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Study on The Effect of Reldan (Chlorpyrifos-methyl) on Health and Growth of Nile Tilapia *(Oreochromis niloticus)* Fingerlings

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ABSTRACT

In this study 350 *Oreochromis niloticus* fingerlings were used to determine the 96 hrs LC_{50} of Reldan, the effect of $^1/_{10}$ and $^1/_{20}$ 96 hrs LC_{50} on health condition and growth performance of tested fish and the recovery of fish exposed to $^1/_3$ 96 hrs LC_{50}.

Our results revealed that, the 96 hrs LC_{50} of Reldan in tested fish was 2.27 mg/L. The exposed fish showed abnormal swimming movement, sluggish, restlessness followed by convulsion, the respiration rate was gradually slow, the fish lying down with loss of equilibrium and did not respond to escape reflex, congestion of all internal organs with excessive mucous on gills. In addition, dark colouration of the skin with thick layer of mucous and enlargement of liver in fish exposed to $^1/_{10}$ and $^1/_{20}$ 96 hrs LC_{50} . There were a significant decrease in growth performance accompanied with hematological and biochemical changes. Detection the residues of Reldan in muscles of tested fish with histopathological changes in gills and liver of fish exposed to $^1/_{10}$ and $^1/_{20}$ 96 hrs LC_{50} . Fish exposed to $^1/_3$ 96 hrs LC_{50} of Reldan were recovered after one week.

INTRODUCTION

Pesticides have contributed to dramatic increase in crop yields and in the quantity and variety of the diet. Also, they have helped to limit the spread of certain diseases. They can cause injury to human health as well as to the environment *(1)*.

Recently, the extensive use of pesticides for the control of aquatic weeds have caused the environmental pollution especially contamination of the aquatic system. Pesticides may contaminate water through its direct application for control of aquatic weeds and insects, percolation, run off from agricultural land, drift from aerial and land application, discharge of industrial wastes and from draining up of equipments used for their formulation *(2)*.

Organophosphate insecticides are oily and relatively insoluble in water. They could be absorbed through the skin, gills and digestive tract of fish. Toxicity of organophosphates was derived from their ability to inhibit acetyl cholinesterase, enzymes, essential in the degradation of acetylcholine *(3)*. Continuous accumulation of these pesticides may induce several changes in fish, resulting into pathological condition *(4)*. It can also alter the normal activity of fish by changing their physiology *(5)*.

Reldan (Chlorpyrifos-methyl) is a highly toxic organophosphorus insecticide widely used in agriculture. Beside its use on stored grains the WHO recommended it for aquatic use *(6)*. The 96 hrs LC_{50} of chlorpyrifos on the juvenile of *Tilapia guineensis* was 0.002 mg/L *(7)*.

In the present study , the experiments were conducted to investigate the effect of Reldan on health and growth of *Oreochromis niloticus* fingerlings.

MATERIAL AND METHODS

A total number of 350 *Oreochromis niloticus* fingerlings were used. Fish was obtained from Abbasa Fish Hatchery, Sharkia

province. Fish were apparently healthy and free from any skin lesions and external parasites. Fish were kept in a glass aquarium (80 x 30 x 40 cm) for 15 days, for acclimatization before start of experiment. Reldan was obtained from Dow Agro Sciences Company, Indiana, USA.

Glass aquaria, each, 80 X 30 X 40 cm provided with aerator, thermostatically controlled heater, thermometer and filled with dechlorinated tap water were used.

Determination of 96 hrs LC_{50} of Reldan in *Oreochromis niloticus* fingerlings

A total number of 200 *oreochromis niloticus* fingerlings with 10.26 ± 0.07g average body weight were divided into 20 equal groups (each group contain 10 fish) kept in glass aquaria. All groups of tested fish, except control group, were exposed to different concentrations of Reldan (Table 1).

The experimental fish were observed daily. The clinical signs and post mortem lesions of the affected fish as well as the mortality rate were recorded ***(8).*** The 96 hour LC_{50} was determined ***(9)***.

The effects of Reldan concentrations ($^1/_{10}$ and $^1/_{20}$ 96 hrs LC_{50}) on health and growth of *Oreochromis niloticus* fingerlings

A total number of 90 fish, with 10.15-10.45 g mean body weight were used. The fish were divided into 3 equal groups, each with two replicates at density of 15 fish per aquarium. The fish of the first group kept as control, while fish of the second and third groups were exposed to $^1/_{10}$ and $^1/_{20}$ 96 hrs LC_{50} respectively. The experiment was conducted for 8 weeks .

