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Assessment of Cytotoxic and Genotoxic Effect of the Textile Dyes on *Allium cepa*

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Abstract

The aim of this study was to evaluate cytotoxic effect of the textile dyes (Red HE48, Blue GL, Green HE4B and Violet Brown gmr (sulphur) on *Allium cepa* as a test organism. The effect of dye was assessed at the doses of 1mg, 3mg and 5mg/100ml concentration at the exposure time of 24 h in root tip cells of *A.cepa*. 500 cells for each dose were studied and the rate of mitotic index and aberrations were calculated. The result shows that there was decrease in mitotic index with increasing concentrations of textile dyes and abnormality rate was increased with increasing concentrations of textile dyes. Mainly 5mg concentration shows higher rate of aberrations with decrease in mitotic index. Aberrations like anaphase bridge formation, polyploidy, cell with no nucleus, micronucleus, disturbed prophase and stickiness in chromosome were found at the exposure time of 24 h. In addition, the toxicity level was checked by measuring chromosomal length, arm ratio and centromeric index. The result of this study will be useful in detecting the presence and action of mutagenic agents. Therefore, this will set pace for toxicity identification evaluation (TIE) studies.

Keywords

Chromosomal aberrations, Karyotype, Mitotic index, Textile dyes.

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1. INTRODUCTION

Developing countries are facing three major problems which are population, poverty and pollution due to rapid industrialization [1]. The release of untreated waste into the natural water-bodies, water pollution is the major and serious problem. Waste-water has been increases due to increasing the demand of textile products lead to cause pollution [2]. In all industrial sectors, the textile plants are the most water contaminating plant which leads to cause serious health problems. During the textile wet processing, industries utilize more volumes of water and chemicals for scouring, dyeing, printing and finishing processes [3] consequently, entering into the environment. Moreover, during the operation

time of manufacturing, labors are also exposed to these dyes. Thus, the evaluation of genotoxicity of such pollutants is a crucial parameter for the ecological system.

Dye is a substance having extensive coloring capacity widely employed in the textile industry. These dyes having chemicals along with salts, carboxymethyl cellulose, calcium stearate and other compounds [4]. It includes variety of structure such as metal complex dyes, acidic, reactive, anthraquinone-based, basic, disperse, azo and diazo [3]. In the textile industry, during dyeing and finishing process up to 200,000 tons of dyes are lost into effluents [5]. The presence of minute amounts of dyes extremely affects quality and transparency of natural water-bodies [3]. Dyes that escapes the conventional waste water treatment and remain is in the environment as a result of their high stability to light, temperature, water, detergents, chemicals and soap etc [6].

Identification and evaluation of toxic compounds is done by using various test organisms. Higher plant comprises a significant material for testing genetic alterations acquired through the environmental pollutants [7]. The plant as a test-organism has been suggested by various environmental agencies such as WHO (World Health Organization) UNEP (United Nations Environmental Program) etc [8]. The *Allium cepa* is a test showing good correlation to other test systems and excellent test organism commonly adopted for finding environmental pollution [9]. It is produced as a plant having economic significance in the country [10]. The environmental monitoring system, the root tip observation is a quick and susceptible method [1]. Identification of chemical compounds is done by using *A.cepa* as it shows good correlation with the results compared with mammalian test systems [11].

In plant phylogenetic, karyotyping studies have been carried out for more than hundred years [12]. Many plant and animal species have been identified chromosomes via karyotyping [10]. The chromosomes of *A.cepa* have been studied for diversity in size, structure and number. The several substances include colchicines, 8-hydroxyquinoline, para dichlorobenzene, ice cold water and bromonapthalene are used for separating mitotic chromosomes through microtubule formation inhibition [13]. In present study, the effect of Red HE48, Blue GL, Green HE4B and Violet gmr (sulphur) on the onion root tips mainly at 1mg, 3mg and 5mg/100ml concentrations have been studied to check the changes in the cell cycle interphase and the mitotic phase. Thus, the mitotic index was calculated to study normal dividing cell. However, abnormal cells and chromosome length were calculated to investigate the rate of aberration and cell toxicity level correspondingly.

2. MATERIALS AND METHODS

2.1. Chemicals

Para dichlorobenzene was purchased from Sigma Aldrich and Aceto-orecin stain was obtained from Sd fine- Chem limited. All other used chemicals were of analytical grade.

