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Evaluation of Cytotoxic and Genotoxic Effects of Commonly Used Food Additives on the Root Meristem Cells of Allium cepa

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Abstract. The effects of the food preservative sodium benzoate, food sweetener aspartame and food colorant carmoizine have been studied on root tips of *Allium cepa* L. Roots of *A. cepa* were treated with a series of concentrations of food additives for 72 h. The results indicate that these food additives reduced values of mitotic index in meristem cells of *A. cepa* compared with the control samples. All concentrations of these chemicals showed an inhibitory effect on cell division. The frequencies of chromosomal aberrations were generally increased with increasing of substances's concentrations. All studied food additives induced c-mitosis, lagging and vagrant chromosomes and fragments and micronucleuses. Sodium benzoate and aspartame were the reason for the formation of anaphase bridges and diagonal anaphases. Only in the root tips were treated with aspartame were established pulverized chromosomes and multipolar metaphases and anaphases. The research of the cyto- and genotoxic effects of food additives used in food products are relevant, given their wide application and their unclear effect on human health.

Key words: carmoisine, sodium benzoate, aspartame, root meristem cells, *Allium cepa*.

Introduction

Since the middle of the 20th century and still today, the use of food additives is becoming more widespread. Manufacturers use them in various food products, medicines and cosmetics in order to improve their consistency, taste, achieve the desired organoleptic properties, increase their use value and longer shelf life (Leathwood et al., 2007, Carocho et al., 2014). Unfortunately, however, these additives can have adverse health effects (Jarskog, 2006; Kitano et al., 2002).

The use of food additives is regulated in Codex Alimentarius and is justified only when it does not pose a risk to the health of consumers, does not mislead the consumer and gives an advantage to the product concerned, which cannot be achieved in any other economic and technological way (Codex Alimentarius, FAO, 2011).

The legislation of most countries adheres to a list of permitted and prohibited additives and their maximum permitted amounts. As a result of numerous studies on the negative effects of synthetic colours on the human body, their number in the permitted lists is constantly decreasing. It has been shown that regular consumption of azo colours in food can lead to allergic reactions, anemia, binding to blood hemoglobin, kidney and liver diseases, hyperactivity and hyperexcitability in children, asthma, especially if they are used in larger quantities (Amichova et al., 2015; Basu & Kumar, 2015; Oplatowska-Stachowiak & Elliot, 2017).

Toxic, mutagenic and carcinogenic effects of azo colours, including carmoisine, have been found in studies by Zhang & Ma (2013a). Carmoisine is a widely used colouring agent whose application is responsible for altering biochemical markers in murine vital organs, modifying the secondary structure of serum proteins (human serum albumin and bovine serum albumin), and increasing the conformational changes in the DNA of bovine cell lines (Amin et al., 2010; Arvin et al., 2013; Datta et al., 2013).

Carmoisine can cause side effects in asthmatics and people allergic to aspirin, (Martin, 2007). This colorant is banned in the United States (Mahfoz et al., 2010). A study by Macioszek and

Kononowicz (2004) showed that some colouring agents and food additives can cause genotoxic effects in human lymphocyte cells and in the root meristem of *Vicia faba in vivo*. The effect of this synthetic colouring agent on mitotic activity, induction of abnormalities in the course of mitosis and changes in DNA structure was also studied using the *Allium cepa* test system (Mahfoz et al., 2010). The results obtained show that carmoisine causes a decrease in the mitotic index, changes in mitotic phase indices and induces a wide range of chromosomal abnormalities in dividing cells. These changes are accompanied by variation in the content of nucleic acids. Electrophoretic analysis has shown that the colorant has the ability to change the size of proteins (Mahfoz et al., 2010).

Antimicrobial additives (benzoic acid and benzoates, including sodium benzoate) are of limited use and act by destroying the membrane and inhibiting metabolic reactions in the microbial cell (Brul and Coote 1999).

The preservatives benzoic acid and sodium benzoate have been tested in vitro and are considered non-toxic, but some authors have found their clastogenic, mutagenic and cytotoxic effects using the *Drosphila* SMART test, the *Allium* test and human lymphocyte cell lines (Nair, 2001; Yilmaz et al. 2008, 2009; Zengin et al. 2011). Sodium benzoate has also been reported to intercalate with DNA in the nucleus of bovine thymus cells at concentrations up to 4.5x10-5 mol/L (Zhang and Ma 2013b).

