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CYTOTOXICITY OF SOME COMMON FOOD COLOURS ON ROOT MERISTEM OF *ALLIUM CEPA* (L.) AND *HIPPEASTRUM REGINAE* (L.) HERBERT

MANEESHA ABRAHAM, DRISHYA K. REGHUVARAN, VARGHESE M. C., MUKESH SHARMA[1] and VIMAL MOHAN[1]*

Post Graduate Department of Botany, Devamatha College, Kuravilangad, Kottayam, Kerala, India.
[1]Microbiology Laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India.
*E-mail: vimal.mohan@yahoo.com

The synthetic food colours like Brilliant Blue FCF and Carmoisine selected for the present investigation are those widely used in Indian food and pharmaceutical industry. Studies were conducted with different concentrations of the above food colours on root meristems of *Allium cepa* and *Hippeastrum reginae*. Healthy plantlets with young root tips were dipped in the food colour solutions and were studied for mitotic, chromosomal and other nuclear abnormalities after 2, 4 and 6 hours of treatment. The results showed that the food colors are capable of causing chromosomal as well as spindle aberration and other cellular nuclear abnormalities indicating its genotoxicity.

Keywords: *Allium cepa;* Genotoxicity; *Hippeastrum reginae;* Synthetic food colours.

Introduction

In the present world, there is a desperate attempt by man to achieve progress in every field and thereby make life more comfortable. For getting maximum comfort, man devices many methods, which directly or indirectly affect his life adversely. Today man is exposed to various physical and chemical agents. The food, water, air are contaminated with hazardous chemical and physical pollutants.

Modern food preparation practices force man to use synthetic food flavours and preservatives for enhancing flavours, aroma etc. Food additives play an important role in today's complex food supply. These chemicals sometimes do not show any immediate effect on human body parts but may affect germ cells, thus transmitting genetic damage to next generation. Effect of chemical, pesticides, beverages, synthetic food colours and aromatic amines have been reported by many workers[1-2].

Food additive cause adverse reactions although careful investigations show that this is often based on misconception rather than on identifiable adverse reactions. Reaction to tartrazine and carmine have been reported occasionally in sensitive individual. Symptoms include skin rashes, nasal congestion and liver, although the incidence is very low and very rare. Mutagenic effect of Fast Green FCF and Indigo Carmine in respect to *Allium cepa* chromosome was reported by Roychoudhary and Giri[3]. The cytotoxic effect of food flavours and other such substances due to their increased use has developed interest in many workers. These were studied in detail by Reghuvanshi and Massey[4], Singh *et al.*[5] Cytological effects of plant extracts and fungicides were studied in detail by many workers[6-10]. Scientists at the Department of Pediatric Neurology at Yale University found that exposure to a mixture of artificial colours can result in hypersensitivity in young rat pups under certain condition. Brilliant Blue FCF and Carmoisine are extensively used colouring substances in soft drinks, non-alcoholic beverages, sugar confectionary pharmaceuticals, icings, pet food and frozen confectionary. These food colours do not show any immediate effect on human body parts but may effect germ cell thus transmitting genetic damage to next generation. In the present investigation, it seems very interesting to study the cytotoxic effects of Brilliant Blue FCF and Carmoisine on *Allium cepa* and *Hippeastrum reginae* Herb. root meristems.

Materials and Methods

Allium cepa and *Hippeastrum reginae* were collected and grown in sterile sand, were used for the study. The roots of the bulbils, washed in sterile water, were immersed in different concentrations of test colours and pesticides in clean cavity blocks in triplicates. Five different concentrations of the test materials (5, 10, 15, 20 and 25%) prepared in sterile distilled water were used for the treatment. A control was run in sterile distilled water along

Table 1. Effect of Brilliant blue FCF on *Allium cepa* root meristems.

