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Cytotoxic Effects of Permitted Food Dyes on Root Tip Cells of *Allium cepa* L.

Resmi L.

Christian College, Kattakada, Thiruvananthapuram, Kerala, India
Present Address: Department of Botany, University of Calicut, Malappuram, Kerala

Abstract: ***Food additives are substances that are intentionally added to modify the visual appearance, taste, texture, processing or storage life of food. There has been significant controversy associated with the risks and benefits of food additives. The cytotoxic effect of different concentrations of food dyes viz. tartrazine, carmoisine and sunset yellow on the chromosomes of Allium cepaL. was investigated in the present study. Allium ceparoottip cells were treated with each dye at threedifferent concentrations (1%, 2% and 5%) for a period of 2 hours. All concentrations of tartrazine, carmoisine and sunset yellow showed a mitotic inhibitory effect in root tips of Allium cepaand an increase in chromosomal aberrations. Various types of metaphasic and anaphasic aberrations were scored and it was found that metaphasic aberrations were more prominent than anaphasic aberrations. The most observed aberrations induced by the dyes were stickiness at metaphase, tropokinesis, laggards and bridges at anaphase, and stickiness at anaphase. Strap-shaped or elongated nuclei were also observed as a deleterious effect of food dyes. Results of the present study clearly establish the cytotoxic behaviour of tartrazine, carmoisine and sunset yellow.***

Keywords: *Allium cepa L., Carmoisine, Sunset yellow, Tartrazine*

1. Introduction

One of the world's fastest-growing economic sectors, the food industry fosters fierce competition among manufacturers as they work to satisfy growing customer expectations. They, therefore, make an effort to create foods that are appealing from a hygienic, nutritional, and sensory perspective. However, in order to compete, they utilize more food additives, which are defined as any component that is purposefully added to foods in order to alter their physical, chemical, biological, or sensory properties, without any nutritional purpose.Since the 19th century, the amount of processed food has increased, which has increased the use of food additives. There are several food additives in use that may have varying degrees of negative effects on consumers [1]. Colourants, preservatives, emulsifiers, and taste modifiers are all examples of food additives.Colouring dyes are widely used among all food additives.

Dye is a group of food additives that have no nutritional value and are added to food to add colour, which makes the product more appealing and increases market acceptance. Food dyes are commonly utilized in a variety of foods and are typically hydrophilic in nature. Many of these dyes, often known as coal-tar dyes because they include the azo group, were derived from coal tar. The azo group (-N=N-) dyes, such as tartrazine yellow, sunset yellow, and Bordeaux red, are some of the most widely used colours in the food business. The azo group dyes consist of a naphthalene ring joined by an azo bond (N=N) to a second benzene ring. These rings may have a single, double, or triple sulfonic group. These dyes are often used in yoghurt, jellies, ice cream, fillings, liquors, powdered juices, cereals, candies, and dairy items.

Azo compounds are formed from arenediazonium ions conjugated through an azo linkage to highly reactive aromatic hydrocarbon compounds containing two aromatic rings, which are responsible for their intense colours [2]. Tartrazine and carmoisine are nitrous derivatives of azo compounds that can be metabolized to highly sensitizing aromatic amines, such as sulphanilic acid [3, 4, 5].Generally speaking, this class of dyes is debatable in terms of its hazardous activity and piques the curiosity of toxicologists and allergists because it has been proposed that it may be to blame for triggering different immunological reactions that range from hives to asthma. According to estimates, one in 10,000 people have negative responses to azo dyes. Because of this, they are prohibited in many nations, including Japan and England. Due to their comparable colours and frequent use combined in food products, tartrazine and sunset yellow are the most popular dyes in this group. Despite their beneficial industrial applications, these substances have a risk toxicity profile that includes negative side effects as allergy, asthma, carcinogenicity, genotoxicity, cytotoxicity, anxiety, etc. In order to give consumers safe food products and prevent both short- and long-term negative effects, the European Food Safety Authority (EFSA) and the US Food and Drug Administration (FDA) restrict the licenses for utilizing these substances. The use of colours in food products still raises questions about their toxicity despite the regulation demanded by these regulatory organizations. This is because it is very challenging to determine whether the amount of additives used by the food sector complies with the rules as they currently stand [6].

