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THE MITODEPRESIVE AND GENOTOXIC EFFECT OF SOME FOOD COLORANTS ON THE MERISTEMATIC CELLS TO *Allium cepa*

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Abstract

Currently, most processed foods purchased from supermarkets contain one or more food colorants. One of the best-known colorants is the so-called red dye for eggs, used during the Easter religious holiday. The purpose of this paper was to highlight the mitodepresive and genotoxic effect of an assortment of red dye for eggs to meristematic cells of onion (Allium cepa). Three different concentrations (0.5, 1 and 1.5%) were used, the exposure time being 3 hours. The results obtained revealed a strong mitodepresive effect of food colorant to A. cepa, by decreasing of the mitotic index as the concentration of colorant increased. Also, a genotoxic effect has been observed by the occurrence of some chromosomal aberrations and nuclear alterations, such as sticky and vagrants chromosomes, multinucleated cells and cells with nuclear erosion. These results suggest prudence in using the red dye for eggs and finding other alternative and ecological solutions such as, for example, the use of some plant extracts for this purpose.

Key words*: Allium cepa, red dye, mitodepresive, genotoxic, meristematic cells.*

INTRODUCTION

Food colouring is used both in commercial food production and in domestic cooking. Either the synthetic food colours or natural food colours, the colour has always had an important implication on the minds of people as far as food is concerned. Cuisines prepared in attractive colours have immensely lured men folk in all the quarters of the world. It is therefore necessary either to preserve the natural or maintain the characteristic colour of a food product while it is manufactured or stored for future use. A non-attractive colour however makes the food look un- fresh and is likely to be rejected.

According to FDA, a food colorant is "any dye, pigment or substance which when added or applied to a food, drug or cosmetic, or to the human body, is capable (alone or through reactions with other substances) of imparting colour" (FDA, 2016). Synthetic food colorants were largely used, but have been progressively substituted by those obtained from natural origins. Despite natural pigments offer a strong advantage when compared to the synthetic ones, its safety and whole effects needs to be assessed, in order to conclusively demonstrate health improving effects (Martins et al., 2016). Numerous side effects and toxicity, at both medium and long-terms, allergic reactions, behavioural and neurocognitive effects have been related with their use. However, several food additives that were used over decades are no longer currently allowed, due to the real evidences of their side effects, toxicity at medium and long-terms and high frequency of health disturbance incidents.

At present, consumers are becoming more concerned about their health, meaning, firstly, adequate, high quality nutrition (Abebe et al., 2016; Ouis & Hariri, 2018; Righi et al., 2018).

Dyeing eggs for the Easter, a habit which we learned from parents and grandparents and we will pass on from generation to generation, may become dangerous under certain conditions. Since the commercial dye for eggs is obtained industrially, from artificial dyes in general, we considered this cytogenetic study to be appropriate for evaluating the cellular activity of meristematic plant tissues under action of the red dye (one of the most used), using the *A. cepa* species (onion) as the test plant. *A. cepa* has assayed to be best model plant for standard

use in environmental monitoring and cytological analysis (Bonciu et al., 2018; Bonciu, 2018).

MATERIALS AND METHODS

The biological material used was represented by commercially onion bulbs, which were immersed in glasses with water for 72 hours, time required for the meristematic roots occurrence. Prior to initiating the test, the dry bottom plate of the bulbs was removed without destroying the root primordial. When the meristematic roots reached the length of 15-20 mm, they were immersed in dilutions of various concentrations of the red dye for eggs (0.5, 1.0 and 1.5%) for 3 hours, at room temperature. The red dye was purchased from a supermarket and the following ingredients were listed on the label: softened water, azorubine (E 122), glazing agent (glycerine and glucose), acidifier (citric acid E 330 and lactic acid E 270), preservative (sodium benzoate and potassium sorbate), and thickening agent (xanthan gum). A number of 5 onion bulbs were used for each treatment variant as well as an untreated control that was immersed in tap water.

The roots were processed according to the protocol of fixation, hydrolysis and staining to highlight the cytological activity and eventual presence of chromosomal aberrations.