The experimental fish were fed on a basal diet contained 35.4 % crude protein. The amount of food (on dry matter basis) per day was 3 % of fish body weight. The fish were fed 3 times daily. The body gain ***(10)***, body gain percent ***(11)***, feed conversion ratio ***(10)*** and condition factor ***(12)*** were carried out.

Blood samples for haematological and biochemical analysis was collected by the caudal blood vessels ***(13).*** Erythrocytes (RBC_s) and leucocytes (WBC_s) counts ***(14)*** , hematocrite (PCV) ***(15)*** and haemoglobin value (Hb) ***(16)*** were carried out. Serum alanine aminotransferase (ALT) and aspartate aminotranferase (AST) were determined colorimetrically ***(17,18)***. Serum urea ***(19)*** and serum creatinine ***(20)*** were determined. Serum glucose (B.S) level ***(21)*** was determined. Plasma cortisol level was determined using radioimmunoassay ***(22)***.

The Reldan was extracted from muscle ***(23,24)*** and determined by HPLC (Beckman , USA). Histopathological examination of the effected fish tissues were carried out ***(25).***

Recovery response of *Oreochromis niloticus* fingerlings exposed to $^1/_3$ 96 hrs LC_{50} of Reldan

Sixty *Oreochromis niloticus* fingerlings with average body weight 10.62 ± 0.24g were divided into two equal groups, each group contained 30 fish. The first group left as control and fish of second group were exposed to concentration of $^1/_3$ 96 hrs LC_{50} of Reldan for one week ***(26).*** After one week 15 fish from the second group were transferred into other clean aquarium free from any toxicant substance.

The obtained data were statistically analyzed using analysis of variance procedure ***(27).***

Table 1. Preliminary trials for determination of zero and hundred % mortalities in *oreochromis niloticus* fingerlings exposed to different concentrations of Reldan.

Group (n=10)	Concentration of Reldan (mg/L)	Mortality during 96 hours.				Total MR*	Mortality %
		1st day	2nd day	3rd day	4th day		
1	Control	0	0	0	0	0	0
2	0.5	0	0	0	0	0	0
3	0.8	0	0	0	2	2	20
4	1.1	0	0	1	1	2	20
5	1.4	0	0	2	1	3	30
6	1.7	0	1	1	2	4	40
7	2.0	0	0	2	2	4	40
8	2.3	1	0	2	1	4	40
9	2.6	0	2	1	3	6	60
10	2.9	0	1	1	4	6	60
11	3.2	2	1	2	3	8	80
12	3.5	2	1	1	4	8	80
13	3.8	4	3	0	2	9	90
14	4.1	4	4	1	1	10	100
15	4.4	6	3	1	0	10	100
16	4.7	6	3	1	0	10	100
17	5.0	8	2	0	0	10	100
18	5.3	8	1	1	0	10	100
19	5.6	8	1	0	1	10	100
20	5.9	10	0	0	0	10	100

*MR = Mortality rate.

RESULTS AND DISCUSSION

Determination of 96 hrs LC_{50} of Reldan in *Oreochromis niloticus* fingerlings

The results demonstrated in Table 2 revealed that, the 96 hrs LC_{50} of Reldan in *Oreochromis niloticus* fingerlings was 2.27 mg/l. This result disagree with the results previously obtained, 0.002mg/L in Juvenile of *Tilapia guineensis* ***(7),*** 1.57mg/L in *Oreochromis niloticus* larvae ***(28)*** and also 1.79mg/L in Guppy fish *Poecilia reticulata* ***(29)*** . These differences may be attributed to difference in types of fish, species, fish life stages, environmental variation as well as individual variation.

The tested fish showing abnormal swimming movement, sluggish, restlessness followed by convulsion , the respiration rate was gradually slow , the fish lying down with loss of equilibrium and did not respond to escape reflex. Congestion of all internal organs with excessive mucous secretion on gills (Fig. 1). The observed clinical signs are similar to those recorded during exposure to other organophosphorus insecticides, Trichlorfan to Eel ***(30)*** and Malathion to catfish ***(31).*** The previous signs could be attributed to the inhibition of acetylecholine esterase (CHE) enzyme by these group of insecticides , specially at neuromuscular junction which leads to continous muscular contraction until complete exhaustion is attained ***(32, 33).***

The effect of Reldan concentrations ($^1/_{10}$ and $^1/_{20}$ 96 hrs LC_{50}) on health and growth of *Oreochromis niloticus* fingerlings.

