Cytogenetical Studies On The Effects Of Glyphosate On Nile *Tilapia (Oreochromis niloticus) Fish*

Iman E El-Araby
Dept. of Animal Wealth Development, Fac. Vet. Med., Zagazig Univ.

ABSTRACT

Pollution of the aquatic ecosystem is recognized globally as a potential threat to both human and other animal's population which interacts with aquatic environment. Therefore, the aim of this study was to study the cytogenetic effects of the herbicide glyphosate in Nile Tilapia fish. This study was carried out on sixty fish which were divided into 4 equal groups. The first and second groups were kept as control for 96 hours and 45 days respectively. The third group was exposed to 1/10 LC_{50} of glyphosate for 96 hours and the forth group was exposed to 1/20 LC_{50} of glyphosate for 45 days. At the end of the experiment the fish in each group were injected intramuscularly with 0.1% of colchicine at the rate of 1 ml/100 gm body weight for studying any cytogenetic changes in gill cells. About 50 metaphase spreads were screened to detect the chromosomal aberrations. The statistical analysis revealed highly significant ($p<0.01$) effect of glyphosate herbicide in inducing chromosomal aberrations. Different types of structural aberrant were represented by deletion, fragments, breaks, gap and centromeric attenuation, while the numerical aberrations were represented by hyper and hypoploidy. These results concluded that numerical and structural chromosomal aberrations in Tilapia nilotica fish could be a good indicator for environmental pollution which reflected on the human health and could be considered as bio- health hazardous chemicals so it should be use under strict measures.

INTRODUCTION

Fish and shellfish are low in saturated fat; contain high concentrations of protein, omega-3 fatty acids and other essential nutrients. Consequently, they are considered to be an important part of a well-balanced diet to heart health and to children's proper growth and development. Fish provided more than 50% of the animal protein for the population of several countries *(1)*.

Fish are useful experimental models to evaluate the health of aquatic ecosystem and biochemical changes, so it acts as monitor of the environmental pollution. *Oreochromis niloticus* is a good model for toxicological experiments because it has high growth rate, adapts easily to commercial diet, resistant to diseases and injuries resulted from rough handling practice, reproduce well in captivity and finally has good tolerance to wide variety of husbandry condition *(2)*.

Chromosomal and cytogenetic studies on fish have received considerable attention in recent years *(3)*. Fish chromosome data have great importance in studies concerning evolution, systematics, aquaculture and mutagenesis *(4, 5)*.

Pollution of the aquatic ecosystem is recognized globally as a potential threat to both human and other animal's population which interacts with aquatic environment *(6)*. Egypt possesses approximately 5000 km of irrigation and drainage canals *(7)*. Drainage canals were polluted as a result of discharging agricultural drains daily, with insecticides, pesticides, heavy metals, fertilizers, chemicals, sewage and other possible domestic/industrial wastes. The wide indiscriminative use of pesticides in weed and pest control, certainly, results in the pollution of all environmental components including water body *(8)*.

Glyphosate is a broad-spectrum, non-selective systemic herbicide. It can be used on non-cropland and among a great variety of crops. Glyphosate is usually formulated as an isopropylamine salt. While it can be described as an organophosphorus compound, glyphosate is not an organophosphate ester but a phosphanoglycine, and it does not inhibit cholinesterase activity *(9)*.

Glyphosate is highly adsorbed on most soils especially those with high organic content. The compound is so strongly attracted to the soil that little is expected to leach from the applied area. Microbes are primarily responsible for the breakdown of the product. The time it takes for half of the product to break down ranges from 1 to 174 days. Because glyphosate is so tightly bound to the soil, little is transferred by rain or irrigation water. One estimate showed less than two percent of the applied chemical lost to runoff. The herbicide could move when attached to soil particles in erosion run-off. Photodecomposition plays only a minor role in environmental breakdown. In water, glyphosate is strongly adsorbed to suspended organic and mineral matter and is broken down primarily by microorganisms. Its half-life in pond water ranges from 12 days to 10 weeks *(10)*.

