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Mitotic aberrations induced by orange red (a food additive dye) as a potential genotoxicant on root tip cells of onion (*Allium cepa* L.)

*Tripathy, S. K. and Rao, D. A.

MITS School of Biotechnology, 2(P), Infocity Square, Patia, Chandaka Industrial Estate, PO- KIIT Campus, Bhubaneswar-751024, Odisha, India

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Abstract

The dye orange red is an amalgamation of two primary food colourants like caramoisine and sunset yellow. It is used to enhance the market adaptability and commercialization of food items by increasing consumer's attraction. Assuming the potential genotoxicity of the dye, this study was carried out on the root tip cells of onion (*Allium cepa* L.) through assessment of mitotic aberrations (MA) by conventional aceto-carmine staining. Some of the common MA observed was gradual decondensation or little condensation of chromosomal arms in abnormal metaphases and anaphases. These also included unequal cytokinesis and karyokinesis and formation of bi-nucleated cells. Those aberrations were supposed to be due to genotoxic assault of the dye on chromosomal condensation mechanism affecting some structural maintenance of chromosomes (SMC) proteins like condensins and/or cohesins resulting in very unusual long arms or affecting the spindle fiber formation causing disoriented mitosis (DOM). The frequencies of MA showed good correlation with the concentration of dye.

Introduction

Selection and purchase of quality food is the essence of balanced diet. Human body has wide range of nutritional requirements varying according to age, sex and level of activity (Chauhan *et al*., 1999). A number of natural and synthetic dyes are used to enhance the market adaptability of food items by increasing consumer's attraction. They are usually water soluble and extensively used in almost every type of edible preparations like soft drinks, foodstuffs, jams, jellies, sweets, candies, ice-creams, sauces and pickles (Hallagan *et al*., 1995). A wide range of food additives, running into more than 2,500 items are used for preservation and colouring (Toledo, 1999).

Colour additives are dyes, pigments or substances when added or applied to foods and drugs which impart colour for making them palatable (Macioszek and Kononowicz, 2004). The global production of food dyes is more than 8000 tons per year, while India produces 2% of the world production (Das and Mukherjee, 2004). There are three categories of food colours i.e. primary, blended and lake pigments.

The primary colours comprise of dyes not having any evidence of hazardous affects. These are widely used in adding visual appeal to different food items and other utility products for decoration. They are of comprehensive range with wide application in several consumable industries and added to beverages, desserts, confectioneries, canned items, meat and fish products, cheese, syrups, cosmetics, pesticides, writing inks, toothpastes, tablets and capsules. They are Quinoline Yellow, Tartrazine Yellow, Sunset Yellow, Erythrosine, Ponceau 4R, Allura Red, Red 2G, Fast Red E, Caramoisine, Amaranth, Chocolate Brown HT etc *(www.google.com/primary foods)*.

The blended food colours are unique mixture or calculated blend of two or more water soluble colouring agents combined in various ways to produce different varieties of shades for any specific requirement in powder and liquid forms *(www.google.com/blendedfoods)*. These colours are used as food additives in many edible preparations like cakes, biscuits, chewing gums and ice creams etc. The lake pigments find wide application in areas like food stuffs, drugs, pharmaceuticals, cosmetics, beverages, dry mixes, baked goods, confectionaries, dairy products, pet foods and a variety of other products. (Hassan *et al*., 2006) and are commonly known as "lakes". These are dyes dissolved in water, but insoluble in oil and do not have side effects but ingested in large quantity can colour stools. These are more stable than dyes and are ideal for colouring products containing fats and oils or items lacking sufficient moisture *(www.wikipedia.org)*.

