قسم : الطب الشرعى والسموم - كلية الطب البيطرى - جامعتى القاهرة والزقازيق .
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التأثير السمى لمادة الدايكوات على أسماك القرموط والبلطى النيلى

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اجريت التجارب لاظهار التأثير السام للمبيد العشبى المستخدم فى ابادة الحشائش المائية (الديكوات) على نوعين من الأسماك النيلية القرموط والبلطى النيلى ودلت النتائج على أن الجرعة متوسطة السمية لسمك البلطى النيلى ٢٢٠ (١٦٠ - ٢٥٠) جزء فى المليون . ولدراسة تأثير التعرض قصير المدى اعطيت الاسماك من كلا النوعين جرعات يومية تعادل عشر التركيز الذى يسبب ٥٠ ٪ نفوق لمدة خمسة أسابيع ولوحظت الأعراض والتغيرات التشريحية والهستولوجية واظهرت النتائج وجود انخفاض فى عدد كرات الدم الحمراء وكرات الدم البيضاء وكذلك فى نسبة الهيموجلوبين .

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TOXICOLOGICAL EFFECTS OF "DIQUAT" ON CLARAIS LAZERA AND TILAPIA NILOTICA - EGYPTIAN NILE FISH

(With 2 Tables and 6 Figures)

By

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SUMMARY

Experiments were carried out to investigate the toxic effects of the aquatic herbicide "Diquat" on two of most familiar Egyptian Nile fish Clarias lazera and Tilapia nilotica. The LC_{50} of diquat on Tilapia nilotica was 200 (166-250) ppm.

Short-term toxicity studies were carried out on both types of fish by daily inoculating 1/10 LC_{50} dose for five weeks.

Symptoms, post-mortem and Histopathological changes were observed. Our investigation revealed a significant decrease in RBCs.,WBCs. count and Hb concentration during short term toxicity study.

INTRODUCTION

BUTLER (1965), has reported no effect of diquat and paraquat at 1 ppm concentration on estuarine oysters, shrimp and fish.

YOE (1967), reported that traces of weed killers could be detected in water at the end of 12 days but more often residues are below 0.01 ppm within 4-7 days after the treatment.

Diquat was shown by GILDERHNS (1967) to be harmless to blue-gills and did not affect their breeding. CARTER (1965), showed that when trout were exposed to 1 ppm diquat for 16 days, the highest concentration reached in the whole fish was 0.5 to 0.6 ppm and this slowly disappeared on returning the fish to fresh water.

The lowest median tolerance limit (MTL) value recorded for 48 hours exposure was 2.1 ppm for Walleye pike and the highest (125 ppm) for Blueqills (McKENZIE, 1971)).

The object of this work is to asses the effect of one of the mostly used aquatic weed killer herbicides" " Diquat " on two of the most prevalent Egyptian water fish Clarias lazera and Tilapia nilotica during acute and short-term exposure studies.

MATERIAL AND METHODS

Diquat was obtained from I.C.I., Cairo assistance office as formulated compound containing 2 lbs. active ingredient per gallon. The solution is missible with water.

Tilapia nilotica fish of a weight 15-20 gm. and average lenth of 10 cm. was used.

The LC_{50} of Diquat on Clarias lazera (previously determined, SHAABAN et al., 1979) and Tilapia nilotica was calculated after LITCHFIELD & WILCOXON method (1949). Preliminary trials were carried out on 190 Tilapia nilotica Nile fish, divided into 19 groups (Ten fish each). Fish were subjected to different concentrations of diquat begining with 5 ppm up to 1800 ppm, except one group which was used as control.

For test proper 7 groups (Each contain 10 fish), were subjected to different concentrations ranging from 100 ppm up to 600 ppm except the control group. Aquaria aeration and test media was adopted as previously described, SHAABAN et al., (1979).

Short-term toxicity studies of Diquat on Clarias lazera and Tilapia nilotica were carried out by subjecting the fish to 1/10 LC_{50} dose (3.6 ppm for Clarias lazera and 20 ppm for Tilapia nilotica) for five weeks. For Tilapia nilotica, 150 fish were used, 100 for experiment and the rest 50 used as control. In cases of Clarias lazera, 50 fish were used, 30 subjected to 1/10 LC_{50} while 20 fish were used as control.

