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Vol. 13(38), pp. 3881-3891, 17 September, 2014
DOI: 10.5897/AJB2014.13879
Number: 7C5F5C347604
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Abnormal mitosis in root meristem cells of *Allium cepa* L. induced by a fabric dye reactive turquoise blue (Procion MX)

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Received 22 April, 2014; Accepted 12 September, 2014

Reactive Turquoise Blue (Procion MX) is a fabric dye used in small scale cotton fabric industries in various parts of India. The impact of this chemical on human health in the surrounding areas that discharge effluents is of serious concern. This needs to be assessed for short and long term effect on human genome. This investigation was aimed to find mitotic abnormalities as cytological evidence induced by the dye in root tip cells of onion (*Allium cepa* L.) grown in different concentrations: 0.01, 0.05, 0.1, 0.5 and 1.0% (weight per volume) prepared in distilled water in separate treatment schedules for 24 and 48 h. Mitotic aberrations (MA) were scored after staining with 2% acetocarmine by conventional squashing method. Root growth at various concentrations and duration of exposure of the dye were analyzed as macroscopic parameter for testing the cytotoxicity. Percentages of mitotic cells were analyzed as microscopic parameter to find the trend of mitotic indices and depression. Total abnormality of cells in percentage indicated the genotoxic assault of the dye. At higher concentrations, the root tip cells died in 24 h. Highest number of dividing cells with largest mitotic index value was observed at 0.01% exposed for 48 h. The abnormalities of common occurrences observed were unequal cytokinesis/ karyokinesis, formation of micronuclei, bi-nucleated cells and little condensed chromosomal arms in abnormal metaphase, anaphase and telophase. The abnormal mitotic cells were assumed to be due to genotoxic assault of the dye on chromosomal condensation mechanism resulting in very unusual long arms in rapidly dividing meristematic cells.

Key words: Reactive torquoise blue, genotoxicity, *Allium cepa*, mitotic aberration.

INTRODUCTION

Clothes and fabrics form the basis of human aesthetic senses and beauty and are given utmost importance from the beginning of civilization. To make them colourful, attractive and lucrative to customer for better commercialization, an array of various dyes are employed throughout the globe. Dyes are widely used in various industries like textile, rubber,paper, printing, colour photography, pharmaceuticals, cosmetics and

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many more (Raffi et al., 1997) and are normally manufactured from the primary products obtained through the distillation of coal-tar (Tyagi and Yadav, 2001). The textile industry utilizes mostly reactive dyes, which are used in dyeing cellulose fibres and accounts for the largest consumption of dyestuffs, at nearly 80% (Mathur et al., 2005a). India is the second largest exporter of dyestuffs after China (Mathur et al., 2005b). The annual global production of dyes and pigments is more than 0.7 Mt of which 10 to 15% is discharged into the environment without any proper treatment (Vinu and Madras, 2009).

Textile effluents are some of the most troublesome wastewaters to treat effectively because of their low biodegradability, significant toxicity and varied composition (Riga et al., 2005). The synthetic origin and complex aromatic structures of the dyes make them stable and difficult to be biodegraded (Seshadri et al., 1994; Fewson, 1998). Excessive use of these chemicals and ready disposal of their wash-off effluents to nearby agro-fields as well as drinking water resources poses problems on the surrounding biomass. It contaminates soil surface and ground water as well as food chains affecting health of the inhabitants of aquatic and terrestrial environment.

Textile dyes are mixtures of chemicals which contain salts, calcium stearate, carboxymethyl cellulose (CMC) and other unknown chemicals. The textile industry utilises mostly reactive dyes, which are used in dyeing cellulose fibres like cotton which accounts for about 40% of world fibre production (http://www.utexas.edu/centers/natstat/data/pdf). Fibre reactive dyes attach permanently to cellulose fibers using a covalent bond. These molecules carry a chromophore which absorbs various spectrum of light, allowing only certain spectrum to reflect. During the dyeing processes about 10 to 90% of the dye do not bind to the fibers and therefore are released into the environment (Zollinger, 1991; Abadulla et al., 2000). The most obvious impact of the discharge of dye coloured effluent is the persisting nature of the colour. It is stable and fast, difficult to degrade, toxic, rendering the water unfit for its intended use. Such dyestuffs can reach the aquatic environment, primarily dissolved or suspended in water, the conventional treatment of wastewaters from textile mills and dyestuff factories are unable to remove most of the azo and other dyes effectively. The resulting dye effluents may contain some components or moieties that could be toxic, carcinogenic or mutagenic to aquatic life (Suzuki et al., 2001).

Procion is a brand of fibre reactive dyes and Procion MX are a class of cold reactive dyes including Reactive Turquoise Blue which is a widely used cotton linen dyeing chemical in many parts of the world and are extensively used in tie dye and textile industry. It is widely used in cotton linen dyeing industries throughout Australia, Europe and US (http://en.wikipedia.org/wiki/ Reactive_ dye). It has cyclic structure with two chlorine atoms on it and these are the reactive sites that react with OH groups on cellulose fiber to create the strong covalent bonds responsible for the dichlorotazine giving extremely high fastness. The dye in commercial form represents a mixture of different chemicals. It is therefore not possible to know the exact concentration of the dye itself in the powder form of the commercial dyestuff and there could be other components in this mixture that could also be toxic. There may be one or two sulfonate groups on the phthalocyanine ring, two or three dichlorotriazine sections per copper phthalocyanine and the positions of these different items on the phthalocyanine ring are unknown and presumably random. In addition, this dye molecule, especially if kept in solution for several days, tends to 'stack up' to form aggregates of two or more molecules, causing the hue to shift to be more blue and less green. In tie and dye industries, the resulting textile wastewater is of a deep blue colour that affects water quality by inhibiting the penetration of sunlight and thus reducing photosynthetic activity (Lambrecht et al., 2007).