In order to highlight chromosomes and chromosomal aberrations was used the Feulgen-Rossenbeck method and Schiff reagent. Feulgen stain is a staining technique discovered by Robert Feulgen (1924). Depend on acid hydrolysis therefore fixating agents using strong acids should be avoided. The necessity for hydrolysis of tissues with acid in order to obtain the specific reaction with Schiff reagent was demonstrated by Feulgen and Rossenbeck in their original description of the Feulgen stain in 1924. Schiff reagent is prepared by pouring 200 ml of boiling distilled water over 1-g basic fuchsin. Shake thoroughly, cool to 50°C, filter, and add 30 ml 1N HCl to the filtrate. Cool to room temperature and add 1 g potassium metabisulfite Allow the solution to stand overnight in the dark or until a light straw or faint pink colour develops.

The microscopic preparations were performed according to the squash method.

Statistical analysis was done 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 LSD-test at a probability level of 0.05% subsequent to the ANOVA analysis.

The mitotic index was calculated using the following formula:

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

The index of the total abnormalities (TA) was also calculated:

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

Photomicrographs of cells showing chromosomal aberrations and nuclear alterations as well as showing mitosis were taken using the Kruss microscope.

RESULTS AND DISCUSSIONS

The results are illustrated in Table 1. The cytotoxicity level can be determined by the decreased rate of mitotic index. It was found that red dye induced a strong mitodepresive effect in meristematic cells to *A. cepa*. The mitodepresive effect was enhanced as the concentration of red dye increased. Thus, compared to the Control variant, the mitotic index recorded a decrease of over 50%, from 36.42% (V1-Control) to 18.04% (V2), 1.0% (V3) and 9.03% (V4) in all treated variants.

Table 1. Effect of different concentrations of the commercial red dye on the cytological parameters to *A. cepa*

Variants/ Conc. (%)	MI ± SE %	Cells abnormalities frequency (%)				TA (%)
		S	V	MN	NE	
V1 (Control)	36.42±0.68	0	0	1.32	0	1.32
V2/0.5	18.04±0.42*	4.18	2.62	4.21	1.58	12.59*
V3/1.0	12.18±0.39*	6.45	4.01	5.98	2.08	18.52*
V4/1.5	9.03±0.34**	8.34	5.84	11.07	2.64	27.89**

MI = Mitotic index; SE = Standard error; S = Stickiness; V = Vagrants; MN = Multinucleated cells; NE = Cells with nuclear erosion; TA = Total abnormalities; *Significant at level 5% (p=0.05)

The decrease in the mitotic index was positively correlated with increasing concentration of the red dye solutions. According to Panda and Sahu (1985), a decrease of mitotic index below 50% usually has lethal effects. If mitotic index decreases below 22% of control, that it causes sub-lethal effects on test organism (Antonsie-Wiez, 1990). In our study, the mitodepressive effect of red dye may be due to its inhibitory effect on the mitotic cycle during interphase and delaying of spindle formation.

Synthetic colours are a major source of food intoxication and many surveys have been conducted to determine the presence of no permitted food colours in different food products (Vazhangat P. & Thoppil J.E., 2016). In the case of kids' foods, shines colours are also added to attract their attention and make the foods appear attractive and fun. But in most cases, if a food comes in a colour that is not found in nature, excessive consumption can cause health problems.

Food dyes are one of the most widely used and dangerous additives. While the European Union has recently placed regulations on labelling food dyes to inform consumers of the health risks, the United States has no such requirement. Every year, food manufacturers pour 15 million pounds of artificial food dyes into U.S. foods, according to the Center for Science in the Public Interest (CSPI). In the "Food Dyes: A Rainbow of Risks" report, CSPI revealed that nine of the food dyes currently approved for use in the United States are linked to health issues ranging from cancer and hyperactivity to allergy-like reactions and these results were from studies conducted by the chemical industry itself. For instance, Red 40, which is the most widely used dye, may accelerate the appearance of immune system tumours in mice. Almost all the toxicological studies on dyes were commissioned, conducted, and analysed by the chemical industry and academic consultants. Ideally, dyes (and other regulated chemicals) would be tested by independent researchers.

Several studies have been oriented to demonstrate the antimitotic and genotoxic activities of some food additives and pointed out their danger as carcinogens or mutagens. Many authors reported that the mitotic index of *A. cepa* root tips was successively decreased with the increase in different dye concentrations and duration of treatments (Vazhangat P. & Thoppil J.E., 2016).

In our study, all treatments with red dye resulted in a significant increase of the percentage of chromosomal aberrations and nuclear alterations (Figure 1).

Thus, the index of the total abnormalities (TA) recorded an increase in all treated variants, from 1.32% (Control) to 12.59% (V2), 18.52% (V3) and 27.89% (V4). The increase in the TA index was positively correlated with increasing concentration of the red dye solutions.

