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Full Length Research Paper

Genotoxicity induced by 4-Nonylphenol in adult and embryos of *Clarias gariepinus*

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The presence of the genetic toxic chemicals in the aquatic environment increased. This study aimed using the random amplified polymorphic DNA (RAPD-PCR) assay to investigate the genotoxic effects of 4-nonylphenol in adults and embryos of African catfish *Clarias gariepinus*. After exposure of adults and embryos to sublethel concentrations of 4-nonylphenol (0, 0.05, 0.08, and 0.1 mg/l) for 15 days, DNA was extracted for RAPD assay. The RAPD pattern from catfish exposed to 4-nonylphenol displayed some changes in polymorphism band patterns including disappearance and appearance of bands. Cluster method was used to indicate the distinct distance between the band patterns of exposed fish and the control. In conclusion, the RAPD-PCR is method can be used as an investigation tools for the evaluation of the genetic damage of the catfish induced by the exposure to 4-nonylphenol.

Keywords: 4-nonylphenol, genotoxicity, embryos, *Clarias, gariepinus,* DNA.

INTRODUCTION

Environmental pollution is now attracting increasing attention. Some genotoxic agents can not only injure the integrity of the genome but also affect the expression of DNA directly or indirectly (Shugart and Theodorakis, 1994). Degree of DNA integrity has been proposed as a sensitive indicator of genotoxicity and an effective biomarker for environment monitoring (Shugart, 1990). 4-nonylphenol is a highly toxic chemical (Mekkawy et al., 2011; Sayed et al., 2011; Sayed et al., 2012a; Sayed et al., 2012b). The dispersion of 4-nonylphenol in the environment has increased over the past decades due to its widespread industrial use. It is a by product of nonylphenol ethoxylate (NPE) which has been found in aquatic environments, particularly in sediments, sewage and river water (Clark et al., 1992; Tsuda et al., 2000; Rivero et al., 2008). 4- Nonylphenol (NP) is more stable and persistent in the environment (Sakai, 2001; Uguz et al., 2003) and many studies report NP's estrogenic effects on different organisms (Sone et al., 2004; Sayed et al., 2012a). Evaluations of many environmental estrogenic –like compounds indicated that nonylphenol produced negative effects on human health and wildlife thought water, food, air and skin contaminations (Rivero et al., 2008; Sayed et al., 2012b). There are a limited number or reports concerning the effects of NP on genotoxicitiy, especially on aquatic organisms (Reviro et al., 2008; Sayed et al., 2012a).

It has been reported that organic chemicals are known to be biotransformed by fish giving rise to potentially genotoxic intermediates able to induce genetic alterations in exposed organisms, which can cause somatic or hereditary defects (Peters et al., 1997; Lyons et al., 1997; lyons et al., 1999). Various contaminants in polluted water are reported to be capable of interacting with the

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DNA of living cells and therefore cause genotoxic effects, such as DNA base modifications, strand breaks, depurination and cross-linkages (Frenzilli et al., 2004). There is interest in assessing the impact of genotoxic compounds released into the aquatic environment (Mekkawy et al., 2011; Sayed et al., 2011; Sayed et al., 2012a; Sayed et al., 2012b). It is difficult to evaluate the genotoxicity of an environmental sample using traditional chemical analysis (Walker, 1996). A number of methodologies are now available to ecotoxicologists in order to evaluate the impact of genotoxic compounds upon marine and freshwater species. These include molecular, biochemical and cytological techniques that have been used as biomarkers for assessing the biological impact of pollution (Frenzilli et al., 2004; Mekkawy et al., 2011; Sayed et al., 2011; Sayed et al., 2012a; Sayed et al., 2012b). Among the different techniques used to detect genotoxic damage is the randam amplified polymorphic DNA (RAPD-DNA), developed by Williams et al., (1990) and Welsh and McClelland (1999) and it is able to detect DNA damage (strand breaks) at tissue level. RAPD is a powerful technique that involves the amplifications of random segments of genomic DNA using PCR and Damage to the genomic DNA resulted in changes of the binding site and PCR product, and furthermore altered the electrophoresis pattern (Savva, 1996; Bacerril et al., 1999), so that these results made this technique possible to use to detect the genotoxicity of pollutants (Atienzar et al., 1999, 2000). Several studies have indicated the RAPD as useful method for detecting DNA damage in both laboratory and field studies both with aquatic invertebrates and fish (Savva, 1998; Atienzar et al., 2002; Atienzar and Jha, 2004; Atienzar and Jha, 2006). Many authers reported the RAPD assay to detect genomic DNA alterations induced by several DNA-damaging agents such as benzo[a]pyrene (Castano and Becerril, 2004; Atienzar and Jah, 2004), cyclophosphamide and dimethoate (Zhiyi and Howen, 2004), heavy metals (Cambier et al., 2000; Enan, 2006), mitomycin C (Becerril et al., 1999a, b), 4-n- nonylphenol and 17-β estradiol (Atienzar et al., 2002), chysotile asbestos (Yoshida et al., 2001), UV radiation (Kumar et al., 2004; Atienzar et al., 2000) or x-rays, and radio nuclides (Theodorakis et al., 2001).

The use of fish biomarker as indices of pollution effects are of increasing importance and can permit early detection of environmental problems (Frenzilli et al., 2004; Mekkawy et al., 2011; Sayed et al., 2011; Sayed et al., 2012a; Sayed et al., 2012b). Catfish is a commercially important warm water fish which is distributed allover the world. *Clarias gariepinus* (Burchell, 1822) is belongs to family air-breathing or labyrinth catfishes (Clariidae) (Burgess, 1989). *Clarias gariepinus* is the native species of the Africa. This species has been used in aquaculture because of its faster growth rate, resistance to disease and possibility of high stocking density (Rahman et al., 1995). Therefore the aim of this study is to investigate the genotoxicity of NP and Its effects on adult catfish *Clarias gariepinus* and its embryos.

MATERIALS AND METHODS

Specimen collection

Specimens of adult Catfish *C. gariepinus* were collected from the River Nile at Assiut and then were transported to Fish Biology Laboratory of Zoology Department, Faculty of Science, Assiut University. The fish (500–1200 g) were fed on a commercial pellet diet (3% of body weight per day) and kept together in 100 l rectangular tanks containing tap water (conductivity 2000 ls/cm; pH 7.5; oxygen 88–95% saturation; temperature 27-28 ºC; photoperiod 12:12 light: dark). After 2 week acclimatization, fishes were used for the experimental setup.

4-nonylphenol

Nonylphenol was obtained from Sigma- Aldrich (Schnelldrof, Germany) with purity 99.3%.

Gamete collection

Mature African catfish, *Clarias gariepinus* (weight of 900-1500g) were collected from the River Nile at Assuit, Egypt and transported to the Fish Lab, Zoology Department, Assuit University. The criteria applied for the selection of spawners were those described by De Graaf and Janssen (1996). The catfish were kept in 100-L glass tanks to be acclimatized for two-week period at 27-29 ºC, PH=7.56, dissolved oxygen 88-94% saturation. The photoperiod was a 12-hour light to 12-hour dark cycle and the catfish were fed on a commercial pellet diet (3% of the body weight/day).