The observed symptoms due exposure of Diquat on Clarias lazera and Tilapia nilotica were noted and recorded during LC_{50} determinations as well as during short-term study for five weeks by daily dosing of 1/10 LC_{50}. Dead or

killed fishes by transection of the spinal cord, were examined for post-mortem findings. Tissue specimens from different organs was subjected to histopathological examination.

Blood samples were taken weekly from six Clarias lazera fish by heart puncture, the technique was applied by removing the operculum (gill arch) and inserting a micro-pipette in the heart and withdrawing blood. Blood was also obtained from the caudal artery. The R.B.Cs., W.B.Cs as well as Hb % were calculated (GRADWHOL, 1956).

RESULTS

Preliminary trials for LC_{50} determination of diquat in Tilapia nilotica fish showed that mortality started at 45 ppm, while the 100% mortality was at 600 ppm.

The test proper indicated that, the LC_{50} with 19/20 confidence limits was 200 (160 - 250) ppm of formulated Diquat (Table I).

Haematological investigation showed that, Diquat causes decrease in both R.B.Cs. count and Hb concentration, while W.B.Cs. count was increased (Table II).

TABLE (I)

Solution of the dose effect curve of Diquat to Tilapia nilotica.

Dose ppm	Response	Observed %	Expected %	Observed minus expected	Contribution to $(Chi)^2$
100	1/10	10	9	1.0	0.0012
120	3/10	30	16	14.0	0.1400
150	5/10	50	30	20.0	0.2000
170	4/10	40	38	2.0	0.0018
200	3/10	30	50	20.0	0.1600
600	10/10	100	98.6	1.1	0.0058

TABLE (II)

Effect of short-term exposure for five weeks at 1/10 LC_{50} dose on R.B.Cs., W.B.Sc. count and haemoglobin level of Clarias lazera Nile fish (Mean ± S.E.).

Mean ** ± S.E.	Control	Time post exposure (week) 1	2	3	4	5
R.B.Cs. (Millions/ C.mm.)	2.954±0.013	2.06 ±0.086*	1.685±0.032*	1.29±0.014*	1.168±0.014*	1.04 ±0.024*
W.B.Cs. (Thousands/C.mm.)	1.442±0.008	7.283±0.133	6.483±0.121	6.15±0.152	5.60 ±0.06	5.266±0.053
Hb (gm%) 100 ml.	8.535±0.035	1.54 ±0.033	1.663±0.029	1.33±0.035	1.243±0.05	1.171±0.014

* : Significant at $P < 0.05$

** : Each mean represent the values of 6 fish except the conrol (20 fish).

Symptoms:

The symptoms observed in Clarias lazera fish after dosing were nervous movements. The fish stand vertically in the water with its mouth opened over the water surface before it dies. Emaciation and ascitis appeared in case of short-term toxicity study.

In case of Tilapia nilotica, the fish before it dies was swimming on its back on the water surface with increased demand for oxygen which is manifested by accelerated movements of the gill cover (Operculum).

Post-mortem Findings:

In case of Clarias lazera, the abdominal cavity contained excess of serous fluid "ascitis". The liver was pale in color, enlarged with subcapsular haemorrhage, the mucosa of the stomach was hyperemic and the kidney was congested and friable. Gills were highly congested, spleen dark in colour and enlarged.

The P.M. finding in case of Tilapia nilotica fish were mainly congestion of all visceral organs, the digestive tract was empty from food particles. The gills were congested.

Histopathology:

I- Clarias Lazera:

The liver showed congestion and focal areas of coagulative necrosis and vacuolization of hepatocytes especially under the capsule (Fig. 1). Congestion of the spleen and hyperplasia of lymphoid tissues specially at dose level of 70 ppm or higher. Congestion of coronary blood vesseles and perivascular oedema with slight leucocytic infiltration of epicardium. Congestion of the renal vessels with hyaline droplets in tubular lumen or in the luminal border of cytoplasm of epithelial cells (Fig. 2). The gut showed desquamation of epithelial cells with collection of exsudate in the lumen, the deep layer of submucosa appeared oedematous (Fig. 4).

Hyperplasia of the epithelium lining. The gill filaments were associated with an increased number of mucous secretory cells. With increasing the dose gill filaments were completely destroyed. Some gill arches showed hyperplasia of epithelial tissues covering the fine filaments or in between the filaments. Congestion of the gill arch was also noted.

