

Hematological and Neurotoxic Effects of Endosulfan Pesticide on Common Carp *Cyprinus carpio*

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Summary

The present study including determined blood picture by measuring red blood cells count, hemoglobin concentration, packed cell volume and white blood cells in common carp *Cyprinus carpio*, as well as description behavior and growth of carp fish. In order to estimate LC50 used 240 fingerlings of common carp *Cyprinus carpio* were exposed to 0.0008 μ g/L, 0.0010 μ g/L, 0.0011 μ g/L, 0.0012 μ g/L, 0.0013 μ g/L, 0.0014 μ g/L and 0.0015 μ g/L. The LC₅₀ of endosulfan was 0.0012 μ g/L for 48h of exposure. Fish behavioral were recorded that showed abnormalities after exposure to the various endosulfan concentrations such as increase swimming activity, hypersensitivity, jerky movement, violent movements, loss of equilibrium, hyperactivity, increase operculum movement, frequent jumping, swimming at the water surface, erratic swimming, spiraling, convulsion, escape attempts from the aquarium with respiratory stress and decrease in respiratory rate as well as a significant decrease at (P<0.05) in body weight of all treated groups. The result of blood picture showed a significant reduction in red blood cells count, hemoglobin concentration, packed cell volume values while the number of white blood cells was showed a significant increase in its values.

Key word : Endosulfan , Hematology, Pesticide, *Cyprinus carpio*

التأثيرات الدمية والسلوكية العصبية لمبيد الاندوسلفان في اسماك الكارب العادي *Cyprinus carpio*

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الخلاصة

هدفت الدراسة الحالية معرفة تأثير مبيد الاندوسلفان في اسماك الكارب الاعتيادي عن طريق وصف سلوك ونمو الاسماك ودراسة الصورة الدمية المتضمنة حساب عدد كريات الدم الحمراء وتركيز خضاب الدم و حجم الخلايا المرصوصة وعدد كريات الدم البيض فضلاً عن وصف سلوك ونمو الاسماك. لغرض تحديد التركيز المميت الوسطي أستعمل 240 أصبعية من الاسماك للتركيز 0.0008 و 0.0010 و 0.0011 و 0.0012 و 0.0013 و 0.0014 و 0.0015 L/ μ g ، وكان التركيز المميت الوسطي 0.0012 μ g/L أثناء 48 ساعة. أظهرت النتائج زيادة فعالية الاسماك وفرط التحسس وحركة متقطعة وحركة انفعالية وفقدان التوازن وزيادة حركة الغطاء الغلصمي والقفز المتكرر والسباحة على سطح الماء بصورة لولبية غير طبيعية والتشنج وصعوبة التنفس. أظهرت نتائج التحليل الأحصائي انخفاضاً معنوياً بمستوى P<0.05 في معدل أوزان الاسماك. أظهرت نتائج حساب عدد الخلايا الحمر وتركيز خضاب الدم وحجم الخلايا المرصوصة انخفاضاً معنوياً في معاييرها بينما سجلت عدد الخلايا البيض زيادة معنوية في قيمها.

مفاتيح الكلمات : الدمية , الاندوسلفان , اسماك الكارب.

Introduction

Pesticides are well recognized as an economic approach for controlling pests in agriculture and horticulture. Endosulfan (C₉H₆Cl₆O₃S), an OC compound belonging to cyclodiene group is extensively used as a broad- spectrum pesticide to treat a wide variety of invertebrate pests and bird repellent on fruit crops in more than 70 countries (1). Endosulfan is an organochlorine insecticide and acaricide, and acts as a contact poison in

a wide variety of insects and mites. Endosulfan is persistent, with a half-life in water of up to six months under anaerobic conditions (2), and a half-life in soil under aerobic conditions of up to six years (3). Fish can be exposed to the insecticide when dissolved in water by absorption through the gills, skin and by contaminated food. Fish gills are the first organ that adsorbs the toxicants and contaminants which are then distributed via blood system into other organs. Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxicant, its concentration and duration of exposure.