Natural and synthetic dyes used in food stuffs

*Corresponding author.
Email: *sbskt04@rediffmail.com, sbskt04@yahoo.com, sbskt04@gmail.com*
Tel/Fax: +91-674-2741584, 6537796

have become potential suspects for causing cancer (Tsuda *et al.*, 2001; Das and Mukherjee, 2004). International Agency for Research on Cancer (IARC, 1983) classified some of these dyes as possible/ probable mutagens and/or carcinogens. According to IARC (1983) mentioned by Hassan (2010), some of the food additives/colouring agents were genotoxicants causing chromosomal aberrations in mammalian cells including human cells. Caramosine and tartrazine induced chromosomal aberrations and sister chromatid changes (SCEs) in mouse and rat bone marrow cells showed a significant increase in higher concentration (Giri *et al*., 1990). Some food colours and their side effects have been investigated in animal studies (Tsuda *et al*., 2001) reporting their mutagenicity which varies widely with the dose applied. The individual response varies according to dose, age, gender, nutritional status, genetic factors and long term exposure to low doses (Sasaki *et al*., 2002). Chromosomal aberration analysis of animals as well as comet assay is used as the most useful assay to detect potential genotoxicity of chemicals (Blasczyk *et al*., 2003). There is a need to boost application of legislative control restricting the use of harmful colours in food stuffs to check certain permitted non-hazardous items (Tripathi *et al*., 2007). Rao and Sudershan (2008) attempted to assess the risk of food colours in India. Most of the colours bind directly to DNA and cause both structural and numerical anomalies (Hamdy *et al*., 2000; Mpountoukas *et al*., 2010).

A comprehensive review of the genotoxicity of food colours, drugs and cosmetic dyes was published by Coombs and Haveland-Smith (1982) and Giri (1991) and supported later on by others (Sweeney *et al.*, 1994; Durnev *et al.*, 1995; Hamdy *et al.*, 2000; Tsuda *et al*., 2001; Tema Nord, 2002; Blasczyk *et al*., 2003; Das and Mukherjee, 2004; Macioszek and Kononowicz, 2004; Hassan *et al*., 2006; Tripathi *et al*., 2007; Rao and Sudershan, 2008; Abdelmigid, 2009; Hassan, 2010; Mpountoukas *et al*., 2010; Swaroop et al., 2011; Montaser and Alkafafy, 2013).

Tests involving plants have been widely used for detecting the genotoxicity of chemical compounds and for in situ monitoring of environmental genotoxic contaminants (Grant, 1982; Plewa, 1985). Levan (1938) introduced *A. cepa* test to find out the affect of colchiciene on mitosis. Adhvaryu and Shah (1979) reported that successful operation of cell division depends on programmed organization of many macromolecule synthesizing processes. *A. cepa* has been widely accepted for evaluation of genotoxicity and the assay is well known and commonly used in many laboratories in good correlation with results from other established test systems (Fiskesjo, 1985). Cabrera *et al*. (1994) validated the use of plants for evaluation of environmental pollutants. Emphasis has been given to use of alternative experimental organisms 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).

A. cepa has relatively large monocentric chromosomes and accepted as suitable test organism for the study of environmental mutagenesis (Rank and Nielsen, 1997; Kong and Ma, 1999; Moraes and Jordao, 2001; Patra and Sharma, 2002). Kar (1992), studied the affect of textile mill effluents, Badr and Ibrahim (1987) revealed stickiness in chromosomes attributed to improper folding of chromosome fibers. Rank and Nielsen (1993) employed a modified *A. cepa* test as a tool in the screening of genotoxicity of complex mixtures and Ma *et al.* (1995) employed similar test with micronucleus assay to assess the clastogenic effect of environmental pollutants where as Abdelmigid (2009) employed *V. faba* beans as indicator to determine genotoxic effect of synthetic dyes used as food additives.