The results demonstrated in Table 3 revealed that, the mortality rate of fish exposed to $^1/_{10}$ (0.227 mg/L) and $^1/_{20}$ (0.114 mg/l) 96 hrs LC_{50} of Reldam are 26.6% and 16.6% respectively. The tested fish appeared sluggish and not respond to tested reflexes , dark colouration of the skin which covered with thick layer of mucous (Fig. 2).

Congestion of internal organs and enlargement of liver, were the most recorded post-mortem changes. These finding are similar to that previously obtained in carp ***(34)*** and some fresh water fish ***(35).***

There was a significant decrease in body weight, body gain and weight gain percent in fish exposed to $^1/_{10}$ followed by fish exposed to $^1/_{20}$ 96 hrs LC_{50} of Reldan. Feed conversion ratio was higher in fish exposed to $^1/_{10}$ 96 hrs LC_{50} of Reldan. Condition factor was decreased with decrease Reldan concentration (Table 4). These results are nearly agree with those previously recorded in cutthroat trout ***(36),*** fry of *Macropodus caponus* ***(37)*** and *Pontius stigmor* ***(38).***

The results demonstrated in Table 5 revealed that a significant decrease in RBCs, WBCs, Hb, PCV and cortisol level while a significant increase in urea , creatinine, ALT, AST and B.S in fish exposed to $^1/_{10}$ and $^1/_{20}$ 96 hrs LC_{50} of Reldan. Similar results were recorded in *Heteropneutstus fassili* ***(39),*** *Tilapia guineensis* ***(7)***, Carp ***(40)***, *Oreochromis mossambicus* ***(41)*** and *Channa Punctatus* ***(42)*** . These may be attributed to inhibition of erythropoiesis by orghanophosphorus insecticides or increase the rate or erythrocyte destruction ***(39)*** . The decrease of plasma cortisol may be due to the occurrence of corticosteroid feed back inhibition on adrino corticotropin hormone ***(43,44).*** Orghanophosphorus insecticides affect secondary adrenergic reaction leading to hypersecretion of adrenaline which stimulate the breakdown of glycogen to glucose ***(45).*** The increase in urea and creatinine may be due to the decrease of glomerular filteration rate of kidney and tubular dysfunction ***(46).*** The increase of ALT and AST activities in exposed fish to Reldan may be due to liver damage or increasing protein metabolism ***(42).***

Our results cleared that, the residues of Reldan in muscles of fish exposed to $^1/_{10}$ 96 hrs LC_{50} was higher than the fish exposed to $^1/_{20}$ LC_{50} (Table 6). The chloropyrifos is known to bioaccumulate in the tissue of tested organism ***(47).***

Histopathological findings in fish exposed to $^1/_{10}$ and $^1/_{20}$ 96 hrs LC_{50} of Reldan were nearly similar. Liver showed disorganization of some acini, moderate aggregation of melanomacrophage and esinophilic granular cells in hepato – portal area and hydropic degeneration of some hepatocytes (Fig. 3). The gill filaments showed moderate congestion of the blood vessels and capillaries, partial desquamation of the epithelium , focal proliferation of macrophages and infiltration of lymphocytes (Fig. 4). These results were similar to that previously reported in *Cirrhinus mrigala* ***(48)*** and in mosquito fish ***(49).***

Recovery response of *Oreochromis niloticus* fingerlings exposed to $^1/_3$ 96 hrs LC_{50} of Reldan

The fish exposed to $^1/_3$ 96 hrs LC_{50} (0.76 mg/L) of Reldan appeared sluggish, swim near water surface with opened mouth, the fish didn't response to tested reflexes, the skin covered with thick layer of mucous in addition to congestion of kidney , liver and spleen. After recovery period the fish react well and rapidly to escape reflex as well as the viscera and gills were normal.