2.2. Sample Collection

Allium cepa were collected from local market of Kolhapur, Maharashtra and textile dyes were procured from textile industry Ichalkaranji, Maharashtra, India.

2.3. Allium cepa root tip Assay

The dried external roots of *A.cepa* were removed and placed in coupling jar containing distilled water (D/W) for germination as a control set. For examination of the dyes namely Red HE48, Blue GL, Green HE4B, Violet brown gmr (sulphur) were diluted in the concentration 1mg, 3mg and 5mg/100ml of distilled water (D/W). The base of *Allium cepa* bulb were soaked in the above prepared stocks and allowed for germination for 24 h. After germination, root tips were cut and soaked in D/W for 2 min, then hydrolyzed in 1N hydrochloric acid at 60^0C for 3 min. After that, they were again washed with D/W for 2-3 times to remove the traces of hydrochloric acid and finally blot by filter paper for sucking excess of liquid. Two root tips of about 1-2 mm were then squashed on each slide, stained with 2% aceto-orcien for 10 min and cover slip was carefully lowered on to the slide to exclude air bubble. The cover slips were sealed on the slides with nail polish to prevent drying out of the slide by the heat of microscope [14].

2.3.1. Microscopic examination

Total 500 cells were taken for interphase and dividing phase counting. The cells were counted at each concentration and were analyzed at 10X for mitotic index and at 40X and 100X for the aberrations. The mitotic index was calculated as the number of dividing cells per 500 cells and the frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored at each concentration.

2.3.2. Mitotic squash preparation for karyotyping

The dried external roots of *A.cepa* were removed and the base of onion was placed in distilled water as the disk just touching water and allowed for sprouting as a control set. For test samples, the four dyes namely Red HE48, Blue GL, Green HE4B and Violet Brown gmr (sulphur) were diluted in 5mg/100ml in D/W. The base of *Allium cepa* were soaked in the above prepared stocks and allowed for germination for 24 h.

After germination, the root tips were cut and placed in para dichlorobenzene for 4 h at 8-10^{0}C, then keep it at room temperature for 2 h. Thereafter, the root tips were hydrolyzed in 1N hydrochloric acid at 60^{0}C for 3 min. The hydrolyzed root tips were rinsed in three changes of D/W and excess fluid was blotted with filter paper. About 1-2 mm of root tips was cut off and placed in a drop of 2% aceto-orcien covered with cover slip for 10 min to stain the cells. Subsequently, the cells were squashed by placing layers of blotting paper onto the coverslip and by pressing the thumb slightly. The cells well examined under Trinocular microscope.

2.3.3. Karyotype Analysis

The number of chromosomes per cell was calculated. The long arm length, short arm length and total length of chromosome was measured using software Ikaros. Centromeric index was determined as long arm length of chromosome divided by total length of chromosome and Arm ratio was determined by long arm length of chromosome divided by short arm length of chromosome [12].

3. RESULTS AND DISCUSSION

3.1. Mitotic index and percentage of aberrations

3.1.1. Red HE 48, Blue GL, Green HE4B and Violet brown gmr (sulphur)

Because of the rapid development of textile industries, amount of waste water having toxic chemicals is getting discharged into fresh water of river and ocean ultimately disturbing the environment. The cytotoxic substances have capability to block normal dividing cycle of the cell that is to interfere with mitosis. Thus, the evaluation on *A.cepa* has advantage that it shows similarity to mammalian cells [9]. In this connection, study of effect of routinely used textile dye on the *A. cepa* for cytotoxic and genotoxic effects is an essential study.

The mitotic cells were counted in the root meristems at 24 h of incubation. In control set, the mitotic index was found to be 83±2.64. After dye treatment to the *A.cepa* root tips mitotic index was decreased. For three different concentrations of dyes (1, 3 and 5 mg/100ml) the mitotic index was observed to be 58±1.50, 44±1.52 and 35.6±1.21 whereas, percentage of aberration was 3.52±0.32, 6.68±0.69 and 12.36±0.67 respectively. One of the best mutagenicity related endpoints are chromosome breaks and micronucleus because the cells do not repair these genetic alterations [8]. Mitotic index was decreased, and percentage of aberrations was increased as the concentration of the dye was increasing (Table 1). Incubation of *A.cepa* roots in the Red HE48 dye cause the change in structure of chromosome at lower and higher concentration. This was manifested by disturbed prophase, anaphase bridge formation, strap nucleus and polyploidy in chromosome (Fig 1). The dye induced aberrations such as; multipolar anaphase, polyploidy, disturbed prophase, sticky chromosome, c-metaphase, Anaphase bridge formation, Telomere puffing, micronucleus, dead cells, cells with no nucleus, apoptic cells and micronucleus was observed which suggests the toxic level of cytotoxic substance. The Aneuploidy risk is indicated by c-metaphase, high level of toxicity is indicated by sticky chromosome which causes death of the cell [1].

