CYTOGENOTOXICITY EVALUATION OF WATER CONTAMINATED WITH SOME TEXTILE AZO DYES USING RAPD MARKERS AND CHROMOSOMAL ABERRATIONS OF ONION (*Allium cepa*) ROOT CELLS

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Environmental problems are one of the most important dangers threatening to human, animal health and the ecological balance. Most of the water system (river and lack) are commonly used for purposes, such as irrigation of fields, landscape, public parks and also as drinking water. On the other hand, it is reported that the use of wastewater for the irrigation of agricultural fields harms the mitotic division of plant and in turn wipes out the plant due to some substances contained within this water. If these plants are consumed as food, it may influence human health adversely. The chemicals profile of plants grown on such fields could give rise to serious consequences such as allergy at early ages, respiratory disorders, coronary and cancer in middle ages (Carita and Marin-Morales, 2008).

Higher plants constitute an important material for genetic tests to monitor environmental pollutants. However, this feature is due to the possibility of assessing several genetic endpoints range from point mutation to chromosomal aberrations in cells. Among the higher plants species, the most frequent ones used to evaluate environmental contamination are *Allium cepa*, *Vicia faba* and *Zea mays*. But, still among these species, *Allium cepa* (Onion) has been considered an efficient test organism to indicate the presence of mutagnic chemicals (Fiskesjo, 1985).

Onion (*Allium cepa* L.) is very suitable for genotoxic studies and list some of its advantages: The root growth dynamics is very sensitive to the pollutants; the mitotic phases are very clear in the onion; It has a stable chromosome number; Diversity in the chromosome morphology; Stable karyotype; Clear and fast response to the genotoxic substances and spontaneous chromosomal damages occur rarely. Therefore, this test has become well established for the determination of the genotoxic substances in various environments. Various chromosome and chromatide damages in the root meristeme cells of the onion (*Allium cepa* L.), which serve as biomarkers for the different types of environmental pollution (Rank and Nielson, 1994).

Azo dyes are the largest group of synthetic chemicals that are widely used by the textile, leather, cosmetics, food

coloring and paper production industries. Studies demonstrated that azo dyes are cytotoxic to cells, because they induce the formation of micronucleated cells, and multilobulated and extremely condensed nuclei, besides inducing endoreplication and binucleated cells (Matsuoka *et al.*, 2001). All the cytotoxic effects observed for azo dyes might be due to the direct action of dyes on the cells or, especially, to the formation of metabolites resulting from the azo bond reduction (Chung and Stevens, 1993). Metabolites can react with the DNA molecule, damaging both its structure and function (Oliveira *et al.*, 2010). Higher plants constitute an important material for testing genetic alterations brought about by environmental pollutants. They are also currently recognized as excellent bioindicators of cytotoxic, genotoxic and mutagenic effects of environments contaminated by toxic substances (Yi and Meng, 2003).

Different parameters of *Allium cepa* such as root shape, growth, mitotic index and chromosomal aberrations can be used to estimate the cytotoxicity, genotoxicity and mutagenicity of environmental pollutant (Amin, 2002).

Mitotic index is considered a parameter that allows estimating the frequency of cellular division and the reduction of mitotic activities has been used frequently to trace substances that are cytotoxic (Marcano *et al.*, 2004).

RAPD analysis presently otters the greatest chance of detecting small genetic differences, since a larger proportion of the genome can be sampled than with other techniques. RAPDs are a comparatively fast and economical technique (Bachmann, 1997).

To avoid the shortfalls of RAPD analysis, all reactions should be repeated, and all reactions should be analyzed on the same gel for a reliable scoring of presence and absence of bands. For the investigation of closely related species or different accessions of all species were proceedings on the assumption that all fragments of identical size are homologues (Kresovich *et al.*, 1992).

The objective of this study were to evaluate the genotoxic effects of the textile azo dye contaminated irrigation agricultural water using the *Allium cepa* chromosome aberration assay and DNA alteration using RAPD-PCR technique.

MATERIALS AND METHODS

Textile azo dyes

The commercial textile azo dyes, Reactive Lanasol Black B (RLB), Eriochrome Red B (RN) and 1, 2 metal complexes I. Yellow (SGL) were obtained from local industrial company, Egypt. These dyes were selected on the basis of their structural diversity and frequent use in local textile industries. The chemical structures of used dyes were shown in (Table 1). The stock solution of dyes (1 g/100 ml) was prepared by dissolving in distilled water and filtration through Whatmann No. 5 filter paper.

Sampling

Onions bulbs (*Alleium cepa* 2n=16) of the brown variety of average size (2-2.5 cm diameter) were obtained commercially at the Fayoum market, Egypt. The dry bulbs were later used for the test.