The residues of Reldan in muscles of *Oreochromis niloticus* fingerlings exposed to $^1/_3$ 96 hrs LC_{50} were 1.00 ppm and 1.35 ppm at 3 days and 7 days respectively. After recovery period, the residues of Reldan decreased to 0.035 ppm and 0.0071 ppm at 3 days and 7 days respectively (Table 7). This result is nearly consistent with that previously recorded ***(26, 50, 51).***

Table 2. Actual determination of 96 hours LC_{50} of Reldan in *oreochromis niloticus* fingerlings.

Group	Concentration of Reldan (mg/L)	Number of dead fish after 96 hours	a	b	a X b	∑ (a x b)
1	Control	0	0	0	0	
2	0.5	0	0.3	0	0	
3	0.8	2	0.3	1	0.3	
4	1.1	2	0.3	2	0.6	
5	1.4	3	0.3	2.5	0.75	
6	1.7	4	0.3	3.5	1.05	
7	2.0	4	0.3	4	1.2	
8	2.3	4	0.3	4	1.2	
9	2.6	6	0.3	5	1.5	
10	2.9	6	0.3	6	1.8	
11	3.2	8	0.3	7	2.1	
12	3.5	8	0.3	8	2.4	
13	3.8	9	0.3	8.5	2.55	
14	4.1	10	0.3	9.5	2.85	
						18.3

$$96\ Lc_{50} = \text{highest dose} - \frac{\sum(a \times b)}{n}$$

$$= 4.1 - \frac{18.3}{10} = 2.27 \text{mg/L}$$

a= Constant factor between two successive doses.
b= The mean of dead fish in the groups.
n = Number of fish in each group
Σ= The sum of (a x b).

Table 3. The effect of different Reldan concentrations on behavioural response and mortality of *Oreochromis niloticus* fingerlings.

Group	Concentration of Reldan (mg/L)	Observation (week)																Mortality rate at the end	
		1st		2nd		3rd		4th		5th		6th		7th		8th			
		ER	MR	ER	MR	ER	MR	ER	MR	ER	MR	ER	MR	ER	MR	ER	MR	No	%
1	Control	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0	0	0
2	1/10 LC_{50} (0.227)	+++	0	+++	1	++	0	++	2	++	0	++	3	+	1	+	1	8	26.6
3	1/20 LC_{50} (0.114)	+++	0	+++	0	+++	0	+++	1	+++	1	++	2	++	0	+	1	5	16.6

ER= escape reflex
MR= mortality rate
+++ = fish respond well to escape reflex and showing normal activity and movement.
++= fish moderately respond to escape reflex and showing sluggish activity and movement
+ = fish not respond to escape reflex and showing sluggish movement.

Table 4. Effect of Reldan concentrations ($^1/_{10}$ Lc_{50} and $^1/_{20}$ LC_{50}) on growth performance of *oreochromis niloticus* fingerlings.

Parameters \ Group (n=30)	1 Control	2 $^1/_{10}$ LC_{50} (0.227 mg / L)	3 $^1/_{20}$ LC_{50} (0.114 mg / L)
Initial body weight(g)	10.15 ± 0.62^a	10.20 ± 0.25^a	10.45 ± 0.53^a
Initial total body length (cm)	8.5 ± 0.65^a	8.5 ± 0.65^a	8.5 ± 0.38^a
*Final body weight(g)	32.35 ± 1.40^a	24.8 ± 0.37^b	27.7 ± 0.76^b
**Final total body lengt(cm)	12.50 ± 0.57^a	11.7 ± 0.49^a	12 ± 0.47^a
Weight gain (g)	23.2 ± 0.84^a	$14.6 \pm .17^b$	17.25 ± 1.26^b
Weight gain %	229.7	143.1	165.07
Feed consumed (g)	22.47 ± 0.88^a	19.19 ± 0.52^b	$20.47 \pm .088^b$
Feed conversion ratio	$0.97 \pm .01^a$	1.31 ± 0.02^a	1.19 ± 0.03^a
Condition factor			
a. at start.	1.64	1.66	1.70
b. at end.	1.70	1.548	1.6
Percent of change condition factor (% of initial value)	+ 6	- 11.15	-10

Means within the same row bearing different superscripts are significant at $p \leq 0.05$.

* Final body weight after 8 week.

** Final total body length after 8 week.

Table 5. Effect of different concentrations ($^1/_{10}$ and $^1/_{20}$ 96 hrs LC_{50}) of Reldan on some haematological and biochemical parameters in *oreochromis niloticus* fingerlings .