After germination, the *A.cepa* root tips were treated with different concentrations of Blue GL

dye. After 24 h of incubation, the mitotic index of control sample was counted as 83±2.64 while, with increased concentration of dyes from 1, 3 and 5 mg/100 ml the mitotic index apparently decreased to 47.73±3.60, 31±1 and 20.86±0.75 with percentage aberrant cell of 9.84±1.40, 18.55±0.50 and 33.42±4.40 respectively. Taking together, Mitotic index was lowest, and percentage of aberrant cell was highest for 5mg concentration as compared to 3mg. Hence, it was significant increase in aberration rate (Table 2). Incubation of *A.cepa* in Blue GL shows change in structure of chromosome; picnosis, scattered prophase, diagonal anaphase, telophase clumping, polyploidy, reduced number of chromosome and cells with no nucleus (Fig 2). Apoptosis may be due to the process of cell elimination, without bursting of plasma membrane [8].

In case of Green HE4B, the mitotic cells were counted was 83±2.64 in control samples. Whereas, mitotic index decreased from 53.13±3.13, 37.8±1.70 and 22.6±0.69 with percentage aberration was 9.17±0.78, 17.2±0.39 and 26.90±2.16 respectively for the concentration of 1, 3 and 5 mg/100ml of dye. Therefore, it was showing highest mitotic index at lower concentration of 1 mg compared to 3 and 5 mg (Table 3). The aberrations are clearly seen in the fig 3. Incubating *A.cepa* with Green HE4B causes abnormalities in chromosome such as; Strap nucleus, stickiness of chromosome in metaphase, Telophase bridge formation, unipolar anaphase, polyploized interphase and cell with no nucleus

The mitotic cells were counted after germination; the Mitotic index was 83±2.64 in control. Dye effect resulted as decrease in mitotic index and increase in percentage of aberrant cell. At 1mg/100ml violet brown gmr (sulphur) dye, 65.06±3.10 mitotic index was observed, and percentage of aberrant cell was 6.55±1.24. While, at 3mg/100ml, Mitotic index and percentage of aberrant cell was 54.73±2.0, 13.36±1.18 respectively. However, mitotic index and percentage of aberrant cell for 5 mg/100ml was found to be 37.86±1.80, 18.46±2.05 correspondingly. Mitotic index was higher for 1mg concentration and percentage of aberrant cell was higher for 5mg concentration (Table 4). There were considerable changes in chromosome structure such as; Multipolar Anaphase, c-mitosis, Anaphase bridge formation and telomere puffing etc (Fig 4). According to Ananthakrishnan et al. (2013), anaphase bridges might be caused due to formation of dicentric chromosomes due to breaking and reunion of chromosome. In comparision, MI was higher for Red HE48 and Violet brown gmr (sulphur) than Blue GL and Green HE4B. Decrease in mitotic index in the treated cells may be due to blocking of DNA synthesis, arrestation of mitotic phases or may be due to inhibition of G2 phase of the cell cycle [11]. The results obtained in present study indicate that the dyes Blue GL and Green HE4B were more toxic than Red HE48 and Violet brown gmr (sulphur). The Mitotic index decreased with increasing concentrations of textile dyes. Our results are supported by earlier studies obtained after treating *A. cepa* with cadmium [15], squezzed garri extract [16], *M. elengi* bark extract [9] and industrial effluent from paint and textile [1].