A. cepa test system is widely used to assess potential cytotoxic and genotoxic affect of water and water bodies like rivers, lakes, wells, etc contaminated with domestic and industrial wastewaters (Smaka-Kincl *et al.*, 1996; Steinkellner *et al*., 1998; Monarca *et al*., 2000, 2002 a, b and 2003). Cytotoxicity and environmental pollution (El-Shahaby *et al*., 2003) have been assessed by same test system giving similar results as observed in *in vivo* animal cytotoxicity tests (Chauhan *et al*.,1999; Vicentini *et al*., 2001 and Teixeira *et al*., 2003). Tartar *et al*. (2006) reported genotoxicity of avenoxan on two different species of *Allium* (*A. cepa* and *A. sativum*). Kumari *et al*. (2009) assessed the mutagenicity of water samples and observed most frequent chromosomal abnormalities i.e. c-metaphases, sticky chromosome, chromosome breaks and losses, bridged anaphases, multipolar anaphases and micronucleated and binucleated cells. Similarly, El-ghamery *et al*. (2000) revealed the action of Atrazine as an Indicator of cell division on chromosomes and Dane and Dalgic (2005) determined the phytotoxic and genotoxic affects of different pesticides. Thais *et al*. (2007) reported the mechanism of micronuclei formation in polyploidized cells of *A. cepa* exposed to trifluralin. Based on the availability of vast literary wealth

on dye, experimental plant model, technique and possibility of detecting the suspected genotoxicity, the experiment was designed. The purpose of this study was to evaluate the potential genotoxicity of orange red dye en route mitotic aberration (MA) assay by analyzing mitotic cell division in growing root tip cells treated at various concentrations.

Materials and Methods

Test Dye

Keeping in view of the potential environmental impact due to introduction and random use of food additive dyes, orange red was selected for this study to assay its genotoxic affect on *A. cepa* root tip cells. The dye was purchased from the local market of Bhubaneswar, Odisha, India which is manufactured by Jani Industries Ltd. 2, Krishnakunj, 291, Samuel Street, Mumbai, India. It is used mostly as a food colouring agent in sweets and bakery preparation and is used in maximum 85% of all food items. It contains some ingredients like caramoisine and sunset yellow.

Caramoisine or azorubine is a synthetic red dye of azo group. IUPAC name of caramoisine is disodium (E)-4-hydroxy-3[(4-sulfonatonaphthalen-1-4) diazenyl naphthalane-1 sulfonate] commonly used in UK but prohibited in Japan, Norway and US. The IUPAC name of sunset yellow is disodium-6-hyroxy-5-[4-sulfophenyl)azo]-2-naphthalene sulfate and chemical formula is $C_{16}H_{10}N_2Na_2O_7S_2$. In addition, to these primary colourants orange red also contains 16% NaCl. The blend is soluble in water and possesses affinity for amphoteric fibers while lacking direct dyes affinity to cellulose fibers (Durnev *et al*., 1995; Hamdy *et al*., 2000; Das and Mukherjee 2004; Mathur *et al*., 2005a; Hassan, 2010 and *www. google. com/primary food colour /blended food colour*).

Various concentrations of orange red dye (w/v) were prepared in distilled water (i.e.-0.3, 0.15, 0.03, 0.015 and 0.003 %) and applied to the growing root tips of *A. cepa* in separated glass tubes of 10-20 ml capacity for various durations i.e. - 48 h to induce mitotic aberrations *in vivo* and for 24 h and 48 h to assess mitotic index by recording the fraction of dividing cells. A single duration of exposure (48 h) was applied to induce MA based on Tripathy *et al*. (2013) reporting affect of endosulfan on *A. cepa* which showed that in case of 48 h of exposure the result were more prominent. Very few abnormalities were recorded in case of 24 h and 72 h of exposure and it was presumed that, probably the cell cycle *A. cepa* root tip cells (eukaryotic) corresponds to 24 h and cells exposed for 24 h or 72 h are either too early or too late to score the actually affected cells.

Experimental organism or plant material

A. cepa was selected as plant model to detect MA due to spindle poisoning effect as potential genotoxicity induced by orange red in root tip cells. For this purpose, 4-10 presoaked onion bulbs in distilled water of weight range 15 to 50 g with mean value of 27.56 ± 0.59 g were employed for each treatment schedule. The bulbs were purchased from local vegetable market of Damana, Bhubaneswar, Odisha, India in 6 (six) different slots of 100 to 250 g. As per information from the local bulk vendors of *A. cepa* it was confirmed that the bulbs were collected from different farmers of various harvest slots from different parts of Odisha, India ranging from 6 months to 2 years.