Glyphosate residues were detected in Egyptian plants after 30 days post-application *(11)*, also the muscle of catfish and Nile Tilapia, collected from different market in Sharkia governorate, contained a high level of glyphosate *(12)* and was higher than its maximum residue level in cattle, poultry and rabbit meat *(13)*. The 96 hours LC50 of glyphosate was determined in *Oreochromis aureus* and was 5 and 6 ppm for fingerling and adult respectively *(14)*.

The genotoxicity of glyphosate has been investigated in different assays *(15)*. It induced a significant increase in chromosomal aberrations as fragment, bridges, multipolar or polyploidy anaphase *(16)*. Glyphosate has caused DNA damage and/or micronucleus induction in the neotropical fish (*Prochilodus lineatus*) *(17)*, the fish Tilapia rendalli *(18)*, goldish (*Carassius auratus*) *(19)*, and European eel (*Anguilla anguilla*) *(20)*.

Therefore, the aim of this study was to study the cytogenetic effects of the herbicide glyphosate in Nile Tilapia fish.

MATERIALS AND METHODS

1. Glyphosate

Chemical class/use:phosphonoglycine, herbicide.

Physical Properties

- Appearance: Glyphosate is a colorless crystal at room temperature.
- Chemical Name: N-(phosphonomethyl) glycine.
- CAS Number: 1071-83-6.
- Molecular Weight: 169.08.
- Water Solubility: 12,000 mg/L at 25 °C.
- Solubility in Other Solvents: insoluble in common organics (e.g., acetone, ethanol, and xylene).
- Melting Point: 200 °C.
- Vapor Pressure: negligible.
- Partition Coefficient: -3.2218 - -2.7696.
- Adsorption Coefficient: 24,000 (estimated)

Trade name: Roundup *(21)*

2. Fish used in the experiment

A total number of 60 healthy Nile Tilapia fingerlings weighing 20-25 gm were obtained from the Arab Egyptian company for fish resources, Sharkia province, Egypt, transported to the Animal Wealth Development Department laboratory, Faculty of Veterinary Medicine, Zagazig University. The fish were maintained into well-aerated glass aquaria (about 100 liters capacity) filled with dechlorinated tap water which changed every other day, fed on a basal diet at level of 3% of the total body weight, and were acclimatized to the laboratory conditions for 2 weeks.

3. Cytogenetic studies

Sixty fish were divided into 4 equal groups. The first and second groups were kept as control for 96 hours and 45 days respectively. The third group was exposed to 1/10 LC_{50} of glyphosate (5 mg/L) *(22)* for 96 hours and the forth group was exposed to 1/20 LC_{50} of glyphosate (2.5 mg/L) *(22)* for 45 days. At the end of the experiment the fish in each group were injected intramuscularly with 0.1% of colchicine at the rate of 1 ml/100 gm body weight for studying any cytogenetic changes in gill cells.

Clear and excellent metaphase figures were observed at oil immersion objectives and were photographed at each of the dose levels, including control. When possible, one of each type of chromosomal aberration seen was photographed and illustrated. The observed

chromosomes in each cell were classified whether they normal or if they possess any chromosomal or chromatid aberrations. Also, the types of aberrations and the times these specific aberrations were encountered in each cell were taken note of. All the data gathered were recorded under their corresponding dose levels.

4. Statistical Analysis

The data obtained were statistically analyzed by Chi–square analysis test *(23)*.

RESULTS AND DISCUSSION

The present investigation was conducted to evaluate the *in vivo* chromosomal aberrations in *Tilapia nilotica* fish treated with glyphosate.