Determine effective concentration (EC_{50}) of Textile azo dyes and allium root growth test.

For the determination of EC_{50} a series of bulbs were grown inside 100 ml beakers containing tab water at a temperature of 28±2°C. After 24 hours, the bulbs with uniform root growth were selected and placed in 100 ml beakers filled with different concentrations of selected textile azo dyes (100, 200, 300, 400 and 500 µg/mL and tab water (negative control) in the dark for 96 hours at 28±2°C. During the experiment, the test solutions were changed every 24 h instead of aeration. On the fourth day, root lengths were measured for each group (control as well as treatment group) and mean values were calculated. Taking mean root length of control as 100%, lengths of different treatment groups were plotted against test concentrations and the point on the graph which showed 50% growth was designated as EC_{50} concentration. (Fiskesjo, 1985). The relative reduction of root length was calculated as the percentage of the deviation from the control (T/C%).

Allium cepa root chromosomal aberration assay

The onion bulbs were washed under tap water after each treatment and at the end of the exposure period; the root tips from each treated onion bulb and control were cut and fixed in ethanol: glacial acetic acid (3:1, v/v) as the method of Grant (1983). The roots were transferred to 70% alcohol and stored in refrigerator until use. The root tips were hydrolyzed in 1 N HCl at 60°C for five minutes. Then the roots were washed in distilled water three times. Two root tips were then squashed on each slide, stained with acetocarmine for 10 min and cover slips carefully lowered on to exclude air bubble. The cover slips were sealed on the slides with clear fingernail polish as suggested by Grant (1982).

Microscopic examination

All slides were coded and cells were screened under a light microscope for mitotic index, mitotic phase and chromosomal aberrations. Three slides were examined per onion for each group included five onions and 100 cells per slide were scored. Photomicrographs of some aberrant cells were taken. The phase indices were estimated as number of cells in each mitotic phase over the number of dividing cells expressed in percentage. Similarly, the percentage abnormal cells were calculated as the number of aberrant cells over the number of dividing cells.

The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored at EC_{50} of each dye according to Bakare *et al.* (2000)

Mitotic index

The mitotic index (MI) was expressed as the number of divdining cells per mitotic stage according to the method was described by Fiskesjo (1985). The MI of each treatment group was compared with the negative control group. The mitotic index and mitotic inhibition were determined from the scores obtained for dividing cells based on these formulae:

$$\text{Mitotic Index (MI)} = \frac{\text{number of dividing cell in the treatment} \times 100}{\text{Total number of cell}}$$

$$\text{Mitotic Inhibition} = \frac{\text{Mitotic index of control} - \text{Mitotic index of treatment} \times 100}{\text{Mitotic index of control}}$$

Genomic DNA isolation from root tips of Allium cepa

Samples of treated and control roots of bulbs of *Allium cepa* were frozen in liquid nitrogen, ground with mortar and pestle, and total genomic DNA was isolated by genomic DNA and Total RNA Co-extraction KIT (Spin -column) BioTake Corporation. Purity of DNA was determined by measuring its optical density in spectrophotometer at 260 nm/280 nm ratios and quality of DNA samples was checked by loading them on 0.8% agarose gel and observed it on UV illuminator.

RAPD fingerprinting

Random Amplified Polymorphic DNA (RAPD) was performed using primers OPA01, OPA02, OPA03, OPA04, OPA05, OPA06, OPA07, OPA08, OPA09 and OPA10. PCR was carried out in a reduced volume of a 25 μL reaction mixture containing Tris HCl 10 mM, KCl 50 mM, $MgCl_2$ 3.5 mM, dNTP 0.3 mM, Primer 1 μM, AmpliTaq 0.5 U, 100 ng DNA. The amplification started with a denaturation step of 5 min at 94°C, followed by 35 cycles consisting of 1 min at 94°C, 1 min at 36°C, 2 min at 72°C and ended with a final elongation step of 10 min at 72°C. PCR products were separated in a 1.5% agarose gel, stained with ethidium bromide and visualized under UV light, PCR reaction products were comparison, DNA molecular size marker (100 bp) was used for each agarose gel and the ladder is composed of eleven individual DNA fragments: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 and 1500 bp).

Computer assisted analysis of the RAPD-PCR fingerprinting patterns was performed using RAPD distance software package, version 1.4 (Armstrong *et al.*, 1994). Similarity of the band profiles was based on Excoffier matrix (Excoffier *et al.*, 1992). The correlation coefficient was used to compare the number of the DNA patterns obtained. The clustering of the strains was determined by the Unweighted Pair Group Method using Arithmetic Average (UPGMA). This experiment was also conducted to confirm if extra band appears or disappears in majority of *Allium cepa* germinating exposed to different concentrations of selected azo dyes.