Concentration	Time	Total Cell Studied	Total dividing cell	Mitotic Index	Interphase	Lesion	Nucleus pushed	Strap shaped	Prophase	Abnormality	Metaphase	Clumping	Polyploid	Disorienatation	Anaphase	Bridge	Telophase	Bridge	Binucleate	Abnormalcell	% of abnormality
	2	420	111	26.42%	309	0	0	3	44	0	29	0	0	0	27	0	11	0	0	0	0
Control	4	418	85	20.33%	333	0	0	1	35	0	23	0	0	0	21	0	6	0	0	0	0
	6	417	67	16.06%	350	0	0	3	23	0	16	0	0	0	13	0	5	0	0	0	0
	2	422	108	25.59%	314	18	0	4	62	8	27	7	0	0	15	3	4	0	0	40	9.47%
5%	4	418	83	19.85%	335	22	0	7	46	9	24	4	0	1	10	0	3	0	1	44	10.53%
	6	406	62	15.27%	344	28	0	4	33	11	22	5	1	4	7	3	0	0	0	57	14.04%
	2	412	94	22.81%	318	24	0	2	52	10	28	4	0	4	12	2	2	0	0	46	11.17%
10%	4	408	78	19.11%	330	32	0	1	47	12	23	3	0	8	8	1	0	0	0	57	13.97%
	6	402	61	15.17%	341	42	0	2	40	13	14	2	0	2	7	3	0	0	0	64	15.92
	2	418	84	20.09%	334	35	0	0	46	15	22	3	0	2	14	6	2	0	0	61	14.60%
15%	4	413	76	18.40%	337	39	0	3	41	12	23	4	0	7	12	3	0	0	0	68	16.46%
	6	406	58	14.28%	348	48	0	4	35	10	16	8	0	3	7	3	0	0	0	76	18.71%
	2	417	78	18.70%	339	37	0	2	47	13	23	4	3	3	8	4	0	0	0	66	15.83%
20%	4	415	63	15.18%	352	52	0	2	37	8	22	4	2	4	4	2	0	0	0	74	17.83%
	6	409	54	13.20%	355	58	0	8	38	15	14	4	1	5	2	1	0	0	0	90	22.00%
	2	404	48	11.88%	356	42	0	3	20	10	21	3	2	4	7	4	0	0	0	68	16.83%
25%	4	410	45	10.97%	365	49	0	4	24	11	17	4	1	5	4	2	0	0	0	75	18.30%
	6	412	38	9.22%	374	64	0	5	26	12	12	5	1	2	0	0	0	0	0	92	22.33%

Table 2. Effect of Brilliant blue FCF on *Hippeastrum reginae* root meristems.

Concentration	Time	Total Cell Studied	Total dividing cell	Mitotic Index	Interphase	Lesion	Nucleus pushed	Strap shaped	Prophase	Abnormality	Metaphase	Clumping	Polyploid	Disorienatation	Anaphase	Bridge	Telophase	Bridge	Binucleate	Abnormal cell	% of abnormality
	2	512	104	20.31%	408	0	0	0	70	0	28	0	0	0	5	0	3	0	0	0	0
Control	4	514	105	20.42%	409	0	0	0	74	0	24	0	0	0	5	0	2	0	0	0	0
	6	508	100	19.68%	408	0	0	0	69	0	27	0	0	0	3	0	1	0	0	0	0
	2	517	102	19.72%	405	28	1	0	57	8	27	2	0	0	15	0	5	1	0	40	6.79
5%	4	506	98	19.36%	408	33	0	2	64	12	24	3	0	0	12	0	5	2	0	52	12.74%
	6	502	94	18.72%	408	34	0	5	70	13	22	6	0	0	7	2	3	1	0	61	12.15%
	2	505	100	19.80%	405	27	0	8	65	9	18	6	0	0	13	2	6	0	0	52	10.29%
10%	4	506	96	18.97%	410	34	0	7	71	14	14	5	0	0	8	3	3	0	0	63	12.83%
	6	500	94	18.805	406	46	0	3	67	18	18	4	1	6	6	0	1	0	0	72	14.40%
	2	505	94	18.61%	411	44	2	4	69	11	16	2	0	2	7	2	2	1	0	68	13.46%
15%	4	504	95	18.84%	409	48	3	3	75	14	13	2	0	2	5	0	2	0	0	72	14.28%
	6	515	95	18.44%	420	49	1	5	77	17	15	4	0	0	3	0	0	0	0	79	15.33%
	2	506	88	17.39%	418	52	0	0	72	19	12	2	0	0	4	0	0	0	0	77	15.27%
20%	4	514	85	16.53%	429	54	2	7	69	18	14	2	1	1	2	0	0	0	0	80	15.56%
	6	508	72	14.17%	436	60	0	2	57	19	12	3	0	2	2	0	1	0	0	86	16.93%
	2	502	70	13.94%	432	64	0	3	56	15	12	4	0	0	2	1	0	0	0	87	17.33%
25%	4	504	64	12.69%	440	72	0	4	52	16	8	2	0	0	4	0	0	0	0	94	18.65%
	6	505	60	11.88%	445	74	0	10	49	19	8	2	0	3	3	1	0	0	0	109	21.58%

Table 3. Effect of carmoisine on *Allium cepa* root meristems.