Exposure to genotoxic agents may result in mutation, metabolic disorder, damage embryos and reduced fertility *(24)*. The use of genotoxicity testing is essential for the assessment of potential livestock toxicity so that, hazard can be controlled. Cytogenetic analysis of chromosomes has been employed as a biological dosimeter to estimate the effect of genotoxic agents (pollutants) on fish and very useful for direct detection in somatic cells *(25, 26)*.

Tilapia fish had been used as monitor for the cytogenetic studies as it has the following criteria which are; mitotic activity is sufficient to score statistical number of metaphase, easily available (catching, buying, raising, culturing) in laboratory condition and it is easily to be accommodating to the external environment *(27)*.

As fish may act as organisms for study aquatic pollution, several species have been successfully used as test materials for detecting genotoxic activity in the aquatic environment *(28, 29)*. Analysis of metaphase chromosomes in fish for the occurrence of chromosome aberrations in order to detect as well as quantify the extent of genotoxicity or point mutation induced by an agent has been proved to be useful only in a few fish models *(30, 31)*.

1.Normal Chromosomal picture in the fish

The metaphase chromosomes of the *Tilapia nilotica* under study were properly identified. The diploid number (Figure 1) of chromosomes was 22 pair *(32)*.

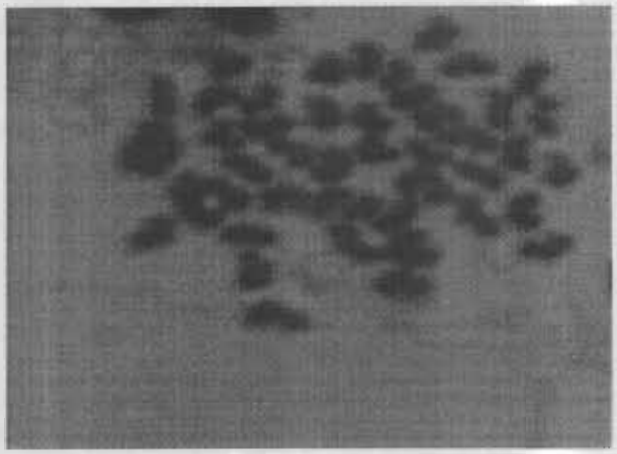

Fig. 1. Metaphase spread of Tilapia nilotica fish showing normal chromosomal number (Giemsa stain, X=1000).

2. Chromosomal Aberrations in the fish treated with 1/10 LC_{50} of glyphosate for 96 hrs

A total number of 1000 metaphase figures were used to investigate the cytogenetic effects of control and 1/10 LC_{50} glyphosate for 96 hrs in *Tilapia nilotica* fish. Table 1 showed that, the total chromosomal aberrations in the control and treated group were 42 and 114 (8.4% and 22.8%) respectively. The Chi–square analysis showed highly significant differences between the control and the treated group.

The different types of numerical and structural chromosomal aberrations in control and 1/10 LC_{50} glyphosate treatment for 96 hrs group were presented in Table 2. The statistical analysis revealed highly significant ($p<0.01$) effect of glyphosate herbicide in inducing chromosomal aberrations. Different types of structural aberrant were represented by deletion, fragments, breaks, gap and centromeric attenuation, while the numerical aberrations were represented by hyper and hypoploidy (Figures 2, 3).

Table 1. Total chromosomal aberrations 1/10 LC_{50} of Glyphosate for 96 hrs

Group	Normal metaphase Figures	Abnormal metaphase Figures	% of abnormal figures
Control	458	42	8.4%
1/10 LC_{50} (96 hrs) of Glyphosate	386	114	22.8%
Chi-Square Value		38.28**	

