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# Cytotoxicity of Sunset Yellow and Brilliant Blue food dyes in a plant test system

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**Abstract.** Dyes used in the food industry are an important class of food additives and are often used to make processed food more visually appealing, especially to children. The purpose of this paper was to evaluate the cytotoxic effect of Sunset Yellow (SY) and Brilliant Blue (BB) food dyes on the root meristematic cells of *Allium cepa* L. The root tip cells of onion were exposed to aqueous solutions of dye in concentration of 50 ppm, 100 ppm and 200 ppm, respectively 150 ppm, 300 ppm and 600 ppm BB for 24 hours, at room temperature. Cytogenetic tests reveal a decrease of the mitotic index and an increase of various chromosomal aberrations following food dyes treatments, in a concentration-dependent manner. Types of chromosomal aberrations were varied; thus, were observed cells with irregular kinetics of chromosomes, ring chromosomes, laggards, sticky chromosomes and micronuclei. Exposure of onion roots tips to both food dyes showed a large number of cells in prophase and low number of cells in anaphase, regardless of dyes concentration. The obtained results suggest caution in the consumption of foods that contain these two types of dyes and finding healthier alternatives for food coloring.

**Keywords:** allium assay, cytotoxicity, food dyes, mitodepressive.

## INTRODUCTION

In the technological process, especially after heat treatment, many food products lose or change their natural color. Sometimes, the color changes during storage of the product, and these changes negatively affect the commercial appearance of food and reduce the sensory quality and consumer acceptance. In addition, foods, especially sweets, are more attractive to consumers, mainly children, if they are beautifully colored.

Dyes used in the food industry are chemical compounds responsible for the attractive colorful appearance of the food. Their widespread use is determined by the increasing demand of buyers for the aesthetic qualities of the food on the market. Synthetic dyes are also called artificial dyes. They do not exist as such in nature and are obtained by chemical synthesis. The solubility

in water is due to the presence of an acid group (anionic dyes) and an amine group (cationic dyes), respectively (EFSA).

Synthetic dyes are classified into: azodyes (–N = N–): (congo red, methyl yellow, sunset yellow; tartrazine); triarylmethane group: (brilliant blue, brilliant green) xanthenics: (erythrosine); quinoline: (quinoline yellow); indigo group: (indigotine, indigo). Azodyes represent the most numerous class of food dyes, they account for over 50% of the world's production of dyes, although so far no azoderivative has been found in nature. Azodyes cover the entire spectrum of colors and satisfy practically the needs of coloring for any substrate, having representatives in all applicative classes. Ultra-processed foods indeed often contain mixtures of additives. They represent about 330 authorized compounds in the European Union (Database on Food Additives).

Sunset Yellow (E-110) is a water-soluble food dye, widely used in the confectionery industry. It has the chemical formula $C_{16}H_{10}N_2Na_2O_7S_2$, molar mass 452.38 g/mol and the melting point is 300°C. Brilliant Blue (E-133) is a synthetic organic compound used mainly as a blue dye for processed foods, but also drugs, food supplements and cosmetics. Its chemical formula is $C_{37}H_{34}N_2Na_2O_9S_3$ and the molar mass is 792.85 g/mol (Database on Food Additives).

Higher plants are suitable systems for a wide range of toxicological tests applicable to the assessment of risks to the environment, ecosystems (Geras'kin et al. 2011) and, in some cases, to animals (Arung et al. 2011). The bioassays with plants have been considered quite sensitive and simple in comparison to animal bioassays in the monitoring of the cytotoxic and genotoxic effects of chemical compounds (Gomes et al. 2013; Iganci et al. 2006). From this point of view, *Allium cepa* L. (onion) has been indicated as an efficient test system for cytogenotoxicity assessment (Mert and Betül 2020; Bonciu et al. 2018; Samanta et al. 2012; Metin and Bürün 2008; Evseeva et al. 2001). Also, *Allium* assay has advantages such as low costs and showing good correlation with mammalian test systems (Özgün Tuna-Gülören et al. 2021; Rosculete et al. 2019).

With the mandatory mention of all ingredients on the food label, more and more consumers are wondering if the presence of different additives can affect their health. This study aimed to analyses the cytogenotoxic effect of Sunset Yellow (SY) and Brilliant Blue (BB) (two of the most used food dyes) in *A. cepa* root meristematic cells.

