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Cytotoxic Effects of Food Colour (Kesar Yellow) in Plant Cell Division

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Abstract:

A study was made on the cytotoxic effects of food colours (Kesar yellow) on meristematic cells of root tips in *Allium cepa L.*Here plant test-system *Allium cepa* was used in vivo. A decreased cell division rate was established and deviations from the normal mitosis in the result of chromosome mutations were registered. The cytological observations revealed that with increasing concentrations of food colour sunset yellow the mitotic index reduced to variable degrees. Mitotic index decreased in a dose dependent manner. The highest dose of 0.5mg/ml was the most cytotoxic. The mutagenic effects observed in cells treated with the chemical include C-metaphase (no spindle fibres), sticky chromosomes, lagging chromosomes or chromosome fragments at anaphase and telophase, multipolar anaphases. However at concentration of 0.1mg/mL and 0.5mg/mL multipolar anaphase chromosomes and sticky chromosomes were predominant.

Keywords: Cytogenetic effect, fragment chromosome, telophage bridge, acentric chromosome,

Introduction

Food additives have been used to preserve foods for centuries. Salt, being one of the first additives, was primarily used to preserve meats due to its antimicrobial properties. Additives have long evolved since simply being salt. Modern day food additives are complex chemical formulas in high demand due to our need to mass produce and globally transport food items while maintaining nutritional value, marketability and freshness. **Natural food color** is any dye, pigment or any other substance obtained from vegetable, animal, mineral, or source capable of coloring

food drug, cosmetic or any part of human body, colors come from variety of sources such as seeds, fruits, vegetables, algae & insect [1].

The Food and Drug Administration is the agency responsible for the approval of new additives. The probable amount of the preservative that will be consumed with the food product, or amount of any substance formed in or on the food resulting from use of the preservative; The cumulative effect of the preservative in the diet; The potential toxicity (including cancer-causing) of the preservative when ingested by humans or animals [2].

A number of plant bioassay techniques had been developed to evaluate the toxic effect of chemicals on human health because plant chromosomes are relatively large and respond to treatment with mutagens in a similar way to mammals and other eukaryotes[3]. Among these assays, the *Allium* root meristems is a sensitive test that has often been used for the determination of cytotoxic and/or genotoxic effects of various substances [4, 5]. The test has been shown to have a good correlation with tests in other living systems; hence, results obtained from *Allium* test are usually handled with care, because it could serve as an indicator of toxicity of the test materials [6]. The usefulness of root tips of *Allium cepa* as a test system for monitoring the genotoxic effects of test materials was demonstrated by Fiskesjo (1985)[7]. Different concentrations of chemicals were subjected to *Allium* test to check their cytotoxic effects on the chromosome activities of *Allium cepa*.

Another approach is to screen the chemicals used in food for cytotoxicity using human and other cell lines [8]. This methodology has the advantage of using target cells whilst avoiding whole animal experiments [9]. The methodology can be developed to be fairly rapid (a result can be obtained after 12 hours incubation) cover most toxic substances and the cytotoxicity can be expressed quantitatively against an untreated control[10].

In the present study the selected colour was Kesar yellow. The cytotoxic effect of these two food colors were studied here by taking Mitotic indices in onion root tip cells.

Materials and Methods

Onion root tip harvestation and slide preparation

Healthy and equal-sized bulbs chosen from a population of the common onion *Allium cepa* L. (2n = 16), were used as study material. These are considered one of the best biological models for the study of cytotoxicity [6, 11].

Before starting the experiment, the scales of the bulbs and the brownish button plate were removed, and the ring of the root primordial was left intact. The bulbs were allowed to germinate roots by placing them in beakers filled with distilled and sterilized water along with the experimental chemical at different concentrations. The root tips treated in distilled water was used as control. After treatment, the root tips of control and experimental samples were thoroughly washed in distilled water. Chromosome preparations were made using squash technique. Onion root tip were harvested and made it to tiny tips with the help of blade and taken in a clean watch

glass. 1 N HCl was added for 2 -3 minutes to make the root tip soft. 8-9 drops of LAO stain was added for 30 – 45 minutes. 1-2 drops of acetic acid was added to remove the excess stain. Cover slip was placed without introducing any air bubble. Blunt end of the pencil was used to squash the root tips, excess of acetic acid was removed using the blotting paper. Different stages were observed under the microscope. Approximately 1, 000 cells from 1 root tips from 1 bulb was analyzed to score the frequency of mitotic index (MI) and chromosomal aberrations (CA). The values are expressed as mean ± SE. The statistical significance between control and experimental data were analyzed [11].