Concentration	Time	Total Cell Studied	Total dividing cell	Mitotic Index	Interphase	Lesion	Nucleus pushed	Strap shaped	Prophase	Abnormality	Metaphase	Clumping	Polyploid	Disorienatation	Anaphase	Bridge	Telophase	Bridge	Binucleate	Abnormal cell	% of abnormality
	2	425	114	26.82%	311	0	0	0	54	0	30	0	0	0	18	0	12	0	0	0	0
Control	4	422	108	25.59	314	0	0	0	63	0	27	0	0	0	12	0	6	0	0	0	0
	6	414	84	20.28%	330	0	0	0	55	0	18	0	0	0	8	0	3	0	0	0	0
	2	420	112	26.66%	308	18	0	3	80	12	18	4	0	0	8	0	6	2	0	39	9.28%
5%	4	417	87	20.86%	335	14	0	8	64	10	12	6	0	3	7	0	4	0	0	41	9.83%
	6	408	74	18.13%	334	19	2	5	60	14	6	3	0	1	6	0	2	0	0	44	10.78%
	2	418	95	22.00%	326	22	0	4	68	18	14	0	0	4	10	2	0	0	0	50	11.96%
10%	4	414	85	20.53%	329	27	0	4	70	20	8	4	0	3	5	1	2	1	0	60	14.49%
	6	412	82	19.905	330	32	0	6	72	24	4	2	0	0	4	1	2	0	0	65	15.77%
	2	420	84	20.00%	336	30	1	4	71	20	10	4	0	2	3	0	0	0	0	61	14.52%
15%	4	415	76	18.31%	339	38	0	3	66	18	8	3	0	1	2	0	0	0	0	63	15.18%
	6	408	62	15.19%	346	42	2	4	53	24	7	3	0	2	2	0	1	1	0	77	18.87%
	2	418	75	17.94%	343	44	1	5	64	22	7	2	0	2	4	2	0	0	0	78	18.66%
20%	4	412	60	14.56%	352	49	0	7	52	24	4	1	0	2	2	0	0	0	0	83	20.14%
	6	408	54	13.23%	354	52	0	4	47	28	2	1	0	0	3	1	2	0	0	86	21.07%
	2	410	52	12.68%	358	52	2	6	42	18	5	3	0	0	5	3	0	0	0	81	19.75%
25%	4	408	48	11.76%	360	54	0	8	39	17	5	2	0	1	2	0	2	1	0	83	20.34%
	6	400	34	8.50%	366	62	2	4	33	19	2	1	0	0	1	0	0	0	0	88	22.00%

Table 4 Effect of Carmoisine on *Hippeastrum reginae* root meristems.

Concentration	Time	Total Cell Studied	Total dividing cell	Mitotic Index	Interphase	Lesion	Nucleus pushed	Strap shaped	Prophase	Abnormality	Metaphase	Clumping	Polyploid	Disorienatation	Anaphase	Bridge	Telophase	Bridge	Binucleate	Abnormal cell	% of abnormality
Control	2	524	118	22.50%	416	0	0	0	79	0	28	0	0	0	8	0	3	0	0	0	0
	4	515	105	20.38%	410	0	0	0	74	0	24	0	0	0	5	0	2	0	0	0	0
	6	508	98	19.29%	410	0	0	0	69	0	25	0	0	0	0	0	1	0	0	0	0
5%	2	522	102	19.54%	420	14	0	0	67	10	26	4	0	3	6	1	3	0	1	33	6.32%
	4	516	96	18.60%	420	27	0	2	65	13	24	5	0	4	5	0	2	0	0	51	9.88%
	6	504	98	18.25%	412	26	0	0	65	16	23	3	0	7	4	0	0	0	0	52	10.31%
10%	2	512	104	20.31%	408	31	2	5	74	9	22	3	0	4	6	0	2	0	0	54	10.54%
	4	506	100	19.76%	406	37	4	3	75	17	20	4	0	2	3	0	1	0	0	67	13.24%
	6	514	98	19.06%	416	36	8	5	67	16	24	5	2	3	4	2	3	1	0	78	15.18%
15%	2	505	94	18.61%	414	35	8	2	67	14	22	4	0	4	3	2	2	0	0	69	13.68%
	4	501	92	18.36%	409	40	7	5	78	18	20	5	0	4	2	0	0	0	0	79	15.76%
	6	506	88	17.39%	418	49	3	3	66	23	18	6	0	3	3	1	1	0	0	88	17.39%
20%	2	508	72	14.17%	436	52	0	4	49	15	19	4	0	2	2	0	2	1	0	78	15.35
	4	500	70	14.00%	430	55	0	5	48	18	20	5	2	3	2	0	0	0	0	88	17.60%
	6	501	68	13.57%	433	60	1	5	49	19	18	6	0	3	2	0	1	0	0	94	18.76%
25%	2	507	64	12.62%	443	64	2	2	44	11	18	2	0	4	2	1	0	0	0	86	16.96%
	4	503	54	10.73%	449	65	10	4	65	10	19	3	0	4	0	0	0	0	0	96	19.08%
	6	503	48	9.54%	455	68	12	6	32	15	16	4	1	6	0	0	0	0	0	112	22.26

with the experiments and the pesticide treated plants as a positive control.