For collection of semen, male fishes were anaesthetized with 200 mg/l Ms_222 (tricaine methane sulphonate, Crescent Research Chemicals, Phoenix, Arizona, USA) buffered with 800 mg/l sodium bicarbonate and one of the testes was removed surgically. Alternatively the fish were killed and the whole gonads were removed. Testes were cleaned from the blood by surgical towels. The sperms from the testes were pressed through a mesh fabric into a sterile dry petri dish and used directly for dry fertilization. For collection of eggs, ovulation was artificially induced. Females were injected intra-peritoneal with pellets (gonadotropin-releasing hormone analogue, GnRHa, D-Ala6, Pro9 Net) containing 2.5-3.0 mg of water soluble dopamine antagonist metoclopramide (Interfish Ltd, Hungary) dissolved in 0.65% NaCl. One pellet was used per Kg

body weight. 10-11 hours after injection, the fish were stripped and the eggs were collected in clean dry plastic containers; dry fertilization was considered.

Experimental setup

First experiment

The adapted adult fish classified into four groups (10 fish per each): control, 4-nonylphenol-treated group (for15 day/ for 0.05mg/l day), 4-nonylphenol-treated group (for 15 day/for 0.08mg/l day), and 4-nonylphenol-treated group (for 15 day/for 0.1 h/ day). The conditions of the experiment were as that of acclimatization with changing the entire tap water everyday. 4-nonylphenol concentrations were according to Mekkawy et al. (2011).

Second Experiment

After artificial spawning for mature male and mature female untreated fishes, and dry fertilization occurred, the fertilized eggs At age of 18 h-PFS were exposed to different dosed of 4-nonylphenol (0.08mg/l; 0.05mg/l and 0.1mg/l) to calculate the incubation period and hatching rate. Also, at 29 h-PFS other fertilized eggs were exposed to different doses of 4-nonylphenol (0.08mg/l; 0.05mg/l and 0.1mg/l) to calculate the mortality rate at 30, 32, 37 h-PFS.

Third Experiment

Mature female of catfish was exposed to 0.1mg/l 4-nonylphenol for one week then artificial spawning occurred using untreated mature male. The fertilized eggs were used to calculate fertilization rate, hatching rate, mortality rate at 4, 30, and 50 h-PFS respectively and also samples taken for RAPD-PCR analysis and malformations studies at different developmental stages.

DNA extraction

NA extracted from liver and embryos using QIA amp DNA mini kit (50) Cat. No. 51304 from GIAGEN Gmbh, D-40724 Hilden,Germany. 60 mg of the tissue or embryos pooling reserved at -80 ºC were cutted into small pieces then added to 1.5 ml micro centrifuge tube containing 180 µl PBS. Homogenized using tissue Ruptor then added 100 µl ATL. 20 µl of protekinase K added mixed and incubated at 56 ºC until complete lysis. 4 µl RNase added, mixed and incubated for 2 min at room temperature, centrifugation then added 200 µl buffer AL then centrifugation again. 200µl ethanol (95-100%) added to the sample, mixed and centrifuged. The mixture was applied to QIAamp MINI spin column then centrifuged at 8000 rpm for 1 min and the filtrate discarded. 500 µl buffer AW for washing added to the column the centrifuged at 8000 rpm and the filtrate discarded. Washing again and centrifuged at 14000 rpm for 3 min. finally placed the spin column in a clean 1.5 ml microcentrifuge tube and discarded the tube containing the filtrate then 200 µl buffer AE incubated at room temperature for 1 min centrifuged at 8000 rpm for 1 min and the collection tube contained purified DNA. The amount of DNA was estimated using Spectrophotometer GeneQuant 1300 from (Healthcare Bio-Science AB, SE-75184 Uppsala, Sweden). Procedures of DNA extraction according to QIAamp DNA Mini Handbook 11/2007 (tissues part pg. 33-36).

Rapid-PCR analysis

Ten primers (obtained from Applied Biosystems UK. 7 Kingsland Grange, Woolston, Warrington, Cheshire, WA 1 4SR) were initially screened for consistently reproducible and scoreable bands (Table 3 and Figure 4) according to Saad et al., (2009). The only one was suitable and more scoreable bands giving used to determine genetic differences among fish exposed to different doses of 4-nonylphenol is 260110- 54b primer with sequence (5'- CCACAGCAGT-3') and MW (g/mol) =2997. PCR reaction was prepared according to the instructions provided with Go Tag® Green Master Mix; Cat. No. M7122 purchased from Promega Co. USA. The RAPD reaction was performed in a total volume of 25 µl [2 µl (20pmol) primer, 2 µl purified DNA, 12.5 µl master mix and 8.5 µl DNA, RNA free water]. The reaction conditions involved initial denaturation of DNA for 5 minutes at 94 ºC, 40 cycles of 1 min denaturation at 94 ºC, 1 min annealing at 28 ºC, 2 minutes extention at 72 ºC and 10 minutes cycle at 72 ºC for final extention. 4 µl of the PCR products were separated on 1 % agarose gels using 100bp Ladder DNA marker (100bp-300bp) purchased from Axygen Biosciences Co. stained with ethedium bromide, run in 1X TBE buffer at a constant voltage of 100 V. the bands were visualized with a UV trans illuminator (IVA-Vilber Lourmat, KAISER RS1, Germany) provided with video camera (CCD module N50, 49 mm, Japan) and documented using an Biodocanalyze (BDA) software ver. 2.64.8.1 (Biometra, D-37079 Göllingen, Germany).

Data analysis

All gels were analyzed using Gel-Pro Analyzer package (Media Cybernetica 1993-97) and the statistics ver. 8 soft ware. Data were recorded as presence (1) and absence (0) of bands from the gel photographs. This data was then introduced to statistics ver. 8 soft ware package in

Table 1. Total DNA (ng/µl) of liver of adult *C. gariepinus* and developmental stages under different doses of 4-nonylphenol.

Lanes / DNA Fractions	Control	0.05 mg/l 4-nonyphenol	0.08mg/l 4-nonylphenol	0.1mg/l 4-nonylpenol
Female	58.43 ± 2.55 a (55.9- 61)	33.80 ±3.65 b (31.4 -38)	33.17 ± 2.97 b (29.8 – 35.4)	29.30 ± 2.85 b (26.8 – 32.4)
Male	53.77 ± 3.26 a (50.32 – 56.8)	28.63 ± 1.15 b (27.5- 29.8)	25.43±1.26 b (24.3- 26.8)	12.03 ± 2.05 c (10 – 14.1)
4h-PFS*	66.10 ± 2.46 a (63.5 – 68.4)	63.47 ± 1.5 a (62 - 65)	58.2 ± 1.9 b (56.3 -60.1)	54.97 ± 2.2 b (52.4 – 56.3)
17h-PFS	90.33 ± 0.95 a (89.3 – 91.2)	43.93 ± 1.15 b (43.1 – 45.2)	41.30 ± 1.53 b (40.2 – 42.5)	31.27 ±4.96 c (28.2- 37)
37h-PFS	108.8 ±1.64 a (107- 110.2)	72 ± 2.81 b (69.4 -75)	65.9 ±3.9 c (61.8- 68.5)	44.7 ± 2.16 d (42.3- 46.5)

Table 2. Total DNA (ng/µl) of different developmental stages of adult *C. gariepinus* obtained from fertilization of exposed to 0.1 mg/l 4-nonylphenol (for a week) and unexposed male (Third experiment).