RESULTS AND DISCUSSION

Allium root growth test/determine EC_{50} of textile azo dyes

The results in Table (3) showed that, the mean number and growth of roots decreased with increasing the concentration of textile azo dye. The average length of roots was 3.96 cm. after 96 hours of growth in the control and the dose - response results obtained between the concentrations of different dyes and *Allium* roots growth determined the effective concentration (EC_{50}) value which retards about 50% root growth as 500 µg/ml for RLB, RN and SGL. The root length at the EC_{50} was 2.0 cm (RLB), 1.92 cm (RN) and 2.11 cm (SGL). Higher plants such as *A. cepa* are accepted as admirable genetic models to evaluate genotoxic effects such as disturbances in the mitotic cycle and the results of the study reflected the utility of root tips of cells of *A. cepa* for monitoring the genotoxic effects of textile azo dyes.

Root growth decrease over 45% indicates the presence of toxic nature of substances having sublethal effects on plants (Wierzbicka, 1999).

Mitotic index (MI)

MI serves as an important parameter of cytotoxicity in environmental biomonitoring studies. The MI of samples exposed to EC_{50} (500 µg/ml) of selected azo dyes for RLB, RN and SGL was 10.8, 10.3 and 8.8, respectively. The MI of treatments was decreased compared to control (15.7) Table (4). This effect suggests that azo dyes treatment had some effects on cell division of *A. cepa*. This may be due to abnormal conditions of the cells induced by the treatments.

Mitotic index was used as an indicator of cell proliferation biomarkers which measures the proportion of cells in the mitotic phase of the cell cycle. Hence, the decrease in the mitotic index of *A. cepa* meristematic cells could be interpreted as cellular death.

Low mitotic index may be reflecting a direct genotoxic effect of textile azo dyes. Therefore the mitotic index was analyzed in this study to determine the genotoxicity of three selected textile azo dyes treatment on *A. cepa*. The mitotic inhibition percentage of azo dyes at the EC_{50} concentration of RLB, RN and SGL (31.2, 34.4 and 44.0, respectively) as compared to the control (Table 4).

The cytotoxic level of a test chemical/compound can be determined based on the increase or decrease in the mitotic index (MI), which can be used as a parameter of cytotoxicity in studies of environmental biomonitoring (Smaka-Kincl *et al.,* 1996). Sudhakar *et al.* (2001) reported that, reduction in MI may be due to the inhibition of DNA synthesis or the blocking in the G2 phase of the cell cycle.

A mitotic index decrease below 22% of negative control causes lethal effects on test organism while a decrease

below 50% has sub lethal effects and is called cytotoxic limit value. Several investigators have used MI as an endpoint for the evaluation of genotoxicity or antigenotoxicity of different chemical treatments. The reduction of the mitotic index might be explained as being due to the obstruction of the onset of prophase, the arrest of one or more mitotic phases, or the slowing of the rate of cell progression through mitosis (Panda and Sahu, 1985; Sharma *et al.*, 2012).

Allium cepa root chromosomal aberration assay

Several types of chromosome aberrations were considered in the four phases of cell division (prophase, metaphase, anaphase and telophase) to evaluate chromosomal abnormalities. The abnormalities of chromosomes could be due to the blockage of DNA synthesis or inhibition of spindle formation.

The photomicrographs in Fig. (1) show the presence of chromosomal aberrations consisting of abnormal vacuoles, anaphase bridge, C-metaphase, polyploidy interphase nucleus, metaphase sticking, micronucleus, lagging chromosome, disoriented metaphase, mature cell division showing puffing, strap nucleous, multipolar anaphase, telophase with one end puffing, vagrant chromosome. The percentage of aberrant mitotic cells due to the genotoxicity of textile azo dyes was different from that of the control. The frequencies of total alterations in control and treated onions were 0.85, 11.1, 10.3 and 15.1, respectively. The total chromosomal aberrations induced were in the order: SGL > RLB > RN (Table 5).

Chromosomal aberrations provide an important measure of the genotoxicity potential of textile dyes and effluents (Jadhav *et al.*, 2011). In genotoxicology studies of genotoxicity of pollutants sample, the use of the plant model, *Allium cepa* has several advantages given that it is easy to manipulate, it is sensitive to rapid response bioassays, it is cheap, and, most importantly, it has a good correlation with models that use mammalian cells for this type of study (Fiskesjö, 1997; Chaparro *et al.*, 2010).

Stickiness observed in the treated onion roots may be due to physical adhesion of the proteins of the chromosome (Patil and Bhat, 1992). However, Mercykutty and Stephen (1980) reported that this stickiness may be interpreted as a result of depolymerisation of DNA, partial dissolution of nucleoproteins, breakage and exchanges of the basic folded fibre units of chromatids and the stripping of the protein covering of DNA in chromosomes. According to Fiskesjo (1985), sticky chromosomes indicated a highly toxic, irreversible effect, probably leading to cell death.