Results

Cells treated with chemical with higher doses had reduced mitotic indices compared with cells treated with water which was indicative of inhibition of cell division by these food additives. The cytotoxicity of the chemical increased with dose. The highest dose of 0.5mg/ml was the most cytotoxic. The average of mitotic index of each dose of chemical was analysed by bar and line chart (Table1, 2, 3, 4, 5, 6).

The mutagenic effects observed in cells treated with the chemical include C-metaphase (no spindle fibres), sticky chromosomes, lagging chromosomes or chromosome fragments at anaphase and telophase, multipolar anaphases (Fig 1, 2, 3, 4).

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Table 1: The results of the cytotoxicity determination are presented in Table 1, 2, 3, 4, 5 and 6

Table 1A- Control

field	interphase	prophase	metaphase	Anaphase	telophase	total	MITOTIC INDEX	Average of MI
1	225	19	5	6	5	260	0.134615385	
2	243	29	4	7	6	289	0.15916955	
3	213	26	6	5	2	252	0.154761905	
4	230	27	7	6	1	271	0.151291513	
5	241	21	6	9	3	280	0.139285714	0.163220011
6	226	29	3	7	2	267	0.153558052	
7	234	33	4	6	2	279	0.161290323	
8	219	32	3	6	3	263	0.16730038	
9	219	42	6	8	3	278	0.212230216	
10	246	46	9	3	3	307	0.198697068	

Table 1B- Concentration of food colour 0.1mg/ml

Field	interphase	prophase	metaphase	Anaphase	telophase	total	MITOTIC INDEX	Average of MI
1	248	22	0	2	0	272	0.088235294	
2	189	10	1	1	1	202	0.064356436	
3	272	12	1	1	0	286	0.048951049	
4	262	13	2	1	2	280	0.064285714	
5	262	24	2	1	1	290	0.096551724	
6	277	12	1	1	1	292	0.051369863	0.067284413
7	262	13	0	0	1	276	0.050724638	
8	235	15	2	0	0	252	0.067460317	
9	199	16	0	2	3	220	0.095454545	
10	231	9	0	2	0	242	0.045454545	

Table 1C- Concentration of food colour: 0.2mg/ml

field	interphase	prophase	metaphase	anaphase	telophase	total	MITOTIC INDEX	Average of MI
1	196	9	0	2	0	207	0.053140097	
2	209	10	1	0	0	220	0.05	
3	197	11	1	0	0	209	0.057416268	
4	196	9	0	1	0	206	0.048543689	
5	231	13	0	1	1	246	0.06097561	
6	230	20	0	1	1	252	0.087301587	0.063908353
7	209	10	0	1	0	220	0.05	
8	237	12	1	2	0	252	0.05952381	
9	197	17	1	0	0	215	0.08372093	
10	237	21	1	1	0	260	0.088461538	