A synergistic effect of sodium benzoate and food colouring agents, expressed in hyperactivity in children and students was found in a study by Beezhold et al. (2014), which confirms the need for further studies.

Flavoring agents are additives used to change the taste of food and are divided into 3 subgroups: sweeteners, natural and synthetic flavours and flavour enhancers.

Aspartame is a widespread low-calorie sweetener. Extensive studies have been conducted on aspartame, and while some ensure its safety (Jeffrey and Williams, 2000; Mukhopadhyay et al., 2000), others have drawn alarming conclusions (Ashok et al., 2013; EFSA 2013; Rycerz & Jaworsaka-Adamu, 2013). Other studies have shown that long-term consumption of aspartame can lead to hepatocellular damage and changes in liver antioxidant status and behaviour in rats (Abhilash et al., 2011; Ashok et al., 2013).

The cytotoxic and genotoxic effect of aspartame concentrations of 100, 300 and 400 mg/l was established by De la Cruz et al., (2013) in the root meristem cells of onion bulbs. The authors found a decrease in the mitotic index depending on the concentration of the sweetener. The progressive increase in nuclear bud formation as a function of the increasing concentration of aspartame proves the possible genotoxicity of this sweetener. Nuclear buds are probably the result of the elimination of excess genetic material obtained from the processes of polyploidization and hyperamplification of DNA, which subsequently become micronuclei (Fernandes et al., 2007; Prieto et al., 2008).

There are certain advantages associated with the use of plant test systems in procedures for cytogenetic analysis and screening for toxic and mutagenic effects of various substances in the environment (Grand, 1999; Iganci et al., 2006; Leme & Marin-Morales, 2008; Samoilov et al., 2019). Compared to other tests that use animals or cell cultures, the *Allium* - test turns out to be less complicated and expensive and more sensitive, and the *Allium cepa* species is indicated as an effective system for assessing cytotoxicity (Leme & Marin-Morales, 2008; Gomez et al., 2013; Nunes et al., 2011), which facilitates the analysis to detect changes in the intensity of cell division (Kuras et al., 2006; Tabrez et al., 2011) and damage to the structure of DNA (Carita & Marin-Morales, 2008; Herrero et al., 2012).

Studies of the cyto- and genotoxic effects of colouring agents, preservatives and sweeteners used in food products are relevant, given their wide application and their unclear effect on human

health. For our country, such studies have not been conducted, which determines the interest in developing a combined approach for cytogenetic characterization of their impact in vivo. The purpose of this study is the assessment of cytotoxic and genotoxic effects of the commonly used food additives carmoisine (E122), sodium benzoate (E211) and aspartame (E951) on the root meristem cells of *Allium cepa*.

Material and Methods

For the purposes of the study, untreated bulbs of Allium cepa, Asenovgradska kaba variety with a diameter of 2.5-3 cm were used, provided by the Maritsa Vegetable Crops Research Institute in the city of Plovdiv. The food additives were purchased from Trimart Ltd. and accompanied by an analytical certificate for use in the food industry. The solutions of the colorant carmoisine, the preservative sodium benzoate and the sweetener aspartame (E951) with different concentrations presented in Table 1.

Table 1. Concentrations of the tested food additives (by Prajitha & Thoppil, 2016; Yilmaz et al., 2008; De la Cruz et al., 2013).

Carmoisine (E122)	Sodium benzoate (E211)	Aspartame (E951)
0.005% (0.05 g/l)	50 mg/l	400 mg/l
0.01% (0.1 g/l)	100 mg/l	600 mg/l
0.1% (1 g/l)	200 mg/l	800 mg/l
1% (10 g/l)	500 mg/l	1000 mg/l

Five bulbs per each concentration were placed in tubes with a volume of 50 ml containing food additive solutions, away from direct sunlight for 72 hours. In the control sample, distilled water was used. After the treatment period, the sprouted roots were separated from the bulbs with the help of a scalpel, fixed with a Clarke's fixative and stored in 70% ethanol at 4˚C.

Acetocarmine-stained root apical meristem was used to prepare the temporary microscopic slides. From each experimental sample and from the control one, 5 preparations were prepared, which were observed under a light microscope at a magnification of x400 and pictures were taken with a digital camera for microphotography. At least 1000 cells for each preparation were analyzed.