Lanes / DNA Fractions	30h-PFS*	40h-PFS	50h-PFS	72h-PFS	96h-PFS	120h-PFS	144h-PFS
0.1mg/l nonylphenol	144.4 ±2.163 a (142 – 146.2)	174.9± 1.353 b (173.5– 176.2)	181.47± 2.53 c (179.2–184.2)	166.4 ± 2.11 d (164 -168)	143.4 ± 1.57 a (142 – 145.1)	137.7 ± 1.99 e (135.4 – 139)	96.73 ± 1.86 f (94.6 – 98)

* h-PFS; hours postfertilization stages.
* Different letters indicates there is a significant difference at (p≤0.05)

order to calculate the relationships between 4-nonylphenol doses affecting fish. According to the binary values (0, 1) and using unweighted pair-group average and Squared Euclidean distances the dendrogram was constructed.

RESULTS

DNA extraction

Suitable method for DNA extraction was used to obtain high purity and integrity DNA. Concentration of DNA extracted were measured using spectrophotometer Gene Quant 1300 and illustrated in table (1, 2). As table (1) shows, significant decrease in the concentrations of genomic DNA with increase of 4-nonylphenol doses in both male and female adult catfish

Table 3. Primer codes, Sequences, molecular weight (g/mol) and Length (bp) of the tested primers

No.	Primer code	Primer sequence	MW(g/mol)	Length (bp)	Total band number
1	260110-54A	5'- TCGGCGATAG-3'	3068.1	10	0
2	260110-54B	5'- CCACAGCAGT-3'	2997	10	4-9
3	260110-54C	5'- TTCGAGCCAG-3'	3028	10	0
4	260110-54D	5'- GTGAGGCGTC-3'	3084.1	10	0
5	260110-54E	5'- CCGCATCTAC -3'	2948	10	2
6	260110-54F	5'- GATGACCGCC-3'	3013	10	0
7	260110-54G	5'- CTCACCGTCC-3'	2923.9	10	0
8	260110-54H	5'- AAAGCTGCGG-3'	3077.1	10	0
9	260110-54I	5'-TGTCATCCCC-3'	2938.9	10	2
10	260110-54J	5'- GTTGCCAGCC-3'	3004	10	0

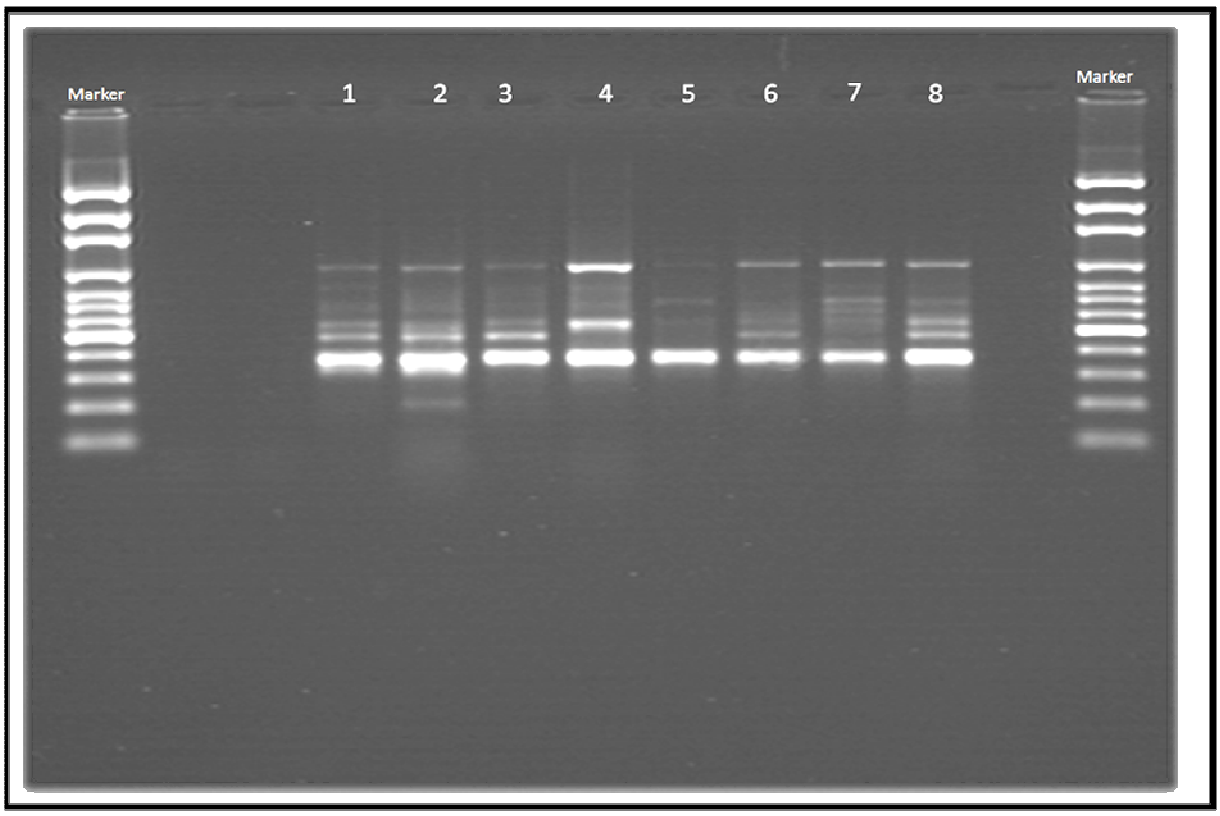

Figure 1. The electrophoresis pattern of the catfish *Clarias garepinus* genomic DNA exposed to 4-nonylphenol using RAPD-PCR with single primer no 2. lane 1, male fish control; lane 2, female fish control; lane 3, male fish exposed to 0.08 mg/l nonylphenol, lane 4, female fish exposed to 0.08 mg/l nonylphenol; lane 5, male fish exposed to 0.05 mg/l nonylphenol; 6, female fish exposed to 0.05 mg/l nonylphenol ; lane 7, male fish exposed to 0.1 mg/l nonylphenol; lane 8, female fish exposed to 0.1 mg/l nonylphenol.

Clarias gariepinus. Also, decrease in the amount of genomic DNA was recorded in the embryos at different developmental stages. In the early developmental stages obtained from fertilization of female exposed to 0.1 mg/l 3-nonylphenol for one week and unexposed male, increase in the concentration of genomic DNA in the 30, 40 and 50h-PFS embryos but decrease in more advanced-aged embryos (table 2).