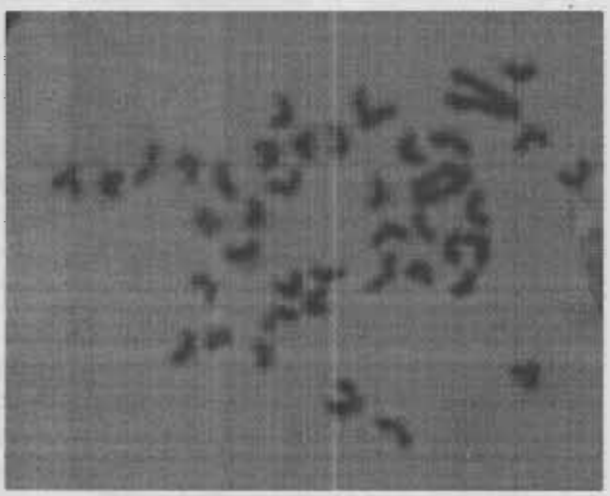

Fig. 2.Metaphase spread of Tilapia nilotica fish showing aneuploidy (Giemsa stain, X=1000).

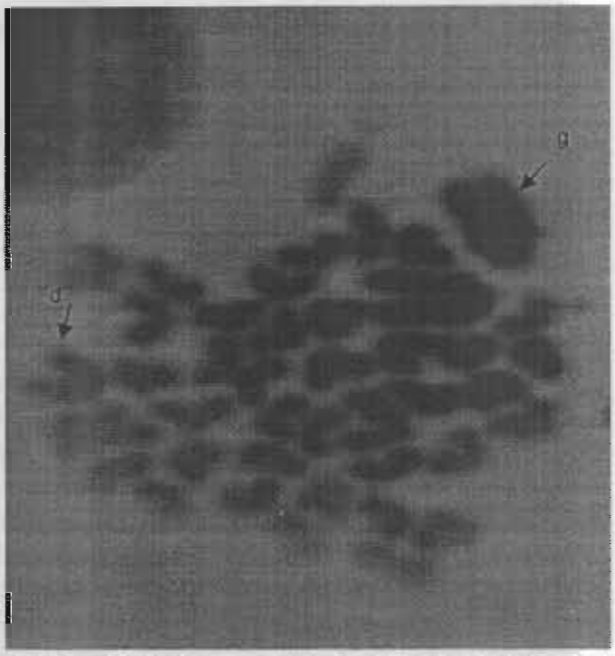

Fig. 3. Metaphase spread of Tilapia nilotica fish showing gap and deletion (Giemsa stain, X=1000).

Table 2. Different types of chromosomal aberrations in control fish group and 1/10LC_{50} of glyphosate for 96 hours

Group	Total examined metaphase	Numerical aberrations								Structural aberrations											
		Poly.	%	Aneu.	%	Total	%	G.	%	B.	%	D.	%	F.	%	C.A.	%	R.	%	Total	%
Control	500	2	0.4	4	0.8	6	1.2	11	2.2	5	1	6	1.2	10	2	4	0.8	-	-	36	7.2
1/10LC_{50} Of glyphosate/ (96 hrs)	500	15	3	22	4.4	37	7.4	20	4	15	3	8	1.6	12	2.4	10	2	2	0.4	77	15.4
χ^2_{cal}		8.617 **		11.912 **		21.871 **		2.131 N.S		4.133 *		0.072 N.S		0.046 N.S		1.811 N.S		0.501 N.S.		15.965 **	

Poly. = Polyploidy. neu. = Aneuploidy , = Gap. , = Break. D. = Deletion.
F. = Fragment. A. = Centromeric Attenuation. R. = Ring. $^2_{cal}$= Chi – square Value.
N.S. =Non-significant. - **=Highly-significant. - *= Significant.

3.Chromosomal Aberrations in the fish treated with 1/20 LC_{50} of glyphosate for 45 days

To evaluate the chronic effect of glyphosate on *Tilapia nilotica* fish, a total number of 1000 figures were investigated. Table 3 showed that the total chromosomal aberrations in the control and treated group were 58 and 136 (11.6% and 27.2%) respectively. The Chi – square analysis showed highly significant differences between the control group and the treated group.