Numerous azo dye groups have been discovered to be carcinogenic and to cause genetic abnormalities in people. In experiments, it was discovered that administering certain coloured pigments to rats caused them to develop a variety of metabolic abnormalities. Many azo compounds were discovered to be carcinogenic in animals in various short-term genotoxicity testing. Some of the colouring pigments can also result in other chromosomal abnormalities.Genotoxicity research aims to identify substances that can disturb genetic material and result in chromosomal or gene alterations. To determine the genotoxic potential of various chemicals, numerous test techniques, particularly *in vitro* systems, have been

developed. Results of tests for genotoxicity are frequently interpreted as signs of mutagenic effects. Animal and plant assays will react to chemicals differently, but this will be because their metabolisms are different. The results of plant bioassays can, to some extent, identify possible risks to human health.

Among plant test systems, *Allium cepa*is one of the most commonly used species for the study of chromosomal aberrations. *A.cepa* test was introduced by Levan (1938)[7] to study cytogenetic short-term bioassay and has proven to be a useful tool in basic research to detect the chromosomal aberrations caused by chemicals and complex mixtures such as industrial wastewater [8].*A.cepa*test represents an alternative first-tier assay to experiments on animals for preliminary toxicity screening [9].*A. cepa* has large and low chromosome number, high proliferative rate of meristematic cells, inexpensive assay and do not require an elaborate laboratory. facilities. Plants constitute an important material for testing genetic alterations brought about by environmental chemicals. The *Allium cepa*has been used as an efficient model organism in genetic tests for chromosome aberration assays. The genotoxicity of many food dyes has been evaluated using *A. cepa* as an indicator. Plant bioassays are quite sensitive and simple in comparison to animal bioassays to assess the genotoxicity and cytotoxicity of a chemical compound.

Therefore, due to the wide industrial use of food dyes in the colouration of foods, there is a need for additional studies and the deep involvement of researchers in the evaluation of the effect of these dyes on a cellular level[10]. Considering the *Allium cepa*system as an appropriate bioassay for evaluation of cytotoxicity of chemical compounds, the objective of this study was to analyze the cytotoxic effect of three food dyes tartrazine yellow, carmoisine, and sunset yellow on *Allium cepa*L. root meristematic cells.

2. Materials and Methods

For studying the cytological effects of food colours, permitted food dyes; Tartrazine (CI. 19140), carmoisine (CI.14720) and sunset yellow were used. The common onion (*Allium cepa*, 2n=16) was used as the test system which is an important bulb crop vegetable enjoying worldwide distribution.

Food dyes
The food dyes of the present investigation were obtained as food colours with common name 'Lemon Yellow' and 'Orange Red'. They were bought from a supermarket at Kattakada, Thiruvananthapuram. These colours are manufactured byManju chemicals, madras, having the brand name "Tiger brand". The weight of each bottle was 10 grams and all were having the ISI mark which is a guarantee of good quality prescribed by Indian Standards Institution. The Lemon Yellow and Orange Red are synthetic water-soluble food colours made from chemicals obtained from coal tar. These are widely used in soft drinks, food items such as bakery products and sweets.

Lemon yellow
Lemon yellow is an orange-yellow powder soluble in water. Its ingredients are Tartrazine (C.I. 19140) and NaCl. Tartrazine is a bright orange-yellow coloured,water-soluble azo-dye which is used as food, drug and cosmetic dye.

Orange red
It is a dark red powder which is also water-soluble, giving a dark red solution. It contains two azo-dyes, Carmoisine (C. I. 14720) and Sunset yellow FCP (C. I. 15985) and NaCl.