Selection of PCR primers

RAPD-PCRs were performed on genomic DNAs extracted from six individuals of control fish to suppress the intra-population genetic polymorphism potentially revealed by RAPD. Ten different random primers (table 3) were used by Saad et al., (2009) used in this selection. Among the ten primers used, three gave more than one

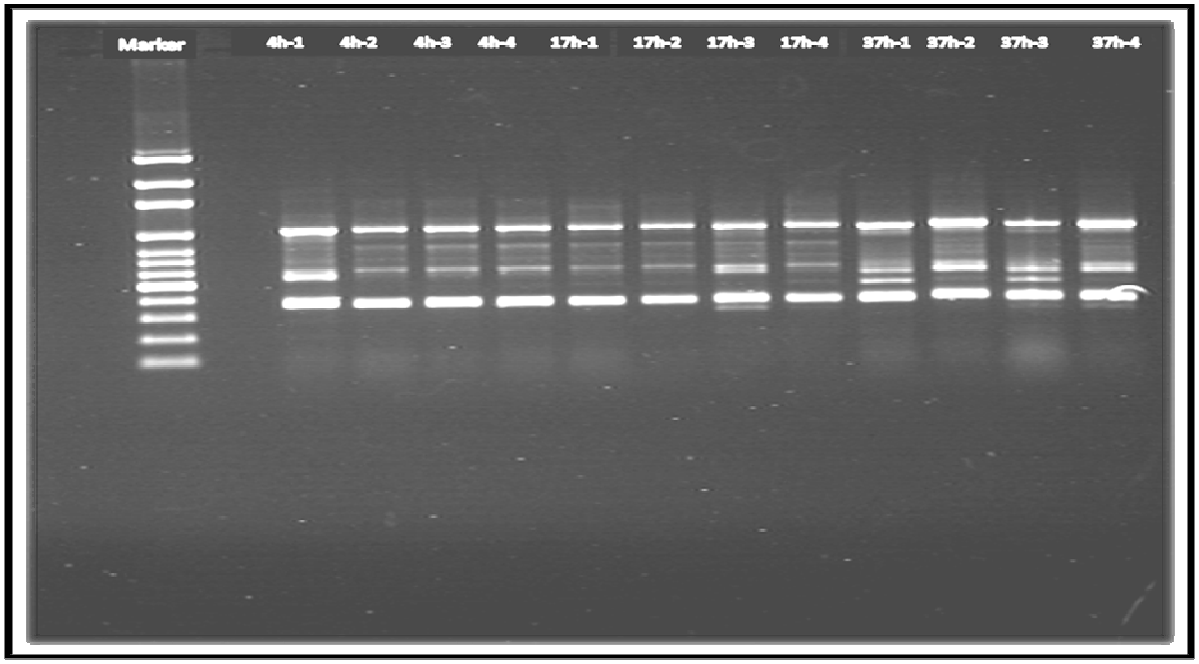

Figure 2. Random amplified polymorphic DNA profiles of a pool of genomic DNAs from *Clarias gareipnius* embryos fish exposed 4-nonylphenol.4h-1, 4-hour post fertilization stage control; 4h-2, 4-hour post fertilization stage exposed to 0.08mg/l nonylphenol; 4h-3, 4-hour post fertilization stage exposed to 0.08mg/l nonylphenol; 4h-4, 4-hour post fertilization stages exposed to 0.1 mg/l nonylphenol; 17h-1,17-hour post fertilization stage control; 17h-2, 17-hour post fertilization stage exposed to 0.05mg/l nonylphenol; 17h-3, 17-hour post fertilization stages exposed to 0.08 mg/l nonylphenol; 17h-4, 17-hour post fertilization stages exposed to 0.1 mg/l nonylphenol. 37h-1,37-hour post fertilization stage control; 37h-2, 37-hour post fertilization stage exposed to 0.05mg/l nonylphenol; 37h-3, 37-hour post fertilization stages exposed to 0.08 mg/l nonylphenol; 37h-4, 37-hour post fertilization stages exposed to 0.1 mg/l nonylphenol.

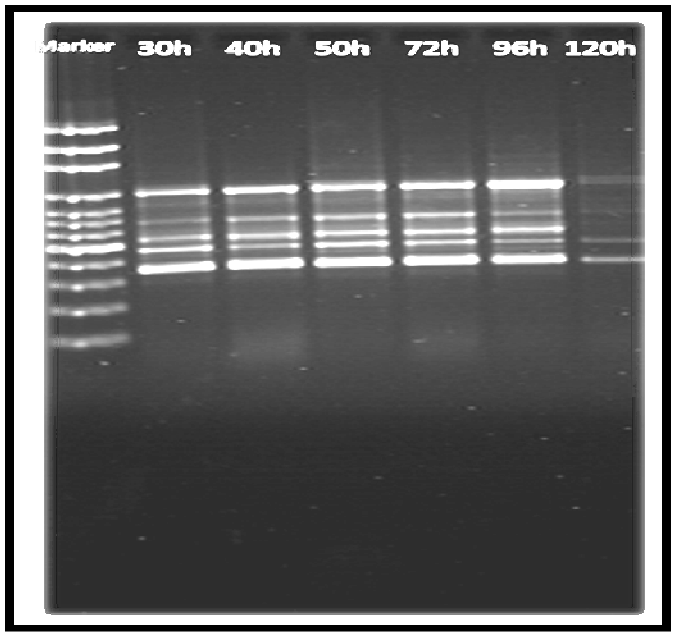

Figure 3. Random amplified polymorphic DNA profiles of a pool of genomic DNAs from *Clarias gareipnius* embryos obtained from fertilization of unexposed male and exposed female to 0.1mg/l for a week 4-nonylphenol. 30h, 30h-postfertilization stage; 40h, 40h-postfertilization stage; 50h, 50h-postfertilization stage; 72h, 72h-postfertilization stage; 96h, 96h-postfertilization stage; 120h, 120h-postfertilization stage.

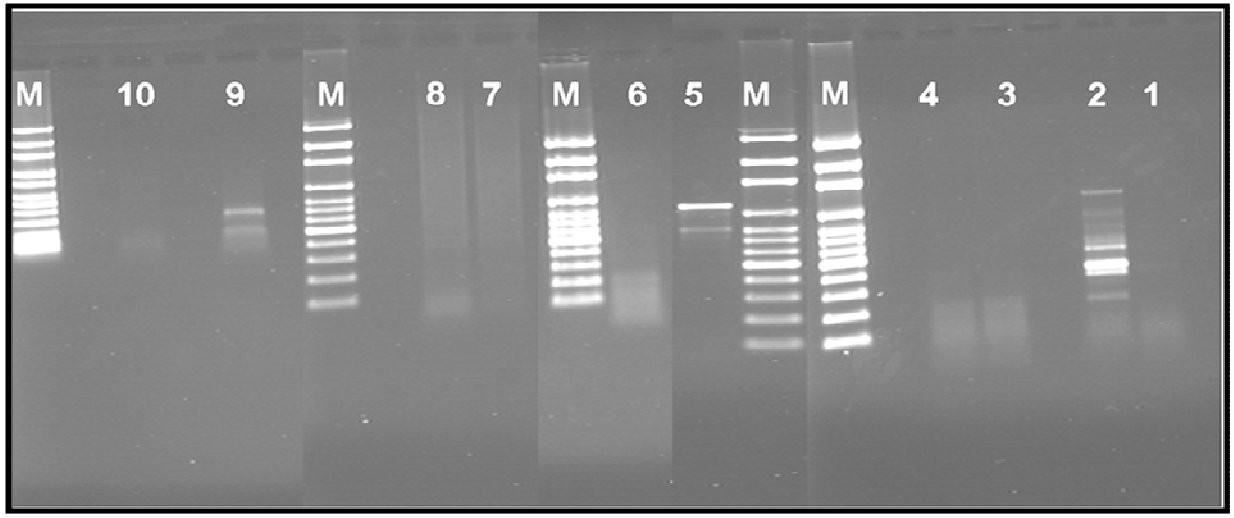

Figure 4. The electrophoresis pattern of the catfish *Clarias garepinus* genomic DNA of liver control, M, marker; 1, primer 1; primer 2; 3, primer 3; 4, primer 4; 5, primer 5; 6, primer 6; 7, primer 7; 8 primer 8; 9, primer 9 and primer 10.