3.1.2. Karyotype analysis

The molecular level effect of hazardous textile dye is abnormal interphase or mitosis or blocking of the cell cycle in interphase. A chromosome measurement study reveals that, total length of the chromosome was normally 148.28µm for control set [12]. In our present investigation, *A.cepa* germination in distilled water shows total chromosome length 148.28µm, with mean 9.26µm having total chromosome number 16 (Table5) (Fig 5). While, *A. cepa* when exposed to textile dyes at 5mg/100ml concentration shows that total length of Red HE48 and Violet Brown gmr (sulphur) was 121.55µm and 146.02µm which was nearly same as observed by Ahmet Okumu and Lutful Hassan, (2000) (Table 6, 9) (Fig 6, 9). *A.cepa* treated with dye Blue GL at 5mg/100ml shows total chromosome length 279.49µm, with mean 17.46µm (Table 7) (Fig 7). *A.cepa* treated with Green HE 4B shows total chromosome length 252.44µm and mean 15.77µm (Table 8) (Fig 8). The chromosome length was increased when *A.cepa* treated with dye Blue GL and Green HE4B as compared with control set. Hence the dye Blue GL and Green HE4B shows high level of toxicity as compared with Red HE48 and Violet brown gmr (sulphur). The increase or decreases in length of

chromosome is might be due to some addition or deletion of base pairs which further might cause mutation. Such mutation may be attributed by morphological or altered chemical constituents.

Table 1: Red HE48

Sr. no	Treatment	Number of cells counted	Number of dividing cells	Mitotic cells	Percentage of aberrant cell
1	control	500	415±13.22	83±2.64	-
2	1mg	500	292.33±7.50	58±1.50	3.52±0.32
3	3mg	500	223.33±7.63	44±1.52	6.68±0.69
4	5mg	500	178±6.08	35.6±1.21	12.36±0.67

Table 2: Blue GL

Sr.no	Treatment	Number of cells counted	Number of dividing cells	Mitotic index	Percentage of aberrant cell
1	control	500	415±13.22	83±2.64	-
2	1mg	500	238.66±18.03	47.73±3.60	9.84±1.40
3	3mg	500	155±5	31±1	18.55±0.50
4	5mg	500	104.33±3.78	20.86±0.75	33.42±4.40

Table 3: Green HE4B

Sr. no	Treatment	Number of cells counted	Number of dividing cells	Mitotic index	Percentage of aberrant cell
1	Control	500	415±13.22	83±2.64	-
2	1mg	500	265±15.69	53.13±3.13	9.17±0.78
3	3mg	500	189±8.54	37.8±1.70	17.2±0.39
4	5mg	500	113±3.46	22.6±0.69	26.90±2.16

Table 4: Violet Brown gmr (sulphur)

Sr. no	Treatment	Number of cells counted	Number of dividing cells	Mitotic index	Percentage of aberrant cell
1	Control	500	415±13.22	83±2.64	-
2	1mg	500	328.66±16.44	65.06±3.10	6.55±1.24
3	3mg	500	273.66±10.01	54.73±2.0	13.36±1.18
4	5mg	500	189.33±9.01	37.86±1.80	18.46±2.05

Table 5: Control

Chromosome no	(p) in µm	(q) in µm	pq µm	CI	AR
1	5.95	6.76	12.71	0.53	1.13
2	6.27	6.27	12.55	0.49	1
3	3.54	6.60	10.14	0.65	1.86
4	4.66	5.95	10.62	0.56	1.27
5	3.54	4.99	8.53	0.58	1.40
6	2.25	3.70	5.95	0.62	1.64
7	5.31	6.44	11.75	0.54	1.21
8	4.02	5.63	9.66	0.58	1.40
9	2.57	4.02	6.60	0.60	1.56
10	2.57	4.50	7.08	0.63	1.75
11	4.18	4.66	8.85	0.52	1.11
12	3.54	4.34	7.88	0.55	1.22
13	2.09	5.79	7.88	0.73	2.77
14	2.41	3.70	6.11	0.60	1.53

15	4.66	6.44	11.10	0.58	1.38
16	3.05	7.72	10.78	0.71	2.53

Total chromosome length: 148.28µm, mean: 9.26µm

Table 6: Red HE48

Chromosome no	(p) in µm	(q) in µm	pq µm	CI	AR
1	3.70	5.47	9.17	0.59	1.47
2	2.25	7.40	9.66	0.76	3.28
3	2.73	5.63	8.37	0.67	2.06
4	2.89	5.79	8.69	0.66	2.00
5	2.73	5.79	8.53	0.67	2.12
6	2.73	5.15	7.88	0.65	1.88
7	3.86	4.18	8.05	0.51	1.08
8	2.73	4.99	7.72	0.64	1.82
9	2.57	4.99	7.56	0.66	1.94
10	3.05	5.15	8.21	0.62	1.68
11	3.05	3.86	6.92	0.55	1.26
12	2.89	3.38	6.27	0.53	1.16
13	2.57	3.70	6.27	0.59	1.43
14	1.61	4.66	6.27	0.74	2.89
15	2.09	3.86	5.95	0.64	1.84
16	2.25	3.70	5.95	0.62	1.64

