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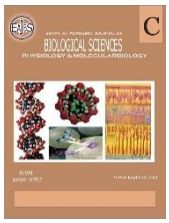

Cytotoxicity and Mito-depressive Effect of Synthetic Lemon Flavour Food Dye on *Allium Cepa* Root Tips

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ABSTRACT

Synthetic food dyes have garnered significant attention due to potential health risks, including cytotoxicity, allergenicity, and even carcinogenicity observed in animal models. Given the widespread use of artificial food dyes in the food industry and potential concerns regarding their safety, this investigation could explore the influence of a lemon-flavored food dye on *Allium cepa* L.. Onion root tip meristems were exposed to various concentrations (0%, 0.25%, 0.5%, 1%, and 2%) of food dye in aqueous solutions for 24 hours at room temperature. A concentration-dependent detrimental effect was observed on all evaluated morphological parameters, including root number, root length, dry weight, and wet weight, with the control group exhibiting the highest values and the 2% treatment group displaying the lowest. Mitotic analysis revealed normal cell division in the control group, but increasing food dye concentrations induced a spectrum of chromosomal aberrations, suggesting disruption of spindle fibers and chromosome cohesion defects. The control group displayed the highest mitotic index (MI) of 9.2, with a positive correlation observed between increasing food dye concentration and the frequency of mitotic abnormalities (0.3 in control vs. 16.8 at 2% treatment). The most prevalent abnormality was multipolar anaphase (5.4 at 2% treatment), while anaphase bridge displayed the lowest incidence (1.7 at 2% treatment). This is evidenced by the significant decrease in root growth parameters (length and number) and (MI), a key indicator of cell division activity. Furthermore, the observed chromosomal aberrations, including sticky metaphase, C-mitosis, laggard chromosomes, anaphase bridges, and multipolar anaphase, are strong indicators of potential genotoxicity.

INTRODUCTION

Given the significant role of visual appeal in consumer food choices, food colorings play a vital role in the food industry. Food colorings, encompassing pigments and dyes, are additives used to enhance the visual appeal of food and beverages which are available in various forms (liquids, pastes, powders, gels), water-soluble synthetic colors dominate their use (Hallagan *et al.,* 1995; Kumar *et al.,* 2018). Their applications are widespread, including processed seafood, baked goods, confectionaries, beverages, and even healthcare products like soaps and cosmetics (Dwivedi & Kumar, 2015; Floriano *et al.,* 2018; Vidal *et al.,* 2018; Sheetal *et al.,* 2019; Khan *et al.,* 2020). Beyond microbiological quality, consumer perception of food heavily relies on sensory attributes like color, alongside taste, texture, and nutritional value. Notably, color and the use of artificial dyes rank among the most crucial sensory factors influencing food choice (Sorouraddin & Saadati, 2010; Prajitha & Thoppil, 2016)

Synthetic food dyes have garnered significant attention due to potential health risks, including cytotoxicity, allergenicity and even carcinogenicity observed in animal models (Hossain *et al.,* 2002; Rangan & Barcelous, 2009; Micic *et al.,* 2014; Sheetal *et al.,* 2019; Farheen *et al.,* 2021). Additionally, research suggests these dyes can disrupt cell division (mitotic index) and cause chromosomal aberrations (genetic abnormalities) (Hossain *et al*,.2002). However, public knowledge regarding the safety of food dyes often remains limited, potentially leading to the inadvertent use of unauthorized and potentially harmful dyes in food preparation (Hossain *et al.,* 2002). This highlights the need for continued research on the effects of synthetic food dyes and increased public awareness regarding their potential risks.

MATERIALS AND METHODS

Plant Material and Root Growth:

Commercially available onion bulbs (*Allium cepa* L., 2n = 16 chromosomes) were purchased from a local store in Zakho, Kurdistan Region, Iraq. To promote root growth, the bulbs were placed in small jars containing distilled water, with the basal plate resting on the water surface. The jars were maintained in a laboratory setting at room temperature (21°C) for several days until the roots reached a length of approximately 1-2 cm.