The term c-mitosis was coined by Levan (1938) and described that colchicines prevents the assembly of the spindle fibers and results in scattering of the chromosomes over the cells. (El-Ghamery *et al.*, 2003).

Chromosome Bridge is formed by stickiness of chromosomes which made their separation and free movements complete and thus they remained connected by bridges breakage and fusion of chromosomes and chromatids, the stickiness of chromosome and subsequent failure of free anaphase separation, and unequal translocation or inversion of chromosome segments (Gomórgen, 2005).

Vagrant chromosomes were caused by unequal distribution of chromosomes with paired chromatids in which resulted from nondisjunction of chromatids in anaphase. In vagrant chromosome, a chromosome moves ahead of from its chromosomal group toward poles and leads to the unequal separation of number of chromosomes in the daughter cells (Sondhi *et al.*, 2008).

Lagging chromosomes resulted due to failure of the chromosomes to get attached to the spindle fiber and to move to either of the two poles (Turkoglu, 2007).

Besides to the types of chromosome aberrations, the formation of micronucleus in interphase cells was determined. The induction of micronucleus in root meristem cells of *A. cepa* is the manifestation of fragments or vagrant chromosomes (Yi and Meng, 2003). MN can be a formed as a result of acentric fragments or entire chromosomes not incorporated to the main nucleus during the cell cycle. The exceeding DNA of a cell may originate a bud and which gives rise to a micronucleus and it is subsequently expulsed as a mini cell, which constitute small cytoplasmatic portions bearing a small nuclear content. (Turkoglu, 2007).

Random amplified polymorphic DNA (RAPD)

Ten random primers were used in the present study to identify the RAPD profiles of genomic DNA from root tips of *allium cepa* untreated and treated of textile azo dyes and study the relationship between genotoxicity effect of dyes and change in DNA fingerprint. These primers generated reproducible and easily securable RAPD profiles (Fig. 2) with a number of amplified DNA fragments ranging from 3 to 8 amplicons per primer (Table 6). In the present study, the total number of fragments produced by the 10 primers was 54 with an average number of 5.4 amplicons per primer. The number of amplified DNA fragments was scored for each primer. Primer OPA03 was amplified the highest number of amplicons (8), while the lowest number was 3 with the primers OPA02 and OPA06. The number of polymorphic amplicons per primer ranged from 1 to 7 amplicons. The UPGMA dendrogram generated from the similarity values is shown in Fig. (3). This dendrogram grouped the control and treatments into two main clusters, the first cluster contained the control. On the other hand, the second cluster contains three treatments RLB, RN and SGL. It was divided into two main sub clusters; the first one contained SGL, while the second subcluster contained the other dyes RLB and RN.

Assay of RAPD is suitable for any extracted DNA of sufficient quality, allows rapid analysis of a large number of samples. As arbitrary primers are used, specific details of DNA damage or the genome sequence in organisms are not needed. Furthermore, no radioactivity or enzymatic degradation of PCR products is required prior to analysis (Atienzar *et al*., 1999). Savva (1998) showed that, changes in DNA fingerprint (i.e. band patterns) observed reflect DNA alterations in genome from single base changes (point mutations) to complex chromosomal rearrangements and that DNA fingerprinting offers a useful biomarker assay in assessment of genotoxicity.

Effect of textile azo dyes on RAPD bands pattern

RAPD fingerprints showed substantial differences between unexposed and exposed *Allium cepa* root tips to EC_{50} concentration of tested textile azo dyes, with apparent changes in the number and molecular size of the amplified DNA fragments (Fig. 2). In all cases, polymorphisms were due to the loss and/or gain of amplified bands in the treated samples compared with the control. Table (8) showed the number of appearing and disappearing bands in EC_{50} concentrations of the three textiles azo with the ten primers. One new band (500, 300, 600 and 250 bp) was appeared with primers 0PA01, OPA07, OPA08 and OPA 10, respectively in 400 ug/ml of RLB dye, meanwhile, one new band (450 bp and 200 bp) appeared with OPA-01 and OPA10 primer in 500 ug/ml of RN dye and three new bands (600, 500 and 300 bp) were appeared with OPA07. One new band at 400 ug/ml SGL dye concentration appeared with OPA01, OPA03, OPA09 and OPA10, also the primers OPA 04 and OPA07 were appeared two and three new bands, respectively.