Total chromosome length: 121.55µm, mean: 7.59µm

Table 7: Blue GL

Chromosome no	(p) in µm	(q) in µm	pq µm	CI	AR
1	8.69	9.56	18.35	0.52	1.10
2	8.05	10.14	18.19	0.55	1.25
3	9.33	10.14	19.48	0.52	1.08
4	9.49	9.66	19.15	0.50	1.01
5	9.17	9.98	19.15	0.52	1.08
6	7.88	10.46	18.35	0.57	1.32
7	7.24	12.55	19.80	0.63	1.73
8	8.69	12.39	21.09	0.58	1.42
9	6.76	13.36	20.12	0.66	1.97
10	5.31	11.43	16.74	0.68	2.15
11	4.66	13.36	18.03	0.74	2.86
12	3.70	11.59	15.29	0.75	3.13
13	5.47	9.98	15.45	0.64	1.82
14	5.79	8.05	13.84	0.58	1.39
15	6.11	8.21	14.32	0.57	1.34
16	5.95	6.11	12.07	0.50	1.02

Total chromosome length: 279.49µm, Mean: 17.46µm

Table 8: Green HE4B

Chromosome no	(p) in µm	(q) in µm	pq µm	CI	AR
1	4.34	7.40	11.75	0.62	1.70
2	5.63	7.40	13.04	0.56	1.31
3	7.24	7.56	14.81	0.51	1.04
4	6.60	6.76	13.36	0.50	1.02
5	5.95	9.33	15.29	0.61	1.56
6	4.50	7.72	12.23	0.63	1.71
7	9.17	9.98	19.15	0.52	1.08
8	8.85	10.46	19.32	0.54	1.18
9	4.02	9.33	13.36	0.69	2.32
10	6.76	8.53	15.29	0.55	1.26
11	6.76	8.05	14.81	0.54	1.19
12	7.40	7.40	14.81	0.52	1
13	7.24	9.17	16.42	0.55	1.26
14	6.44	8.53	14.97	0.56	1.32
15	4.99	5.47	10.46	0.52	1.09
16	5.79	11.10	16.90	0.65	1.91

Total chromosome length: 252.44µm, Mean: 15.77µm

Table 9: Violet Brown gmr(sulphur)

Chromosome no	(p) in µm	(q) in µm	pq µm	CI	AR
1	2.73	4.34	7.08	0.61	1.58
2	2.89	4.34	7.24	0.59	1.50
3	2.57	2.73	5.31	0.51	1.06
4	3.38	3.86	7.24	0.53	1.14
5	3.86	5.63	9.49	0.59	1.45
6	3.54	5.79	9.33	0.62	1.63
7	5.79	5.79	11.59	0.49	1
8	5.95	6.44	10.78	0.59	1.08
9	5.31	6.27	11.59	0.54	1.18
10	3.86	6.76	10.62	0.63	1.75
11	5.15	5.63	10.78	0.49	1.09
12	4.83	5.15	9.98	0.51	1.06
13	4.18	4.66	8.85	0.52	1.11
14	2.57	5.63	8.21	0.68	2.19
15	3.22	4.34	7.56	0.57	1.34
16	4.99	5.31	10.30	0.51	1.06

Total chromosome length: 146.02µm, Mean: 9.12µm

Fig 1: Mitotic effect of Red HE48 on root tip cells of *A.cepa*; a) Disturbed prophase b) Polyploidy c) Anaphase bridge formation d) Strap nucleus e) Apoptic cells f) Multipolar Anaphase