The bulbs were taken out after 2, 4 and 6 hours of treatments, the roots cut, washed in distilled water and squashed in 2% acetocarmine. For each treatment, temporary slides were prepared and observed under microscope for abnormalities. The mitotic index and the abnormality index were calculated as follows -

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\text{Abnormality index} = \frac{\text{Number of abnormal cells}}{\text{Total number of cells}} \times 100$$

Results and Discussion

The cytological observations from the treated root tip cells revealed that the aqueous solutions of different concentration of Brilliant Blue FCF and Carmoisine have a strong mitodepresive effect on *Hippeastrum reginae* and *Allium cepa* root tips. Treatment with all five concentrations of Brilliant Blue FCF and Carmoisine not only reduced the frequency of dividing cell but a wide spectrum of chromosomal and spindle abnormalities were recorded in the treated root. The root tips placed in distilled water showed normal cell division.

The root tips of *A. cepa* treated with five different concentration of Brilliant Blue FCF and Carmoisine shows decrease in mitotic index which directly proportional to the concentration and duration of treatments (Table 1 & 3). The mitotic index in controlled condition is 26.42% and it reduced to 9.22 % when the root tips were treated for 6 hrs with 25 % aqueous solution of Brilliant Blue FCF. In the case of 25 % Carmoisine, mitotic index was reduced to 8.5% after 6-hour treatment. *A. cepa* root tips treated with both food colours showed almost the same effect on reduction of mitotic index with concentration and treatment time.

The root tips of *H. reginae* treated with different concentration of Brilliant Blue FCF and Carmoisine at different time duration reduced the mitotic index. The mitotic index in controlled conditions was 20.31%. It reduced to 11.88% when the root tip was treated with 25 % Brilliant Blue FCF for 6 hrs. The mitotic index was 9.54 % when the root tip was treated with 25 % carmoisine for 6 hrs. In the case of *H. reginae* root tips, Carmoisine was more mitodepressive than Brilliant Blue FCF.

Both food colours showed increase in abnormality when the root tips of both *A. cepa* and *H. reginae* were treated with increasing concentration and time duration. The numbers of dividing cells were also reduced. At greater concentration, anaphase and telophase cells were not seen. In the case of *A. cepa* root, total abnormality index was 22.33% (Table 1) when treated with Brilliant Blue FCF and was 22% (Table 3) with Carmoisine. The root tips of *H. reginae* showed abnormality index of 21.58% (Table 2) when treated with Brilliant Blue FCF and 22.26% (Table 4) when treated with Carmoisine. Abnormalities were noticed in all stages of cell cycle and the percentage of each is given in tables 1-4. In this study, it was observed that both Brilliant Blue FCF and Carmoisine have an adverse effect on the cytogenetic equilibrium of both plants.

Synthetic food colours play an important role in modern food industry. These food colours may do harm to man and thereby create environmental problems. These food colours may result in the genetic loss of plants and other living organisms. Notable abnormalities encountered were metaphase clumping, polyploid like cells, chromosome bridge, strap shaped nuclei, binucleate cells and nuclei pushed towards the periphery of the cells.

Mitotic depression - Cells treated with Brilliant Blue FCF and Carmoisine showed an immediate effect on the dividing cells by reducing the mitotic index. The frequency of division was further reduced at long time treatment. Such mitodepressive effect of various chemicals have been reported by several investigators such as Mercykutty and Stephen[11] and Singh[12] in *A. cepa*, Raghuvanshi and Massey[4] in barley; Roychoudary and Giri[3] in *A. cepa;* Singh *et al.*[5] in *Vicia faba*; Kumar and Sharma[13] in legumes; Datta *et al.*[14] in *Chrysanthemum*; Yadav and Saxena[15] in *Allium cepa.*

The process of mitosis and eduration of cell cycle are altered by a number of factors. Khilman[16] noted that disturbance in DNA synthesis and oxidative phosphorylation are responsible for inhibition of mitosis. In the present investigation, Brilliant Blue FCF and Carmoisine treated root tips of *A. cepa* and *H. reginae* showed concentration and time dependant decrease in mitotic index. This is an indication of the inhibitory effect of Brilliant Blue FCF and Carmoisine on DNA synthesis in *A. cepa* and *H. reginae.* It may also be possible that the synthetic food colour have an inhibitory effect on synthesis of some proteins or RNA necessary for mitosis at GI- phase of interphase. Protein inhibition by Brilliant Blue FCF and Carmoisine might have taken place as the concentration and time duration increase which in turn reduced the number of dividing cells.