Appearance of new PCR products occurred because some oligonucleotide priming sites could become accessible to oligonucleotide primers after structural change or because some changes in DNA sequence have occurred due to mutations (resulting in new annealing events), and/or large deletions (bringing two pre-existing annealing sites closer), and/or homologous recombination (two sequences that match the sequence of the primer). Apparent bands may also be the results of genomic template instability related to the level of DNA damage, the efficiency of DNA repair and replication (Atienzar *et al.*, 1999).

Disappearing bands are likely to be due to changes in oligonucleotide priming sites, originated from rearrangements and less likely from point mutations and DNA damage in the primer binding sites. The disappearance of PCR products mainly affected the high molecular weight bands because the chances of obtaining DNA damage increased with the length of the amplified fragment (Enan, 2006; Liu *et al.*, 2009).

SUMMARY

The *Allium cepa* assay is an efficient test for chemical screening and *in*

situ monitoring for genotoxicity of environmental contaminants. This test has been used widely to study genotoxicity of many chemicals pollutions revealing that these compounds can induce chromosomal aberrations in root meristems of *Allium cepa.* In this study, we aimed to determine genotoxic effects of some textile azo dyes by using the *Allium cepa* chromosome aberrations test and random amplification of polymorphic DNA (RAPD) analyses. The onion (*Allium cepa* L.) roots were exposed to different concentrations of three textile azo dyes. The mitotic index of samples exposed to EC_{50} (500 µg/ml) of selected azo dyes for RLB, RN and SGL was 10.8, 10.3 and 8.8, respectively. The results indicated that the root length of *Allium cepa* reduced with an increasing azo dye concentration. A random amplification of polymorphic DNA (RAPD) analysis from the extracted DNA was carried out using ten 10-base pair random primers. Ten primers produced 54 bands between 100-1600 base pairs in gel electrophoresis. The number of disappearing bands in profiles was differenced from one to five bands of azo dyes treatment compared to total bands in control and new bands were appeared in treatments.

Obtained results from this study revealed that the total chromosomal aberrations and RAPD profiles were performed as useful tool for detection and biomarker assays for the evaluation of genotoxic effects on textile azo dyes polluted plants.

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Table (1): Chemical structures and some information of textile azo dyes used in this study.

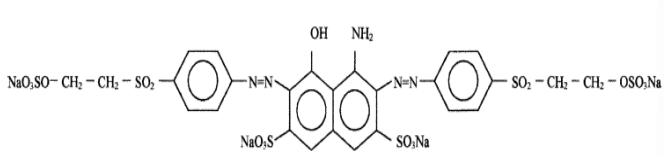
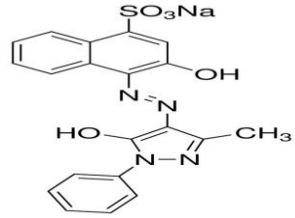
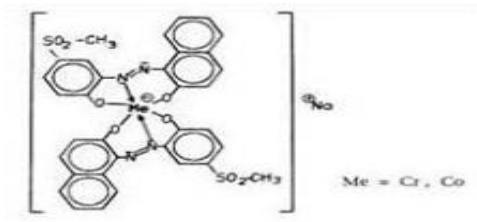
Dye name	Chemical structure	M.W.
Lanasol black B		991.82
Eriochrom red B		446.41
1, 2 metal com-plex1. Yellow		620.00

Table (2): Nucleotide sequence of ten 10 mer primers used for the random amplification of polymorphism DNA.

S/N	Primer name	Primer sequence	G+C %
1	OPA-01	5′ - CAGGCCCTTC-3′	70
2	OPA-02	5′ -TGCCGAGCTG-3′	70
3	OPA-03	5′ -AGTCAGCCAC-3′	60
4	OPA-04	5′ -AATCGGGCTC-3′	60
5	OPA-05	5′ -AGGGGTCTTG-3′	60
6	OPA-06	5′ -GCTCCCTGAC-3′	70
7	OPA-07	5′ -GAAACGGGTG-3′	60
8	OPA-08	5′ -GTGACGTAGG-3′	60
9	OPA-09	5′ -GGGTAACGCC-3′	70
10	OPA-10	5′ -GTGATCGCAG-3′	60

Table (3): Effect of different concentration of textile azo dyes on root number, root length and relative reduction of root length (T/C %) of *Allium cepa* bulbs.

Treatments	Concentrations µg/ml	Roots			Root growth inhibition%	EC_{50}
		Mean number of roots	Mean length of roots (cm)	T/C (%) for roots length		
Control	-	36	3.96	100	-	
RLB	100	34	3.48	88	12	500 µg/ml
	200	32	3.32	84	16	
	300	31	2.28	57	43	
	400	30	2.22	56	44	
	500	26	2.00	51	49	
RN	100	32	3.56	90	10	500 µg/ml l
	200	31	3.42	86	14	
	300	29	2.43	61	39	
	400	26	2.11	53	47	
	500	24	1.92	48	52	
SGL	100	34	3.65	92	8	500 µg/ml
	200	32	3.51	86	14	
	300	31	3.23	82	18	
	400	29	3.14	79	21	
	500	27	2.11	53	47	

Table (4): Mitotic index and phase indices of *Allium cepa* root tips cells exposed to EC_{50} concentrations of different textile azo dyes.