Chromosomal structural changes andmitotic index
Healthy bulbs of common onion *Allium cepa*(2n=16) were placed in pots containing sandy soil. Pots were placed in sunlight and watered thoroughly. When the roots were about 1-2cm in length, the rooted bulbs were used for the treatment. The first series of experiments were conducted with the food colour lemon yellow (Tartrazine).The cytological effect of the dyes on the root tip cells of *Allium cepa*was studied with three concentrations of 1% (1g in 100ml distilled water), 2% and 5%for 2 hours and the solutions were taken in clean vials of about 10ml each. The rooted bulbs were thoroughly washed and were placed above the mouth of vials for 2 hours. After 2 hours, the roots were washed properly and dried and fixed in freshly prepared Carnoy's fluid. As each time Carnoy's fluid (3:1, alcohol-acetic acid mixture) was prepared freshly and root tips were fixed. After 24 hours, the root tips were washed and hydrolysed with 1N HCl at 60^0C for 5-6 seconds and dried. Afterwards, they were squashed in 2% acetocarmine and two slides were studied in detail and tabulated. Photomicrographs of selected chromosomal aberrations were taken from temporary preparations and the experiment was repeated with the food colour Orange-red. The effect of food dye on the mitotic index was calculated by;

Mitotic Index = Number of dividing cells / Total Number of cells x 100

3. Results and Discussion

In the present investigation, cytological effects of the three permitted food dyes Tartrazine, Carmoisine and Sunset yellow FCF on plant cells were studied, taking *Allium cepa*root cells as the test system. *A. cepa* is considered an ideal model for determining genotoxicity due to high sensitivity of plant cells to environmental stress [11].Cytological analysis of treated cells revealed that the tested food dyes are capable of producing a wide range of abnormalities, and also, they are having a mitodepressive effect on dividing cells in *A. cepa*. The structural and numeric changes in chromosomes have become an important trend for evaluating the genotoxicity of food chemicals, pollutants, heavy metals, water samples and pesticides. Generally,a change in the structure of the chromosome was considered as a good marker of genomic damage. It was reported that nearly all azo chemical colours and their oxidative end products have carcinogenic or mutagenic potential and can cause modification of DNA [11].

The effect of the dyes was studied at three different concentrations (1%, 2% and 5%) for 2 hours duration. It was observed that above 5% concentration the dyes were highly

toxic to the *A. cepa*cells. At 10% concentration, no cell division could be encountered and noticed a complete inhibition of cell division.

Effect of tartrazine, carmoisine and sunset yellow on the mitotic index of root tip cells

In *A. cepa*, the somatic complement consists of 16 chromosomes (2n=16). The mitotic index was recorded to be 19.02% in the control set with no chromosomal anomalies. Mitotic index significantly decreased with increasing concentrations of tartrazine, carmoisine and sunset yellow. Mitotic indices at different doses of tartrazine, carmoisine & sunset yellow have been shown in Table 1. At the lowest concentration (1%) of tartrazine, carmoisine and sunset yellow, the mitotic indices were reduced to 12.34 and 13.04, respectively (Table 1). However, further increases in the concentration of these food additives resulted in a decline in the mitotic index in a dose-dependent manner. At a 5% concentration of tartrazine, carmoisine and sunset yellow, the mitotic index was greatly reduced and found to be 2.26 and 3.14, respectively. The present study clearly showed that tartrazine was more mitoinhibitory in comparison to carmoisine and sunset yellow. Present results are in positive agreement with researchfindings in which genotoxicity of food azo dye sunset yellow was evaluated using root meristematic cells of *Brassica campestris*in which a highly significant reduction in mitotic index and increase in chromosomal aberrations was reported [12]. A similar kind of result was also observed when genotoxicity of some common food preservatives (butylated hydroxytoluene, butylated hydroxyanisole, sorbic acid, propyl gallate and sodium nitrate) was evaluated using *A. cepa* as test model [13].

Table 1: Effect of tartrazine, carmoisine and sunset yellow on the mitotic index in the root tip cells of *A. cepa*

Treatments	Concentration (%)	Mitotic Index
Control	-	19.02
Lemon yellow (Tartrazine)	1	12.34
	2	7.14
	5	2.26
Orange red (Carmoisine & Sunset yellow)	1	13.04
	2	6.01
	5	3.16