Parameters \ Group	1 Control	2 $^1/_{10}$ LC_{50} (0.227 mg / L)	3 $^1/_{20}$ LC_{50} (0.114 mg / L)
Hb (g/dL)	5.9 ± 0.17^a	4.63 ± 0.15^b	5.47 ± 0.42^{ab}
RBC_s (10^6 / µL)	1 ± 0.02^a	0.575 ± 0.01^b	0.965 ± 0.01^a
WBC_s (10^3 / µL)	28 ± 0.28^a	22 ± 0.57^b	24 ± 1.15^c
PCV (%)	23.6 ± 0.23^a	19.4 ± 0.86^b	23.2 ± 0.34^a
Urea (mg / dL)	3.00 ± 0.57^c	15.00 ± 0.86^a	13.00 ± 0.28^b
Creatinine (mg / dL)	0.3 ± 0.57^a	0.22 ± 0.01^a	$0.19 \pm .005^a$
ALT (u / L)	8.00 ± 0.57^c	28.00 ± 0.57^a	22.00 ± 1.15^b
AST (u / L)	10 ± 0.57^c	36 ± 0.57^a	32 ± 1.15^b
B.S (mg / dL)	38 ± 1.15^b	50 ± 0.57^a	45 ± 1.73^a
Cortisol (nmol)	89.7 ± 0.63^a	56.00 ± 6.65^c	68.00 ± 2.08^b

Means within the same row bearing different superscripts are significant at $p \leq 0.05$.

RBCs = Erythrocytes counts. WBCs = Leucocytes counts Hb= Haemoglobin

PCV= Packed cell volume B.S = Blood sugar

ALT = alanine aminotransferase

AST= aspartate aminotransferase

Table 6. Residues of Reldan in muscles of *oreochromis niloticus* fingerlings exposed for 8 weeks

Group \ Period	Residues of Reldan (ppm)	
	4 Week	8 Week
Control	0.0	0.0
$^1/_{10}$ LC_{50} (0.227 ppm)	0.81	1.00
$^1/_{20}$ LC_{50} (0.114 ppm)	0.239	0.438

Table 7. Residues of Reldan in muscles of *oreochromis niloticus* fingerlings exposed to $^1/_3$ 96 hrs LC_{50}.

Group (n =30) \ Period	Exposure period		Recovery period	
	3 days	7 days	3 days	7 days
Control	0.0	0.0	0.0	0.0
$^1/_3$ LC_{50} (0.76 mg/L)	1.00	1.35	0.035	0.0071

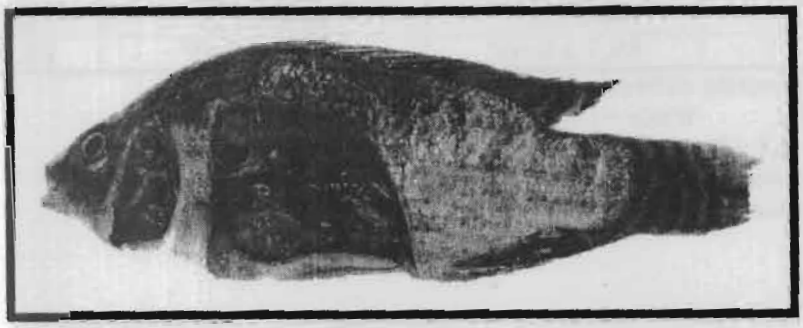

Fig. (1): *Oreochromis niloticus* fingerlings exposed to acute dose of Reldan, showed congestion of internal organs with mucous secretion on gills .