The effect of different concentrations of substances on the intensity of cell division was determined by calculating the mitotic index according to the following formula:

$$\text{IM}\,(\%) = \frac{N'}{N} \times 100,$$

Where: IM is the mitotic index calculated in%,
N′ is the number of dividing cells, and
N is the total number of cells analyzed.

The genotoxic effect of food additives was analyzed using an anaphase method and a micronucleus mutagenicity test. The frequency of chromosomal aberrations in the meristem cells of *Allium cepa* was calculated as a percentage of the number of dividing cells and relative to the total number of analyzed cells according to the formulas:

$$CAI^{N'}(\%) = \frac{CA}{N'} \times 100,$$

Where: CAI $^{N'}$ is the frequency of chromosomal aberrations relative to the number of dividing cells, calculated in %,
CA is the number of cells with chromosomal aberrations, and

N' is the number of dividing cells.

$CAI^N(\%) = \frac{CA}{N} \times 100$, Where: CAI ^{N} is the frequency of chromosomal aberrations relative to the total number of cells, calculated in %,
CA is the number of cells with chromosomal aberrations, and
N is the number of all cells analyzed.

Different types of aberrations have been reported at different stages of cell division - lagging and 'vagrant' chromosomes, acentric fragments, chromosome bridges, micronuclei, C-mitosis and asynchronous mitosis.

The spectrum of chromosomal aberrations was analyzed as an indicator of the specificity in the mutagenic action of food additives.

Comparisons between treated and control samples were subjected to statistical analysis using Student's t-test to assess the significant differences between them.

Results

The effect of carmoisine (E122), sodium benzoate (E211) and aspartame (E951) on the intensity of cell division, expressed by the value of the mitotic index in the various experimental sample and in the control sample is presented in Table 2.

Table 2. Values of mitotic index and phase indexes (%) (X±SD) in the control and experimental samples treated with different concentrations of carmoisine (E 122), sodium benzoate (E 211) and aspartame (E951). Legend: *p<0.05, **p<0.01, ***p<0.001.

Food additives		Mitotic index %	Phase indexes %			
			Prophase index	Metaphase index	Anaphase index	Telophase index
E 122	0.05 g/l	48.65±11.34	92.96±3.27	2.81±1.3	1.5±0.79	2.72±1.5
	0.1 g/l	48.56±5.41*	89.34±4.17	4.22±1.96	3.26±1.31	3.17±1.35
	1 g/l	50.85±4.37	89.57±1.86	4.02±0.5	3.59±0.82	2.81±0.93
	10 g/l	31.12±11.1***	90.24±4.16	5.21±2.88	2.06±1.04	2.49±1.51
E 211	50 mg/l	34.83±10.68***	86.73±12.59	6.04±4.48	3.08±2.35	4.16±5.4
	100 mg/l	33.79±10.3***	99.79±0.35	0.21±0.35	0	0
	200 mg/l	35.69±4.72***	100±0	0	0	0
	500 mg/l	27.95±7.26***	100±0	0	0	0
E 951	400 mg/l	52.88±3.59	92.26±2.74	3.26±1.8	2.7±0.89	1.43±0.68
	600 mg/l	44.8±5.88***	91.18±3.57	4.23±1.54	2.91±1.78	1.67±0.79
	800 mg/l	45.28±4.12**	95.76±1.23	1.78±0.66	1.91±0.5	0.55±0.4
	1000 mg/l	33.95±3.33***	100±0	0	0	0
Control		51.58±1.5	93.42±2.91	2.51±1.16	1.9±0.96	2.17±0.92

The data presented in Table 2 show that in all experimental samples (except the lowest concentration of sweetener) the values of the mitotic index are lower than the value in the control sample. The preservative sodium benzoate has the strongest effect on reducing the intensity of cell division. Significant differences in mitotic index values were found at all preservative concentrations studied and at the highest dissolved carmoisine concentration. At a concentration of

aspartame higher than 600 mg/l, the intensity of cell division is significantly reduced in the experimental samples.

The genotoxicity study was performed taking into account structural aberrations of chromosomes and other mitotic abnormality, most often associated with changes in DNA helix and changes in the structure of the dividing spindle, which are visualized as different changes in chromosome behaviour during different stages of mitosis. The analysis of the type and frequency with which the disorders are observed allows characterization and interpretation of the mutagenic effects of the chemical compounds.

The total number and frequency of chromosomal aberrations among dividing cells and among all analyzed cells in the experimental and control samples are presented in Table 3.