Table 4. Molecular weight (in bp) identification of DNA fractions in (in percent) of liver of adult *C. gariepinus* under different doses of 4-nonylphenol in comparison with control.

Lanes / DNA Fractions	Marker (bp)	control		0.05 mg/l 4-nonyphenol		0.08mg/l 4-nonylphenol		0.1mg/l 4-nonylpenol	
		Male	Female	Male	Female	Male	Female	Male	Female
r1	3000								
r2	2000								
r3	1500								
r4	1000	1107 (6.94)	1107 (6.36)	1143 (6.22)	1107 (19.30)	1143 (4.19)	1179 (10.52)	1179 (10.28)	1179 (8.40)
r5	800	867 (4.28)		822 (4.67)	867 (5.35)			889 (4.26)	
r6				733 (4.29)	733 (6.12)	733 (13.08)	733 (6.19)	767 (8.74)	750 (6.34)
r7	700	717 (7.36)	700 (10.62)				667 (2.80)	683 (6.28)	
r8	600	586 (8.41)		600 (7.95)	586 (19.90)	600 (8.68)	617 (6.69)	617 (13.71)	600 (12.95)
r9	500	514 (14.72)	514 (20.16)	514 (19.63)			529 (14.67)		514 (13.60)
r10	400	390 (58.29)	380 (54.88)	380 (57.24)	390 (49.32)	380 (74.06)	400 (59.19)	390 (59.73)	400 (58.71)
r11	300								
r12	200		215 (7.98)						
r13	100								

Table 5. Molecular weight (in bp) identification of DNA fractions in (in percent) during early developmental stages of the African catfish *Clarias gariepinus* under different doses of 4-nonylphenol in comparison with control

Lanes / DNA Fractions	Marker (bp)	4h-PFS*				17h-PFS				37h-PFS			
		Control	0.05mg/l	0.08mg/l	0.1mg/l	Control	0.05mg/l	0.08mg/l	0.1mg/l	Control	0.05mg/l	0.08mg/l	0.1mg/l
r1	3000												
r2	2000												
r3	1500												
r4					1143 (29.41)	1167 (28.22)	1167 (29.17)	1167 (23.45)	1190 (30.91)	1190 (24.70)	1238 (33.41)	1238 (18.74)	1190 (32.74)
r5		1095 (30.38)	1119 (29.80)	1119 (32.10)						1095 (6.45)		1119 (4.66)	
r6	1000										982 (4.08)	1000 (1.07)	
r7								927 (3.82)	927 (7.53)			945 (1.80)	945 (3.00)
r8		855 (2.96)	873 (8.25)	873 (8.72)	891 (8.16)	909 (7.24)	909 (7.6)	873 (3.02)			891 (2.31)	909 (1.28)	891 (3.61)
r9	800	786 (2.06)				771 (4.96)	771 (5.34)	771 (7.94)	786 (4.40)	800 (5.11)	818 (4.31)		800 (3.14)
r10	700	729 (6.89)	743 (3.56)	743 (3.28)	757 (4.86)			686 (7.60)	686 (9.50)	700 (5.97)			
r11			643 (13.99)	643 (14.74)	657 (13..31)	671 (11.26)	671 (13.53)	643 (16.35)	643 (4.32)	643 (10.16)	671 (20.52)	657 (22.68)	657 (16.27)
r12	600	575 (21.28)			513 (3.98)	525 (3.54)	525 (3.81)	525 (2.07)	538 (4.91)	550 (11.13)	563 (3.24)	563 (10.46)	550 (2.71)
r13	500	480 (1.68)						430 (33.44)	430 (38.43)	430 (36.48)	450 (32.13)	440 (34.86)	430 (35.26)
r14	400	375 (34.76)	392 (44.40)	392 (41.15)	392 (40.28)	392 (44.78)	410 (40.48)	367 (6.30)				375 (4.44)	375 (3.26)
r15	300												
r16	200												
r17	100												

*h-PFS; hours postfertilization stages.

Table 6. Molecular weight (in bp) identification of DNA fractions in (in percent) during early developmental stages of the African catfish *Clarias gariepinus* obtained from exposed female to 0.1mg/l 4-nonylpheno fertilized with unexposed male.

Lanes / DNA Fractions	Marker (bp)	30h-PFS*	40h-PFS	50h-PFs	72h-PFS	96h-PFS	120h-PFS
r1	3000						
r2	2000						
r3	1500						
r4		1100 (18.38)	1175 (21.81)	1175 (19.94)	1200 (23.27)	1225 (34.61)	1275 (18.52)
r5	1000	917 (8.5)	983 (5.46)	983 (6.38)	1000 (5.55)	917 (2.98)	983 (8.83)
r6	800	743 (11.38)	786 (12.48)	786 (15.35)	817 (14.36)	817 (8.51)	850 (9.99)
r7	700						729 (7.34)
r8			638 (19.93)	638 (17.70)	650 (17.82)	663 (18.36)	675 (7.10)
r9	600	590 (17.40)				580 (7.61)	580 (15.25)
r10	500	510 (16.58)	550 (11.02)	550 (13.89)	570 (12.25)	520 (3.86)	510 (5.38)
r11	400	393 (28.01)	430 (29.30)	430 (26.75)	440 (26.74)	450 (24.07)	450 (27.58)
r12	300						
r13	200						
r14	100						

*h-PFS; hours postfertilization stages

band and the only one from these used is the number two (260110-54B) which gave 4-9 bands and evidenced significant differences in both control and contaminated fishes (figure 4).

Analysis of 4-nonylphenol-induced genotoxicity

The main changes observed in the RAPD profiles (Figure 1, 2, 3) have resulted both in the appearance and disappearance of different bands with variations of their intensity as well (table 4, 5, 6).

The RAPD band patterns produced by the 260110-54B primer with sequence 5'-CCACAGCAGT-3' were recorded by UV tans illuminator and documented using a Biodocanalyzer software. Figure 1, 2 and 3 give an overview of the RAPD band patterns obtained with 260110-54B primer in the independent experiments.

As Figure 1 and table 4 shown, in the control fish the RAPD-PCR profile shows six permanent bands (fractions) in male and female at different molecular weight. The number of bands in fishes exposed to 0.05 mg/l of 4-nonylphenol as the same of control with little change in molecular weight while in fish exposed to 0.08 mg/l of 4-nonylphenol two bands disappeared from male but no change in female. Table 4 shows the difference in the molecular weight of similar bands and also in the new ones.