Multinucleated cells and stickiness were the dominant abnormality induced after treatment, especially at higher concentrations. Stickiness is an irreversible chromosomal aberration and reflects high toxicity of tested solutions.

Frequency of multinucleated cells recorded values between 1.32% (Control), 4.21% (V2), 5.98% (V3) and 11.07% (V4). Also, the frequency of cells with stickiness abnormalities was 4.18% (V2), 6.45% (V3) and 8.34% (V4). Regarding the cells with chromosomal aberrations type vagrant, their frequency ranged between 2.62% (V2) and 5.84% (V4). The frequency of nuclear erosion abnormalities was 1.58% (V2), 2.08% (V3) and 2.64% (V4) (Figure 2). In Figure 3 are shown some cytogenetic abnormalities identified in meristematic roots of *A. cepa* exposed to commercial red dye.

The results obtained indicated that red dye induced a strong mitodepresive and genotoxic effect in meristematic cells to *A. cepa*, by reduction of the mitotic index and occurrence a several cytological abnormalities.

These results suggest prudence in using the chemical red dye for eggs painting. Alternatively, natural extracts from various plants can be used (red beet juice, pomegranate juice, red onion peels, red peony petals etc.).

Natural food colours are preparations obtained from foods and other edible natural source materials obtained by physical and/or chemical extraction resulting in a selective extraction of the pigments relative to the nutritive or aromatic constituents. They come in many forms consisting of liquids, powders, gels and pastes.

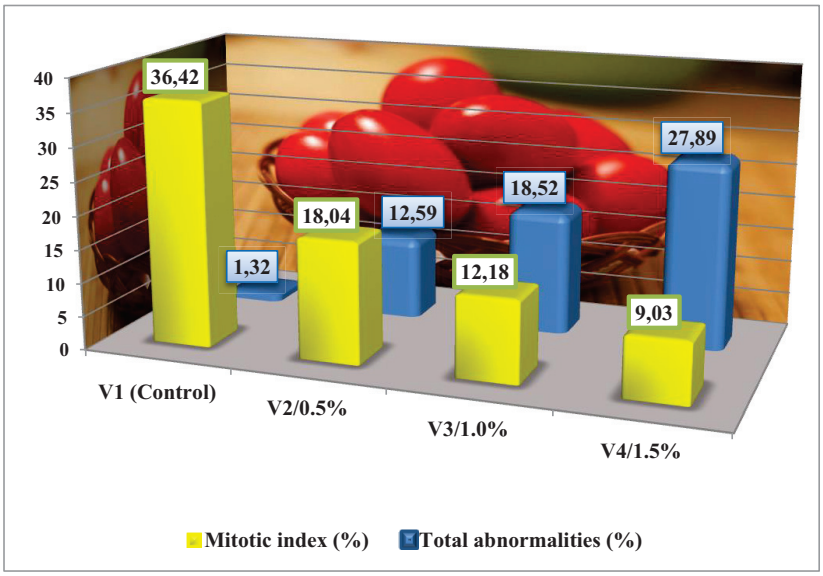

Figure 1. The mitodepresive and genotoxic effect of red dye on the meristematic cells to *A. cepa*