Table 3. Total number and frequency of chromosomal aberrations among dividing cells and among all analyzed cells in the experimental and control samples (X±SD) of carmoisine (E 122), sodium benzoate (E 211) and aspartame (E951).

Food additives (concentration)		**Total number of chromosomal aberrations**	**Frequency of chromosomal aberrations among:**	
			Dividing cells	**All analyzed cells**
E 122	0.05 g/l	8±2*	1.66±0.72***	0.76±0.2***
	0.1 g/l	12.8±2.59***	2.58±0.4***	1.25±0.25***
	1 g/l	12.2±3.96***	2.3±0.81***	1.16±0.36***
	10 g/l	12.2±4.38***	4.09±1.55***	1.18±0.42***
E 211	50 mg/l	10.8±5.02**	3.77±3.66**	1±0.46***
	100 mg/l	4.4±9.84	1±2.24	0.43±0.97
	200 mg/l	5.4±3.51	1.5±0.94**	0.52±0.35*
	500 mg/l	-	-	-
E 951	400 mg/l	9.6±4.56*	1.65±0.9***	0.85±0.38***
	600 mg/l	12±4***	2.67±1.07***	1.16±0.39***
	800 mg/l	12.2±1.3***	2.53±0.43***	1.13±0.1***
	1000 mg/l	-	-	-
Control		5.6±2.88	0.53±0.29	0.27±0.24

The total number of chromosomal aberrations in all colorant concentrations was significantly higher than the number of aberrations in the distilled water samples. The lowest concentration of carmoisine showed an average of 8 aberrations per microscopic slide, and in the three higher concentrations the number of cells with different aberrations in the chromosomes or in the structure of the dividing spindle is on average from 12.2 to 12.8.

An interesting dependence was found in the samples with different concentrations of the preservative E211. At the lowest concentration, an average of 10.8 aberrations per microscopic slide were detected. In the next two concentrations (100 mg/l and 200 mg/l) the number of aberrations was 4.4 and 5.4. These values are lower than the value in the control sample, although the differences are insignificant. This can be explained by the low value of the mitotic index and index of metaphase, and by the lack of dividing cells in anaphase and telophase (Table 2). The registered aberrations in these slides are micronuclei and those associated with disorders in the formation of the dividing spindle (C-mitosis).

At the highest concentration of the preservative, dividing cells are found only in prophase. This is also observed in the highest concentration of the sweetener. The dates in Table 2 show that sodium benzoate in concentrations above 200 mg/l and aspartame in concentrations above 1000

mg/l block cell division in prophase. In the lower concentrations of aspartame, an average number of chromosomal aberrations from 9.6 to 12.2 per slide were registered.

More precise indicators of the genotoxic effect of food additives in the cells of the growing root meristem of onion are the percentages of aberrations to the number of dividing cells and the total number of analyzed cells.

At all concentrations of food additives studied, the frequency of chromosomal aberrations was significantly higher than in the control sample. The presence of dividing cells only in prophase, without chromosomal aberrations, is the reason why in the highest concentrations of preservative (E211) and sweetener (E951) no values were recorded for these two indicators.

The specificity in the mutagenic action of the analyzed substances is expressed in the spectrum of chromosomal aberrations that provoke.

All three studied additives induce C-mitosis, lagging and vagrant chromosomes and fragments and micronuclei. Sodium benzoate and aspartame cause the formation of anaphase bridges and diagonal anaphases. Only in the cells of the roots treated with aspartame, pulverized chromosomes and multipolar metaphases and anaphases are found.

Discussion

The mitotic index is the indicator that correlates directly with the intensity of cell division. According to Fiskesjo (1985), the study of cell proliferation activity, expressed by the mitotic index, can detect the presence of cytotoxic effects from various environmental factors (Fiskesjo, 1985). In all experimental samples the values of this indicator are lower than its value in the control sample. An exception is found only in samples with a concentration of aspartame 400 mg/l, but the differences between this value and the one registered in the control sample are insignificant.

At a concentration of aspartame higher than 600 mg/l, the intensity of cell division is significantly reduced in the experimental samples. The preservative sodium benzoate reduces cell proliferation the most. Significant differences in mitotic index values were found at all studied preservative concentrations, as well as at the highest dissolved carmoisine concentration.