RAPD- PCR profile of a pool genomic DNAs from *C. gariepinus* embryos exposed to 4-nonylphenol at 4 hour-post fertilization stage (4h-PFS) shows different pattern from control, where the fraction (r_9) disappeared in the exposed embryos in comparison with control while the fraction (r_{11}) appeared in the exposed group in comparison with control. In the 17h-PFS exposed to 0.08 and 0.1 mg/l of 4-nonylphenol new fractions appeared (r_7, r_{10}, r_{13}) in comparison with these exposed to 0.05 mg/l of 4-nonylphenol and control (Figure 2 and Table 5). Also, 37h-PFS exposed to 0.08 mg/l and 0.1 mg/l of 4-nonylphe-

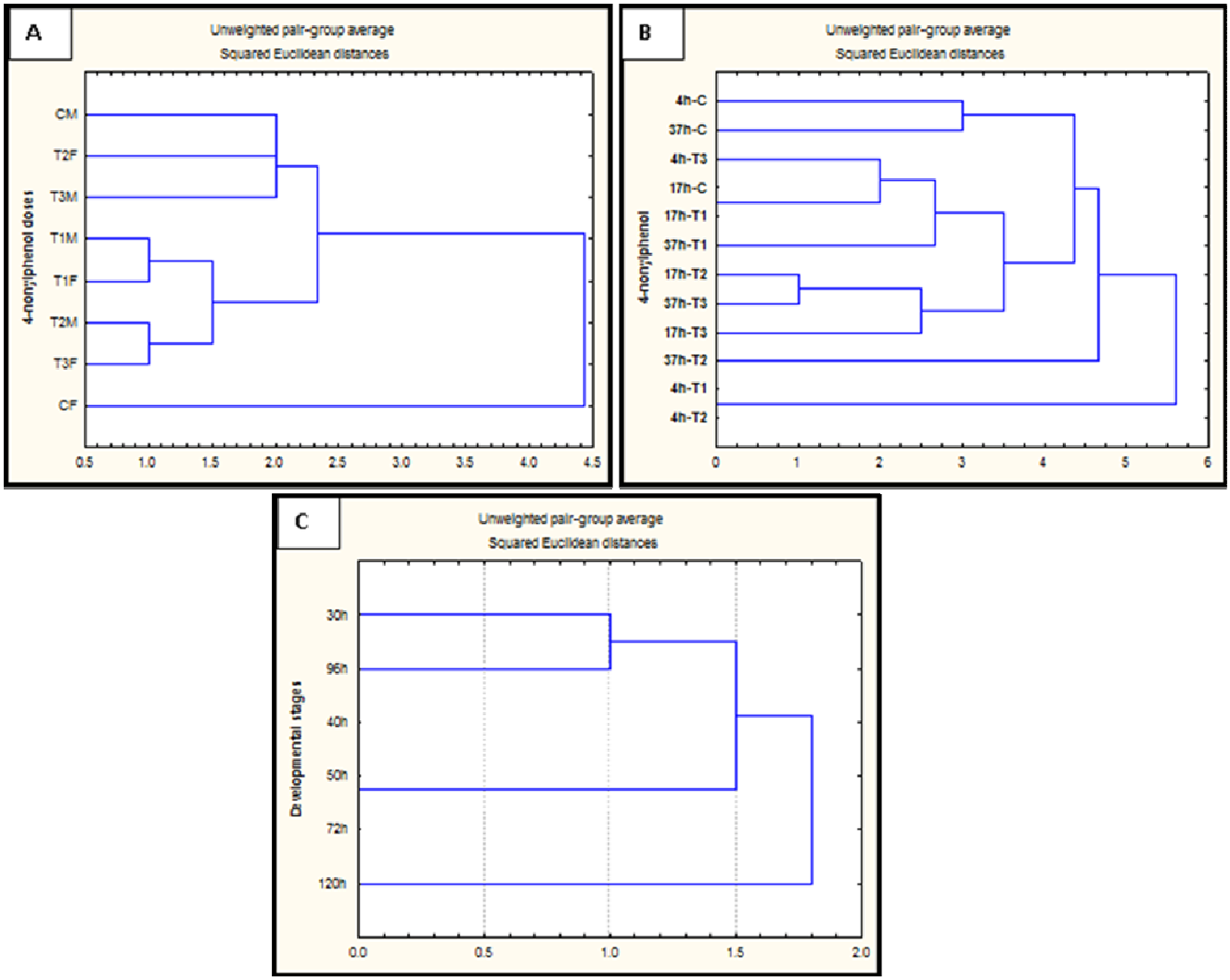

Figure 5. Dendrogram representing the relationships among applied doses of 4-nonylphenol on adults catfish and their developmental stages based on RAPD analysis. (A) CM; male control, CF; female control, T1M; male exposed to 0.05mg/l nonylphenol for 15 days, T1F; female exposed to 0.05mg/l nonylphenol for 15, T2M; male exposed to 0.08mg/l nonylphenol for 15, T2F; female exposed to 0.08mg/l nonylphenol for 15, T3M; male exposed to 0.1mg/l nonylphenol for 15, T3F; female exposed to 0.1mg/l nonylphenol for 15. (B) 4h-C; 4-hour postfertilization stage control, 4h-T1; 4-hour postfertilization stage exposed to 0.05mg/l nonylphenol, 4h-T2; 4-hour postfertilization stage exposed to 0.08mg/l nonylphenol, 4h-T3; 4-hour postfertilization stage exposed to 0.1mg/l nonylphenol, 17h-C; 17-hour postfertilization stage control, 17h-T1; 17-hour postfertilization stage exposed to 0.05mg/l nonylphenol, 17h-T2; 17-hour postfertilization stage exposed to 0.08mg/l nonylphenol, 17h-T3; 17-hour postfertilization stage exposed to 0.1mg/l nonylphenol, 37h-C; 37-hour postfertilization stage control, 37h-T1; 37-hour postfertilization stage exposed to 0.05mg/l nonylphenol, 37h-T2; 37-hour postfertilization stage exposed to 0.08mg/l nonylphenol, 37h-T3; 37-hour postfertilization stage exposed to 0.1mg/l nonylphenol. (C) 30h; 30-hour postfertilization stage, 40h; 40-hour postfertilization stage, 50h- 50-hour postfertilization stage, 72h; 72-hour postfertilization stage, 96h; 96-hour postfertilization stage, 120h;120-hour postfertilization stage.

nol new fractions appeared (r_6, r_7, r_8, r_{14}) while r_{10} disappeared.

In the third experiment, the early developmental stages of the African catfish *C. gariepinus* obtained from exposed female to 0.1 mg/l of 4-nonylphenol for a week then fertilized with unexposed male are shown (Figure 3, Table 6). RAPD- PCR profile showed disappearance each of fraction r_7 in the 30h-PFS, 40h- PFS, 50h- PFS, 72h- PFS, and 96 h- PFS fraction r_8 in 30h-PFS and fraction r_9 in 40h- PFS, 50h- PFS, 72h- PFS.

Dendogram in Figure 5a shows the relationship among applied doses of the 4-nonylphenol on adult fish and among the 4-nonylphenol and the developmental stages (Figure 5b). The pattern of relationship between the early developmental stages summarized (Figure 5c)

DISCUSSION

Our previous studies indicated the dangerous effects of

4-nonylphenol on hematological indices (Mekkawy et al., 2011), biochemical parameters (Sayed et al., 2011), endocrine disruption (Sayed et al., 2012a) and histological alterations (Sayed et al., 2012b). The present study indicated the damage DNA concentrations and alterations on bands patterns in comparison with control. In this study, the potential use of the RAPD method for the detection of DNA effects induced by NP on adults and embryos was demonstrated. Atienzar et al., (2002) has been reported that, the most significant advantages of the RAPD method lie in its speed, applicability to any organism, potential to detect a wide range of DNA damage and mutations, and the only allows aquatic a quantitative assessment of the DNA effects.