Human being is exposed to low levels of such toxic substances by direct or indirect way via irrigation, drinking, bathing, pisciculture and consuming many aquatic food species throughout his/her life time. The health hazard posed by such dyes is a major concern all the time. The immediate detrimental effects of these hazardous chemicals on human health are apparent while other long range effects are only dimly perceived. Concern for genotoxicity caused by environmental pollutants has led to the development of several biological tests for detecting and identifying genotoxicants in air, water and soil. According to Wollin and Gorlitz (2004), several azo dyes have shown genotoxicity by studying human keratinocytes (HaCaT cells).

Over the past decade, issues of animal use and care in toxicology research and testing have become one of the fundamental concerns for both science and ethics. Emphasis has been given to use of alternatives to mammals in testing, research and education (Mukhopadhyay et al., 2004). Organisms used in mutagenesis testing should be selected using criteria that permit a realistic evaluation of the potential of a suspected mutagen to induce changes in genetic material such as structural and/or numerical modification of chromosomes resulting in chromosome aberrations (Matsumoto et al., 2006).

Plants are direct recipients and are important model for genetic tests and environmental monitoring and use of plants for evaluation of environmental pollutants was validated (Cabrera et al., 1994). According to Vieira and Vicentini (1997) the effect of mutagens on eukaryotic nuclei can be assessed cytologically by observing inhibition of cell growth or division, interruption of metaphase for the induction of numerical and structural changes of chromosomes.

Chromosome identification is essential for biotechnolo-

gical studies including genome analysis, somatic hybridization and ploidy manipulation (Yamamoto and Tominaga, 2004). Since chromosomes are physical entity of the genetic system it is naturally assumed that any agent affecting the chromosomes will also lead to heritable genetic change.

As the sizes of chromosomes in *A. cepa* is comparatively larger than many other plants and eukaryotes and the number of chromosomes is less (2n =16), Grant (1982) reported *Allium* test as one of the excellent assay for mutagenesis assay. The common onion is one of the most outstanding higher plant recommended by United States Environmental Protection Agency (USEPA) and the American Society for Testing and Materials (ASTM) in 1982 and 1994 respectively for use as an excellent and alternative first-tier indicator for safety evaluation of cytogenetic and mutagenic effects of drinking water and environmental pollutants as their root length inhibition and chromosome aberration bioassay are sensitive, cost effective and valid indicator of toxicity test for routine monitoring of water pollution having good correlation with other test systems involving genotoxicity (Rank, 2003; Babatunde and Bakre, 2006; Olorunfemi et al., 2011). The test is a fast and inexpensive method, easy to handle, gives reliable results, comparable with other tests performed in mammalian systems is in high concurrence with similar assay in bone marrow cells in rats (Grover et al., 1998).

The Reactive Turquoise Blue is a chemical of regular use in small scale cottage industries of cotton fabrics in various parts of India with special reference to Western and Southern Odisha for handlooms works, but its genotoxicity due to waste water discharged on surrounding biomass in terms of any cytological end point has not been analyzed till to date. The purpose of our study is to evaluate the genotoxic effect of reactive turquoise blue (Procion MX) under various concentrations by analyzing mitotic aberrations in growing root tip cells of *A. cepa*.

MATERIALS AND METHODS

Test dye

The material used in this study is a dye that is, reactive turquoise blue (Procion MX) which is assumed to be a potential genotoxicant on plant model. The dye was purchased from the local market of Bargarh, Odisha, India which is used mostly by the weaver community residing in nearby areas for tie and dye techniques. It is a product of Atul Textiles and Imperial Chemical (ATIC) industry, Rajasthan, India.

Experimental plant organism

The experimental organism employed was *A. cepa*. It is one of the most extensively used biennial plants (Kovatch, 2003) and oldest cultivated vegetables (Fritsch and Friesen, 2002; Phillip and Jenderek, 2003). Cytotoxicity and environmental pollution (El-Shahaby et al., 2003) have been assessed by *A. cepa* root tip system, which is known to give similar results to *in vivo* animal cytotoxicity tests (Teixeira et al., 2003; Vicentini et al., 2001).

Methods

Various concentrations of the dye (weight per volume) in distilled water (0.01, 0.05, 0.1, 0.5 and 1.0%) were prepared and applied to the growing root tips of *A. cepa* (N = 6 to 10 for each treatment) in separate glass tubes of 10-20 ml capacity and incubated for various durations that is, 48 h to induce mitotic aberrations *in vivo* and for 24 and 48 h to assess mitotic index by recording the proportion of dividing cells. The procedure involved original form of *A. cepa* test (Fiskesjso, 1985) where root growth was initiated in tap water. Standard protocol (Fiskesjo, 1985) was followed with slight modification in the treatment schedule for duration of exposure and concentration of dye (w/v). The method used was similar to the method of Asita and Matebesi (2010).

The bulbs presoaked in distilled water were subsequently germinated in sand trays and grown *in situ* in different test tubes containing various concentrations of dye for 24 and 48 h. The test tubes with diluted test dye in distilled water for each bulbs were filled every day to compensate evaporation. When the root growth reached the length of 1 to 2 cm the tips were cut, fixed and preserved. The cut root tips were fixed in 1:3 aceto-alcohols (Carnoy's fixative) for 24 h and then stored in 70% alcohol for future use. The bulbs grown in tap water served the purpose of control. No positive control experiment employing mitomycin-c or cyclophosphamide was performed. Conventional squash preparation was adopted following the acid hydrolysis of cellulosic cell wall in 1 N HCl followed by warming at 60°C. Staining was done in 2% aceto-carmine in 45% glacial acetic acid (v/v) followed by rubbing (mordenting) in rust free iron needle to visualize the scorable stages under microscope (Das, 1986; Kar, 1992; Rank and Nielsen, 1997; Dane and Dalgic, 2005; Tartar et al., 2006).

Scoring

The slides were viewed under the binocular light microscope (Olympus CX 31) using the 100 X objective lens with oil immersion. Photographs of some representatives selected stages were taken by a 14.2 mega pixel Canon Cyber Shot Digicam. A total of 300-700 cells were scored per slide mounting to an average of 3979 cells (2220 to 4717). Mitotic indices (MI) and mitotic depression (MD) were calculated following the procedure of Das (1986) and Kar (1992). The mitotic index (MI) in percentage was calculated as number of dividing cells / total number of cells scored × 100. Similarly, the mitotic depression (MD) was calculated as {MI (control)-MI (treated) / MI (control)} × 100. The proportion of specific cell abnormalities such as abnormal prophase, metaphase and anaphase was calculated in terms of percentage of the type of abnormality out of the total number of cells counted.