Figure: 1

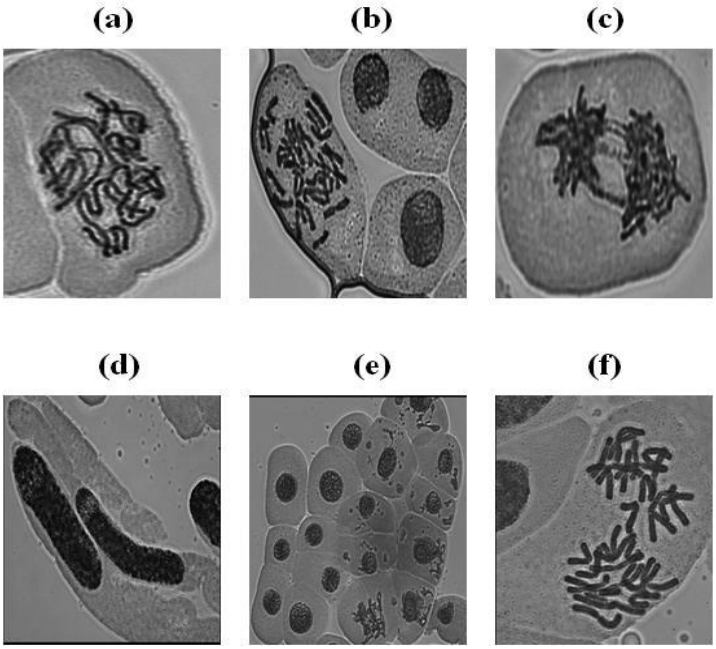


Fig 2: Mitotic effect of Blue GL on root tip cells of *A.cepa*; a) Picnosis b) Scattered prophase c) Diagonal Anaphase d) Telophase Clumping e) polyploidy f) Reduced number of chromosome g) Polyploidy h)Disturbed Anaphase

Figure:2

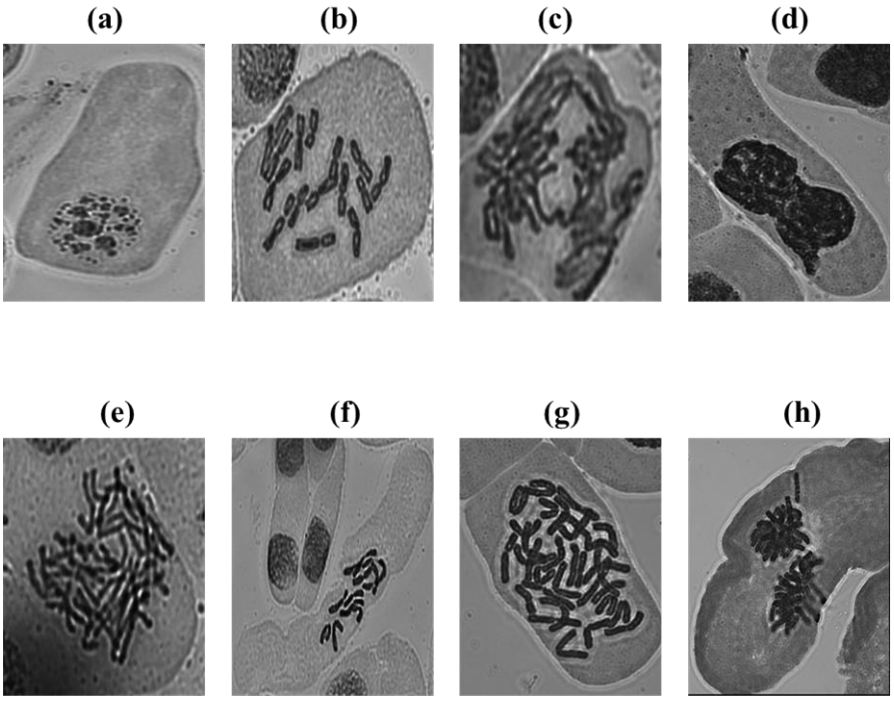


Fig 3: Mitotic effect of Green HE4B on root tip cells of *A.cepa*; a) Strap nucleus b)Sticky chromosome c) Telophase bridge formation d) Unipolar Anaphase e) Polyploized interphase f) cells with no nucleus g) cell with micronucleus h) fragmented chromosome.