During short term toxicity, all organs appeared normal except liver and gills. The liver showed congestion and hepatic cells swollen with vacuolated cytoplasm having dots or threads like acidophilic cytoplasm. The cells have a signet ring appearance. Hyperplasia of bile ducts was also observed. The epithelial lining of gill arch and gill filament was desquamated in additon to destruction of the villae. The underlying connective tissues slightly infiltrated with leuocytes (Fig. 3).

II- Tilapia Nilatica:

Liver congestion and hepatic cell vacuolization were common observation (Fig. 5), in different doses in addition to lymphocytic aggregation at dose 600 ppm. The gut showed no alteration. In short-term exposure to level of 1/10 LC_{50} for five weeks the kidney showed congestion and cloudy swelling. Gills showed desquamation of epithelial cell lining and congestion of connective tissue cord, (Fig. 6).

DISCUSSION

The ecological effects of herbicides on aquatic system are still poorly documented at present time (NEWBOLD, 1974). However, recent work justifies the present concern that ecological change does not occur in many of fresh water systems where herbicides are commonly used (WALSH et al., 1971).

During our experimental work, formulated product (2 lb, active ingredient per gallon) was used in quatic ecosystem since the degree of toxicity is altered by different types of formulation and active ingredient alone (MAYHEW, 1955). The aim of the LC_{50} determination was to compare the effect of Diquat on two types of popualr commonly used Nile Fish in Egypt and to use it as a starting point for short-term exposure studies. In addition to the preceding fact, the value of its determination is important especially because Diquat is only used once or twice per year, a procedure which may not be able to produce chronic form of toxicity.

BATHE et al., (1974), classifed plant protection products on the basis of 96 hours LC_{50} value in fish into highly toxic (LC_{50} 0.5 ppm), toxic (0.5-5 ppm), slightly toxic (5-50 ppm) and non toxic (LC_{50} : 50 ppm or more). According to previous classification Diquat may be listed under slightly toxic to Clarias lazera and non toxic to Tilapia nilotica.

RICHARDSON (1969), recorded that the LC_{50} od Diquat to Salmogairdnerii to be 90 mg/l. (24 hours exposure). Our figure are inagreement with other investigatiors which showed the low toxicity of Diquat to fish (BUTLER, 1965; HILSENHOFF, 1966; YOE, 1967 nd GILDERHNS, 1967).

The LC_{50} in natural population of Nile fish may be a subjected to different factors interfering such as the presence of mud which is normally negatively charged and dipyridilium compounds (Paraquat and Diquat) are strongly cationic and are strongly adsorbed (KNIGHT and TOMLINSON, 1967).

The effects of this weedicide on blood constituents need further investigation to clarify its toxic action on blood constituents. Histopathological changes were specially observed in the spleen and livers of poisoned fish.

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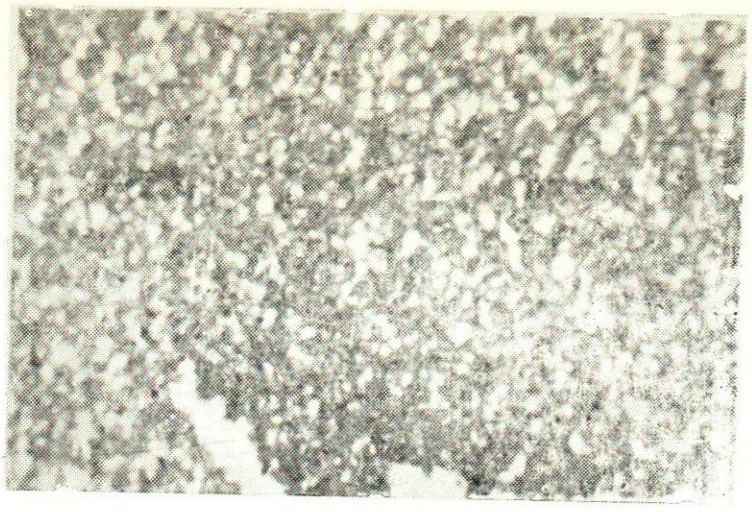

Fig. 1 : Liver of Clarias lazera showing coagulative necrosis in addition to vacalization of hepatocytes x 100