Statistical analysis

The mean value with standard deviation (SD) for each root length was calculated from values obtained from individual bulbs and it was compared with the corresponding control values and student's 't' test was conducted to ascertain if the differences were statistically significant or not for root growth and for total cell abnormality.

RESULTS AND DISSCUSSION

This study was based on two parameters namely-

Table 1. Effect of Reactive Turquoise Blue on MI and MD in root tip cells of *A. cepa*

Parameter	C	T1	T2	T3	T4	T5
Concentration (mg/L)	0	1.0	0.5	0.1	0.05	0.01
Duration (h)	48	48	48	48	48	48
Number of Bulbs	4	6	3	5	3	3
Number of cells scored (N)	4413	2220	4508	4000	4020	4717
Number of dividing cells (n)	160	45	91	159	163	218
Mitotic Indices (MI)	3.62	2.02	2.01	3.97	4.05	4.62
Mitotic depression (MD)	_	44.19	44.47	-9.66	-11.87	-27.62

C= Control in distilled water; Mitotic index (MI) = n/N X100; Mitotic Depression (MD) = [MI (Control)-MI (Treatment)/MI (Control)] X100. T_1, Treatment 1 of onion root tips at a concentration of 1.0 mg/L of Reactive Turquoise Blue dye; T_2, treatment 2 of onion root tips at a concentration of 0.5 mg/L of Reactive Turquoise Blue dye; T3, treatment 3 of onion root tips at a concentration of 0.1 mg/L of Reactive Turquoise Blue dye; T_4, treatment 4 of onion root tips at a concentration of 0.05 mg/L of Reactive Turquoise Blue dye; T_5, treatment 5 of onion root tips at a concentration of 0.01 mg/L of Reactive Turquoise Blue dye.

Table 2. Root growth (mm) in *A. cepa* bulbs treated with Reactive Turquoise Blue (Procion MX) (Mean± SD).

Treatment	C1	C2	T1 (A)	T1(B)	T2 (A)	T2 (B)	T3 (A)	T3 (B)	T4(A)	T4 (B)	T5(A)	T5(B)
Conc. (mg/L)	0	0	1.0	1.0	0.5	0.5	0.1	0.1	0.05	0.05	0.01	0.01
Duration (h)	24	48	24	48	24	48	24	48	24	48	24	48
Number of Bulbs	4	4	7	6	5	3	5	5	4	3	3	3
Mean Root Length ± SD	5.80 ± 0.35	6.00 ±0.44	5.40 ± 2.16	5.80 ± 1.28	5.90 ± 0.84	7.40 ± 1.80	6.60 ± 1.42	6.80 ± 2.27	4.90 ± 1.06	6.30 ± 0.24	4.10 ± 0.56	5.40 ± 1.36
t values												
(p=0.005)	NA	NA	NS	NS	NS	*	NS	*	NS	NS	NS	NS
(p=0.05)			NS	NS	NS	**	NS		NS	NS	NS	NS

C_1, Control-1; C_2, Control-2; * Significant at p=0.005; ** Significant at p=0.05; NS, not significant; NA, Not Applicable. T_1, Treatment 1 of onion root tips at a concentration of 1.0 mg/L of Reactive Turquoise Blue dye; T_2,Treatment 2 of onion root tips at a concentration of 0.5 mg/L of Reactive Turquoise Blue dye; T3, Treatment 3 of onion root tips at a concentration of 0.1 mg/L of Reactive Turquoise Blue dye; T_4, Treatment 4 of onion root tips at a concentration of 0.05mg/L of Reactive Turquoise Blue dye; T_5, Treatment 5 of onion root tips at a concentration of 0.01 mg/L of Reactive Turquoise Blue dye.

cytogenetic and root growth. It was observed that, at higher concentrations that is, - 1.0 and 0.5%, the onion root tip cells could not survive and died in 24 h causing the tips to dry. The dried root tips were presumed to be due to death of cells caused by evaporation of the liquid or cytotoxic nature of chemical. The tips became hard, rough and difficult to cut for preservation. The results are presented in form of observation (Tables 1 to 3, Figures 1 to 5 and Plates 1 to 6).

Table 1 as well as Figures 1 and 2 describe the effect of dye on MI and MD. An average of 3-6 bulbs were used for each treatment and the number of cells scored (N) for MI calculation ranged from 2220 to 4717 (mean = 3979). The

Table 3. Percentage of abnormal mitotic cells induced by Reactive Turquoise Blue.

Treatment number	C	T1	T2	T3	T4	T5
Concentration (mg/L)	0.0	1.0	0.5	0.1	0.05	0.01
Duration (h)	48	48	48	48	48	48
Abnormal Cells (%)	0.11	1.48	3.20	2.42	2.81	3.18
% of Abnormality						
A.P	0.00	0.31	0.59	0.62	0.59	0.73
A.M	0.04	0.49	0.68	0.92	1.16	1.34
A.A	0.02	0.63	0.37	0.70	0.79	0.96
A.T	0.04	0.04	0.24	0.10	0.24	0.12
t values for total cell abnormality						
(p=0.005)	NS	NS	*	NS	NS	*
(p=0.05)	NS	NS	NS	NS	NS	**
Bi or multi nucleated cells	0.00	0.00	1.48	0.02	0.00	0.00

C, Control; AP, abnormal prophase; AM, abnormal metaphase; AA, abnormal anaphase; AT, abnormal telophase. T_1, Treatment 1 of onion root tips at a concentration of 1.0mg/L of Reactive Turquoise Blue dye; T_2, Treatment 2 of onion root tips at a concentration of 0.5 mg/L of Reactive Turquoise Blue dye; T3, Treatment 3 of onion root tips at a concentration of 0.1 mg/L of Reactive Turquoise Blue dye; T_4,Treatment 4 of onion root tips at a concentration of 0.05 mg/L of Reactive Turquoise Blue dye; T_5, Treatment 5 of onion root tips at a concentration of 0.01mg/L of Reactive Turquoise Blue dye.