Treatment	No. of cells analyzed	No. of dividing cells	MI%	MI Inhibition %	No. of divining cells and mitotic index per mitotic stages							
					Prophase		Metaphase		Anaphase		Telophase	
					No. of dividing cells	MI%	No. of dividing cells	MI%	No. of dividing cells	MI%	No. of dividing cells	MI%
Control	1500	236	15.7	-	106	7.08	54	3.60	32	2.1	44	2.9
RLB 500 µg/ml	1500	162	10.8	31.2	72	4.80	35	2.30	26	1.7	29	1.9
RN 500 µg/ml	1500	155	10.3	34.4	68	4.50	31	2.06	23	1.5	33	2.2
SGL 500 µg/ml	1500	132	8.8	44.0	57	3.80	29	1.90	19	1.3	27	1.8

Table (5): Effect of treatment on chromosome aberrations in root tip cells of *Allium cepa*.

Treatment	No. of dividing cells	Chromosomal aberrations						Other*	Total aberration cells %
		Bridge	c-mitosis	stickiness	lagging	micronuclei	vagrant		
Control	236	-	-	1	-	-	-	1	0.85
RLB 500 µg/ml	162	2	6	4	2	-	1	3	11.10
RN 500 µg/ml	155	-	8	3	-	2	2	1	10.30
SGL 500 µg/ml	132	5	7	3	1	1	-	3	15.10

* (abnormal vacuoles- polyploidy interphase nucleus- Disoriented metaphase - Mature cell division showing puffing - Strap nucleus).

Table (6): Random primers showing polymorphism among *Allium cepa* root tip cell

Primer	No. samples	Total band obtained	Polymorphic band	Monomorphic band	% Polymorphic
OPA01	4	7	5	2	71
OPA02	4	3	1	2	33
OPA03	4	8	6	2	75
OPA04	4	7	7	0	100
OPA05	4	4	1	3	25
OPA06	4	3	2	1	67
OPA07	4	7	6	1	86
OPA08	4	4	3	1	75
OPA09	4	5	3	2	60
OPA10	4	6	5	1	83
Total		54	39	15	675
Average		5.4	3.9	1.5	67.5

Table (7): The number and molecular sizes of appearing and disappearing bands by random primers in control and treated onions bulbs roots.

Primer name	Total bands in control (bp)	Treatments					
		RLB 400 µg/ml		RN 500 µg/ml		SGL 400 µg/ml	
		New band (bp)	Disappearing of control band (bp)	New band (bp)	Disappearing of control band (bp)	New band (bp)	Disappearing of control band (bp)
OPA01	900, 600, 350, 250, 50	500	600, 350	450	600, 50	500	600, 350, 50
OPA02	1000, 900, 850, 600	-	-	-	1000, 900	-	900
OPA03	1600, 1400, 1000, 900, 650, 400, 250	-	1600, 1400, 1000, 900, 650	-	1600, 1400	500	1000, 650
OPA04	1500, 700, 600, 550, 250	-	1500, 700, 600	-	700, 250	1200, 750	550, 250
OPA05	1100, 400, 300, 250	-	4000	-	400	-	400
OPA06	1100, 1000, 600	-	-	-	-	-	-
OPA07	550, 350, 250, 100	300	550, 250, 100	600, 500, 300	550, 250, 100	600, 500, 300	550, 250, 100
OPA08	450, 300	600	-	-	-	-	-
OPA09	900, 500, 400, 250	-	900, 500	-	900, 500	100	900, 500
OPA10	1400, 1000, 700, 600, 350	250	1400, 700	200	1400, 1000, 700, 600	200	1400, 700