Food Dye Treatment:

Freshly sprouted onion bulbs were exposed to four different concentrations of lemon yellow food colorant (E102) for 24 hours at room temperature. The test solutions included 0% (control), 0.25%, 0.5%, 1.0%, and 2.0% concentrations. All solutions were prepared by diluting the food colorant with distilled water. Following the incubation period, the root tips (approximately 1 cm in length) were excised and fixed in Carnoy's fixative for 24 hours.

Chromosome Staining and Microscopic Analysis:

After fixation, the root tips were incubated in 1 N HCl for 6 minutes in a 60°C water bath to facilitate chromosome spreading. The root tips were then stained with Giemsa stain for 12 minutes. Subsequently, approximately 1 mm root tip segments were mounted on microscope slides and observed under 1000x magnification (Sharma & Sharma, 2014). Each experiment was replicated three times to ensure data reliability.

Mitotic Index Calculation:

The mitotic index (MI) was determined by dividing the number of cells undergoing cell division (mitosis) by the total number of cells observed. This method adheres to the established protocol described by Fiskesjo (1985).

Statistical Analysis:

Data obtained for the mitotic index were subjected to statistical analysis using SPSS software version 20 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was performed to assess the overall significance of treatment effects on the mitotic index. Post-hoc analysis was

conducted using Duncan's multiple-range tests to identify statistically significant differences between specific treatment groups. Data are presented as mean ± standard error of the mean (SEM). A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Exposure to varying concentrations of synthetic lemon flavor significantly reduced all measured morphological parameters of *Allium cepa* roots, including root number, length, dry weight, and wet weight (Fig. 1).

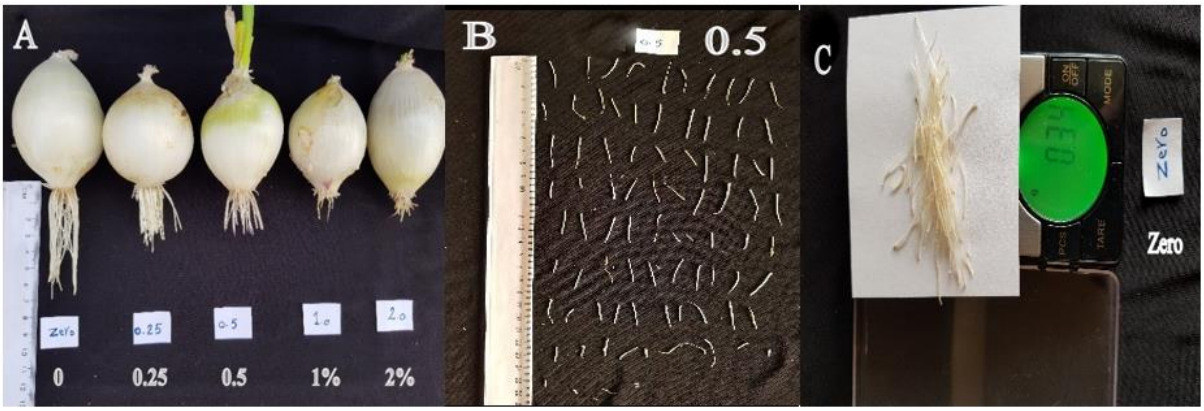

Fig. 1: Effect of the synthetic lemon flavor on *Allium cepa* roots. A; represented onion root growth in different concentrations of synthetic lemon flavor B; represented an example for measurement of the number and length of roots (0.5%). C; represented an example for measurement of the weight of root (Zero).