Table 3. Total Chromosomal aberrations 1/20 LC_{50} of glyphosate for 45 days

Group	Normal metaphase Figures	Abnormal metaphase Figures	% of abnormal figures
Control	442	58	11.6%
1/20 LC_{50} of glyphosate	364	136	27.2%
Chi-Square Value		37.918**	

Table 4, showed the different types of both numerical and structural chromosomal aberrations as well as the Chi – square values for control and 1/20 LC_{50} glyphosate for 45 days. The highest type of chromosomal aberration was polyploidy as numerical aberrations (5.8%) and deletion as structural aberrations (5.6%). On the other hand, the lowest type of chromosomal aberration was ring chromosomes by 0.6% (Figure 4).

Similar effect was previously recorded on *O. niloticus* fish exposed to copper sulfate and lead acetate. Chromatid deletion, stickness and fragments were more frequent than other chromosomal aberrations *(33)*. Also, exposure of *Channa punctata* (2n=32) to mercuric chloride, arsenic trioxide and copper sulphate pentahydrate for a week, showed that kidney cells revealed chromatid and chromosome breaks, chromatid and chromosome gaps, along with ring and di-centric chromosomes. The findings depict genotoxic potential of these metals even in sublethal concentrations *(34)*.

Enhancement in the frequency of chromatid breaks, acentric fragments, centromeric fusions, aneuploidy, condensation, sticky plates and ring, were recorded in *Mystus glulio* fish exposed to a Lambda-cyhalothrin compared with those in the tap water control *(35)*. Chromosomal aberrations frequency increased by the increase of concentrations of herbicide, whipsuper *(36)*.

A significant increase in the frequency of deletions in grass carp fish exposed to carbarmate insecticide was recorded *(37)*. A high significant increase in chromosomal deletion in the kidney cells of grass carp exposed to insecticides was also observed *(8)*.

Chromosomal aberrations result from abnormalities in DNA duplication during the S phase; this may be due to the interference of the pollutants with nucleotide synthesis *(8)*, leading to malformation of DNA molecules *(38)*, or arise as a consequence of miss-repair or of miss-replication of damaged DNA *(39)*.

Table 4. Different types of chromosomal aberrations in control fish group and 1/20LC_{50} of glyphosate

Group	Total examined metaphase	Numerical aberrations								Structural aberrations											
		Poly.	%	Aneu.	%	Total	%	G.	%	B.	%	D.	%	F.	%	C.A.	%	R.	%	Total	%
Control	500	5	1.00	9	1.8	14	2.8	13	2.6	7	1.4	9	1.8	8	1. 6	5	1.00	2	0.4	44	8.8
1/20 LC_{50} Of glyphosate	500	29	5.8	18	3.6	47	9.4	22	4.4	18	3.6	28	5.6	11	2.2	7	1.4	3	0. 6	89	17.8
χ^2_{cal}		16.106 **		2.436 N.S		17.877 **		1.895 N.S.		4.103 **		9.093 **		0.215 N.S.		0.084 N.S.		0.00 N.S.		16.789 **	

Poly. = Polyploidy. Aneu. = Aneuploidy = Gap. B. = Break. D. = Deletion.
F. = Fragment. A. = Centromeric Attenuation. R. = Ring.
$X^2_{cal=}$ Chi – square Value. N.S. =Non-significant. - =Highly-significant. - *= Significant.