Methods

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) as per its recommended dose used in food items. 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. Conventional squash preparation was adopted following the acid hydrolysis of cellulosic cell wall in 1 N HCl followed by warming at 60ºC. 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 (Levan,1938; Grant, 1982; Badr, 1983; Fiskesjo 1985; Das 1986; Mohapatra *et al*., 1986; Badr and Ibrahim 1987; Kar, 1992; Rank and Nielsen, 1993 ; Rank and Nielsen, 1997; Chauhan *et al.,* 1999; El-ghamery *et al*., 2000; Patra and Sharma, 2002; El-Shahaby *et al*., 2003; Dane and Dalgic 2005; Tartar *et al*., 2006 and Tripathy *et al*., 2013).

Scoring of slides

The slides were viewed under binocular light microscope (Olympus CX 31) using the 100X objective in Cedar oil immersion. Photographs of some selected representative stages were taken by a

14.2 mega pixel SONY CYBER SHOT Digicam to find abnormal cells with respect to MA.

Data analysis

The mitotic index (MI) was calculated as (Number of dividing cells / Total number of cells scored) x 100. Similarly, the mitotic depression (M.D) was calculated as {MI (Control)-MI (Treated) / MI (Control)} x 100. In addition, the proportions of specific cell abnormalities for metaphases and anaphases were calculated in terms of % out of the total number of cells counted.

Statistical analysis

The mean value with standard deviation (SD) for each root length was calculated from individual bulbs and it was compared with the corresponding control value and student's 't' test was conducted to ascertain statistical significance for root growth as well as for total cell abnormality.

Result

The common types of genotoxic effects observed in this experiment were chromosomal bridge in anaphase and abnormal uncoiling of chromosomes during anaphase and metaphase. The MA included irregular and transverse orientation of chromosomes in spindle apparatus. In some metaphases, chromosomes were arranged diagonally. In anaphases, chromosomal bridge and orientation problem in pole were observed.

Table-1 describes the effect of orange red on mitotic indices and depression in various treatment schedules depicted by Figures-1 and 2. Number of bulbs applied for each treatment ranges from 4-10 A total of 673 to 5244 cells were scored in 4-10 slides per treatment for calculating MA and the numbers of cells observed are 4407 on an average per treatment. Maximum number of cells scored is 5244 in 0.15%. The maximum number of dividing cells (146) is from 5244 number of dividing cells with M.I. corresponding to 2.78. Maximum M.I. value of 3.26 is calculated at treatment schedule of 0.03% concentration. Similarly, the minimum number of dividing cells is in control (n=3450, M.I. = 2.63). The mitotic depression (M.D.) is positive in all cases due to high M.I. value (4.01) of control. It is maximum 34.41 in 0.015% and minimum 18.7 in 0.03%. The trend of M.I. and M.D. is not very clear but exhibits a rough trend of M.I. and M.D. with increasing concentration.

The mean root length varies from 3.9 ± 1.38 to 6.6 ±1.48 mm (Table 2 and Figure 3). At 24h treatment schedule, maximum root length is 5.9 ±1.22 mm at 0.015% and minimum at 0.3%. Similarly at 48 h, maximum root length is 6.6 ±1.48 mm at 0.015% and minimum 4.5±1.07 mm at 0.3%. Table 3 and Figure 4 show the degree of abnormality of cells whereas figure 5 presents each type of cell abnormality. In control experiment, total cell abnormality is 0.26%. In dye treated experiments for 48 h exposure, it ranges between 0.69 ± 0.007% to 1.32% ± 0.67. The "t" values for root growth (Table-2) are not significant in many cases except in treatment at 0.15 and 0.015% in both 24 and 48 h duration of exposure and at 0.003% in 48 h of exposure at 0.05 and 0.005 level of probability (P = 0.05 and 0.005).. Similar