Intentional misuse of endosulfan for killing fish (4). Endosulfan is very toxic to fish, even compared to other organochlorine pesticides such as dichlorodiphenyl-trichloroethane (DDT) (5). The present work aims to study the acute toxicity of endosulfan through the following: Determining LC₀, LC₅₀, LC₁₀₀ and safety concentration (SC) values for 48h. Study the clinical signs (behavior of fish such as movement, food intake and survival rate and the growth, food ratio). Study the effect on hematological picture which including: RBCs, WBCs, Hb and PCV.

Materials and Methods

The study was conducted at the Fish diseases laboratory, Veterinary Medicine College, University of Baghdad. A total of 240 Fingerlings of common carp *Cyprinus carpio* ranging 12-14 cm in total length and 40-50 gm in weight, were obtained from Al-Talbe hatchery and acclimated to laboratory conditions for 15 days before the commencement of the experiment. The fish acclimatized in aquarium measuring 80x40x30 cm and were supplied with oxygen using tap water in the aquarium to reach 70 L. Physico-chemical characteristics were measured such as temperature ranges between 21-24°C, pH ranges between 7-7.8, Fish were fed twice daily with commercial pellets with ratio of 4% of initial body weight per day, having 28% crude protein.

Determination of median lethal concentration of endosulfan seven different concentrations of treatment of endosulfan was used; each concentration was added 3 times to each group. The concentration at which 50% mortality of fishes occurred after 48h was selected as the medium lethal concentration (LC₅₀). The LC₅₀ concentration for 48h was calculated by the probit analysis method. In this study, LC₀ and LC₁₀₀ were determined also and the observation of toxic symptoms such as movement, respiration, swimming, food intake and response to the outer effects was recorded.

The concentrations that using in this experiment were 0.0008µg/L, 0.0010µg/L, 0.0011µg/L, 0.0012µg/L, 0.0013µg/L, 0.0014µg/L and 0.0015µg/L. During the experiment period the observation of toxic symptoms such as stress, movement, respiration, swimming, responses to the outer effects. Blood collection was done via cardiac puncture technique, blood was withdrawn directly from heart after the experiment end by a sterile disposable syringe (needle gauge 23), and blood was transferred into a tube containing EDTA solution for hematological tests, and counting (RBC, WBC and PCV).

Results are expressed as $M \pm SE$. Statistical analysis of data was performed on the basis of one-way analysis of variance (ANOVA I) for experiment Group differences were determined using least significant difference (LSD).

Results and Discussion

Median Lethal Concentration Measurement: The estimated LC₅₀ by (Probit method) for endosulfan of *Cyprinus carpio* was shown in Table (1). In the acute toxicity test, approximately 1 h after exposure to the various lethal endosulfan concentrations, the fishes showed behavioral abnormalities such as: increase jerking, frequent jumping, erratic swimming, spiraling, and convulsion, escape attempts from the aquarium, loss of

equilibrium, molting, color changes and paralysis. Results showed that no mortality of fishes in the control treatment. The acute toxicity of endosulfan concentrations on *Cyprinus carpio* at different exposure period and the mortality percentages are shown in Fig (1).

The mortality of the fish indicated that the toxicity and mortality of endosulfan increased with endosulfan concentrations and exposure. At the low endosulfan concentration (0.0010 and 0.0011µg/L), mortality was found after 24 h of exposure, While at higher concentrations (0.0015 µg/L), mortality occurred after 1 h of exposure. However, the mortality of *Cyprinus carpio* exposed to either low or high concentrations of endosulfan remained constant after exposure for 48 h. The present study determined the LC₅₀ of endosulfan (0.0012µg/L) and LC₀ (0.0008µg/L), LC₁₀₀ (0.0015µg/L), SC (0.0006µg/L) during 48h.