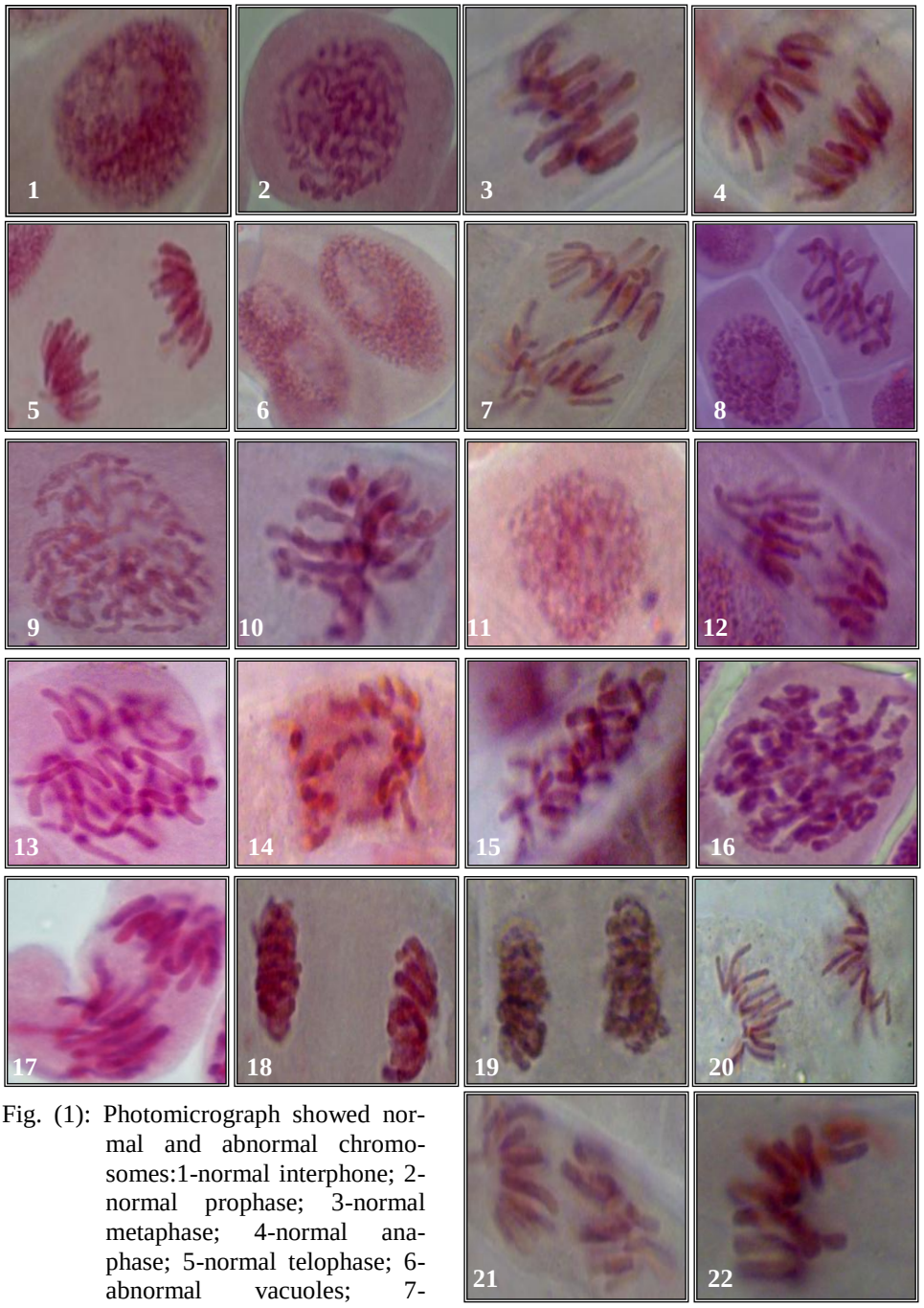


Fig. (1): Photomicrograph showed normal and abnormal chromosomes:1-normal interphone; 2-normal prophase; 3-normal metaphase; 4-normal anaphase; 5-normal telophase; 6-abnormal vacuoles; 7-Anaphase bridge, 8-C-metaphase, 9-polyploidy interphase nucleus, 10-Metaphase sticking, 11-Micronucleus, 12-.Lagging chromosome, 13-Disoriented metaphase, 14-Lagging chromosome, 15-Mature cell division showing puffing, 16-sticky metphase, 17-spindle disturbance at anaphase, 18-Strap nucleus, 19-Multipolar anaphase, 20-Telophase with one end puffing and 21,22-vagrant chromosome.