The analysis of root morphology revealed a concentration-dependent detrimental effect of lemon flavor on *Allium cepa* L. Root length in the control group (0% lemon flavor) was significantly greater ($p < 0.01$) compared to all treatment groups (Table 1). The control group exhibited a mean root length of 8.43 cm, while the 2.0% lemon flavor treatment group displayed the lowest root length (0.6 cm). Similarly, the number of roots, root wet weight, and root dry weight all exhibited a statistically significant ($p < 0.01$) decrease with increasing lemon flavor concentration (Table 1). The control group displayed the highest values for these parameters: number of roots (60), root wet weight (3.53 g), and root dry weight (1.83 g). Conversely, the 2.0% treatment group exhibited the lowest values: number of roots (18.67), root wet weight (0.8 g), and root dry weight (0.23 g). These findings suggest a concentration-dependent inhibition of root growth by lemon flavor.

Table, 1: The effect of the synthetic lemon flavor on root number; root length, dry weight and wet weight of *Allium cepa* roots.

Treatments	Root Length (cm)	Number of Roots	Wet Weight (g)	Dry Weight (g)
Control	8.43 ±0.54 a	60.00 ± 7.00 a	3.53 ± 0.87 a	1.83 ± 0.47 a
0.25%	4.47 ± 0.43 b	67.67 ± 5.24 a	2.77 ± 0.18 a	1.37 ± 0.27 a, b
o.5%	3.60 ± 0.26 b	62.67 ± 12.71 a	1.17 ± 0.22 b	0.47 ± 0.18 b, c
1.0%	2.30 ± 0.17 c	33.33 ± 2.33 b	1.30 ± 0.26 b	0.63 ± 0.27 b, c
2.0%	0.60 ± 0.17 d	18.67 ± 2.40 b	0.80 ± 0.12 b	0.23 ± 0.09 c
Significance	***	**	**	*

Note: *= significant at p<0.05%, **= significant at p<0.01%, ***= significant at p<0.001%.

These findings align with Farheen *et al*. (2021) who reported detrimental effects of food colour additives on onion root tip morphology with increasing concentration. Similarly, the present study demonstrates a concentration-dependent reduction in root length, number, dry weight, and wet weight of *Allium cepa* L. roots exposed to lemon yellow food colouring (Table 1). These observations support the established notion that food colour additives (FCAs) can be phytotoxic (toxic to plants) as reported by Gomes *et al.* (2013), Firbas and Amon (2013), and Farheen *et al.* (2021). The sensitivity of onion root tip length to FCAs highlights its potential as a bioassay tool, as root growth is influenced by both internal cellular processes and external stimuli (Adeyemo & Farinmade, 2013).

Allium cepa L. (2n = 16) exhibited normal mitosis in the control group with proper chromosome alignment and separation (Fig. 2a-d). However, root tips exposed to varying concentrations of lemon yellow food coloring displayed a range of chromosomal abnormalities during active cell division (metaphase and anaphase) (Fig. 2e-i). These included sticky metaphase (chromosome adhesion), C-mitosis (incomplete chromosome condensation), laggard chromosomes (failure to attach to spindle fibers), anaphase bridges (broken or non-disjoined chromatids), and multipolar anaphase (multiple spindle poles). These observations suggest that lemon yellow disrupts spindle fiber function and chromosome condensation, potentially hindering proper cell division in *Allium cepa*.