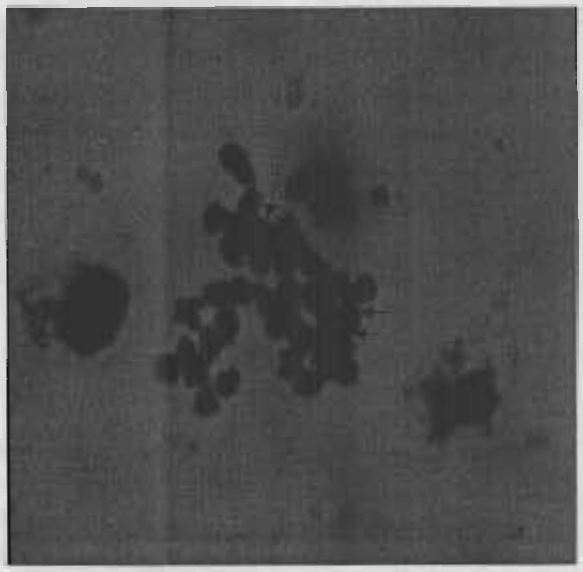

Fig. 4. Metaphase spread of Tilapia nilotica fish showing gap, break, ring, deletion (Giemsa stain, X=1000).

Conclusion

From the results obtained we can concluded that numerical and structural chromosomal aberrations in Tilapia nilotica fish could be a good indicator for environmental pollution which reflected on the human health and could be considered as bio-health hazardous chemicals so it should be use under strict measures.

REFERENCES

1.FAO (2000): FAO Speciications and Evaluations for Plant Protection Products: Glyphosate N-(phosphon omethyl)glycine. Food and Agriculture Organization of the United Nations, Rome. http://74.125.155.132/ custom? =cache:fh Vj5_MxOwJ:www.fao.org/ag/AGP/AGPP/P esticid/Specs/docs/Pdf/new/glypho01.pdf+F AO+Specifications+and+Evaluations+for+P lant+Protection+Products:+Glyphosate+N-(phosphonomethyl) glycine&cd= 1& hl= en&ct=clnk &cl ient=google-coop-np.

2.Badawy EA (1993): Biological studies on Tilapia species as a major component system. Ph.D. Thesis, Zoology Department; Faculty of Science; Zagazig University.

3.Okonkwo JC and Obiakor MO (2010): Karyological and Chromsomal Study of Catfish (*Clariidae, Clarias gariepinus, Buchell* 1822) from Anambra River, Anambra State, Nigeria. Pakistan J. Nurt., 9: 112-115.

4.Amemiya CT (1986): Cytogenetic and Cytosystematic Studies on the Nucleolus Organizer Regions of North American Cyprinid Fishes, Texas A&M University, Ph.D. Thesis.

5.Al-Sabti K (1991): Handbook of Genotoxic Effects and Fish Chromosomes, J Stephan Institute, P.O Box 100, Jamova 39, *Ljubjiana Yugoslavia*)

6. Biney Calamari D, Membe TW, Naeve H, Nyakageni B and Saad MA (1987): Scientific bases for pollution control in African inland waters. FAO fisheries, 369: 9-23.

7.Redding TA and Midlen AB (1992): Fish production in irrigation canals. FAO fisheries Technical Paper. No. 317. Rome, FAO. 1990.111p.

8. Matter EE, ELserafy SS, Zowail M E M and Awwad, MH (1992): Genotoxic effect of carbamyl insecticide (sevin) on the grass carp *Ctenopharygodan idella* (VAL). Egypt. J. Histol., 15 (1): 9-17.

9.US Environmental Protection Agency, (2006): Technical Factsheet on: GLYPHOSATE.

10.Forest Service (1984): Pesticide Background Statements, Vol. I Herbicides. United States Dept. of Agriculture, Agriculture Handbook No. 633).

11.El-Mahy SAA (1986): Studies on the effect of certain herbicides on Panicum repense (L) weed. Master Science Thesis, Faculty of Science, Cairo University.

12.Gergis MT (2001): Herbicides residues in fresh water fish with special reference to the effect of heat treatment on its stability. Master Thesis, Meat Hygiene, Faculty of Veterinary Medicine, Zagazig University.

13.FAO WHO and IPCS (1986): Pesticide residue in food (Evaluations). Part II: Toxicology, received from Monsanto Co. Europe S. A. 15125 Amaroussion Athene-Greece.