Our results indicated that DNA extracted from adults catfish embryos that has been exposed to NP duced RAPD profiles that differed from the control pattern (Figure 1, 2, 3). The genomic DNA as product derived from adults and embryos in this investigation was 12.03-181.47 ng/ µl and the purity and integrity of the template DNA are crucial for RAPD analysis (Zhou et al., 1997). Although, the 50 ng genomic DNA template is the optimal concentration for this RAPD reaction, the amount of genomic DNA used in this study ranged from as little as 12.03 ng to as much as 181.47 ng and this is in agreement of Muralidharan and Wakeland (1993) and Zhou et al., (1997) results. Modifications of the RAPD patterns are due to genomic rearrangements structural changes owing to DNA damage and interactions of DNA polymerase with damaged DNA (Atienzar et al., 2002). Our results are similar with that reported by Atienzar et al., (2002), they reported that both nonylphenol (NP) and 17-β estradiol (E_2) induced the same DNA modifications that had a significant effect on the PCR and led to the new amplified products of an identical size or to the disappearance of an amplicon. Genotoxins are known to interact with genomic DNA at specific sites (Vogelstein and Kinzler, 1992) which lead to hot spot DNA damage and potentially to hot spot mutations (Atienzar et al., 2002). In this context, the results obtained in the present work suggest that DNA effects (DNA damage and mutations) occurred in adults and embryos of African catfish exposed to sublethl concentrations of estrogen NP. That results in agreement with these of Jha et al., (2000), they have been shown low concentrations of xenoestrogen tributylin oxide (0.54- 5.44 µl/l) induced cytogenetic damage in the early life stages of the marine mussel.

Under our experiments conditions, the fingerprinting obtained was clear to been seen by visual investigation. By using selected primer, there exist several amplified bands from 200 to 1000 bp in size and by using anlaysis program there are 4 to 8 fractions in the product amplified. After exposure to 4-nonylphenol the amplified products of genomic DNA revealed some difference from the fingerprinting pattern of normal fish. Our results showed disappearance and appearance of bands after exposure to 4-nonylphenol and that is in agreement with the results of Zhiyi and Haowen (2004) after exposure of Zebrafish to phosphamide and dimethoate equivalent where they stated that increased concentrations of the those genotoxic chemicals and similar to results of Rocco et al., (2010), they reported that changes in the variations of intensity of the bands in the less acquisition of new bands after exposure of *Danio rerio* to pharmacological agents. However, the number of stable bands disappearing is enhanced so that, the genotoxical effect of the 4-nonyphenol exposed is promoted by concentrations.

The cluster method is one of the most effective methods in numerical computation (Zhiyi and Haowen, 2004). Although, the exposure of fish to toxic chemical pollutants can cause DNA damage, mutations that inhibit primer binding or interfere with amplification can be detected as alterations of the pertinent bands in those fish (Bowditch et al., 1993) and this will appear in DNA fingerprinting, so that DNA fingerprinting of control and exposed fish can then be compared and analyzed statistically according to Zhiyi and Haowen (2004). It can calculate the distance between every pair of entities and then summarize the community data sets (Gauch, 1995). Zhiyi and Haowen, (2004) reported that a dendogram construction based on the hierachical clustering method can show the relationships of every sample.

Dendograms constructed by the Unweighted pair-group average (squared Euclidean distances) showed that the fingerprinting of other exposed groups showed a distinct distance from normal group and the fingerprinting of normal fish is not the same and this is may be due to the variability of DNA between individuals (each individual is genetically unique) according to Zhiyi and Haowen (2004).

In conclusion, the result obtained from this study indicates that the 4-nonylphenol is genotoxic in both adults and embryos of African catfish, *C. gariepinus*. Also RAPD-PCR method can be used as an investigation tool for the evaluation of the genetic damage of the catfish induced by the exposure to 4-nonylphenol.

REFERENCES

Cambier S, Gonzalez P, Durrieu G, Bourdineaud J (2010). Cadmium-induced genotoxicity in zebrafish at environmentally relevant doses. Ecoto. Environ. Saft. 73, 312–319.

Atienzar FA, Conradi M, Evenden A, Jha A, Depledge M (1999). Qualitative assessment of genotoxicity using RAPD: comparison of genomic template stability with key fitness parameters in *Daphnia magna* exposed tobenzo(a)- pyrene. Environ.Toxicol.Chem.18, 2275–2282.

Atienzar FA, Cordi B, Donkin ME, Evenden AJ, Jha AN, Depledge MH (2000). Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae, *Palmaria palmata*. Aquat.Toxicol.50,1–12.

Atienzar FA, Cheung VV, Jha AN, Depledge MH (2001). Fitness parameters and DNA effects are sensitive indicators of copper-induced toxicity in *Daphnia magna*. Toxicol. Sci.59, 241–250.

Atienzar FA, Billinghurst Z, Depledge MH (2002). 4-n-Nonylphenoland17-beta estradiol may induce common DNA effects in developing barnacle larvae. Environ. Pollut.120,735–738.

Atienzar FA, Jha AN (2004). The random amplified polymorphic DNA (RAPD) assay to determine DNA alterations, repair and transgenerational effects in B(a)P exposed Daphnia magna. Mutat.Res.552,125–140.

Atienzar FA, Jha AN (2006). The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: acriticalreview.Mutat.Res.613,76–102.

Savva D (1998). Use of DNA fingerprinting to detect genotoxic effects. Ecotoxicol. Environ. Saf. 41, 103–106.

Welsh J, McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18, 7213–7218

Bacerril C, Ferrero M, Sanz F, Castano A (1999). Detection of mitomycin C induced genetic damage in fish cells by use of RAPD. Mutagenesis 14, 449–456.

Zhiyi R, Haowen Y (2004). A method for genotoxicity detection using random amplified polymorphism DNA with *Danio rerio*. Ecotoxicol.Environ.Saf.58, 96–103.

Bowditch BM, Albright DG, Williams JGK, Braun MJ (1993). Use of randomly amplified polymorphic DNA markers in comparative genome studies. Methods in Enzymology 224, 294–309.

Gauch JR (1995). Classification. In: Gauch, J.R. (Ed.), Community Ecology. Cambridge University Press, Cambridge, UK, pp. 173–210.

Savva D (1996). Use of DNA fingerprinting to detect genotoxic effect. Ecotoxicol. Environ. Saf. 41, 103–106.

Shugart L, Theodorakis C (1994). Environmental genotoxicity: probing the underlying mechanisms. Environ. Health Perspect. 102 (12), 13–17.

Walker CH (1996). The use of biomarkers to measure the interactive effects of chemicals. Ecotoxicol. Environ. Saf. 40, 65–70.

Williams J, Kubelik AR, Livak KJ, Rafalski JA, Tinger SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res. 18, 6531–6535.