Table 1: Show LC₅₀ of endosulfan on *Cyprinus carpio* by Probit method

| Conc.µg/L | Log Conc. | Fish No. | Survival Fish | Mortality | Mortality % | Probit No. |
|-----------|-----------|----------|---------------|-----------|-------------|------------|
| 0.0008 | 0.90 | 10 | 10 | 0 | 0 | 0 |
| 0.0010 | 1 | 10 | 8 | 2 | 20 | 4.16 |
| 0.0011 | 1.04 | 10 | 6 | 4 | 40 | 4.75 |
| 0.0012 | 1.07 | 10 | 5 | 5 | 50 | 5.00 |
| 0.0013 | 1.11 | 10 | 3 | 7 | 70 | 5.52 |
| 0.0014 | 1.14 | 10 | 1 | 9 | 90 | 6.28 |
| 0.0015 | 1.17 | 10 | 0 | 10 | 100 | 7.50 |
| Control | | 10 | 10 | 0 | 0 | |

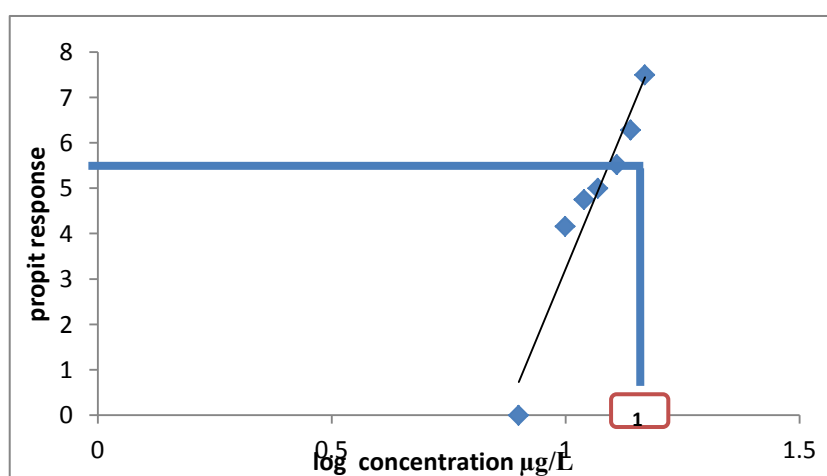


Fig. 1: Linear relationship between probit response and log concentration of median lethal concentration for endosulfan in 48h.

The present study determined the acute toxicity of endosulfan on the *Cyprinus carpio* during 48 h. The LC_{50} of endosulfan was $0.0012\mu\text{g/L}$. In another study, LC_{50} has been established as $0.0016\mu\text{g/L}$ found in *Cyprinus carpio* (6), The endosulfan had 96h LC_{50} of $0.93\mu\text{g/l}$ found in *Macrobrachium rosenbergii*, post larvae (7) and grass shrimp *Palaemonetes pugio* where 96-h LC_{50} was $0.62- 1.01\mu\text{g/L}$, with a range of $0.35-1.43\mu\text{g/L}$ (8).

Fishes behavioral showed abnormalities approximately 1 h. after exposure to the various endosulfan concentrations such as increase swimming activity, hypersensitivity, jerky movement, violent movements, loss of equilibrium, hyperactivity, hyper-excitability, increase operculum movement, frequent jumping, swimming at the water surface, erratic swimming, spiraling, convulsion, escape attempts from the aquarium, hitting to the walls of the aquarium before finally sinking to the bottom (Fig.2). The exposed fishes exhibit tremors and gradual weakening of reflexes leading to imbalance in posture and loss of equilibrium (Fig.3). In due course of time a few fishes start drowning but by sudden somersaulting, regain normal posture and balance temporarily.

Finally, however, they succumb to poison with mouth and operculum wide open, molting of body color changes from silvery white to pale white. At lower concentration, however, the changes in behavior are conspicuous. Results showed fish loss appetite of food. Abnormalities behavior observed in all treated groups but the severity of signs increased with a high concentration of endosulfan.