14. Diab A S, El-Serafy SS, Abd El-Halim, M S and El-Shafey AA (1996): Toxicological biochemical studies of the herbicide glyphosate on Oreochromis aureus. 3rd Vet. Med. Cong. Zagazig 8-10 October. PP. 233-246.

15.De Marco A, De Simone C, Raglione M, Testa A and Trinca S (1992): Importance of the type of soil for the induction of micronuclei and the growth of primary roots of *Vicia Feba* treated with the herbicides atrazine, glyphosate and maleic hydrazide. Mutation Res., 279, 9-13.

16. Rank J, Jensen AG, Skov B, Pedersen LH and Jensen K (1993): Geontoxicity testing of the herbicide roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, Salmonella mutagenicity test and Allium anaphase-telophase test. Mutation Res. 300, 29-36.

17.Cavalcante DG, Martinez CB and Soia SH (2008): Genotoxic effects of Roundup on the *Prochilodus lineatus*. Mutat Res 655 (1-2):41-6.

18.Grisolia CK (2002): A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. Mutat Res., 50-518: 145.

19. Cava T and Konen S (2007): Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay. Mutagenesis 22(4):263-8.

20. Guilherme S, Gaivao I, Santos MA and Pacheco M (2009): Tissue speciic DNA damage in the European eel (*Anguilla anguilla*) following a short-term exposure to a glyphosate-based herbicide. Toxicol Lett 189S:S212: Z15).

21.Caceres-Jensen L, Gan J, Baez M, Fuentes R and Escudey M (2009): Adsorption of glyphosate on variable-charge, volcanic ash-derived soils. J Environ Qual 38(4):1449-57.

22.Abou-Elmagd MM, Ghoniem MH and Diab AS (1998): Some toxicological aspects of glyphosate (Roundup) herbicide on Nile cat fish (*Clarias lazera*). Zagazig Veterinary Journal 26: 15-24.

23.SPSS (2007): SPSS/PC+(2007), for the PC/XT. SPSS INC.

24.Ghaffer AE, Abou-Salem ME and Ashoub MM (1994): Relationship between environmental pollution and incidence of repeat breeder in buffalo-cows. Annals Agric. Moshtoher 32(3): 1715-1728.

25. Yunis JJ (1983): The chromosomal basis of human Neoplasia. Science, 221: 227-236.

26.Radwan H A (1996): Physiological and genetical changes in some freshwater fishes duo to pollution. M.V. Sc.Thesis. Fac.Vet. Med., Zagazig Univ.

27. Seehy A A and Karolin Barakat (1995): A rapid preparation of Tilapia chromosomes-in vivo assessment of aquatic pollution. Egyption J. Genet. Cytology.

28.Hooftman RN and Raat WK (1982): Induction anomalies (micronuclei) in the peripheral blood erythrocytes of the eastern mudminow Umbra pygmaea by ethyl methane sulphonate. Mut. Res., 104: 147-152.

29. Manna GK and Mukherjee PK (1986): Effect of organophosphate insecticide 'malathion' on chromosome cell division and total muscle proteins of cichilid fish, Tilapia, in: G.K. Manna, S.C. Roy (Eds.), Procedings of the Xth All India Congress on Cytol. Gene, Kalyani, West Bengal, 7-10 October, Pers. Cytol. Genet. 5: 225-235.

30. Kligerman AD (1982): Fishes as biological detectors of the effects of genotoxic agents, in: The J.H. Heddle (Ed.), Mutagenicity New Horizons in genotoxic potentiality of the inorganic weedicide, sodium arsenite in

the experimentally treated tilapia fish. J. Freshwater Biol., 2: 147-159.

31.Manna GK (1984): Progress in fish cytogenetics, Nucleus (27): 203–231.

32.Amany M, Zowail M, Ghada Y and Sharafeldin K (2010): Cytogenetical studies on some River Nile species from polluted and nonpolluted Aquatic habitats. Egypt. Acad. J. biolog. Sci., 2 (1): 1-8.