Table 1. Affect of Orange Red on M.I. and (M.D.) in root tip cells of onion

	1	2	3	4	5	6
Treatment No	C*	T1	T2	T3	T4	T5
Conc. (mg/l)	0	0.3	0.15	0.03	0.015	0.003
Duration (h)	48	48	48	48	48	48
No. of Bulbs	10	6	4	5	5	4
No. of cells scored (N)	673	4068	5244	4407	3450	4633
No. of dividing cells (n)	26	114	146	144	91	134
Mitotic indices (M.I)	4.01	2.8	2.78	3.26	2.63	2.89
Mitotic depression (M.D)	_	30.17	30.67	18.7	34.41	27.93

*C: Control

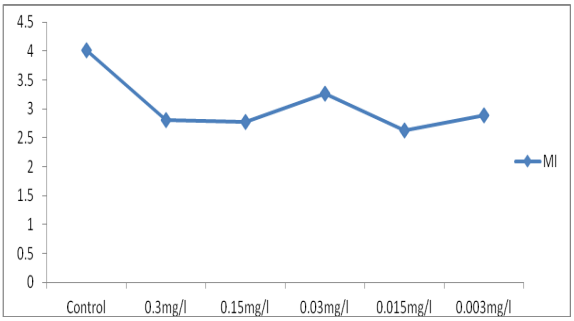


Figure 1. Effect of Orange Red on Mitotic Indices (M.I.) in onion root tip cells

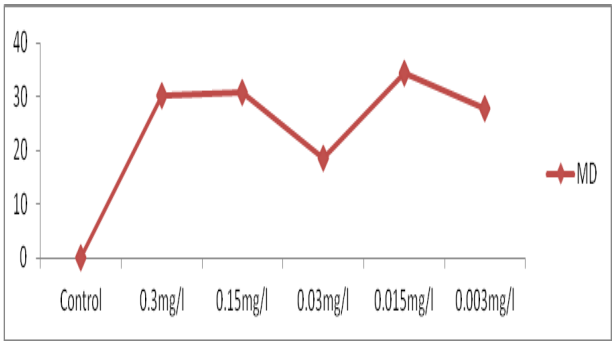


Figure 2. Mitotic depressions (M.D) due to Orange Red in onion root tip cells

Table 2. Root growth of onion bulbs in mm (Mean ± S.D)

	1	2	3	4	5	6	7	8	9	10	11	12
Treatment No	C1	C2	T1	T1	T2	T2	T3	T3	T4	T4	T5	T5
Conc. (mg/l)	0.0	0.0	0.3	0.3	0.15	0.15	0.03	0.03	0.015	0.015	0.003	0.003
Duration (h)	24	48	24	48	24	48	24	48	24	48	24	48
Number of Bulbs	10	10	6	6	4	4	5	5	5	5	4	4
Mean root length	3.7± 1.16	3.4± 0.58	3.9± 1.38	4.5± 1.07	4.2± 1.78	5.0± 2.49	4.7± 0.59	5.8± 1.1	5.9± 1.22	6.6 ± 1.48	4.7 ± 1.60	5.8± 1.29
t values (p=0.005) (p=0.05)	NA	NA	NS NS	NS NS	* *	* *	NS NS	NS NS	* *	* *	NS NS	* *

*Significant Deviations at P = 0.005 and 0.05, C1 and C2: Controls

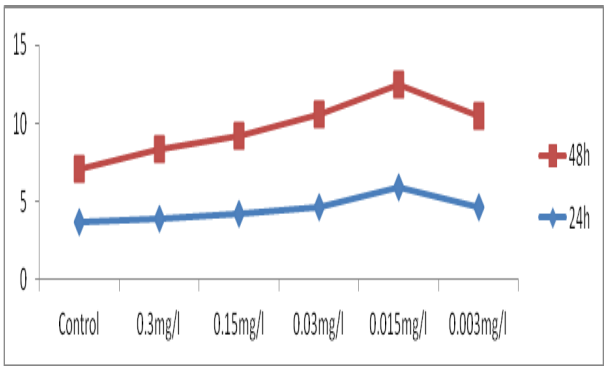


Figure 3. Mean root length (mm) due to affect of Orange Red in onion root tip cells