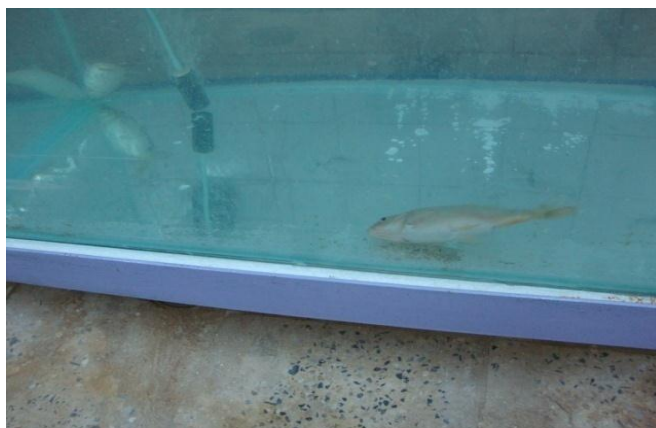


Fig. 2: Show fish sinking to the bottom of the aquarium



Fig.3: Show imbalance in posture and loss of equilibrium of fish

Erratic movements and abnormal swimming are triggered by deficiency in nervous and muscular coordination which may be due to accumulation of acetylcholine in synaptic and neuromuscular junctions (9).

Surfacing phenomena as observed in the fish treated with lethal concentration of endosulfan indicates hypoxic condition. Such surfacing might be to procure definite proportion of its oxygen requirement from the atmosphere (10). Bradbury and Coast (11) reported signs of endosulfan poisoning in fish, which included loss of schooling behavior, swimming near the water surface, hyperactivity, erratic swimming, seizures, loss of buoyancy, increased gill mucus secretions, flaring of the gill arches, head shaking and restlessness before mortality.

Signs of acute Endosulfan poisoning include frequent jumping and erratic opercular movement followed by convulsions (12). Sunderam, (13) found the 96 hr LC50 values vary from 0.014 µg/L for the harlequin fish *Rasborahetero morpha* to 14 µg/L for catfish *Claria sbatrachus*. The 96 hr LC50 values for Australian fish are 2.2 µg/L for the native firetail gudgeon *Hypseleotris gallii*, 2.4 µg/L for the eastern rainbow fish *Melanotaenia duboulayi* and silver perch *Bidyanu sbidyanus*, 0.5 µg/L for golden perch *Macquaria ambigua* and 0.2 µg/L for bony bream *Nematolosarebi* (13).

The LC50 values for exotic species that inhabit the Murray-Darling basin are 3.1 µg/L for mosquito fish *Gambusia affinis*, 0.1 µg/L for carp *Cyprinus carpio* and 1.6 µg/L for rainbow trout *Oncorhynchus mykiss* (14 and 13).

Endosulfan has long been known to be extremely toxic to many fish species, with the harlequin fish *Rasborahetero morpha* apparently most sensitive (24 hour LC50 = 0.02 µg/L). High excitability, loss of equilibrium and spasmodic movement has been reported as general symptoms of endosulfan poisoning in fish (13).

The decrease in the consumption of oxygen is probably the result of alterations of energy metabolism (15). Some studies of the pathological effects caused by chronic exposure to chemical substances evidenced the gradual destruction of gills filaments, killing the fish by asphyxia (16).

Body weight variations are the most sensitive indication of potential toxic effects studied. The physiological indicator may be affected if food is limited or if food consumption of the fish is impaired due to other stress factors. Feeding preferences were affected and consumption of food in fish was impaired and reduced drastically. This was more noticed in sub-lethal exposure periods. For these animals, it might be profitable to decrease their food uptake under toxic environmental conditions to lower the energetic costs of digestion. Depression in appetite is a common response of fish to stress and intermittence of feeding for longer periods, it can have a clear impact on growth and reproduction (17).

The effects on growth or survival rate were observed in fathead minnows *Rasborahetero morpha* continuously exposed for 60 days under flow-through conditions to endosulfan concentrations between 0.04 and 0.4 µg/L (13). Treatment at the high dose of endosulfan induced lower body weights and body weight gains and abdominal cramping in other fish. A substantial growth reduction caused by toxicant stress has important implications for survival in the natural situations. Dembele, (18) indicated that the abnormalities in fish behavior observed in exposure with OC insecticides could be related to failure of energy production or the release of stored metabolic energy, which may cause severe stress, leading to the mortality of the fish. The stress response in some situations may lose its adaptive value, which can result in the inhibition of growth, reproductive failure and immune suppression (19). Body weight may be reduction due to Liver and kidney damage in fish (20).