33.Mohamed MM, El-fiky SA, Soheir YM and Abeer AI (2008): Cytogenetic studies on the effect of copper sulfate and lead acetate.pollution on Oreochromis niloticus fish. Journal of cell Biology. 3(2): 51-60.

34.Yadav KK and Trivedi S P (2009): Chromosomal aberrations in a fish,*Channa* punctata after in vivo exposure to three heavy metals. Mutation Research, Genetic Toxicology and Environmental Mutagenesis 1: 7-12.

35.Velmurugan B Ambrose T and Selvanayagam M (2006): Genotoxicity evaluation of Lambda – cyhalothrin in *Mystus gulio*. Journal of Environmental Biology. 27(2): 247-250.

36.Farag ME, Ramadan AA and Ali MA (2009): Cytogenetic and biochemical effects of whip super herbicide toxicity on Nile tilapia (*Oreochromis niloticus*). nd Proceedings of the 2 Global fisheries and Aquaculture Research. Conference, Cairo International Convention Center, 53: 95-79.

37.Awwad AM (1991): Effect of pollution on haemopoietic cells of fresh water fishes in Qalyobia province. M. Sc. Thesis zoology. Zagazig University, Faculty of Science.

38.Landolt M L and Kocan R M (1983): Fish cell cytogenetics: A measure of the genotoxic effects of environmental pollutants. In Aquatic toxicology (J. O. Nriagu, ed.) 335-352. John Wiley and Sons Ins.

39.Evans HJ (1977): Molecular mechanisms in the induction of chromosome aberration. Scott, D.Bridges, B.A.and Sobier / North Holland, Amsterdam, 57-74.

الملخص العربى

دراسات وراثية خلوية علي تأثير الجليكوفوزيت في اسماك البلطي النيلي

ايمان السيد العربي محمود

قسم تنمية الثروة الحيوانية- كلية الطب البيطري- جامعة الزقازيق

يعتبر تلوث النظام البيئى المائى تهديدا عالميا للبشر و الحيوانات التي تتفاعل مع البيئة المائية. لذا، كان الهدف من هذه الدراسة هو دراسة آثار الغليفوسات السيتوجينية في السمك البلطي النيلي. وقد أجريت هذه الدراسة علي ستين سمكة التي تم تقسيمها الى ٤ مجموعات متساوية تم الاحتفاظ بها. المجموعات الأولى والثانية هي مجموعات ضابطة لمدة ٩٦ ساعة و ٤٥ يوما على التوالي. بينما تعرضت المجموعة الثالثة إلى ١/١٠ LC50 من الغليفوسات لمدة ٩٦ ساعة، وتعرضت المجموعة الرابعة إلى ١/٢٠ LC50 من الغليفوسات لمدة ٤٥ يوما. في نهاية التجربة تم حقن الأسماك في كل مجموعة عضليا بمادة ٠,١ ٪ كولشيسين بمعدل جرام من وزن الجسم 1ml/100 لدراسة أية تغييرات وراثية في الخلايا الخيشومية. و قد لوحظ من خلال التحليل الإحصائي للتجارب المختلفة ان التاثير الحاد و المزمن الغليفوسات ادي الي زيادة معنوية في التشوهات الكروموسومية من الناحية العددية والتركيبية. حيث مثلت التغيرات التركيبية في وجود فقد في قطع كروموسومية، قطع كروموسومية، كسر، فجوه، انفصال السنترومير بينما مثلت التغيرات العددية في زيادة او نقص في العدد الكروموسومى. وخلصت هذه النتائج باعتبار الانحرافات الكروموسومية العددية والتركيبية في السمك البلطي النيلي مؤشرا جيدا للتلوث البيئي الذي ينعكس على صحة الإنسان ويمكن اعتباره الحيوية الصحية للمواد الكيميائية الخطرة، والتي يجب أن يكون استخدامها في إطار تدابير صارمة.