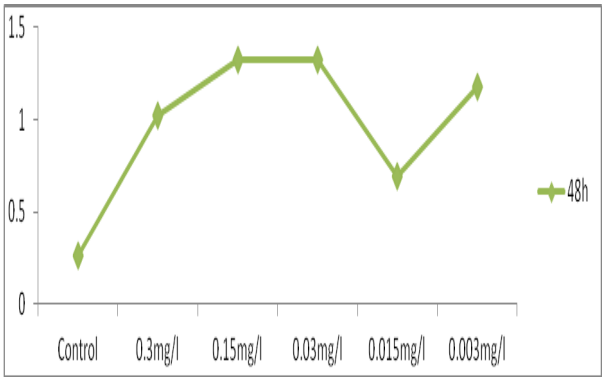


Figure 4. % of Total cell abnormality induced by Orange Red in onion root tip cells

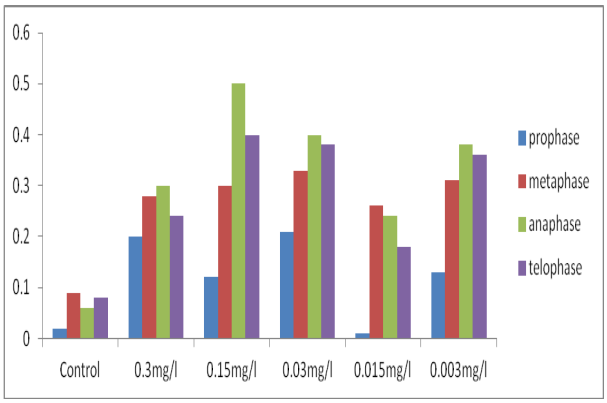


Figure 5. % of each type of MA induced by Orange in onion root tip cells

test performed for total cell abnormality shows significance of "t" value (Table-3) only in case of 0.15 and 0.03%.

Discussion

Generally, induction of structural and /or numerical changes in chromosomes is a common criterion for recognizing potential genetic hazards of a particular agent. The action of dye on *A. cepa* root tip cell can be multiparous as presumed earlier. It might have altered the process or result of cell division, or genome at chromosomal/DNA level. Nayak and Das (1986) reported that, analysis of metaphase chromosome is an important and most widely used method to assess mutagenic potential of a given agent.

Yahagi *et al*. (1988) found several azo dyes and their derivatives to be mutagenic or carcinogenic and suggested that, these effects may involve modifications of DNA. According to Vieira and Vicentini (1997) the effects of mutagens on eukaryotic nuclei can be assessed cytologically by studying inhibition of cell growth or division, interruption of metaphase or the induction of chromosomal aberrations. This study involves observation of macroscopic parameter like root growth and microscopic parameters like MI and total cell abnormality representing MA.

Genotoxicity of food additives depends on their conversion to reactive metabolites and the activation is accomplished by acetyl transferases, widely distributed in animals (King *et al*., 1997). As the dye is a blending of two primary colourants like caramoisine and sunset yellow, affect on root tip cells of *A. cepa* cannot be attributed to any one of them but may be due to combined action of both. Ali *et al*. (2001) reported caramosine induced CA on the bone marrow cells. Tema Nord (2002) reported no evidence for genotoxicity in some *in vitro* and *in vivo* studies for carmoisine. Zaharia and Pavel (2003) reported affect of carmoisine on the frequency of divisional cells, mitotic index, mutagenic process and

Table 3. % of celll abnormality induced in mitotic cells by orange red

	1	2	3	4	5	6
Treatment No	C*	T1	T2	T3	T4	T5
Conc. (mg/l)	0	0.3	0.15	0.03	0.015	0.003
Duration (h)	48	48	48	48	48	48
Abnormal Cells (%) ± SD (t value) Significance (P=0.05)	0.26	1.02 ± 0.045 (0.025) NS	1.32 ± 0.67 (2.68) *	1.32 ± 0.64 (2.68) *	0.69 ± 0.007 (1. 35) NS	1.18 ± 0.5 (1.46) NS
% of Each Abnormality						
A.P	0.02	0.2	0.12	0.21	0.01	0.13
A.M	0.09	0.28	0.3	0.33	0.26	0.31
A.A	0.06	0.3	0.5	0.4	0.24	0.38
A.T	0.08	0.24	0.4	0.38	0.18	0.36