## MATERIALS AND METHODS

### *Plant material*

Clean and healthy onions bulbs were purchased from the Craiova city central market. In order to promote root growth, bulbs were placed in small jars with discoid stem in contact with distilled water, and kept in laboratory, at room temperature (22 ± 2°C) for 72 hours. The onion bulbs with freshly emerged roots were incubated in three different concentrations for each food dyes, for 24 hours, at room temperature. Distilled water has been used as negative control. Five onion bulbs were used for each experimental group.

### *Preparation of different concentrations of dyes*

For each food dye, three different concentrations prepared in distilled water were tested: C1 = 300 ppm, C2 = 150 ppm and C3 = 600 ppm for the testing of BB dye, and C1 = 100 ppm, C2 = 50 ppm and C3 = 200 ppm for the testing of SY dye, respectively. C1 represents the concentration recommended by the manufacturer on the package, and for the other variants we took into account the possibility of using lower doses (half of C1) or higher (twice as much as C1) than those recommended on the package. For the present study the both dyes were bought from one of the Craiova city food store.

### *Microscopic preparations*

After 24 hours of treatment, the onion roots were carefully cut and processed for microscopic preparation.

The biological material were fixed with a mixture of ethanol and glacial acetic acid (Carnoy's solution) in a volume ratio of 3:1 for 24 hours at 4°C in the refrigerator, followed by hydrolysis with 1N hydrochloric acid for 6 minutes at room temperature. Then the onion roots were stained with 10% basic fuchsine solution (Schiff's reagent). Schiff reagent it was composed of basic fuchsine hydrochloride (5 g), hydrochloric acid (100 ml), sodium metabisulfite (10 g), distilled water (900 ml) and activated charcoal (5 g). Its chemical formula is $C_{19}H_{21}N_3S_2O_7 \bullet 4H_2O$.

Slides were prepared and cells were analyzed during the whole cell cycle for cellular and chromosomal aberrations totaling 5,000 cells for each tested dye concentration. The microscopic slides were prepared using the squash technique. For this purpose, after removing the root caps from the stained roots, they were immersed in a drop of 1% acetocarmine on a slide, squashed under a

cover slip and examined microscopically. Five slides for each variant were analyzed for calculating the mitotic index (MI) and the cellular aberration frequency (two roots was used for each slide). The same slides were used to identify the cellular and chromosomal aberrations. All slides were examined using Optika B-290TB microscope with digital camera (Optika manufacturer, Italy).

*Statistical analyses*

The results have been interpreted statistically, using MS Excel 2007. The analysis of variance (ANOVA) was used to assess the significant differences between the control variant and each treatment. The differences between treatment means were compared using the Least Significant Difference (LSD) test at a probability level of 0.05% subsequent to ANOVA analysis.

The mitotic index (MI) was calculated according to Sehgal et al. (2006):

$$MI\ (\%) = \frac{\text{Total number of cells in division}}{\text{Total number of analysed cells}} \times 100$$

The index of the cellular aberrations (CA) which comprising both chromosomal aberrations and nuclear anomalies were also calculated according to Singh (2015):

$$CA\ (\%) = \frac{\text{Total number of aberrant cells}}{\text{Total number of cells in division}} \times 100$$

## RESULTS

Table 1 presents the results of the influence of BB and SY food dyes on the MI and the number of cells in the different mitosis stages in *A. cepa* root tips.

MI decreased with the increase concentration of food dyes solutions. Thus, the intensity of mitotic activity was higher at the lowest concentration of both dyes namely at 150 ppm BB, when MI was 23.9% and at 50 ppm SY, when MI was 16.1%. However, these values are with 22%, respectively with 48% lower than the MI value recorded by the untreated control (30.8%). In the case of SY, a significant mitodepressive effect was recorded at all three tested doses, indicating the high cytotoxic potential of this food dye. The lowest MI value was observed at a concentration of 200 ppm SY (8.5%) i.e. 72.4% lower mitotic activity compared to negative control. It can be appreciated that the tested concentrations of BB and SY induced mitodepressive effect in meristematic root cells of *A. cepa* in a concentration-dependent manner.

Exposure of onion roots tips to both food dyes showed a large number of cells in prophase and low number of cells in anaphase, irrespective of the concentration applied. Thus, compared to the control, the highest number of cells in prophase was registered at the variant BB 150 ppm mg, followed by the variant BB 300 ppm (508 cells) and SY 50 ppm (387 cells). On the other hand, the lowest number of cells in anaphase (43) was observed at SY 200 ppm.