Table 1D- Concentration of food colour: 0.3mg/ml

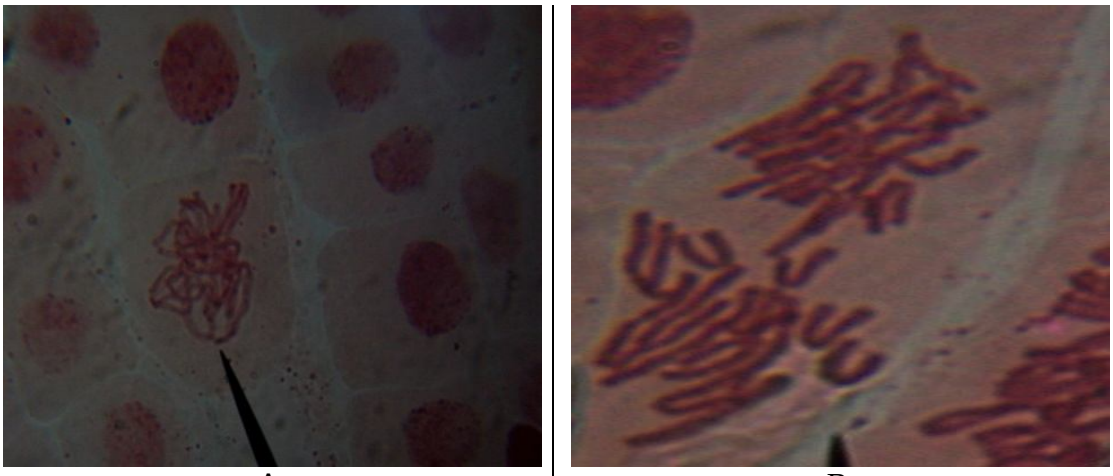
field	interphase	prophase	metaphase	Anaphase	telophase	total	MITOTIC INDEX	Average of MI
1	266	7	1	2	0	276	0.036231884	
2	291	15	2	2	0	310	0.061290323	
3	216	11	1	1	0	229	0.056768559	
4	215	13	3	0	1	232	0.073275862	
5	250	7	0	2	0	259	0.034749035	
6	233	11	0	1	0	245	0.048979592	0.048441853
7	230	6	0	3	0	239	0.037656904	
8	251	8	0	0	1	260	0.034615385	
9	220	10	0	1	0	231	0.047619048	
10	249	8	0	5	1	263	0.053231939	

Table 1E- Concentration of food colour: 0.4mg/ml

field	interphase	prophase	metaphase	Anaphase	telophase	total	MITOTIC INDEX	Average of MI
1	183	7	1	0	1	192	0.046875	
2	194	6	2	0	0	202	0.03960396	
3	274	8	2	1	1	286	0.041958042	
4	240	7	1	0	0	248	0.032258065	
5	194	8	1	0	0	203	0.044334975	
6	266	9	3	0	0	278	0.043165468	0.042214931
7	203	6	1	0	0	210	0.033333333	
8	197	8	2	0	1	208	0.052884615	
9	203	8	1	0	0	212	0.04245283	
10	253	8	1	3	0	265	0.045283019	

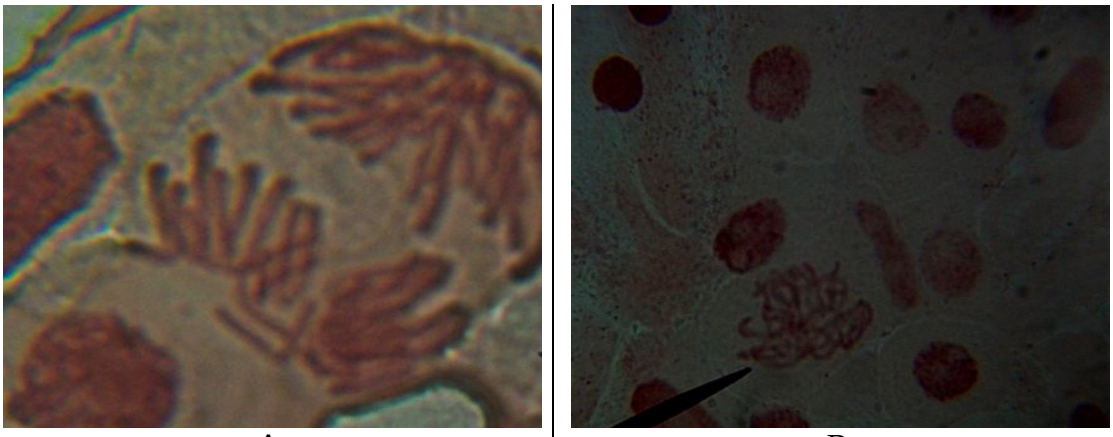
Table 1F- Concentration of food colour: 0.5mg/ml

field	interphase	prophase	metaphase	Anaphase	telophase	total	MITOTIC INDEX	Average of MI
1	199	9	1	1	0	210	0.052380952	
2	199	8	0	1	1	209	0.04784689	
3	261	6	2	1	2	272	0.040441176	
4	243	5	1	1	2	252	0.035714286	0.041869554
5	249	9	2	0	1	261	0.045977011	
6	238	8	1	1	0	248	0.040322581	
7	245	7	1	0	2	255	0.039215686	
8	234	5	1	1	1	242	0.033057851	