Nuclear lesion-A common cytological abnormality observed in the interphase nuclei was the occurrence of nuclei lesion. Lesion were observed at all concentrations and at all time duration. Mercykutty and Stephen[11] reported nuclear lesion in *Allium cepa* roots cells treated

with Adriamycin. According to them nuclear lesions were the result of the inhibitory effect of the chemical on DNA biosynthesis. In the present study the occurence of nuclear lesion might be attributed to the disintegration of DNA histones or non- histones due to the action of Brilliant Blue FCF and Carmoisine at the interphase.

Prophase break - In prophase, the erosion of chromatin was also noticed which could be seen as fine segments. Prophase break has been reported by Sarma and Tripathi[17] in *Chara brauni* treated with 2, 4-D and coumarine. Singh[12] observed erosions in *Allium cepa* root meristem treated with IAA and MH. The decrease in prophase frequency was reported by Sinha and Sinha[18] in *Allium cepa* cells treated with food dye, metanil yellow. Prophase break has also been reported by Yadav and Saxena[15] in *Allium Cepa* treated with Brilliant Blue FCF. Prophase break in the present study could be attributed to the lack of full DNA content due to the action of Brilliant Blue FCF and Carmoisine

Chromosome breakage, stickiness and clumping -The most important result observed in the treated meristematic cells of *Hippeastrum reginae* and *Allium cepa* were stickiness of chromosome at metaphase. Christopher and Kapoor[19] suggested that stickiness is a type of physical adhesion involving mainly the proteinaceous matrix of the chromatin material. They also suggested that stickiness might be resulted from entanglement of chromatin fibers, which fail to condense properly in preparation for mitosis or breakage and exchange between chromatin fibers on the surface of adjoining chromosome or chromatids. Similar results were also reported in Chineese Hamster cells[20] and Adremycin in *Allium cepa* root tip cells[11] with a variety of such chemicals .

Misra[21] reported that calcium ions play an important role in bringing about chromosomal stickiness. Kaushik *et al.*[22] reported the stickiness by the treatment of turmeric in *Vicia faba*. Datta *et al.*[14] reported stickness in *Chrysanthemum* treated with distillery effluent. In the present investigation metaphase clumping could be attributed to the stickiness of condensed chromosome due to the denaturation of proteins in chromosome. Sticky bridge formation at anaphase could be due to the stickiness of chromosomes at metaphase. The clumping was so tight that the chromosomes were not easily separated during anaphase, which resulted in sticky chromosome bridges between the two poles.

Anaphase bridge formation -Various concentration of aqueous solution of Brilliant Blue FCF and carmoisine has not produced a uniform trend in bridge formation. Pandey *et al.*[23] reported chromatin bridge formation at anaphase by various chemicals in different test system. Double and multiple bridges were also noticed by Sharma and Sarbhoy[24] treated with dimethoate in *Pisum*. The present investigation reveals the incidence of reunion of broken chromosomes. Bridges at anaphase might be due to the breakage and reunion of broken chromosome end so that sister chromatids stick together at the ends in the middle and forms bridges when they would separate at anaphase.

Binucleate cell formation - Binucleate cells were not observed in all concentration and timing during present study. At lower concentration (5%) and at lower time duration (2 hrs. & 4 hrs) very few numbers of binucleate cells were observed. The less frequency of binucleate cells at higher concentration and long time duration might be due to the total arrest of cell plate formation as a result of spindle inactivation. Binucleate cells were observed in Aldrex - 30 and Metacid -50 treated cells of Onion[23]. Same observations were reported by Sharma and Sarbhoy[24] with dimethoate in *Pisum sativum* and Kumar and Sharma[13] with aldrin in legumes.

Other abnormalities - Other abnormalities noticed during the present study were nucleus pushed towards the periphery, disorentation, nuclear polymorphism showing spherical and highly elongated strap shaped nucleus. Irregular and abnormal nuclei of varying sizes and shapes were observed with varying frequencies in different concentrations and duration of treatment. Walum *et al.*[25] suggested that toxic substance might cause membrane structure alteration resulting in permeability changes by interference with lipid metabolism. The results show that the chemicals used in the present study behave as potential mitotic poison, which cause metabolic imbalance.

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