit / ml of erythrocytes / hour.

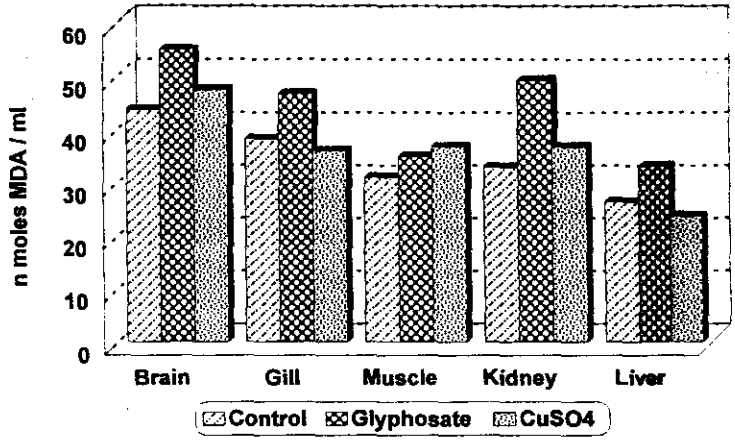
Activities of AChE and BuChE were expressed as μ moles of analyzed substrate / mg protein / min.

Table 2: In vivo effect of glyphosate and CuSO4 on some enzymes of Cyprinus carpio L. liver.

Enzymes	Specific activity (Mean ± SE)		
	Control	Glyphosate	$CuSO_4$
Detoxifying enzymes			
AST (Units / ml)	12.97±0.74^{a}	19.2±0.32^{b}	22.6±1.70^{c}
ALT (Units / ml)	28.50±0.70^{ab}	27.0±1.10^{a}	31.5±0.85^{b}
ACP (μ moles p-nitrophenol / mg protein / min.)	3.02±0.05^{a}	5.3±0.014^{b}	4.4±0.16^{c}
Antioxidant enzymes			
GST (OD / mg protein / min.)	0.023±0.002^{a}	0.027±0.003^{a}	0.028±0.006^{a}
C-ase (Units / gm wt.)	202.3±3.9^{a}	251.3±7.5^{b}	122.6±11.3^{c}
SOD (Units / gm wt.)	370.4	222.2	81.8

Means have the same letter is considered non-significant different, $p < 0.05$.

Fig. 1: Effect of the tested pesticides on the lipid peroxidation in different organs of (*Cyprinus carpio L*)



الملخص العربى

التأثيرات البيوكيميائية لبعض المبيدات على سمك المبروك

نجـــــاة محـــمـــــد عـــــلى

مركز البحوث الزراعية – الصبحية – الإسكندرية

يهدف البحث دراسة تأثير التركيزات (4 ، 8 جزء فى المليون) و التـــى تمثـــل 3/2 مـــن التركيز المميت لـــ 50 % من الأفراد المعاملة بعد 96 ساعة لكل من مبيد الحشائش الجليفوسات والمبيـــد الفطرى كبريتات النحاس على التوالى على بعض النظم الإنزيمية وكذلك عمليـــة الأكســـدة للـــدهن فـــى الأعضاء المختلفة لسمك المبروك.

أوضحت النتائج أن كبريتات النحاس أحدثت تثبيطاً معنوياً لنشاط إنزيم الدلتا أمينـــو لفيولينيـــك أسيد ديهيدراتيز ، بينما لم يتأثر نشاط الإنزيم بالمعاملة بمبيد الجليفوسات. أيضاً فإن نشاط إنزيم الأســـيتيل كولين إستيريز فى المخ قد انخفض انخفاضاً معنوياً بكلا المبيدين ، وعلى العكس من ذلك فإنه لـــم تكـــن هناك فروق جوهرية بين المعاملات وبين الكنترول على نشاط الإنزيم فى السيرم. كما أظهرت الدراسة أن كلا المبيدين أحدثا انخفاضاً غير معنوياً لنشاط إنزيم البيوتيريل كولين إستيريز فى المخ والسيرم. و لقد

حدث زيادة معنوية لنشاط كل من الأسبرتات ترانس أمينيز و الفوسفاتيز الحامضى بينما لم يتغير نشاط إنزيم الالآنين ترانس أمينيز فى الكبد المعامل بكل من الجليفوسات و كبريتات النحاس . وبدراسة تأثير تلك المبيدات على إنزيمات الأكسدة وكذلك عملية الأكسدة للدهن فقد أوضحت النتائج التالى:

أن كلا المبيدين أحدث زيادة فى نشاط كل من إنزيم الجلوتاثيون- إس- ترانسفيريز و إنزيم السوبر أوكسيد ديسميوتيز فى الكبد ، بينما إنزيم الكتاليز فقد زاد معنوياً بالمعاملة بمبيد الجليفوسات وعلى العكس فإن نشاطه انخفض بالمعاملة بكبريتات النحاس وذلك بالمقارنة بالكنترول. أما معدل الأكسدة للدهن فقد زاد فى كل من المخ والخياشيم والكلية والكبد المعامل بمبيد الجليفوسات ، بينما انخفض مع كبريتات النحاس فى كل من الخياشيم والكبد بالمقارنة بالكنترول.