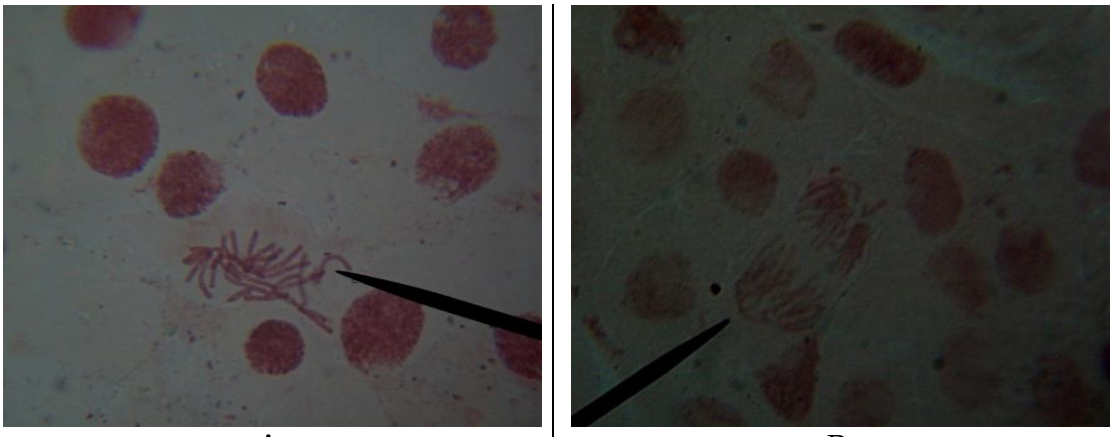
A B

Fig1. (A) *A. cepa* root tip cells treated with 0.5 mg/ml of chemical showing cells with sticky chromosomes (A) and cells with chromosome fragments (B).



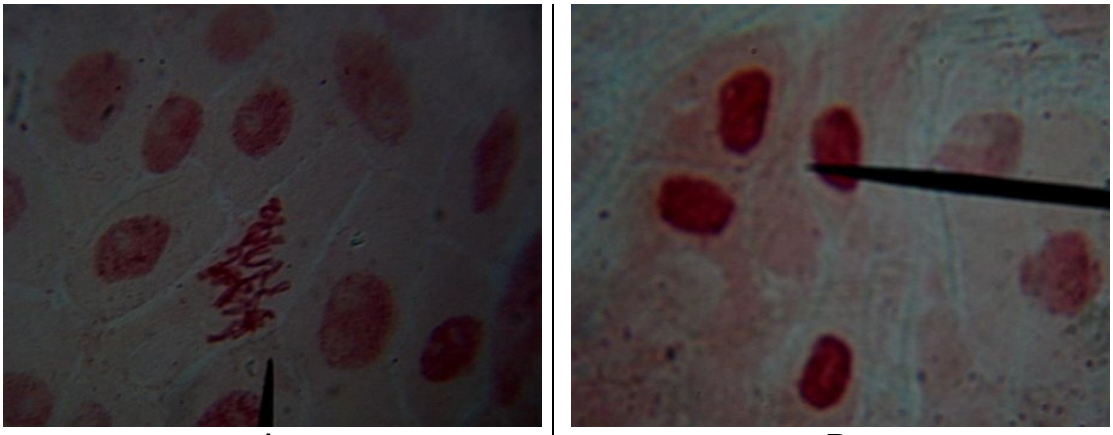
A B

Figure 2. *A. cepa* root tip cells treated with 0.5 mg/ml chemical showing cells with multipolar anaphase (A) and cells with sticky chromosomes(B).