However, the exposure of the meristematic tissues of *A. cepa* to BB and SY also induced genotoxic effects, by increasing the number of cellular and chromosomal aberrations, in a concentration-dependent manner. Thus, as observed in Table 2 and Figure 1, the main cellular aberrations identified were: irregular kinetics of chromosomes (IKC - Figure 1A-B); ring chromosomes (R - Figure 1C); laggard chromosomes (L - Figure 1D); sticky chromosomes (S - Figure 1E) and cells with micronuclei (MN - Figure 1F).

Regarding IKC, the highest values were registered to BB 600 ppm variant (11.20%) and SY 200 ppm vari-

**Table 1.** Total number of analysed cells and Mitotic index (%) in root tips of *Allium cepa* treated with different concentrations of Brilliant Blue and Sunset Yellow food dyes.

| Dyes/ Dose | TCI | TCD | MI± SEM (%) | Cells in Prophase | Cells in Metaphase | Cells in Anaphase | Cells in Telophase |
|---|---|---|---|---|---|---|---|
| Control | 3460 | 1540 | 30.8±0.79 | 510 | 302 | 383 | 345 |
| BB 300 ppm | 4090 | 910 | 18.2±0.48 | 508 | 156 | 114 | 132 |
| BB 150 ppm | 3804 | 1196 | 23.9±0.63 | 730 | 184 | 106 | 176 |
| BB 600 ppm | 4316 | 684 | 13.6±0.41* | 345 | 112 | 68 | 159 |
| SY 100 ppm | 4265 | 735 | 14.7±0.52* | 302 | 193 | 76 | 164 |
| SY 50 ppm | 4192 | 808 | 16.1±0.69* | 387 | 205 | 92 | 124 |
| SY 200 ppm | 4575 | 425 | 8.5±0.38* | 207 | 110 | 43 | 65 |

BB = Brilliant Blue; SY = Sunset Yellow; TCI = Total cells in Interphase; TCD = Total cells in Division; MI = Mitotic index; SEM = Standard error of mean; *Significant at level 5% (p=0.05)
For each treatment were analysed 5,000 cells.

**Table 2.** Type and percentage of cellular aberrations induced by Brilliant Blue and Sunset Yellow food dyes on the meristematic roots of *Allium cepa*.

| Dyes/ Dose | CA (%) | | | | | Total aberrations (%) |
|---|---|---|---|---|---|---|
| | IKC | R | L | S | MN | |
| Control | 1.10 | 0 | 0 | 0 | 0 | 1.10 |
| BB 300 ppm | 7.15 | 3.22 | 3.92 | 8.12 | 4.28 | 26.69* |
| BB 150 ppm | 5.75 | 3.68 | 3.20 | 4.28 | 3.52 | 20.43 |
| BB 600 ppm | 11.20 | 5.16 | 6.05 | 11.14 | 5.20 | 38.75* |
| SY 100 ppm | 10.45 | 4.14 | 6.21 | 10.02 | 5.63 | 36.45* |
| SY 50 ppm | 7.25 | 3.20 | 4.03 | 6.24 | 2.24 | 22.96 |
| SY 200 ppm | 11.08 | 5.14 | 7.02 | 11.10 | 6.35 | 40.69* |

BB = Brilliant Blue; SY = Sunset Yellow; CA = Cellular aberrations; IKC = Irregular kinetics of chromosomes; R = Ring chromosomes; L = Laggard chromosomes; S = Sticky chromosomes; MN = Cells with micronuclei; *Significant at level 5% (p=0.05)
For each treatment were analysed 5,000 cells.

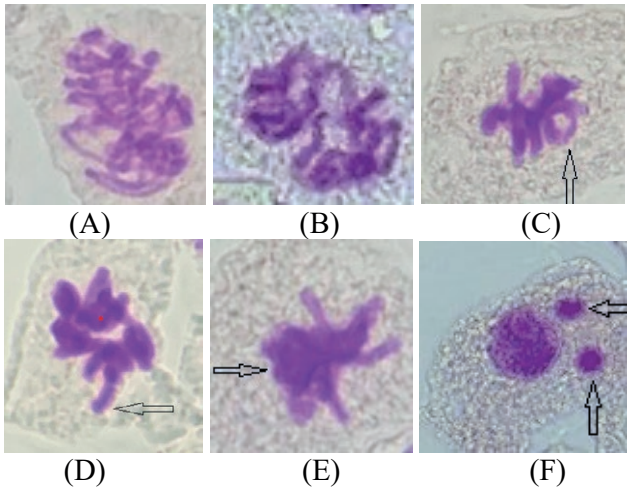


**Figure 1.** Some cellular aberrations identified in meristematic cells of *A. cepa* exposed to Brilliant Blue and Sunset Yellow food dyes: irregular kinetics of chromosomes (A,B); ring chromosome (C); laggard chromosome (D); sticky chromosomes (E); cell with two micronuclei (F).