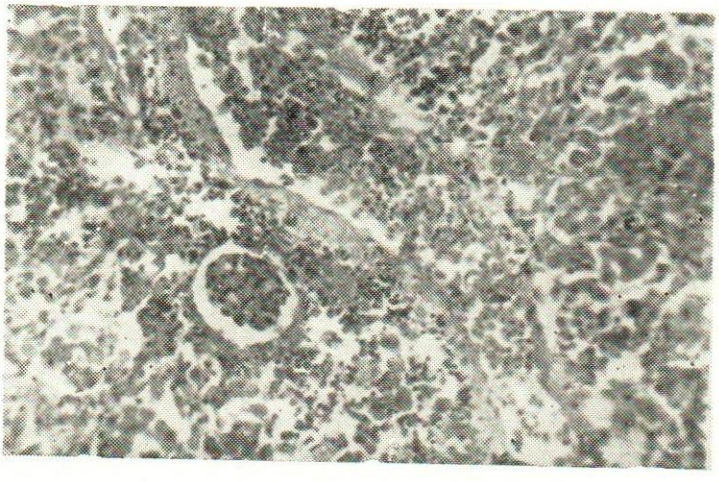

Fig. 2 : Kidney showing hyaline droplets in the lumen of the tubules in Clarias lazera

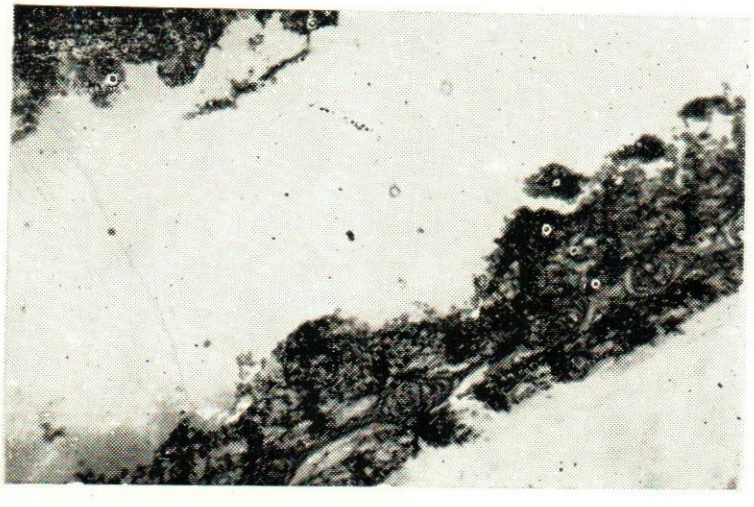

Fig. 3 : Gill fillament showing destruction and desqumation of epthelial cells in Clarias lazera x 100

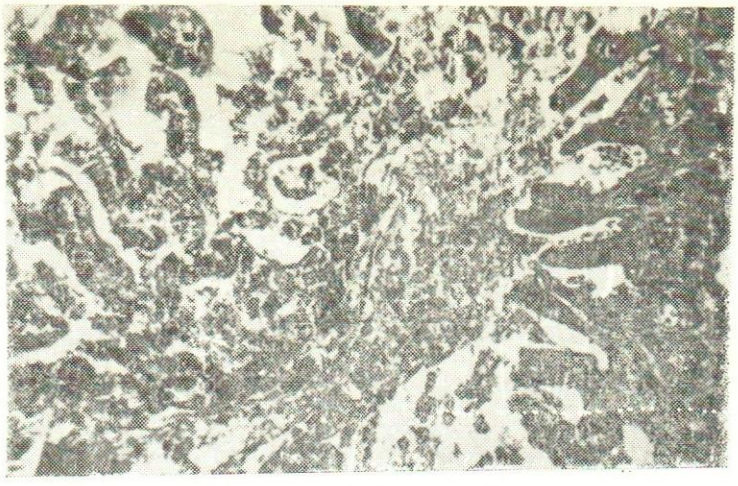

Fig. 4 : Desqumation of epithelium lining the gut in Clarias lazera. x 100

Fig 5 : Vacolization of hepatocytes (Sigmoid ring) appearance of Tilapia nilatica. z 100

Flg. 6 : Desqumation of gill epithelium cells of Tilapia nilatica. x 100