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HAEMATOLOGICAL EFFECTS OF FLURIDONE HERBICIDE ON NILE CATFISH (*Clarias lazera*)

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Ghoneim[1], S.I; Z.H. Zidan[2]; M.A. El-Herawie[3] and M.M. El-Said[3]

ABSTRACT

The influence of fluridone herbicide on haemagram in catfish (*Clarias lazera*) (7 and 28 ppm) for 60 days was studied. The results indicate that fluridone induced significant decrease in erythrocytes count, haemoglobin content and packed cell volume (PCV). The mean corpuscular volume (MCV) was markedly elevated with 7 ppm of fluridone after 60 days of treatment, while the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) did not change. The leucocytes count increased significantly with higher concentration (28 ppm) of fluridone by the end of the experiment. No significant-treatment related changes were observed in heterophils and lymphocytes. The monocytes was elevated significantly with 7 ppm after 60 days of treatment.

Key words: Catfish, Fluridone, Herbicide, Haematological effects

INTRODUCTION

The herbicides have with a few exceptions relatively low toxicities for fish but can cause depletion of oxygen (Hypoxia) (**Murty, 1986**). Hypoxia is of primary interest from the stand point of fish physiology. Fluridone as an aquatic herbicide has a highly effective broad spectrum and is used for control of submerged vascular plants in ponds and lakes. **El-Deen *et al* (1992)** reported that the herbicide diquate bromide caused significant increase in haematocrite value but not in haemoglobin concentration. Chlordane insecticide (0.12 mg/L) induced enhancement in haemoglobin (%), RBCs count, WBCs count and haematocrite value in fresh water fish (*Saccobranchus fossilis*) (***Verma et al* 1979**). The sublethal concentration of zinc sulphate (at 100 ppm) induced haematological changes in *Cliso fasciatus*, where RBCs, WBCs counts and haematocrite values were decreased significantly, while the haemoglobin content remained well within limits observed in control (**Mishra and Srivastava, 1979**). The fish (*Clarias lazera*) were exposed to triad combination of lead, mercury and arsenic at 1/100 of their 72-LC_{50} for 5 weeks, showed a decrease in red blood cells counts while

1- Fac. of Agric., Suez Canal University, Ismalia, Egypt.
2- Fac. of Agric., Ain Shams University, Shoubra El-Kheima, Cairo, Egypt.
3- Central Laboratory of Pesticides, (MOA), Dokki, Cairo, Egypt.

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the white blood cells counts showed significant increase (**El-Nounou *et al* 1997**).

The aim of the present work was to study the haematotoxicity of herbicide fluridone on Nile catfish (***Clarias lazera***).

MATERIAL AND METHODS

1. Fish collection and maintainance

The Nile catfish [*Clarias lazera*] was chosen as the experimental animal to study the toxicity of the herbicide fluridone on its biological system. These fish are the major communities inhabiting the River Nile branches and lakes. The chosen fish is widespread in small, large, shallow and deep canals in which herbicides are applied, can survive in aquaria for a long period. One hundred and fifty five catfish used in this study were collected from Imbaba market in Giza. Their weight was 120 ± 20 g and their mean length was 28 cm. They were transferred alive to the laboratory in tanks containing river water. In the laboratory, the fish were kept under observation in glass aquaria (100 x 50 x 50 cm) containing chloride free-tap water at (23 ± 2°C) and continuous areation was maintained in each aquarium using air pumps. Fish were left for two weeks and fed once daily (2.5% biomass) on special ration formulated in laboratory as follow:

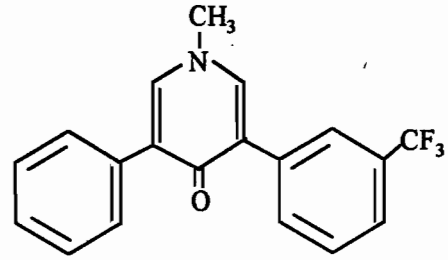
* Beef meat	750	g
* Rice	100	g
* Flour	50	g

Other additives such as plant fat; minerals mixture including sodium bicarbonate and green additives (100 g) were added.

Fish tanks were siphoned daily after feeding to remove feed spellings.