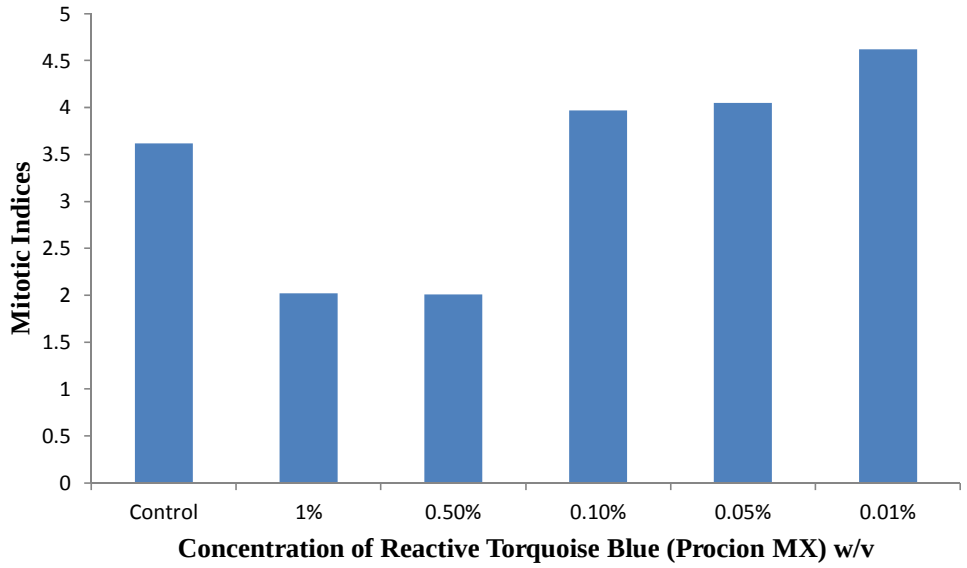

Figure 1. Effect of reactive turquoise blue on mitotic indices (MI) of *A. cepa* root tip cells.

maximum number of cells scored was in the case of treatment with lowest concentration that is, 0.01 mg/L. Highest number of dividing cells (n) was observed (218) with largest MI value of 4.62 in the above treatment. Similarly, the lowest number of dividing cells were observed at 1.0 mg/L (N=2220, MI=2.02) but minimum MI (2.01) was calculated at 0.5 mg/L. A gradual increase with decreasing concentration was observed.

The mean root length varied from 4.10 ± 0.56 to 7.40 ± 1.80 mm (N=6 to 10) and presented in Table 2 and Figure 3. After 24 h, maximum root length was observed at 0.1 mg/L (6.60 ± 1.42 mm) in three replications of 25 each and minimum root length was 4.10 ± 0.56 mm at 0.01 mg/L in three replications of 25 each. Similarly at 48 h treatment schedule, maximum root length was observed (7.40 ± 1.80 mm) at 0.5 mg/L and minimum root length was 5.40 ± 1.36 mm at 0.01mg/L.

Table 3 and Figure 4 show the degree of abnormality of cells at various treatment schedules. Usually under microscope, abnormal divisional stages appeared in

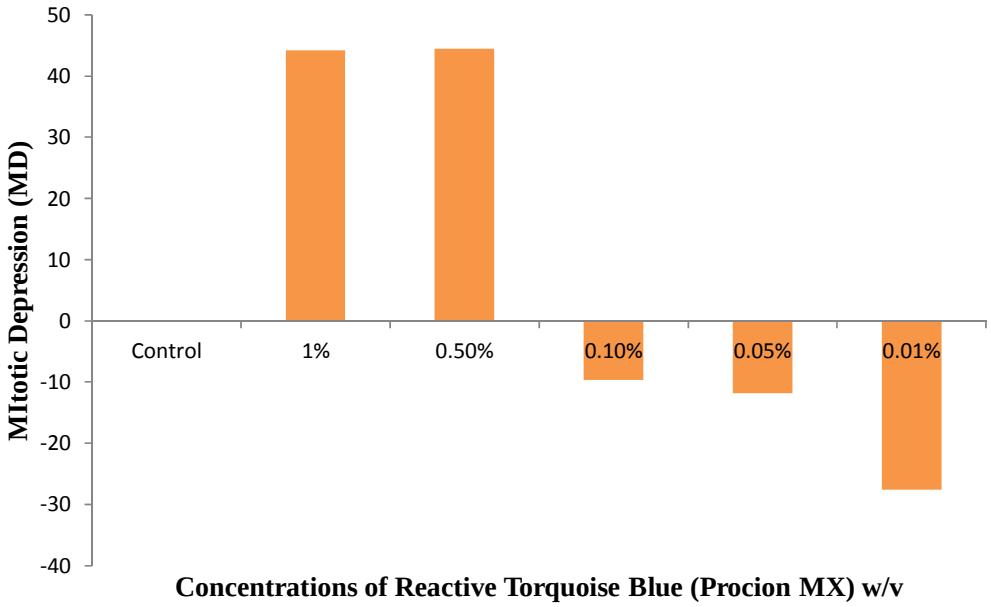

Figure 2. Mitotic depressions (MD) in *A. cepa* root tip cells induced by Reactive Turquoise Blue.