A B

Figure 3. *A. cepa* root tip cells treated with 1 mg/ml of chemical showing cells with chromosome fragments (A) and cells with chromosome lagging (B).



A B

Figure 4. *A. cepa* root tip cells treated with 1 mg/ml of chemical showing cells with

C-metaphase (A) and cells with inhibited mitotic division (B).

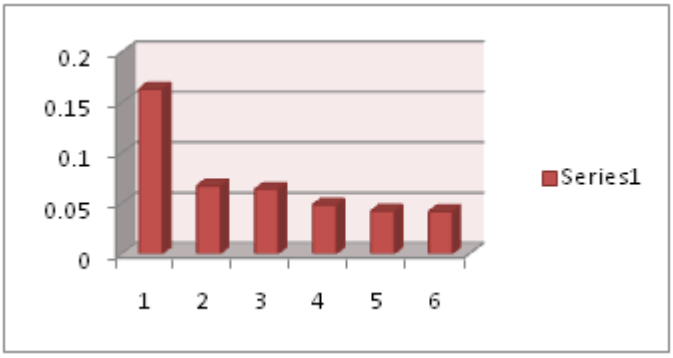


Fig.5. Bar chart showing mitotic index of different concentrations of chemical

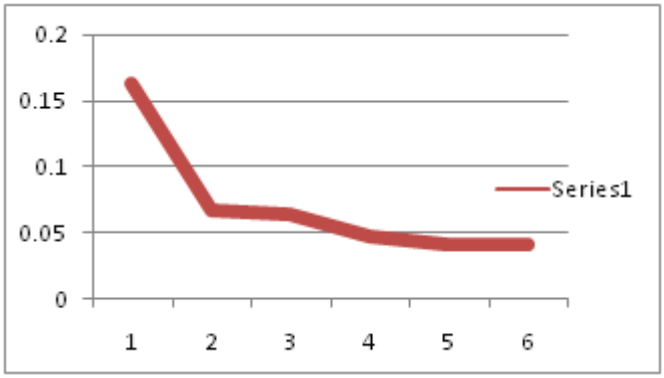


Fig 6. Line chart showing mitotic index of different concentrations of chemical

Discussion

The cytological observations revealed that with increasing concentrations of food colour kesar yellow the mitotic index reduced to variable degrees. Mitotic index decreased in a dose dependent manner as is evident from observations recorded in table 1-table to table 5.

The bar diagram and line chart exhibited pronounced reduction in mitotic index at the dose level of 2mg/mL. The decrease in MI values in the treated onion roots is an indication of cytotoxic substances in kesar yellow which cause inhibition of mitotic activities, while observation of aberrant chromosomes in the treated onion root tips indicate genotoxic effect of kesar yellow on plant cells (E-Shahaby *et al*., 2003) [9].High concentration of kesar yellow were toxic to the cells which may be due to its interference in spindle fibre formation [10] resulting in c-metaphase and multipolar anaphases. Due to the chromosome stickiness, sticky chromosomes at metaphase was observed.chromosomal bridges were observed in anaphase which may resulted due to stickiness.

Mitosis is the result of a number of sequential events, disturbance at any level may result in inhibition of the whole mitotic process(Mishra and Sinha 1979) [10, 11].At high concentration, at and above 0.5mg/mL chromosome fragmentation, C-metaphase, multipolar anaphase, anaphase bridge were observed. However at

concentration of 0.1mg/mL and 0.5mg/mL multipolar anaphase chromosomes and sticky chromosomes were predominant.

The onion bulbs treated with different concentrations of kesar yellow induced a slower rate of root growth [6, 11]. The number of roots developed in the test samples were also highly reduced. Reduction in mitotic index, evidence of chromosomal abnormalities like sticky chromosomes, chromosome fragmentation, C-metaphase, less number of root development in onion bulbs grown in different concentration of kesar yellow, reduction in root length in test onion bulbs compared to onion bulbs grown in water thus reveal the cytotoxic effects of kesar yellow in plant cells.

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