2- Herbicide

Fluridone As 41% formulation [1-methyl-3-3-phenyl-5- (3-trifluoromethyl) phenyl-4- (IH)-Pyridione] was used.



3- Herbicide treatments and fish exposure

Eighty fish were allocated into eight equal groups of ten fish each and fluridone was added to the eight groups in different concentrations to determine the 96-LC_{50}. Seventy-five fish were allocated into experimental groups (A, B, C), each of 25 fish and kept in separate tanks. Fish group (A) were considered as control, while group [B] was kept in tanks containing 7 ppm fluridone. Fish of the third experimental group (C) were kept in tanks containing 28 ppm of fluridone.

4. Water quality index

Water was changed every 5 days and analyzed immediately for pH, total hardness and salinity expressed as water index.

5. Fish sampling and organ weights

Subsequent samples of live fish were taken from the experimental and control groups after 10, 20, 30, 45 and 60 days. The fish were weighed and the total length measured before collection of blood samples. The internal organs were isolated from the visceral cavity and weighed.

6. Haematological change assessments

Fresh blood for haematological parameters was obtained from the Caudal Peduncle. The blood was collected in dry clean tubes containing EDTA [Ethylene Diamine-Tetracetic acid] as anticoagulant (1 mg / ml fresh blood).

6-1. Erythrocytes and leucocytes were counted by using the method of **Blaxhall and Daislyes (1973).**

6-2. Estimation of haemoglobin content (Hb) was done by the cyanomethamoglobin method **(Drabkin and Austin, 1935).**

6-3. Determination of packed cell volume (PCV) (%) was done using the micro-haematocrit technique **(Schalm, 1986).**

6-4. Differential leucocytic count

For the determination of the white blood picture, a microscopic analysis of panoptically stained film **(Hoffman, 1977)** was done.

7. Statistical analysis

All data were subjected to statistical analysis according to the procedure reported by the **Snedecor and Cochran (1980).** Treatments means were compared by the least significant differences (LSD) at the 5% level of probability.

RESULTS

1- Haematological changes in fluridone treated catfish

The fluridone herbicide decreased significantly the erythrocyctes count at 7 and 28 ppm by the end of experiment. (Table, 1). Data in table 1, show that the haemoglobin content was significantly reduced in fluridone treated catfish after 60 days of treatment. Similarly, the packed cell volume (PCV) was decreased significantgly at 28 ppm of fluridone. The lower concentration (7 ppm) did not show any significant differences compared to the control.

Table (2) indicates that, the mean corpuscular volume (MCV) was markedly elevated at 7 ppm of fluridone after 60 days of treatment. The corpuscular haemoglobin (MCH) did not alter significantly after 60 days The highest concentration (28 ppm) caused significant increase in leucocytes count by the end of experiment.

Table (3) indicates that fluridone treatments did not induce, in generally, any changes in heterophils and lymphocyte counts in the treated fish, except, a significant increase in heterophils occured after 80 days and 45 days of exposure, the lymphocytes increased after 60 days with 7 and 28 ppm of fluridone. Also, the treated catfish with the lowest concentration (7 ppm) showed a marked elevation in monocytes count after 60 days.

Table 1. Effect of treatment with different concentrations of fluridone on erythrocytes count, haemoglobin conc., packed cell volume and leucocytes count of male catfish

Treatment	Treatment period (days)					
	10	20	30	45	60	Allover mean of treatment
Erythrocytes count (x 10^6/µl)						
Control (0.0 ppm)	2.706	2.642	3.034	3.032	3.152	2.913
7 ppm	1.650	1.780	2.740	2.658	2.960	2.350*
28 ppm	2.182	1.976	2.774	2.308	2.258	2.300*
L.S.D. of treatment means = 0.2731 No significant of interaction between period of treatment x treatment concentration						
Haemoglobin concentration (g/dL)						
Control (0.0 ppm)	6.082	6.436	6.080	6.540	5.870	6.202
7 ppm	6.012	4.498	5.210	6.254	5.762	5.547
28 ppm	5.396	4.866	5.460	5.202	4.324	5.050
L.S.D. of treatment means = 0.6280 No significant of interaction between period of treatment x treatment concentration						
Packed cell volume (%)						
Control (0.0 ppm)	34.000	31.800	31.000	31.600	26.800	31.040
7 ppm	34.200	37.400*	29.800	31.600	26.600	31.920
28 ppm	22.800	20.200*	24.600	29.200	23.000	23.960*
L.S.D. of treatment means = 2.1358 L.S.D. of interaction between period of treatment x treatment concentration = 4.776						