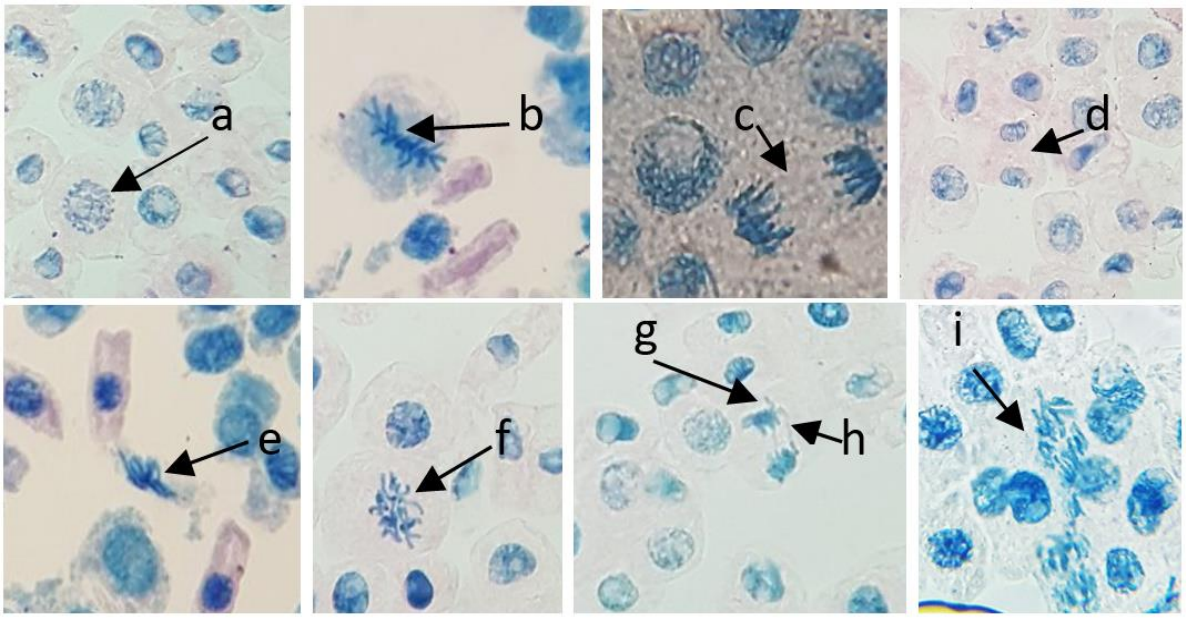

Fig. 2: The different mitotic abnormalities caused by synthetic lemon flavour on *Allium cepa* root tips, a-normal prophase, b- normal metaphase, c- normal anaphase, d- normal telophase, e- sticky metaphase, f- C-mitosis, g- laggard chromosome, h- anaphase bridge, i- multipolar anaphase.

Analysis of *Allium cepa* root tip meristems revealed a clear link between exposure to lemon yellow food coloring and cytotoxicity. Compared to the control group with a mitotic index (MI) of 9.2 (Table 2), all treatment groups exhibited a statistically significant decrease in cell division activity. This suggests that lemon yellow disrupts cellular processes essential for mitosis. Furthermore, the frequency of various mitotic abnormalities, including the most prevalent multipolar anaphase, along with sticky metaphase, C-mitosis, laggard chromosomes, and anaphase bridges, significantly increased

with increasing concentrations of the dye (Table 2). These chromosomal aberrations, indicative of disrupted spindle fiber function and chromosome condensation, could potentially lead to cell cycle arrest or even cell death.

Khan *et al.* (2020) demonstrated that red azo food coloring (FCA) triggered a spectrum of chromosomal abnormalities, including breakage and disorientation during cell division (C-mitosis, metaphase, anaphase), in onion root meristems. Furthermore, their study revealed that red azo FCA significantly inhibited cell division activity, aligning with the concentration-dependent decrease in mitotic index observed in our experiment with lemon yellow food coloring.

Table, 2: The effect of the synthetic lemon flavour on mitotic index and different mitotic abnormalities on *Allium cepa* root tip cells.

Treatments %	Total counted cells	Mitotic Index%	Sticky Metaphase	C-Mitosis	Anaphase bridge	laggard chromosome	Multipolar Anaphase	Total Abnormalities
Control	1018.7±5.2	9.2±0.1 a	0.0±0.0 c	0.0±0.0 c	0.0±0.0 b	0.3±0.3 b	0.0±0.0 b	0.3±0.3 c
0.25	988.7±8.8	5.2±0.3 b	0.7±0.7 b, c	1.0±0.6 b, c	0.0±0.0 b	0.3±0.3 b	0.7±0.3 b	2.7±0.7 c
0.5	1091.0±5.8	3.7±0.1 d	4.3±0.7 a	5.0±1.0 a	0.0±0.0 b	0.3±0.3 b	1.0±0.6 b	10.7±2.0 b
1.0	1014.0±5.6	3.5±0.3 d	2.7±1.2 a, b	2.9±0.5 a, b	1.5±0.3 a	3.9±0.9 a	1.7±0.7 b	12.6±0.8 b
2.0	1086.3±5.5	4.3±0.0 c	2.7±0.7 a, b	3.7±0.9 a	1.7±0.3 a	3.3±0.3 a	5.4±0.7 a	16.8±0.9 a
Significance		***	*	**	***	***	***	***

Note: *= significant at p<0.05%, **= significant at p<0.01%, ***= significant at p<0.001%.