ant (11.08%), when compared to the negative control (1.10%). The lowest values from this point of view can be observed at the BB 150 ppm variant (5.75%), respectively SY 50 ppm variant (7.25%). In the same vein, the appearance of cells with ring chromosomes was dependent by the increase of food dyes concentration. Compared to the negative control, in which no aberrations were identified, the highest values were registered to BB 600 ppm variant (5.16%) and SY 200 ppm variant (5.14%). The lowest values can be observed at the BB

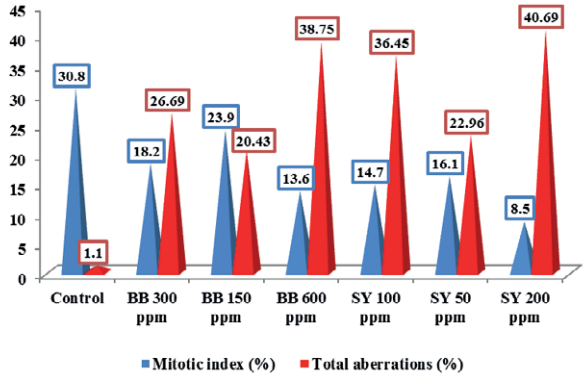


**Figure 2.** Increased of cell aberrations and decreased of mitotic index in meristematic cells of *A. cepa* exposed to Brilliant Blue and Sunset Yellow food dyes.

300 ppm variant (3.22%), respectively SY 50 ppm variant (3.20%).

Regarding laggard chromosomes, the highest values were registered to BB 600 ppm variant (6.05%) and SY 200 ppm variant (7.02%). The lowest values from this point of view can be observed at the BB 150 ppm variant (3.20%), respectively SY 50 ppm variant (4.03%). The identification of other types of cell aberrations (S and MN) in the meristematic tissues of *A. cepa* suggests that their frequency it depends on the food dyes concentration. Thus, the highest S values were registered to BB 600 ppm variant (11.14%) and SY 200 ppm variant (11.10%). The lowest values from this point of view can be observed at the BB 150 ppm variant (4.28%), respectively SY 50 ppm variant (6.24%). On the other hand, the highest MN values were registered to BB 600 ppm variant (5.20%) and SY 200 ppm variant (6.35%). The lowest values from this point of view was observed at the BB 150 ppm variant (3.52%), respectively SY 50 ppm variant (2.24%).

The highest frequency of total cell aberrations was recorded in the variants exposed to the highest concentration of food dyes, namely 38.75% (BB 600 ppm) and 40.69% (SY 200 ppm variant) respectively, this suggesting the genotoxic potential of the two types of food dyes when they are used in high concentrations, as can be seen in Figure 2.

## DISCUSSION

The use of synthetic dyes is preferred because it offers a uniform color intensity, they are stable, easily homogenized in the manufacturing processes and less expensive (Pirvu et al. 2020; Kanarek 2011).

Maximum authorized levels of food additives are set by the European Food Safety Authority (EFSA) and aims to inform and warn consumers against the potential adverse effects of each individual substance in a given food product. Nevertheless, the evaluation, recommendations and regulations has been based only on the currently available scientific evidence which is mainly derived from *in vitro* or *in vivo* experimental research. Thus, essential information regarding the health impact of food additives in humans and the potential effects/ interactions is still missing yet urgently needed (Chazelas et al. 2020).

*Allium* test has been indicated as an efficient test system for cytogenotoxicity evaluation (Samanta et al. 2012; Metin and Bürün 2008; Evseeva et al. 2001). Also, the *Allium* test is considered to be a standard procedure for quick testing and detection of toxicity and pollution levels in the environment (Adesuyi et al. 2018). Different parameters of *Allium cepa* such as root shape, growth, MI, chromosomal aberrations etc. can be used to estimate the cytotoxicity and mutagenicity of environmental contaminants and pollutants (Mert and Betül 2020; Adesuyi et al. 2018; Bonciu et al. 2018; Sutan et al. 2014).