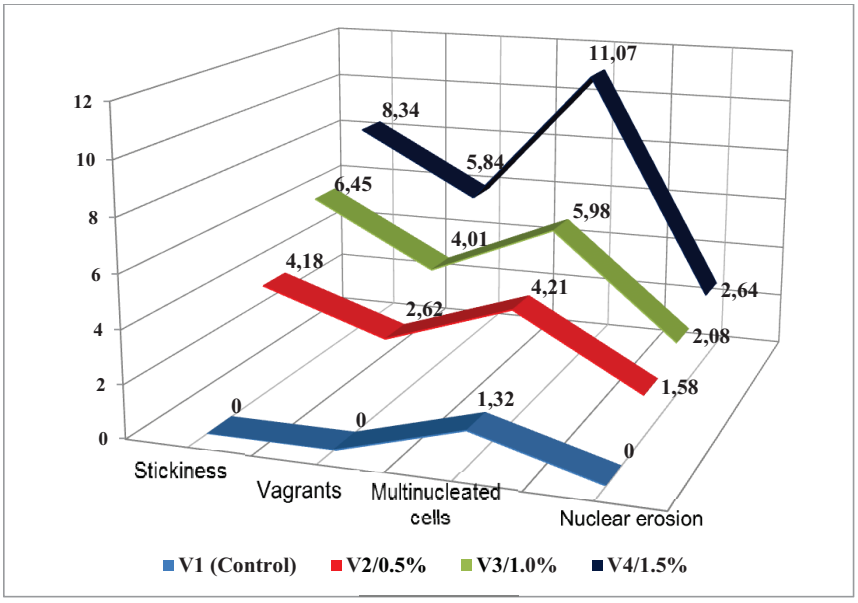

Figure 2. The frequency (%) of aberrant cells in meristematic tissues of *A. cepa* exposed to different concentrations of the commercial red dye

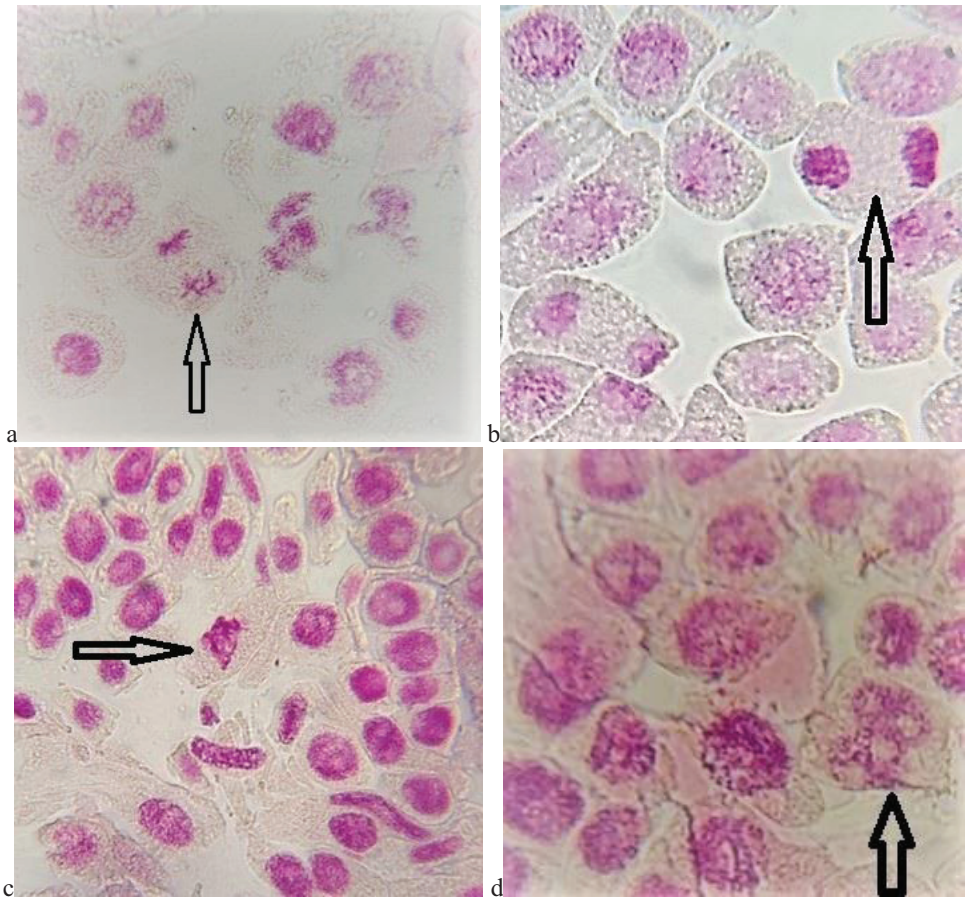

Figure 3. Some cytogenetic abnormalities in meristematic roots of *A. cepa* exposed to commercial red dye: disturbed anaphase with vagrants chromosomes (a); sticky telophase (b); sticky metaphase (c); multinucleated cell (d)

CONCLUSIONS

The cytological results observed in this study suggest that red dye induced a mitodepresive and genotoxic effects in *A. cepa* root tips, by reduction of the mitotic index and occurrence a several cytological abnormalities.

Multinucleated cells and stickiness were the dominant abnormality induced after treatment, especially at higher concentrations.

These results suggest prudence in using the red dye for eggs painting. An alternative solution to avoid the toxic effect of this chemical food colorant is the use of natural herbal extracts such as red beet juice, pomegranate juice, red onion peels, red peony petals etc.

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