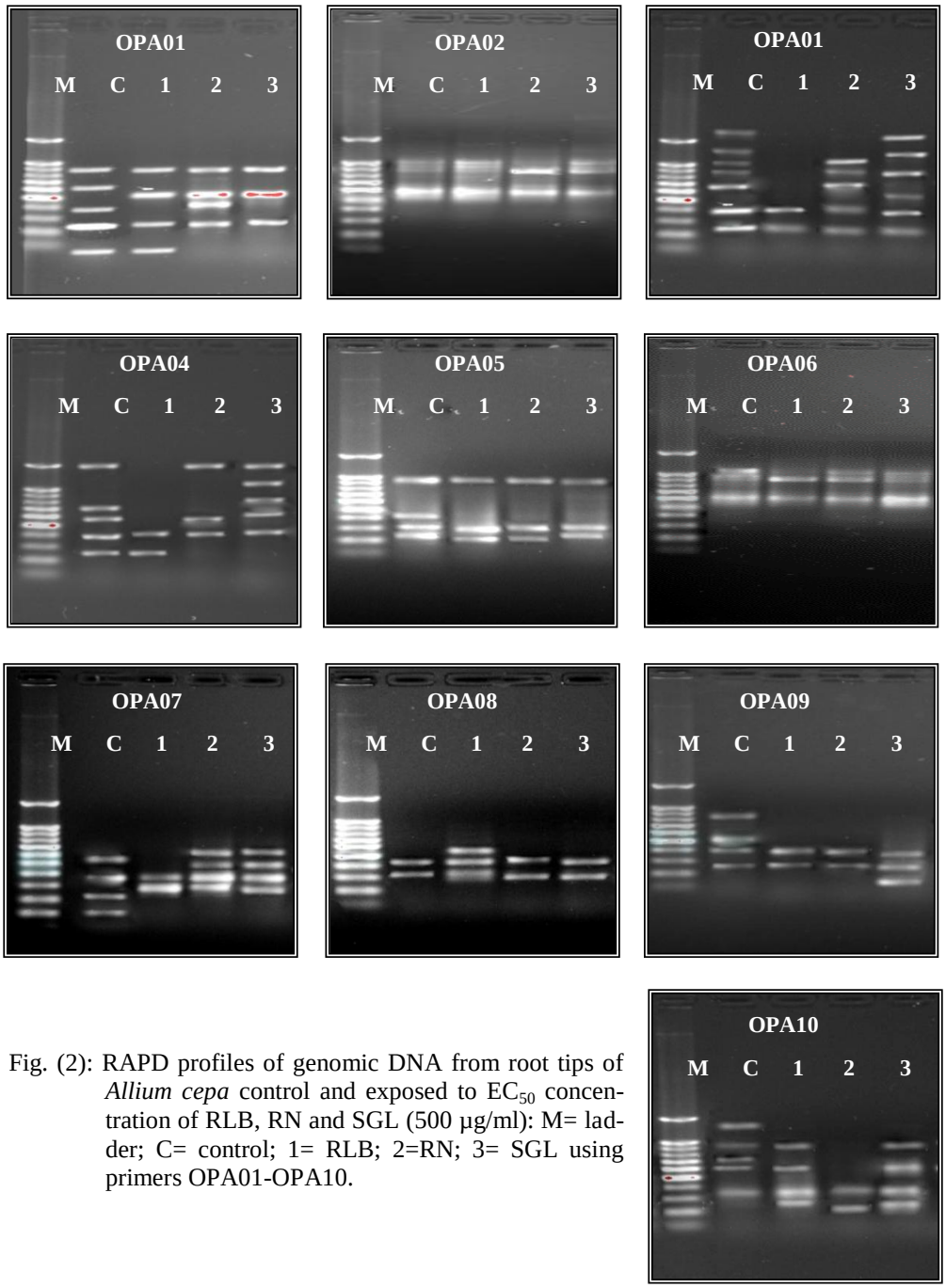


Fig. (2): RAPD profiles of genomic DNA from root tips of *Allium cepa* control and exposed to EC_{50} concentration of RLB, RN and SGL (500 µg/ml): M= ladder; C= control; 1= RLB; 2=RN; 3= SGL using primers OPA01-OPA10.

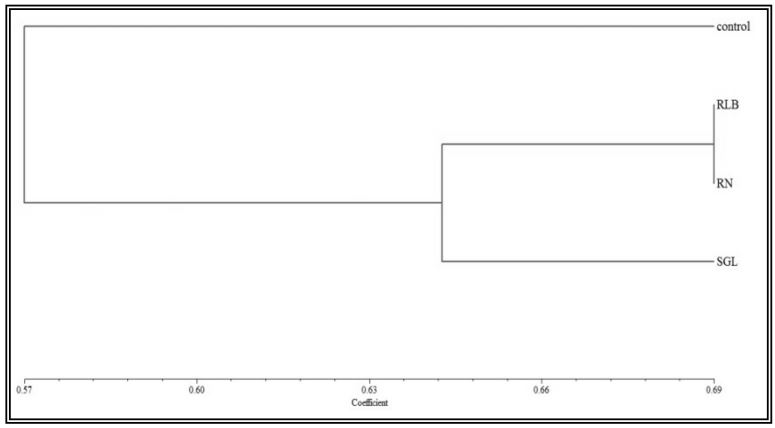


Fig. (3): Dendrogram for control and three textile azo dyes used in this study from RAPD's data using UPGMA and similarity matrices.