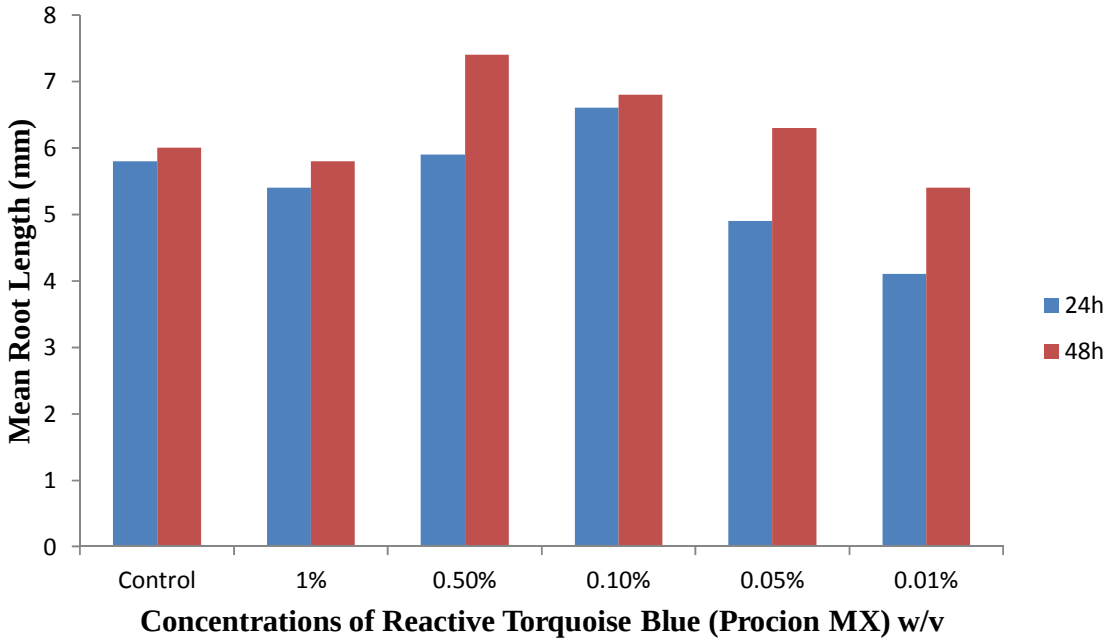

Figure 3. Mean root length (mm) in *A. cepa* due to effect of Reactive Turquoise Blue for 24 and 48 h.

wrong geometric locations and orientations with respect to the equatorial plate of the rectangular or square type cells. Normal prophase or metaphase appeared symmetrical under 100 X magnification with respect to cell boundary and the staining of the nuclear region differed from that of the cytoplasm. Maximum of 3.2% of total abnormal cells were observed at 0.50% and minimum total abnormality observed were 1.48% at a concentration of 1%. The t values for total cell abnormality presented in Table 3 indicated significant deviation from control at 0.5 mg/L at probability level of 0.005 only and significant at 0.01 mg/L at both 0.005 and 0.05 level of probabilities.

Figure 5 presents each type of abnormality in *A. cepa* root tip cells due to different treatment of dye for 48 h. A

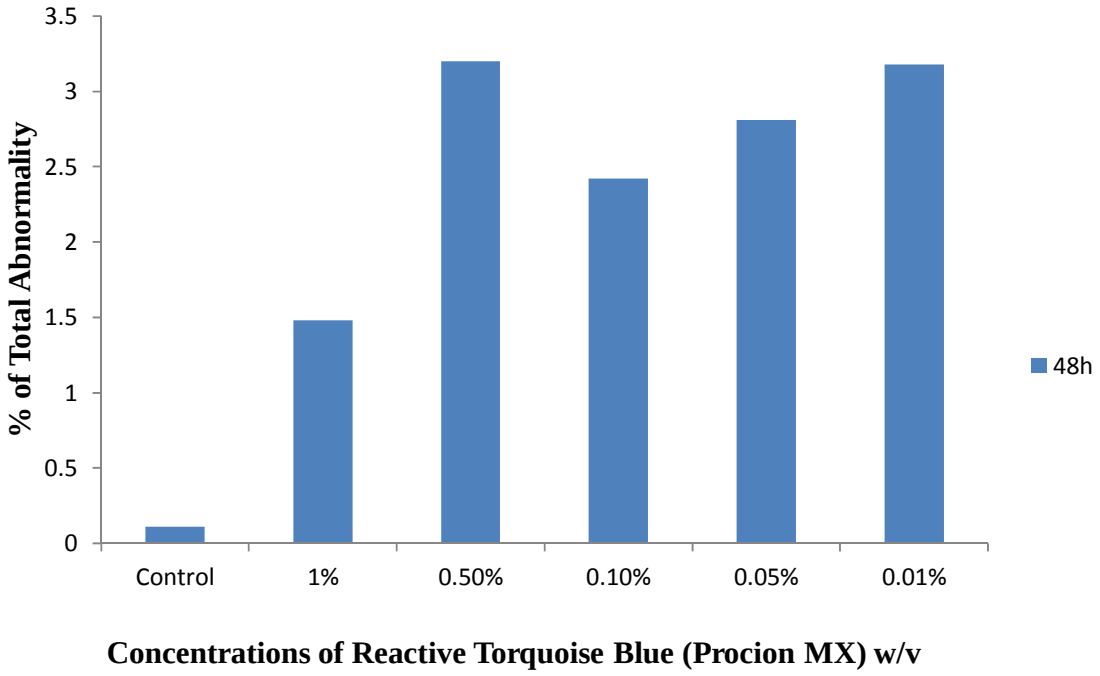

Figure 4. Total abnormality in *A. cepa* root tip cells (%) induced by Reactive Turquoise Blue.