Table 2. Effect of treatment with different concentrations of fluridone on mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration of male catfish

Treatment	Treatment period (days)					
	10	20	30	45	60	Allover mean of treatment
Mean corpuscular volume (Fl)						
Control (0.0 ppm)	124.68	123.06	103.87	105.07	99.516	111.24
7 ppm	210.03	214.42	103.88	122.08	91.818	148.44
28 ppm	104.57	103.75	94.296	129.68	106.08	107.68
L.S.D. of treatment means = 16.311 L.S.D. of interaction between period of treatment x treatment concentration = 34.475						
Mean corpuscular haemoglobin (Pg)						
Control (0.0 ppm)	22.978	24.990	21.018	21.706	19.140	21.966
7 ppm	37.228	27.060	18.928	25.260	19.752	25.646
28 ppm	24.782	24.932	20.756	23.576	19.878	22.785
No significant differences exist between different treatments No significant of interaction between period of treatment x treatment concentration						
Mean corpuscular haemoglobin concentration (%)						
Control (0.0 ppm)	18.712	20.414	19.978	20.558	22.106	20.354
7 ppm	23.490	24.204	22.524	21.028	21.671	21.356
28 ppm	17.962	11.990	17.524	17.830	18.048	18.035
L.S.D. of treatment means = 2.559 L.S.D. of interaction between period of treatment x treatment concentration = 5.726						

Table 3. Effect of treatment with different concentrations of fluridone on differential Leucocytes of male catfish

Treatment	Treatment period (days)					
	10	20	30	45	60	Allover mean of treatment
			Leucocytes count (x 10^6/uL)			
Control (0.0 ppm)	11.274	11.382	12.904	11.120	12.684	11.873
7 ppm	8.030	7.906	12.486	11.444	17.59*	11.492
28 ppm	9.318	9.600	20.186*	13.696	20.39*	14.640*
L.S.D. of treatment means = 1.616.						
L.S.D. of interaction between period of treatment x treatment concentration = 3.64.						
			Heterophils count			
Control (0.0 ppm)	1204.8	1477.0	1401.2	1211.80	1135.0	1285.96
7 ppm	1181.9	971.00	2901.1*	812.40	793.00	1334.91
28 ppm	1921.4	1546.8	1999.4	2399.20*	566.80	1706.72
No significant differences exist between different treatments.						
L.S.D. of interaction between period of treatment x treatment concentration = 1128.939.						
			Lymphocytes			
Control (0.0 ppm)	9938.60	10024.8	9794.00	10576.00	10084.0	10045.48
7 ppm	9478.20	8710.60	10416.2	9794.59	15010.*	10664.04
28 ppm	10321.2	8194.64	11884.6	9741.60	18511.*	11730.75
No significant exist between different treatments.						
L.S.D. of interaction between period of treatment x treatment concentration = 3217.538.						
			Monocytes			
Control (0.0 ppm)	127.600	95.400	262.800	139.800	508.400	226.800
7 ppm	45.500	11.000	163.960	80.200	942.20*	448.572*
28 ppm	36.172	64.960	342.960	84.400	476.800	201.058
L.S.D. of treatment means = 181.7841.						
L.S.D. of interaction between period of treatment x treatment concentration = 406.482.						

2- Water quality index

Data indicate that fluridone caused slight changes of salinity in total hardness, pH and temperature of water (Table, 4). On the other hand, this herbicide changed water salinity than the untreated water.

DISCUSSION

Several haematological parameters were found to be changed as a result of subchronic fluridone toxicity in fresh water catfish (*Clarias lazera*). The decrease in erythrocytes count, haemoglobin content and packed cell volume values (PCV) at higher concentration (28 ppm) of fluridone, may be attributed to the adverse effects of this herbicide on the haematopoietic system (**Muller and Lloyd, 1994**).