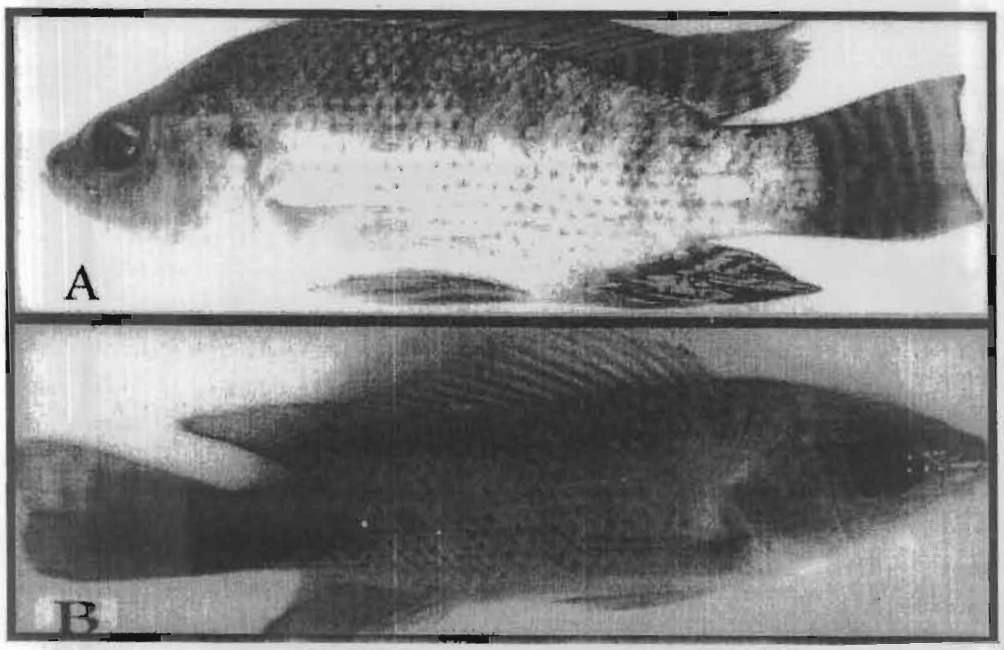

Fig. (2) A: Normal colour (light grey) of *Oreochromis niloticus* .B: *Oreochromis niloticus* exposed to $^{1}/_{10}$ 96hrs LC_{50} of Reldan showed dark colouration of the skin with mucous secretion.

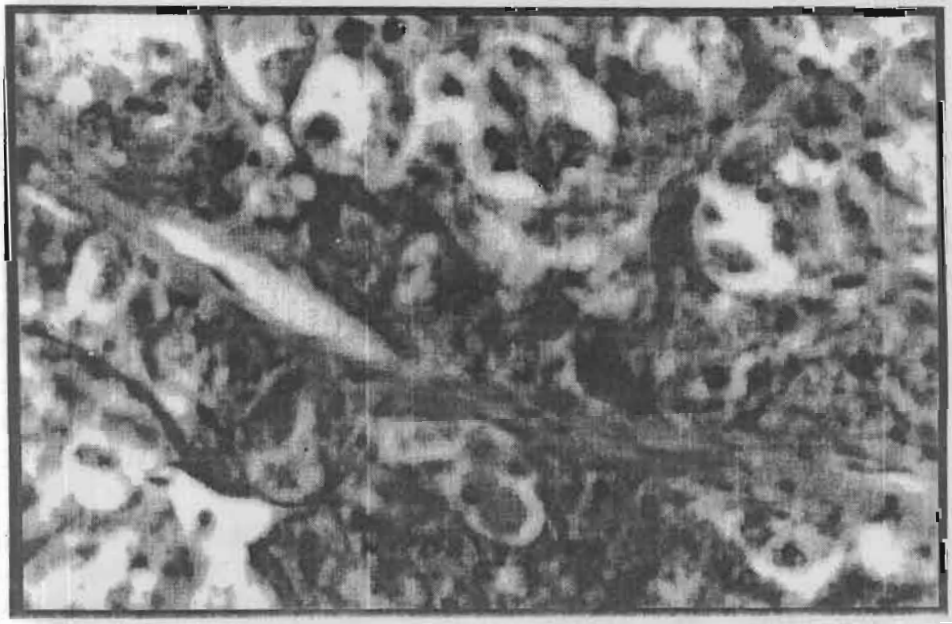

Fig. (3): Section in the liver of *Oreochromis niloticus* exposed to $^{1}/_{10}$ 96 hrs LC_{50} of Reldan showed hydropic degeneration of some hepatocyte with disorganzation of some acini (H& E X 1200).