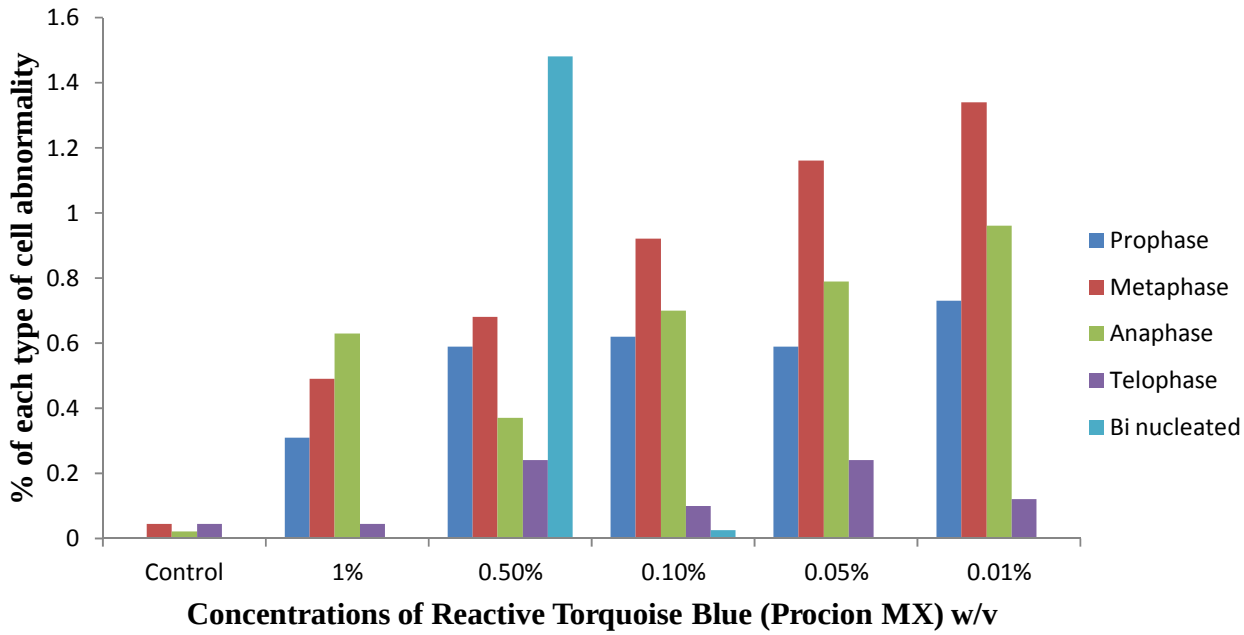

Figure 5. Each type of mitotic aberration (%) in *A. cepa* root tip cells due to Reactive Turquoise Blue.

good correlation was observed between the concentrations of dye and percentage of abnormal metaphase showing an inverse relationship.

Percentage of abnormal prophase and metaphase gradually increased with decreasing concentration of the dye; but, irregular pattern of abnormal anaphase and telo-phase was observed with decreasing concentration. In this study, the t test for root growth observed was found to be insignificant in many cases in comparison to those at control experiments at 0.05 as well as 0.005 level of probability.

Only in case of treatment at 48 h at 0.5% concentration

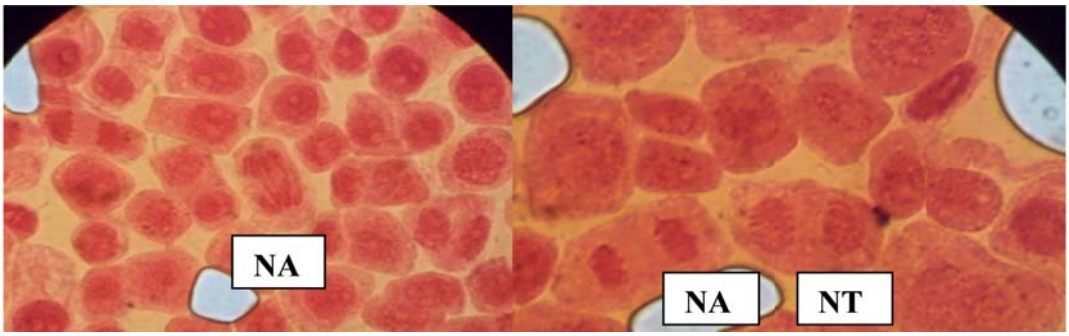

Plate 1. Stages of mitosis in root tip cells of *A. cepa* in control. NA: normal anaphase, NT: normal telophase.

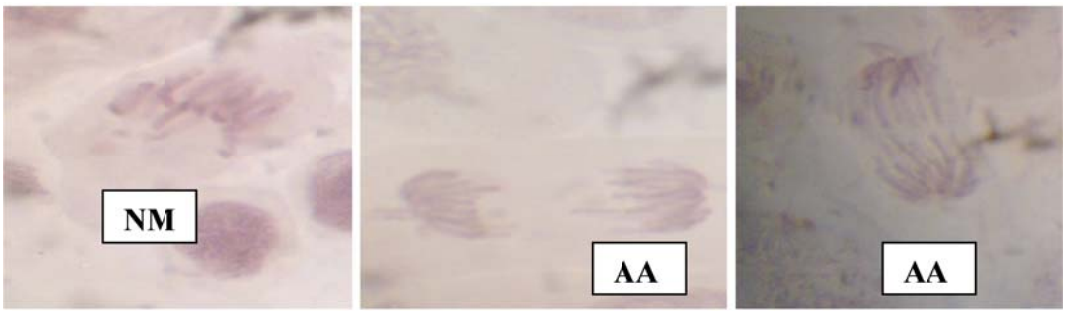

Plate 2. Mitotic aberrations in root tip cells of *A. cepa* in Reactive Turquoise Blue at 0.01%. NM, normal metaphase; AA, abnormal anaphase.