Theodorakis CW, Bickham JW, Lamb T, Medica PA, Lyne TB (2001). Integration of genotoxicity and population genetic analyses in kangaroo rats (*Dipodomys merriami*) exposed to radionuclide contamination at the Nevada Test Site USA. Environ. Toxicol. Chem. 20, 317–326.

Zhou YP, Ge S, Wang XD (1997). RAPD marker. In: Zhou, Y.P., Oe, S., Wang, X.D. (Eds.), The Biomarker in Systemaic and Evalutionary Botany. Science Publisher, Beijing, pp. 30–51.

Frenzilli G, Scarcelli V, Del Barga I, Nigro M, Bolognesi FL, Sturve J (2004). DNA damage in eelpout *(Zoarces viviparus*) from Göteborg harbour, Mutation Research 552 (2004) 187–195

Shugart L (1990). Biological monitoring: testing for genotoxicity, in: J.F. McCarthy, L.R. Shugart (Eds.), Biomarkers of Environmental Contaminationm, Lewis Publishers, Chelsea, pp. 217–227.

Peters LD, Morse HR, Waters R, Livingstone DR (1997). Responses of hepatic cytochrome P450 1A and formation of DNA-adducts in juveniles of turbot (*Scophthalmus maximus* L.) exposed to water-borne benzo[a] pyrene, Aquat. Toxicol. 38, 67–82.

Lyons BP, Harvey JS, Parry JM (1997). An initial assessment of the genotoxic impact of the Sea Empress oil spill by the measurement of DNA adduct levels in the intertidal teleost *Lipophrys pholis*, Mutat. Res. 390, 263–268.

Lyons BP, Stewart C, Kirby MF (1999). The detection of biomarkers of genotoxin exposure in the European flounder (*Platichthys flesus*) collected from the River Tyne Estuary, Mutat. Res. 446, 111–119.

Papadopoulos S, Benter T, Anastassiou G, Pape M, Gerhard S, Bornfeld N, Ludwig WD, Dorken B (2002). Assessment of genomic instability in breast cancer and uveal melanoma by random amplified polymorphic DNA analysis. Int. J. Cancer 99, 193–200.

Castano A, Becerril C (2004). In vitro assessment of DNA damage after short- and long-term exposure to benzo[a]pyrene using RAPD and the RTG-2 fish cell line. Mutat. Res. 552, 141–151.

Enan MR (2006). Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of heavy metals. Biotechnol. Appl. Biochem. 43, 147–154.

Becerril C, Ferrero M, Sanz F, Castano A (1999). Detection of mitomycin C-induced genetic damage in fish cells by use of RAPD. Mutagenesis 14 (5), 449–456.

Yoshida N, Naka T, Sengoku T, Ogawa K (2001). Toxicity and mutagenesis of chrysotile asbestos to Agrobacterium radiobacter. Curr. Microbiol. 42, 398–402.

Kumar A, Tyagi MB, Jha PN (2004). Evidences showing ultraviolet-B radiation-induced damage of DNA in cyanobacteria and its detection by PCR assay. Biochem. Biophys. Res. Commun. 318, 1025–1030.

Jha AN, Hagger JA, Hill SJ (2000). Tributylin induces cytogenetic damage in the early life stages of the marine mussel, Mytilus edulis. Environmental and Molecular Mutagenesis 35,343–350.

Vogelstein B, Kinzler KW (1992). Cancer: carcinogens leave fingerprints. Nature 355,209–210.

Rocco L, Frenzilli G, Zito G, Archimandritis A, Peluso C, Stingo IV (2010). Genotoxic Effects in Fish Induced by Pharmacological Agents Present in the Sewage of Some Italian Water-Treatment Plants Environmental Toxicology DOI 10.1002/tox

Rahman MA, Bhadra A, Begum N, Islam MS, Hussain MG (1995). Production of hybrid vigour through crossbreeding between *Clarias batrachus* Linn. and *Clarias gariepinus* Bur. Aquaculture 138, 125–130.

Burgess WE (1989). The atlas of the fresh water and marine catfishes (*Clarias gariepinus*) aquaculture, 171: 49-64.

Saad YM, Hanafi MSh, Essa MA, Guerges AA, Ali SF (2009). Genetic Signatures of Some Egyptian *Clarias gariepinus* Populations. Global veterinaria 3 (6):503-508.

Rivero CLG, Barbosa AC, Ferreira MN, Dorea JG, Grisolia CK (2008). Evaluation of genotoxicity and effects on reproduction of nonylphenol in *Oreochromis niloticus* (Pisces: cichlidae) Ecotoxicology 17:732–737.

De Graaf GJ, Janssen H (1996). Artificial reproduction and pond rearing of the African catfish Clarias gariepinus in sub-Saharan Africa. FAO, Fisheries Techincal Paper, 362:1–73.

Muralidharan K, Wakeland EK (1993). Concentration of primer and template qualitatively affects products in random amplified polymorphic DNA-PCR. BioTechniques 14, 362–364.

Media Cybernetica (1993-97). L.P.; 8484 Georgia Avenue, Silver Spring, Maryland 20910.

Clark LB, Rosen RT, Hartman TG, Louis JB, Suffet IH, Lippincott RL (1992). Determination of alkylphenol ethoxylates and their acetic acids derivatives in drinking water by particle beam liquid chromatograph/electroscopy. Int J Environ Anal Chem 47:167–180

Sakai A (2001). p-Nonilfenol acts as a promoter in the BALB/3T3 cell transformation. Mutat Res 493:161–166

Sone K, Hinago M, Kitayama A, Morokuma J, Ueno N, Watanabe H, Iguchi T (2004). Effects of 17 B-estradiol, nonylphenol, and bisphenol-A on developing *Xenopus laevis* embryos. Gen Comp Endocrinol 138:228–236

Tsuda T, Takino A, Kojima M, Harada K, Muraki T, Tsuji M (2000). 4-Nonylphenols and 4-terc-octylphenol in water and fish from rivers flowing into lake Biwa. Chemosphere 41:757–762.

Uguz C, Iscan M, Erguven A, Isgor B, Togan I (2003). The bioaccumulation of nonilfenol and its adverse effect on the liver of rainbow trout (*Onchorynchus mykiss*). Environ Res I92:262–270.

Mekkawy IA, Mahmoud UM, Sayed AH (2011). Effects of 4-nonylphenol on blood cells of the African catfish *Clarias gariepinus* (Burchell, 1822). Tissue Cell 43, 223– 229.

Sayed AH, Mahmoud UM, Mekkawy IA (2011). Effects of 4-nonylphenol on metabolic enzymes, some ions and biochemical blood parameters of the African catfish *Clarias gariepinus* (Burchell, 1822). African Journal of Biochemistry Research 5(9), 287-297.

Sayed AH, Mahmoud UM, Mekkawy IA (2012a). Reproductive biomarkers to identify Endocrine disruption in *Clarias gariepinus* exposed to 4-nonylphenol. Ecoto Environ. Saft. 78, 310–319.

Sayed AH, Mahmoud UM, Mekkawy IA (2012b). Histopathological alterations in some organs of adults of *Clarias gariepinus* (Burchell, 1822) exposed to 4-nonylphenol. María-Dolores García (Editor), Zoology, 165-184.