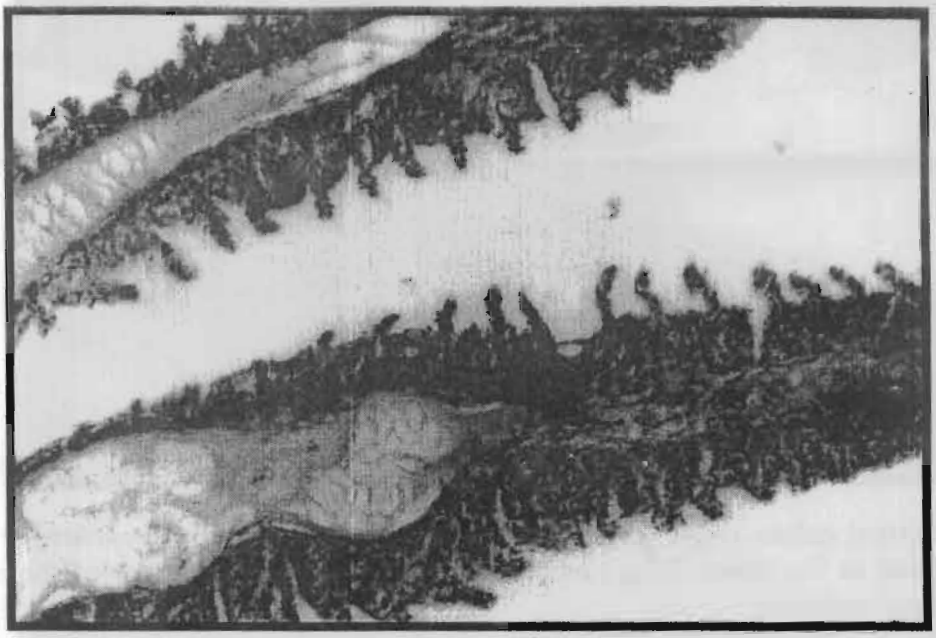

Fig. (4): Gills of *Oreochromis niloticus* exposed to $^{1}/_{10}$ 96 hrs LC_{50} of Reldan showed partial desquamation of the epithelium of gill filament with focal proliferation of macrophage and infilteration of lymphocytes (H& E X 300).

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الملخص العربى

دراسة على تأثير الريلدان (كلوربيريفوس ميثيل) على صحة ونمو إصبعيات البلطي النيلي

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فى هذه الدراسة أستخدم ٣٥٠ سمكة إصبعيات البلطي النيلي لتحديد الجرعة النصف مميتة بعد ٩٦ ساعة للريلدان وكذلك تأثير التركيزات ١/١٠، ١/٢٠ من الجرعة النصف مميتة بعد ٩٦ ساعة للريلدان على الحالة الصحية ومعدلات النمو للأسماك المختبرة. أيضا تحديد الاستجابة الشفائية للأسماك المعرضة لتركيز ١/٣ من الجرعة النصف مميتة بعد ٩٦ ساعة للريلدان.

أوضحت نتائج الدراسة ما يلي :

١- أن الجرعة النصف مميتة بعد ٩٦ ساعة للريلدان فى إصبعيات البلطي النيلي هي ٢,٢٧ ملليجرام/لتر.

٢- وجد أن الأسماك المعرضة للجرعة الحادة من الريلدان تعوم بطريقة غير طبيعية، كسل، توتر وقلق مصحوب بتشنج، بطئ فى معدل التنفس وتفقد الأسماك الوعي والاستجابة لانعكاس الهروب، احتقان في جميع الإعضاء الداخلية مع وجود طبقة مخاط على الخياشيم. بالإضافة إلى تلون الجلد باللون الداكن مع وجود طبقة كثيفة من المخاط مع تضخم الكبد خاصة في الأسماك المعرضة لتركيزات ١/١٠، ١/٢٠ من الجرعة النصف مميتة بعد ٩٦ ساعة للريلدان.

٣- وجد أن هناك انخفاض ملحوظ في معدلات النمو مصحوب بتغيرات في مكونات الدم وتغيرات كيميائية حيوية . أيضاً وجود بقايا للريلدان فى عضلات الأسماك المختبرة مع تغيرات نسيجية مرضية فى الخياشيم والكبد فى الأسماك المعرضة لتركيزات ١/١٠، ١/٢٠ من الجرعة النصف مميتة بعد ٩٦ ساعة للريلدان.

٤- وجد أن الاستجابة الشفائية لاصبعيات البلطي النيلي المعرضة لتركيز ١/٣ من الجرعة النصف مميتة بعد ٩٦ ساعة للريلدان قد تمت بعد أسبوع واحد.