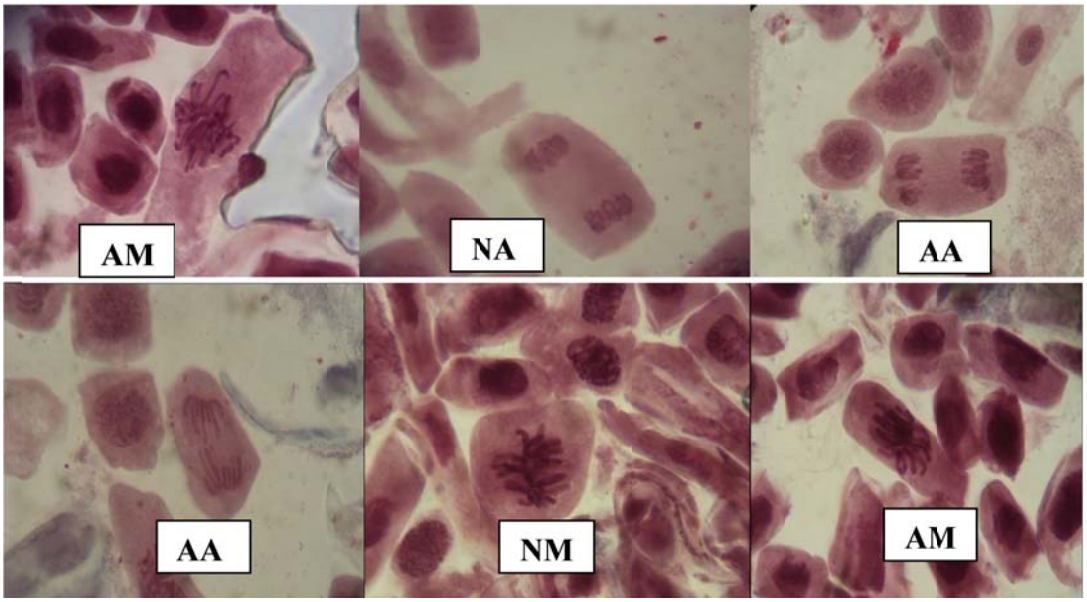

Plate 3. Mitotic aberration in root tip cells of *A. cepa* in Reactive Turquoise Blue at 0. 05%. AM, abnormal metaphase; NA, normal anaphase; AA, abnormal anaphase; NM, normal metaphase.

of the dye the t value showed significant deviation from control value at both 0.05 and 0.005 level of probability.

However, at a concentration of 0.5% of the dye, 1.48% of bi- nucleated cells were observed in comparison to 0.025% of bi-nucleated cells at a concentration of 0.1%.

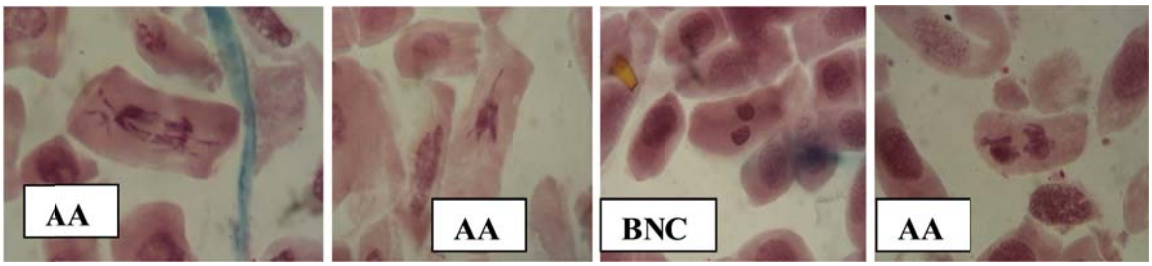

Plate 4. Mitotic aberrations in root tip cells of *A. cepa* in Reactive Turquoise Blue at 0.1%. AA, abnormal anaphase; BNC, Bi-nucleated cells.

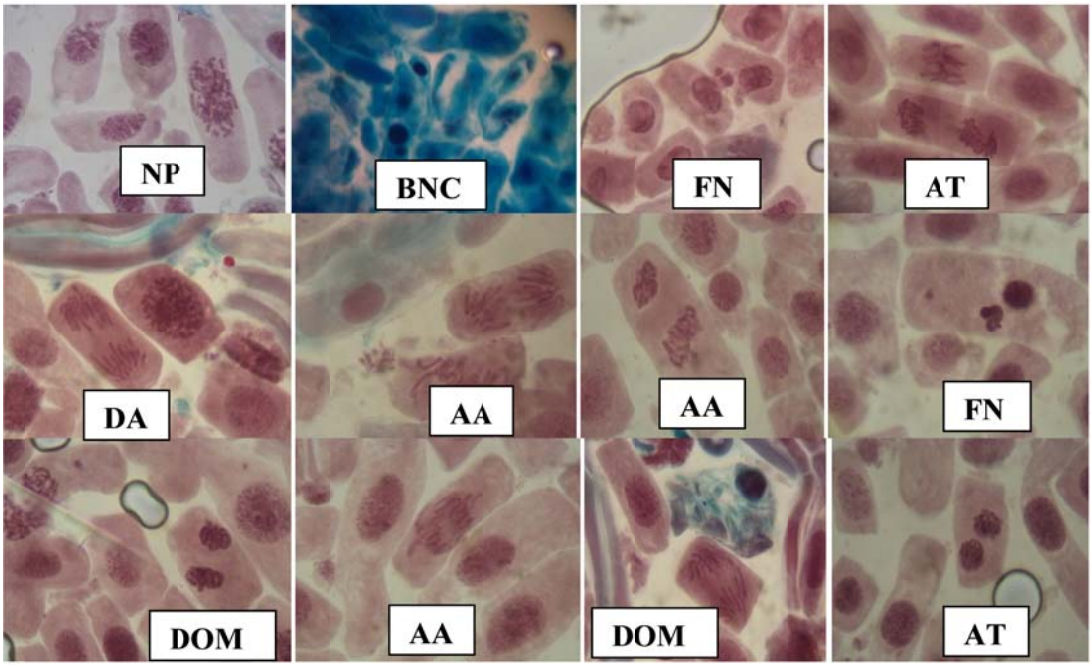

Plate 5. Mitotic aberrations in root tip cells of *A. cepa* in Reactive Turquoise Blue at 0.5%. NP, normal prophase; BNC, Bi-nucleated cells; FN, fragmented nucleus; AT, abnormal telophase; DOA, disoriented anaphase; DOM, disoriented metaphase; AA, abnormal anaphase.