In our study, exposure of onion roots tips to both food dyes showed a large number of cells in prophase and low number of cells in anaphase at all concentrations applied. This might be due to the fact that dye may affect the tubulin and disturbed the mitotic spindle formation. As respects metaphase and anaphase, both mitotic stages showed a decrease in their frequency with increase of dyes concentration. Similar results have been reported by Bhattacharjee (2014) which evaluated the mitodepressive effect of SY using *A. sativum* assay; Onyemaobi et al. (2012), who studied cytogenetic effects of food preservatives on *A. cepa* root tips and Bhattacharjee and Yadav (2005), while studying cytotoxicity of SY on *Vicia faba* root tips.

Some authors reported that the MI in onion root tips was successively decreased with the increase in different dye concentrations and duration of treatments (Gomes et al. 2013; Kanarek 2011). These results are similar to those obtained in present study. Our findings revealed that even at low concentration the both food dyes was cytotoxic for meristematic cells of *A. cepa* with a high significant effect on mitosis. The cytotoxicity level of a test compound can be determined based on the decrease in the MI (Rosculete et al. 2019; Adesuyi et al. 2018; Singh 2015; Liman et al. 2011). Cytotoxicity is defined as a decrease in MI and as increase of frequency of cells with some cellular aberrations like C-Mitosis, multipolar anaphase, sticky and laggards chromosomes (Adesuyi et al. 2018; Singh 2015). Decrease of MI could be due to the inhibition of DNA synthesis (Sudhakar et al. 2001) or due to a block in the G2-phase of the cell cycle (Marcano et al. 2004).

Cytogenetic abnormalities occur under biotic and abiotic stress conditions. Several studies have been oriented to demonstrate the clastogenic effects of some food additives and pointed out their danger as carcinogens or mutagens (Gultekin et al. 2015; Andreatta et al. 2008; Tsuda et al. 2001). On the other hand, some authors claim that chromosomal aberrations may have some benefits in plant breeding. E.g., chromosomal abnormality plants lead to not only gigantic effect, but also increase phytochemical compounds (Ma et al. 2016; Alam et al. 2015).

Many researches have given objects based on the outstanding benefits of chromosomal abnormality to plants. In some studies, the chromosomal abnormalities are regarded as a way to gain elite plant cultivars due to the fact that the increment in plant organs size derived from some of the most significant consequence of chromosomal abnormality (Catalano et al. 2021; Ruiz et al. 2020). As far as ecological perspectives are concerned, the cellular abnormalities enhance biotic and abiotic tolerance to adapt to climate change (Ezquer et al. 2020).

Some chromosomal aberration like stickiness observed in this study may occur as a result of physical adhesion of the proteins of the chromosomes, as Ping et al. (2012) suggested.

In April 2021, California's Office of Environmental Health Hazards Assessment released a ground-breaking, peer-reviewed report concluding that synthetic food dyes (such as Red 40, Red 3, Yellow 5, Yellow 6, Blue 1, Blue 2, and Green 3) negatively affect children's behavior. The final health effects assessment provides authoritative validation of what multiple independent reviews already concluded: that synthetic food dyes can cause or exacerbate behavior problems to some children (CSPI, 2021).

Natural colors are gaining popularity because a natural food dye is a healthier and it does not cause health problems. USDA has certified some natural food dyes that do not cause health problems to consumers (https://sensientfoodcolors.com/en-us/color-solutions/certified-organic-colors/). Most natural food dyes are obtained from fruits and vegetables and contain nutrients that are beneficial to health.

There are simple and handy ways for anyone to easily extract color from some plants, fruits or vegetables, to color some food products. For example, the yellow color can be extracted from saffron soaked in water and then pressed; for different shades of red, purple or even blue, red cabbage or beetroot juice can be used mixed with different percentages of water, etc. Finally, the decision to eat healthier remains with each of us.

## CONCLUSIONS

Brilliant Blue and Sunset Yellow (two of the most used food dyes) exerted mitodepressive and cytogenotoxic effects in meristemtic root cells of *Allium cepa* L., in a concentration-dependent manner.

Results obtained in our study suggest caution in the consumption of foods that contain the two types of dyes and finding healthier alternatives for coloring of some food products. However, additional cytotoxicity studies are needed to add information to these and other previously obtained results in order to deepen the potential risks of these food dyes on a cellular level.

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