Figure: 3

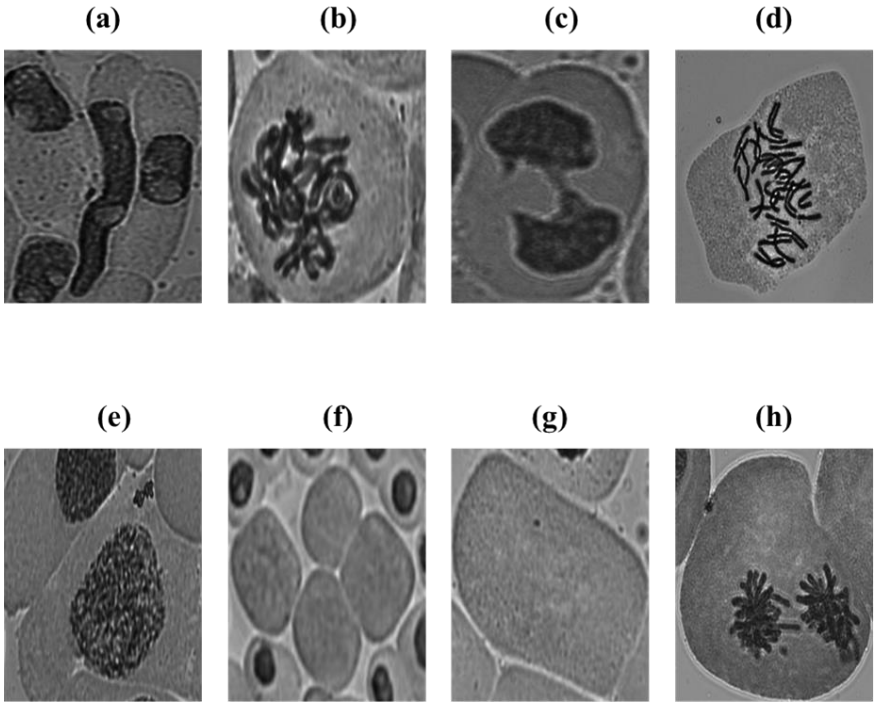


Fig 4: Mitotic effect of Violet brown gmr (sulphur) on root tip cells of *A.cepa*; a)Multipolar anaphase b) c-metaphase c) Anaphase bridge formation d) Telomer puffing

Figure:4

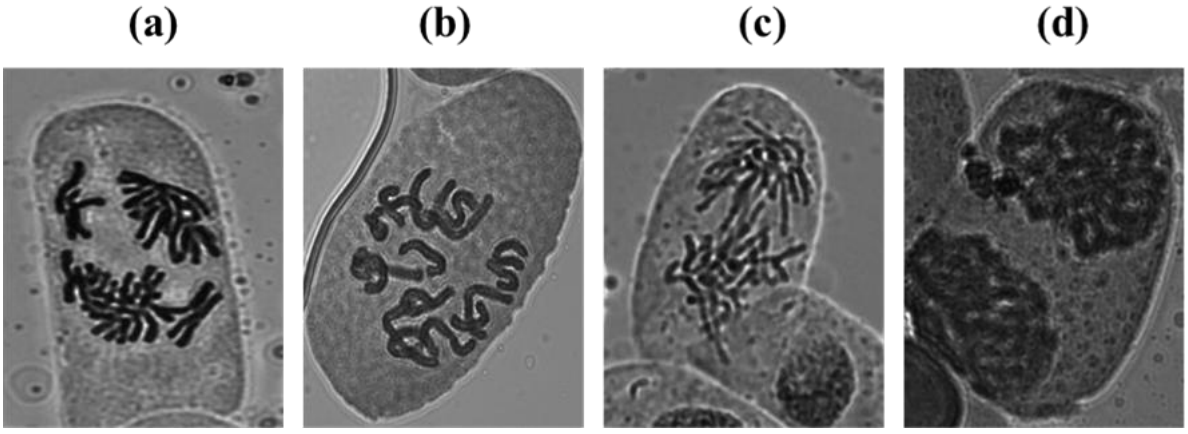


Fig 5: Mitotic metaphase chromosome of *A.cepa*

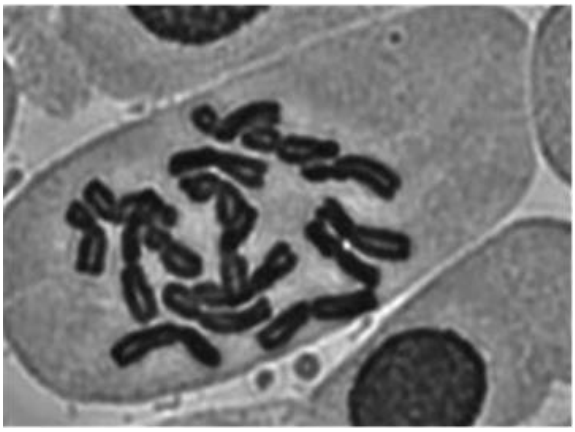


Fig 6: Mitotic metaphase chromosome of *A.cepa* treated with Red HE48.