Effect of tartrazine, carmoisine and sunset yellow on chromosomal aberrations

The result of present investigation clearly suggests the chromotoxicbehaviour of tartrazine, carmoisine& sunset yellow. Tartrazine, carmoisine and sunset yellow have shown a dose-dependent increase in chromosomal aberrations with increasing doses of treatment. The treated root tips showed various types of metaphasic (Table2) and anaphasic (Table3) aberrations at each dose of treatment. One common observation studied in both cases was that metaphasic aberrations were more pronounced than anaphasic aberrations. An increase in the concentration of these two food additives significantly increased the mitotic inhibition and ensures the harmful effect of tartrazine, carmoisine and sunset yellow on the mitotic cell cycle. One of the most frequent cytological abnormalities observed in treated cells was the presence of distinct nuclear lesions in the interphase nuclei (Table4). This was observed in the majority of the treatments. Both single and multiple lesions were noticed.The highest metaphasic abnormality percentage was observed at 5% of tartrazine (12.34%) followed by 5% of carmoisine & sunset yellow (9.27%). The most prevalent aberration noticed was stickiness at metaphase. Anaphase showed lagging chromosomes, tropokinesis and bridges in the treatments in a dose-dependent manner. The highest percentage (46%) of mitotic abnormality was recorded in 5% concentration for all the dyes (Table.5). Khan *et al*., (2020) also observed different kinds of aberrations in meristematic cells of *A. cepa* after treatment with food dyes, metanil yellow and carmoisine, including disorientation at metaphase, metaphase stickiness, anaphase stickiness, anaphase bridge, c-mitosis and chromosome breaks. The stickiness of chromosomes may be due to inhibition of some specific proteins involved in the maintenance of chromosome condensation and segregation. This chromosome stickiness may arise also by some defective metabolic pathways in nucleic acids. The presence of chromosome stickiness relates to highly toxic effects associated with a particular chemical. The anaphase bridge which was also seen as a frequent type of aberration may be likely due to breakage of chromosomes. The disorientation of chromosomes at metaphase, which was seen during the treatment of both azo dyes may be due to the effect of these chemicals on the spindle fibres of microtubules and cause the misalignment of chromosomes at the equatorial plate [11].

The results related to tartrazine yellow dye obtained in the present study indicate that it has anti-proliferative activity action and the potential to cause cellular aberrations, which confirms the results obtained by other researchers in other system tests indicating that this food additive has cytotoxic activity [11].The results obtained in the present research reinforce the importance of the *Allium cepa*system test since, as verified in this study; it presents results similar to those obtained with other bioassays. Even though a plant's metabolism is different, the results of this system-test are excellent cytotoxic analysis parameters, and the observation of chromosomal changes in this vegetable species' cellular cycle has been used as a warning sign to inform people not to consume certain foods and medicines, both synthetic and natural[6].

Table 2: Effect of tartrazine, carmoisine and sunset yellow on metaphase in the root tip cells of *A. cepa*

Treatments	Concentration (%)	Metaphase abnormalities (%) Clumping and stickiness
Control	-	-
Lemon Yellow (Tartrazine)	1	6.02
	2	9.21
	5	12.3
Orange red (Carmoisine & Sunset yellow)	1	4.61
	2	7.21
	5	9.27

Table 3: Effect of tartrazine, carmoisine and sunset yellow on anaphase in the root tip cells of *A.cepa*

Treatments	Concentration (%)	Anaphase abnormalities		
		Tropokinesis	Lagging Chromosome	Multiple Bridges
Control	-	-	-	-
Lemon Yellow (Tartrazine)	1	2.09	0.98	0.32
	2	2.98	1.21	1.88
	5	3.01	1.97	2.71
Orange red (Carmoisine & Sunset yellow)	1	1.91	0.32	0.51
	2	2.01	1.31	1.07
	5	2.87	1.69	1.54

Table 4: Effect of tartrazine, carmoisine and sunset yellow on interphase nuclei in the root tip cells of *A. cepa*

Treatments	Concentration (%)	Interphase abnormalities (%)		
		Single lesions	Multiple lesions	Elongated nuclei
Control	-	-	-	-
Lemonyellow (Tartrazine)	1	5.81	3.01	1.21
	2	12.62	7.92	2.01
	5	18.01	15.61	3.98
Orange red (Carmoisine & Sunset yellow)	1	4.61	2.09	-
	2	15.63	6.25	1.87
	5	19.27	14.26	2.67

Table 5: Effect of tartrazine, carmoisine and sunset yellow on mitosis in the root tip cells of *A.cepa*