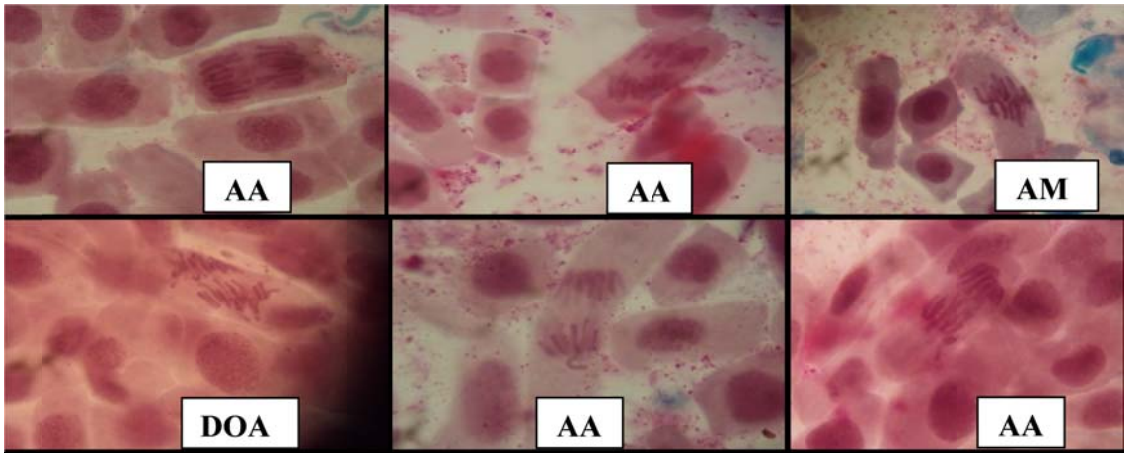

Plate 6. Mitotic aberrations in root tip cells of *A. cepa* in Reactive Turquoise Blue at 1.0%. AA, abnormal anaphase; AM, abnormal metaphase; DOA, disoriented anaphase.

DISCUSSION

Reactive dyes are highly water soluble and are non-degradable in the conventional, biological treatment systems, adsorb poorly to biological solids and therefore remain in the discharged effluents (Epolito et al., 2005). The synthetic dyes used in textile industries are of health concern for various organisms including human. Rajaguru et al. (2001) reported a moderately toxic dye to *Rana hexadactyla* following whole-body exposure to increasing concentrations. The female crab *Spiralothelphusa hydrodroma* after exposure to textile dye effluents at a sub-lethal concentration (66.69%) in two different exposure period shows morphological as well as histological changes in neuro-secretory cells of brain, thoracic ganglia and eyestalks and their contents (Sekar et al. 2008). Birhanli and Ozmen (2005) used frog embryo teratogenesis assay-Xenopus (FETAX) to establish that some reactive dyes have teratogenic potential. Risk of colon and rectum cancers relates mostly to dyes for synthetic fibres (De Roos et al., 2005).

Sahi et al. (1998) used *A. cepa* test system to assess the effects of chromium contamination in the water of an Indian river and showed that at sites where chromium concentration was high there was a reduction in mitotic index and an increase in the rate of mitotic abnormalities, thus confirming the cytotoxic and genotoxic effect of chromium. Similar trends were also observed in the present study (Table 1 and Figure 1). Mitotic activity reduction could be due to the inhibition of DNA synthesis (Sudhakar et al., 2001). In the present study, it was observed that at 0.5 and 1.0% of concentrations, the root tips were dry due to death of cells. The deaths of root cells at such higher concentrations were assumed to be due to interferences of dye in regular cellular activities making them cytotoxic. However in lower concentrations, such deaths of root tip cells were not prominent.

The present study is comparable to similar other studies done earlier by various groups of workers in different non-human organisms (Badr, 1983; Das, 1986; Jain and Sarbhoy, 1988; Kar, 1992; El-Shahaby et al., 2003; Wollin and Gorlitz, 2004). Presence of chromosomal bridges at anaphase might be the result from chromosome stickiness as reported by Badr (1983) caused by clastogenecity. In the present study, the dye affected cell plate formation, which was found to be under great disturbances and the orientation as well as functioning of the spindle was disturbed. In several late anaphases, one group of chromosomes was found to take the extreme terminal position while the other was in the middle. Similar orientations of the two nuclei were also noticed in certain bi-nucleated cells. Occurrence of disoriented chromosomes might have been brought about by action of the dye on the microtubules. The dye might have caused the failure of chromosomes to align at equatorial plate because of the dysfunction of spindle and energy deficiency causing delay in the division of centromeric region which might have also caused distorted chromosome as reported earlier (Jain and Sarbhoy, 1988). Irregular and transverse orientations of chromosomes in equatorial plate were also observed as common form of mitotic aberration. In metaphase, chromosomes were arranged in equatorial plate which was diagonally placed. In anaphase, chromosomal bridge and orientation problem in pole was observed.

The condensation of interphase chromatin to form the compact chromosomes of the mitotic cells is a key event in mitosis, critical in enabling the chromosomes to move along the mitotic spindle without becoming being broken or entangled with one another. The chromatin in interphase nuclei condenses nearly thousand fold during the formation of metaphase chromosome. Cooper and Haussmann (2007) informs that, chromatin condensation is driven by protein complexes called condensins which are members of a class of 'structural maintenance of chromatin proteins' (SMC) that play key role in organization of eukaryotic chromosomes. Another family of SMC proteins called cohesins contributes to chromosome segregation during mitosis. The SMC proteins are recognized as one of the most fundamental classes of proteins that regulate the structural and functional organization of chromosomes from bacteria to humans (Losada and Hirano, 2005; Nasmyth and Haering, 2005).

The final conclusion of this study includes observations of abnormally de-condensed or elongated or stretched chromosomes in numbers of prophases, metaphases and anaphases. Those are considered to be due to disturbance of chromosomal condensation mechanism by the genotoxicant. Disoriented mitosis, decrease in mitotic indices, abnormal divisional stages, and orientation problems of equatorial plate indicating chromosomal disturbances with respect to geometrical orientation indicates genotoxicity of the chemical Reactive Turquoise Blue (Procion-MX). However, further in-depth study involving various chromosomal banding techniques or involvement of molecular markers with protein or nucleic acid specific hybridization after treating with the chemicals in *A. cepa* root tip cells as well as mammalian and non-mammalian cell systems can give further evidence with firm support to prove the cytotoxicity as well as genotoxicity of dye. This may be considered as a preliminary information to create public awareness regarding commercialization, large scale utilization and management as well as disposal of similar potentially genotoxic chemical to the environment.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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