The mean corpuscular volume (MCV) increased significantly at low conc. (7 ppm). This change may be due to swelling of the erythrocytes which occurs whenever fish blood cells are exposed to a hypoxic environment (**Soivio and Nikinmaa, 1981**). These fidnings agree with those reported by **Goss and Wood (1988),** on aluminum in rainbow trout; **Mohamed and El-Hady (1994)** on mercuric chloride and zinc sulphate in carp and **El-Nounou *et al* (1997)** on triad combination of lead, mercury and arsenic in catfish. They found that the RBCs count, haemoglobin content and packed cell volume (PCV) values significantly decreased in treated fish, **El-Nounou *et al* (1997)** also, reported that the erythrocytes count and haemoglobin were decreased significantly, while the haematocrite value showed slight decrease in treated catfish.

These results show a significant increase in leucocytes count (leucocytosis). Such increase was conc. and time dependent response. The present findings agree with that previously reported by **Verma *et al* (1979).** They reported that an increase in leucocytes count occurred in freshwater fish following exposure to chlordane. Also, **El-Nounou *et al* (1997)** found that, the leucocytes count increased significantly after exposure to a triad combination of lead, mercury and arsenic.

The present study indicated the occurrance of lymphocytosis in fish treated with 7 ppm of fluridone which may be due to allergic autoimmune reactions that were caused by the tested compounds on gills and other tissues in fish. (**Bauer, 1982**).

No, changes occurred in the eosinophils count or monocytes following exposure to fluridone.

Table 4. Water quality Index

Fluridone Conc.	Parameter		
	Salinity (ppt)	Total hardness (ppm)	pH
Control	0.28±0.03	222.88±6.04	7.33±0.09
7 ppm	0.31±0.03	218.42±3.94	7.39±0.15
28 ppm	0.36±0.05	219.36±6.25	7.36±0.08

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مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ، ١١(٢) ، ٩٠٣ - ٩١١ ، ٢٠٠٣

التأثيرات الهيماتولوجية لمبيد الحشائش فلوريدون في ذكور اسماك القرموط النيلي المعرضة "*كلارياس لازيرا* "

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سمير غنيم[١] - زيدان هندي[٢] - مصطفي الهراوي[٣] - مجدي محمد السيد[٣]

١- كليـــة الزراعـــة - جامعـــة قناة السويس — الإسماعيلية - مصر

٢- كليـــة الزراعة - جامعة عين شمس - شبرا الخيمة — القاهرة - مصر

٣- المعمل المركزي للمبيدات - وزارة الزراعة - الدقى - الجيزة - مصر

تم دراسة تأثير مركب الفلوريدون (مبيد حشائش) علي صورة الـدم فـي الاسـماك القطنية (قراميط اللايزرا) بعـد تعريضـها لتركيزات ٧ ، ٢٨ جزء في المليـون مـن المركب المختبر لمدة ٦٠ يوما . أشـارت النتائج الي حدوث نقص معنوي فـي عـدد كريات الدم الحمراء وكذلك محتواهـا مـن الهيموجلوبين وكذلك فـي حجـم الكريـات المنضغطة بعد ٦٠ يوم من المعاملة ، فـي حين حدثت زيادة معنوية في قيمة متوسـط حجم كريات الدم الحمراء (MCV) بينما لـم تحدث تغيرات معنوية فـي متوسـط وزن الهيموجلوبين في كريـات الـدم الحمـراء (MCH) وكذلـك فـي متوسـط تركـيز الهيموجلوبين (MCHC) ، بينما حدثت زيادة معنوية في عدد كريات الدم البيضـاء بعـد المعاملة بتركيز ٢٨ جزء في المليون مـن مركب الفلوريدون في نهاية التجربـة (٦٠ يوم) . لم تحدث تغيرات معنوية في عـدد الخلايا المتباينة (heterophils) وكذلك فـي عدد الخلايا الليمفاوية في نهاية التجربة بينما حدثت زيادة معنوية في عدد الخلايا الاكولـة الكبيرة عند التركيز المنخفض (٧ جزء فـي المليون) بعد ٦٠ يوم من المعاملة .

تحكيم: أ.د محمد إبراهيم عبد المجيد
أ.د العيداروس أحمـد جمعه