Results of RBCs count (Cells/ $\mu\text{L} \times 10^6$) in blood of *Cyprinus carpio* which exposed to repeated different concentrations of endosulfan insecticide for three months are shown in Table (2). There was a significant reduction in RBCs count at ($p < 0.05$) of all treated groups values when compared with the control group values. The highest reduction in RBCs count was recorded in T5 group value which treated with a concentration of 0.0005 $\mu\text{g/L}$ while the lowest reduction in RBCs count was recorded in T1 group value which treated with a concentration of 0.0001 $\mu\text{g/L}$. Results of PCV (%) in blood of *Cyprinus carpio* which exposed to a repeated different concentration of endosulfan insecticide for three months are shown in Table (2). Results showed significant reductions in PCV (%) at ($p < 0.05$) of all treatments groups values when compared with the control group value.

The highest reduction in PCV (%) was recorded in T5 group value which treated with a concentration of 0.0005 $\mu\text{g/L}$ while the lowest reduction in PCV% was recorded in T1 group value which treated with a concentration of 0.0001 $\mu\text{g/L}$. The results of hemoglobin (Hb) (gm /dl) are shown in Table (2). There was a significant decrease ($p < 0.05$) in values in all treated groups along the period of the experiment in comparison with the control group value in blood of *Cyprinus carpio* which exposed to a repeated different concentration of endosulfan insecticide. The highest reduction in Hb concentration was recorded in T5 group value which treated with a concentration of 0.0005 $\mu\text{g/L}$ while the lowest reduction in Hb concentration was recorded in T1 group value which treated with a concentration of 0.0001 $\mu\text{g/L}$. Results of WBCs count (Cell/ $\mu\text{L} \times 10^3$) in blood of *Cyprinus carpio* which exposed to repeated different concentration of endosulfan insecticide for three months are shown in Table (2). There were significant elevations at ($p < 0.05$) in T5 group value as compared with the control group value. While There were no significant differences at ($p < 0.05$) among T1, T2, T3 and T4 groups values also, when compared with the control group and T5 group values respectively.

Table 2: The results of hematological tests (WBCs count, RBCs count, Hb concentration and PCV) of *Cyprinus carpio* which exposed to a repeated different concentration of Endosulfan pesticide during experiment period.

| Hematological tests | | | | |
|------------------------------|--|--|-----------------------------|---------------|
| Test Groups | WBCs count Cells/ $\mu\text{L} \times 10^3$ | RBCs count Cells/ $\mu\text{L} \times 10^6$ | Hb concentration (gm/dl) | PCV (%) |
| Control | 25.4±0.9 B | 2.6±0.01 A | 8.4±0.1 A | 31.0±0.5 A |
| T1 0.0001 $\mu\text{g/L}$ | 25.9±1.2 AB | 2.42±0.005 B | 7.8±0.1 B | 29±0.4 B |
| T2 0.0002 $\mu\text{g/L}$ | 26.7±1.2 AB | 2.23±0.004 C | 7.1±0.1 C | 26±0.4 C |
| T3 0.0003 $\mu\text{g/L}$ | 27.3±1.1 AB | 2.04±0.004 D | 6.4±0.1 D | 24±0.3 D |
| T4 0.0004 $\mu\text{g/L}$ | 28.2±1.1 AB | 1.89±0.007 E | 5.9±0.1 E | 22±0.3 E |
| T5 0.0005 $\mu\text{g/L}$ | 28.9±1.1 A | 1.72± 0.006 F | 5.4±0.1 F | 20±0.3 F |

- ❖ L.S.D. value: RBCs=0.014, PCV=0.9, Hbc. =0.3, WBCs=3.1
- ❖ Figures represent mean ± standard error.
- ❖ Different capital letters represent significant difference between groups vertically at $p < 0.05$.
- ❖ n =10

The alterations of the haematological parameters could be used as an important tool for the assessment of pathological conditions of animals. The changes in the haematological parameters of fish are a helpful biomarker for evaluating their health status (21). The endosulfan induced reduction in the blood parameters recorded in the present study. This may be due to haemolysis and/or haemorrhage caused actions of pollutants to the fish (22). In the present study, the haematological parameters were negatively correlated with the endosulfan concentration and the experiment length.