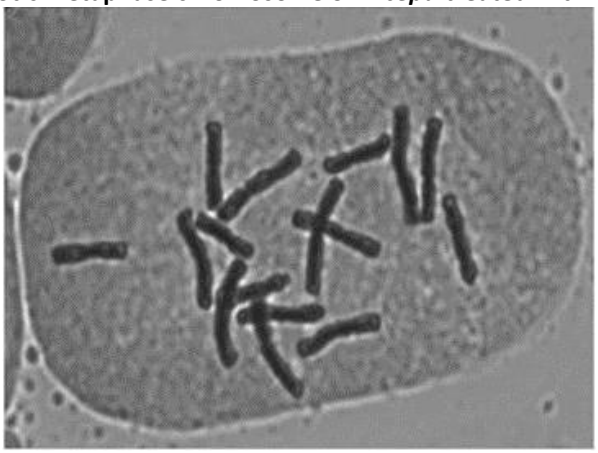


Fig 7: Mitotic metaphase of chromosome treated with Blue GL.

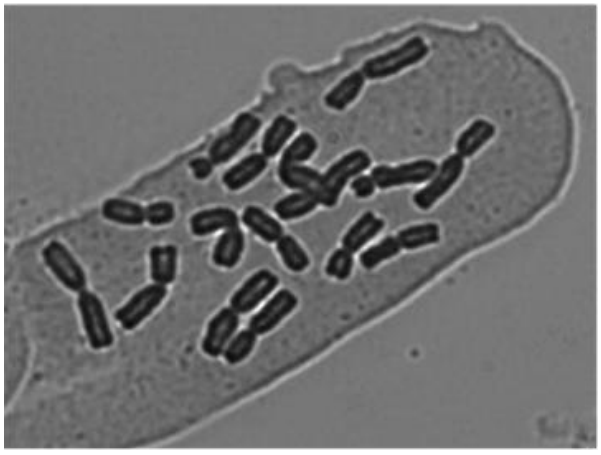


Fig 8: Mitotic metaphase of chromosome treated with Green HE4B

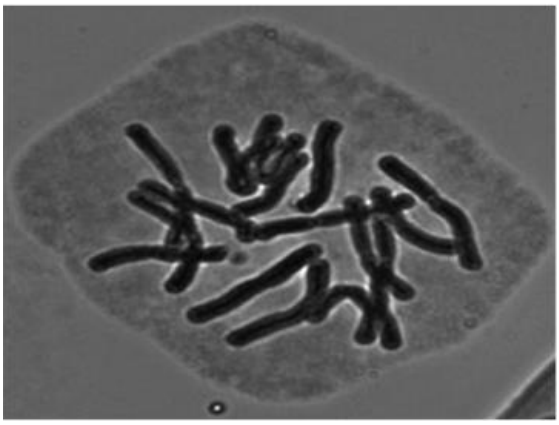
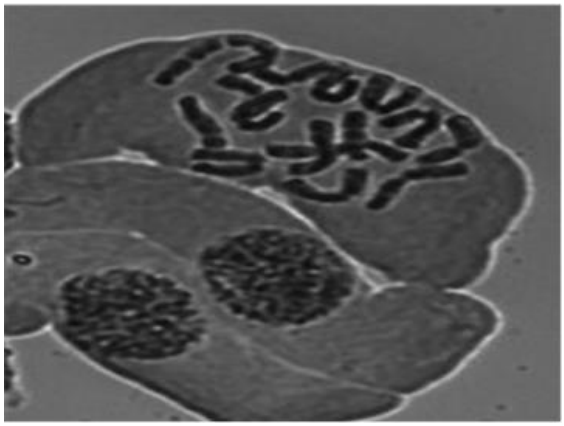


Fig 9: Mitotic metaphase of chromosome treated with Violet brown gmr (sulphur)



4. CONCLUSION

Present investigation of *A.cepa* treated with textile dyes showed mitotic abnormalities. Mitotic index was decreased, and percentage of abnormality was increased as concentration of dyes increases. These aberrations may lead to death of the cell, blocking of G2 phase etc. When we consider mammalian cells, toxic effects of dye may result in mutated or cancerous cells which are life threatening effect. The study of mitotic index, cell aberration and karyotyping proved the toxic effect of textile dye under study. Thus, treatment of textile effluent containing such harmful dyes before discharge in fresh environment is very essential aspect.

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