Treatments	Concentration (%)	Gross abnormality		
		Total no. of cells observed	No. of aberrant cells	Aberrations (%)
Control	-	323	-	-
Lemon yellow (Tartrazine)	1	182	58	31
	2	301	119	39
	5	215	99	46
Orange red (Carmoisine & Sunset yellow)	1	338	86	25
	2	334	108	32
	5	270	126	46

4. Conclusions

The results obtained in the present study indicated cytotoxic activity of the dyes tartrazine, carmoisine and sunset yellow on root meristematic cells of *Allium cepa*. These findings are in agreement with others reported in the scientific literature indicating that the use of these three food additives by the population requires greater scrutiny. Therefore, further studies are necessary with varying doses, exposure times, and system- tests to accurately evaluate the potential risks of the mutagenic agents present in the composition of these food additives.

References

[1] U.S. Food and Drug Administration. (1993). *Everything Added to Food in the United States.* Boca Raton, FL: C.K: Smoley (c/o CRC Press, Inc.).

[2] Solomon T. W. G.,"Organic Chemistry", 6th ed, John Wiley, New York, 1996.

[3] Maekawa A., Matsuoka C., Onodera H., Tanigawa H., Furtura K., Kanno J., Jang J.J., Hayashi Y., Ogiu T., "Lack of carcinogenicity of tartrazine (FD & C Yellow No.5) in the F344 rat", Food and Chemical Toxicology, vol. **25**, pp. 891-896, 1987. 10.1016/0278-6915(87)90281-x

[4] Feng J., Cerniglia C. E., Chen H., "Toxicological significance of azo dye metabolism by human intestinal microbiota", Frontiers in Bioscience (Elite Edition), vol.1, no.4, pp. 568-86, 2012. doi: 10.2741/400.

[5] Amin K.A., Abdel Hameid H., AbdElsttar A.H., "Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats", Food and Chemical Toxicology, vol. 48, pp. 2994-2999, 2010. 10.1016/j.fct.2010.07.039

[6] Gomes K.M.S., de Oliveira M. V. G. A., de Sousa F. R., Carvalho, Menezes C. C.,Peron A. P., 'Citotoxicity of food dyes Sunset Yellow (E-110), Bordeaux Red (E-123), and Tatrazine Yellow (E-102) on *Allium cepa* L. root meristematic cells", Food Sci. Technol, Campinas, vol. 33, no. 1, pp. 218-223, 2013.

[7] Levan A., "The effect of colchicine on root mitosis in *Allium"*, Hereditas, vol. 24, pp. 471-486, 1938.

[8] Odeigah P. G. C., Nurudeen O., Amund O., "Genotoxicity of oil field waste water in Nigeria", Hereditas, vol. 126, pp. 161-167, 1997. https://doi.org/10.1111/j.1601-5223.1997.00161.x

[9] Abdelmigid H.M., "Risk Assessment of Food Coloring Agents on DNA Damage Using RAPD Markers", The Open Biotechnology Journal, vol. 3, pp. 96-102, 2009.

[10] Nunes E.A., de Lemos C. T., Gavronski L., Moreira T. N., Oliveira N. C., da Silva J., "Genotoxic assessment on river water using different biological systems", Chemosphere, vol. 84, no.1, pp. 47-53, 2011. http://dx.doi.org/10.1016/j.chemosphere.2011.02.085

[11] Khan I. S., Ali M. N., Hamid R., Ganie S. A., "Genotoxic effect of two commonly used food dyes metanil yellow and carmoisine using *Allium cepa* L. as indicator", Toxicology Reports, vol. 7, pp.37-375, 2020.

[12] Dwivedi K., Kumar G., "Genetic damage induced by a food coloring dye (sunset yellow) on meristematic cells of *Brassica campestris* L", J. Environ. Public Health 2015, pp. 1-5, 2015. https://doi.org/10.1155/2015/319727.

[13] Pandey H., Kumar V., Roy B.K., "Assessment of genotoxicity of some common food preservatives using *Allium cepa* L. as a test plant", Toxicology Reports, vol.1, pp. 300–308, 2014. https://doi.org/10.1016/j.toxrep.2014.06.002