This results agreement with Jenkins,(23) stated that the reduction in the haematological parameters after sub-lethal endosulfan exposure is the result of the inhibition of erythropoiesis, haemosynthesis, osmoregulatory dysfunction or an increase in the rate of erythrolysis. Although the level of hemoglobin reduced in *Cyprinus carpio* exposed to endosulfan, the erythropenia could be associated with a possible hemolysis (24). Banik, (25) reported that *Anabas testudineus* exposed to 0.00125% of endosulfan are unable to absorb iron efficiently from the intestine leading to a decrease in the hemoglobin formation and then anemia. On the other hand, the reduction in erythrocyte numbers could be related to possible oxidative damage to hemoglobin caused by the lipid peroxidation observed in fish exposed to the low concentration of endosulfan (23)

A reduction in haematological values, indicated anemia in the pesticide exposed fish may be due to erythropoiesis, haemosynthesis and osmoregulatory dysfunction or due to an increase in the rate of erythrocyte destruction in haematopoietic organs (26). In the present study, the decrease in RBCs count during the chronic treatment might be resulted from severe anemic state or haemolysing power of toxicant (endosulfan) particularly on the red cell membrane. Pesticides were found mainly in the erythrocytes (particularly in the erythrocyte content) and plasma and not in the leucocytes, platelets or stroma indicating that they mainly bind with hemoglobin. The reduction in erythrocyte count, PCV and haemoglobin of *Cyprinus carpio* in the present study can be attributed to the following factors, haemodilution of blood due to the damage of fish organs (27) and the haematological parameters PCV, RBCs and Hb, whose changes can be interpreted as a compensatory response that improves the O₂ carrying capacity to maintain the gas transfer, also indicates a change in the water blood barrier for gas exchange in gill lamellae (28).

Erythrocyte level was found to be depressed in fishes subjected to stressful conditions. Changes in the erythrocyte profile suggest a compensation of oxygen deficit in the body due to gill damage and the nature of the changes shows a release of erythrocytes from the blood depots (24).

Inhibition of erythropoiesis and an increase in the rate of erythrocyte destruction in hematopoietic organs are the cause of decrease in RBCs count. Joshi, (29) found a significant decrease of RBCs, Hb and packed cell volume (PCV) in endosulfan exposed fish species and indicated the toxic effect of endosulfan on spleen, liver and anterior kidney.

The decrease in the haemoglobin content in the present study resulted from rapid oxidation of hemoglobin to methaemoglobin or release of O₂ radical brought about by the toxic stress of endosulfan. It is increasingly recognized that xenobiotics which is capable of undergoing redox cycling can exert toxic effects *via* the generation of oxygen free radicals. Matkovics, (30) observed *Cyprinus carpio* a quick decrease in haemoglobin content in response to parquet toxicity .

The PCV values decrease when a fish loses its appetite, or it is diseased or poisoned by pesticides (31). The reduction in the PCV values indicates that the fish suffers from anemia or hemodilution. In addition, an alteration in the fish metabolism would also lead to decrease values of hemotocrite in *Cyprinus carpio* (32).

Similar to the present study, a significant decrease in the red cell count was reported in fingerlings *Cyprinus carpio* exposed to concentrations from 0.26 to 0.78 µg.L of endosulfan (33).

In the present study, although significant variations were observed in terms of leucocyte concentration, the progressive trend of leucocytosis observed in *Cyprinus carpio* exposed to both concentrations of endosulfan could be considered as a cellular response of tissue alteration generated by the insecticide (34) or a high sensitivity of neutrophils to environmental changes (35). The increase in WBCs count in the present study indicates the stress condition of the fish caused by endosulfan which might produce gill damage and other organs.

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