Anaphase, AT: Abnormal Telophase, NS: Not significant at P = 0.05 level of probability, * = significantly different t value from that of control.

the potential to induce chromosomal modifications by cytogenetic analysis and informed alterations in the morphology of somatic chromosomes of Secale cereale (Rye) but no concern with respect to genotoxicity. Mc Cann *et al*. (2007) concluded that exposure to mixture of carmoisine/azorubine resulted in increased hyperactivity in 3-years and 8- to 9-years old children. EFSA (2009) reported that, Azorubine/ Carmoisine had been previously evaluated by Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1983 and the EU Scientific Committee for Food (SCF) in 1984. Amin *et al*. (2010) reported that Tartrazine and Carmoisine in low and high doses adversely alter biochemical markers in vital organs. Montaser and Alkafafy (2013) concluded that, high doses of carmoisine could be harmful to liver which lowers the expression level of some metabolic enzymes. Sunset Yellow is allowed as a food additive in the EU and previously evaluated by the Joint FAO/ WHO Expert Committee on Food Additives (JECFA) in 1982 and the EU Scientific Committee for Food (SCF) in 1984. Sunset Yellow FCF was reported negative in in vitro genotoxicity as well as in long-term carcinogenicity studies. No mutagenic effects were noted in an in vivo bone marrow micronucleus tests in mice and rats after a single oral exposure to 2000 mg/kg body weight (Westmoreland and Gatehouse, 1991). Sweeney *et al*. (1994) indicated that direct-acting oxidative genotoxicity may be induced by reaction products of azo dyes including sunset yellow. It did not display any increase in the number of cells with chromosomal damages (Durnev *et al*., 1995). Mathur *et al*. (2005 a) reported in rats that sunset yellow significantly affects testes and Mathur et al. (2005 b) reported significant and dose-related elevations in total lipid and various lipid fractions. Poul *et al*. (2009) demonstrated a lack of genotoxicity of Sunset Yellow FCF in the gut micronucleus assay in mice after administration by oral gavage 20, 200 or 1000 mg/kg body weight twice at 24 h intervals

Swaroop *et al*. (2011) reported for genotoxicity of eight permitted food colorants including sunset yellow and caramoisine by in vivo studies in human involving cytokinesis block micronucleus (CBMN) assay and informed that, their combinations could cause genotoxicity to human lymphocytes even at the permissible concentration of 100 ppm as per PFA (Prevention of Food Adulteration) Act of India. With this light, the present study evaluates potential genotoxicity of orange red which is comparable to similar other studies done earlier by different workers employing other food additive dyes.

Presence of chromosomal bridges observed at few anaphases might be the result from stickiness as per Badr (1983) causing clastogenicity. Decrease or no significant increase of M.I. at various concentration of dye indicates that orange red dye has mito-depressive effect resulting in inhibition of cell access to mitosis. Significant 't' values for root growth at 0.15% and 0.015% and those for total cell abnormality at 0.15 and 0.03% indicated genotoxicity of dye affecting cell division. It was also confirmed that orange red causes spindle poisoning in onion root tip cells disturbing the normal cell cycle which is in concurrence with Swaroop *et al*. (2011) stating that, the food colourants induce MA in varying concentrations below and above the permissible limit. The chromatin in interphase nuclei condenses nearly thousand fold during the formation of metaphase chromosome. 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