A growing body of research, including these findings, supports the concern that food colour additives (FCAs) can negatively impact *Allium cepa*. Studies by Gomes *et al.* (2013); Bezerra *et al.* (2016), Sheetal *et al.* (2019), Khan *et al.* (2020), Farheen *et al.* (2021), and Bonciu *et al.* (2022), all reported similar aberrations in onion root tips exposed to FCAs. These aberrations likely arise from FCAs' ability to disrupt crucial cellular processes, potentially hindering either cell division or chromosomal synthesis itself. The decrease in mitotic index (MI) and presence of chromosomal abnormalities (sticky metaphase, C-mitosis, laggards, bridges) in *Allium cepa* exposed to lemon yellow food coloring suggests a multi-pronged attack on cell division. This aligns with potential mechanisms like spindle fiber dysfunction (reduced MI, anaphase bridges) due to microtubule activity loss (Panday & Santosh, 2007) and chromosome condensation defects (sticky chromosomes, C-mitosis) caused by DNA misfolding or abnormal structure (Darlington, 1942; Badr, 1986). These disruptions, similar to colchicine's effect (Farheen *et al.,* 2021), could ultimately lead to cell cycle arrest or death (Sheetal et al., 2019). Broken chromosome ends (Ifa, 2009) might further contribute to these abnormalities. While synthetic dyes offer advantages in manufacturing (Kanarek 2011; Pirvu *et al.* 2020; Bonciu *et al.* 2022), their presence in food raises concerns about potential genotoxicity (chromosome damage) to consumers (Kumar and Singh 2017).

Fortunately, numerous natural alternatives exist for food coloring. Simple methods can be employed to extract color from fruits, vegetables, and plants, such as saffron, beetroot juice, and red cabbage. These natural colorants offer a safer and more sustainable solution for the food industry. By promoting awareness and encouraging the use of natural food colorings, we can contribute to a healthier food system for consumers and minimize the potential health risks associated with synthetic dyes.(Bonciu *et al.* 2022).

Conclusion

Our investigation revealed a clear link between exposure to lemon yellow food coloring and cytotoxicity in *Allium cepa* root tips. This is evidenced by the significant decrease in root growth parameters (length and number) and mitotic index (MI), a key indicator of cell division activity. Furthermore, the observed chromosomal aberrations, including sticky metaphase, C-mitosis, laggard chromosomes, anaphase

bridges, and multipolar anaphase, are strong indicators of potential genotoxicity. These abnormalities can disrupt cell cycle progression and potentially lead to cell death, ultimately hindering root development.

While *Allium cepa* serves as a valuable model organism in genotoxicity testing, the observed effects raise concerns about the potential consequences of consuming food products containing synthetic dyes. Further research is warranted to investigate the genotoxicity of lemon yellow food coloring and similar additives in mammalian models to assess their potential human health risks.

Future Research Directions

This study lays the groundwork for further investigations into the genotoxicity of lemon-yellow food coloring and similar synthetic additives. Future research could explore:

The mechanisms by which lemon yellow food coloring disrupts cell division and induces chromosomal aberrations.

The long-term health effects of consuming food products containing synthetic dyes in mammalian models.

The development of more sensitive and robust methods for assessing the genotoxicity of food additives.

Declarations:

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Conflict of interests: The authors declare no conflict of interest.

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Availability of Data and Materials: The data underpinning the findings of this study are accessible upon request from the corresponding author.

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