Plate 1. Under control experiments

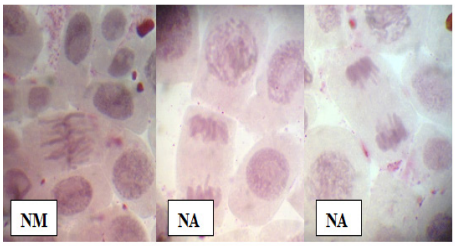


Plate 2. Under treatment with orange red @ 0.3 %

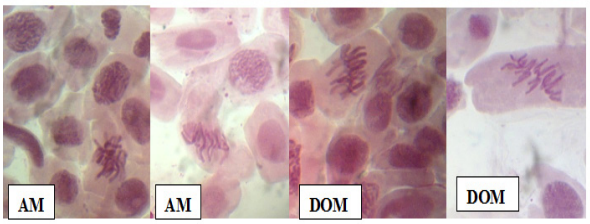


Plate 3. Under treatment with orange red @ 0.03%

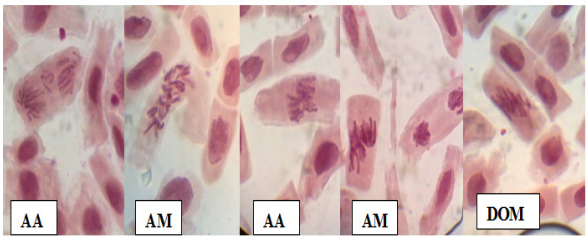


Plate 4. Under treatment with orange red @ 0.003%

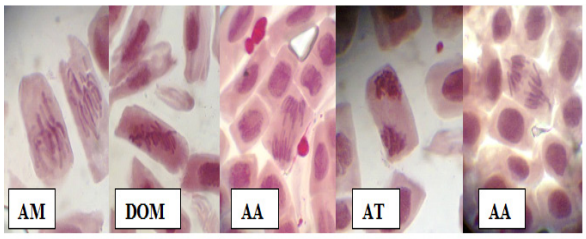


Plate 5. Under treatment with orange red @ 0.15%

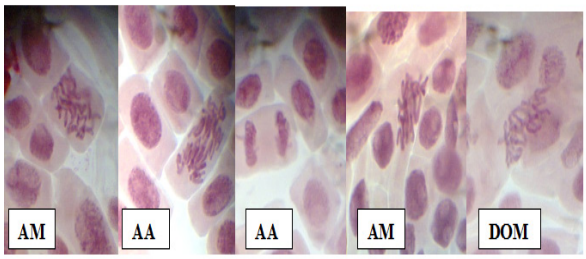
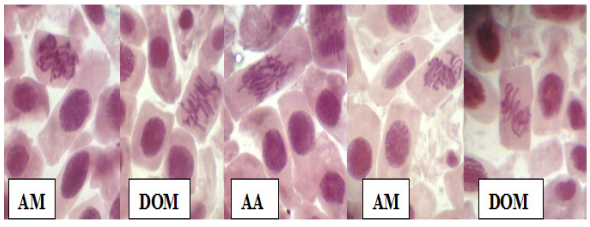


Plate 6. Under treatment with orange red @ 0.015%



with one another.

To describe the chromosomal condensation mechanism during cell cycle, Cooper and Haussman (2007) wrote that, mitosis involves dramatic changes in multiple cellular components, leading to a major reorganization of the entire structure of the cell and 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. In the present study, orange red dye affected cell plate formation, which was found to be under great disturbances and the orientation as well as functioning of the spindle. In several late anaphases, one group of chromosomes was found to take the extreme terminal position while the other was in the middle. Some adjacent cells were also of unequal sizes, some diagonal and transverse metaphases and anaphases were also observed. These indicated abnormal orientation of mitotic apparatus or distorted mitotic spindles causing dis-oriented mitosis (DOM).

Occurrence of disoriented chromosomes might be due to action of orange red on the microtubules (MT) of the spindle fibers. The dye could have cause 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 proposed earlier similarly by Jain and Sarbhoy (1988). However, these observations may be considered as preliminary and it may be further validated by analyzing the effect of orange in other plants and animal models including human being and employing various chromosomal banding techniques and molecular level analysis. This finding may serve as first hand information for workers engaged in related line and to create public awareness among consumers regarding long term consequence of orange red dye on human genome.

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