De la Cruz et al. (2013) reported a cytotoxic effect of aspartame concentrations of 100, 300 and 400 mg/l on the root meristem of onions and a decrease in the mitotic index depending on the concentration of the sweetener, which is confirmed as a trend also in the present study (Table 2) (De la Cruz et al., 2013).

When treating onion seeds for 24 and 48 hours with 0.25%, 0.5%, 0.75% and 1% solutions of carmoisine, Khan et al. (2020) found that increasing the concentration of the colouring agent decreases the mitotic index. In the study we have conducted we found a similar relationship - the highest concentration of carmoisine of 10 g/l (which corresponds to the highest in the study of Khan et al., 2020 of 1%), is the reason for the lowest value of mitotic index.

A lower value of the mitotic index in the experimental samples compared to the control one demonstrates suppression of cell proliferation and hence the growth and development of organisms affected by the test compounds (Hoshina, 2002; Mahfoz et al., 2010). Samoilov et al. (2019) report a synergistic effect of the colorant carmoisine and the sweetener sucrose, expressed in slowing down the growth of onion roots. Inhibition of cell division may be associated with prolongation of cell differentiation time (Fusconi et al., 2006), decreased protein synthesis or other effects on the apical meristem (Webster & MacLeod, 1996).

The reported structural aberrations of chromosomes and other mitotic abnormalities are evidence of the mutagenic effect of food additives on the DNA molecule and the structures that make up the dividing spindle. They are visualized as different changes in the behaviour of chromosomes during the different stages of mitosis. The analysis of their type and frequency allows to characterize the specifics in the action of the chemical compounds on the chromatin and the cell division apparatus.

The percentage of chromosomal aberrations in relation to dividing cells and in relation to the total number of analyzed cells are the lowest in the control sample, respectively 0.53 ±0.29 and 0.27±0.24. Although low in number and frequency, aberrations of the C-mitosis type and vagrant chromosomes were reported in the control samples (Fig. 1 and 2).

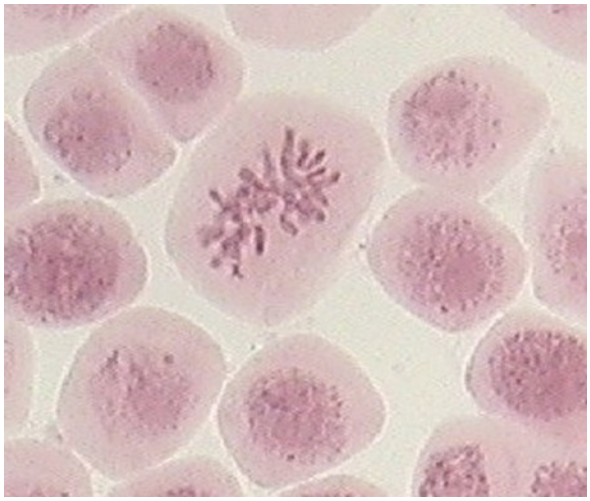

Fig. 1. C-mitosis in control sample of *A. cepa*, magnification x 400.

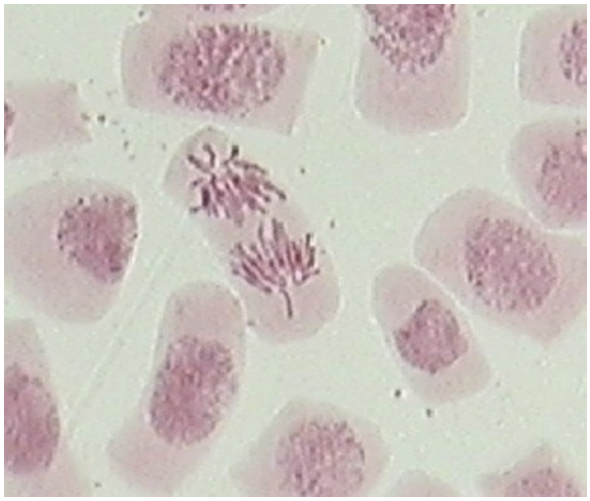

Fig. 2. „Vagrant" chromosome in control sample of *A. cepa*, magnification x 400.

They are due to the automutagenic effect, which is explained by individual characteristics or by peculiarities in the cultivation of the plants. In their study, Azhar et al., 2019 found no chromosomal aberrations in *A. sativum* control cells, but in the *A. cepa* control, micronuclei were found in 0.11% of the cells in interphase.

In all carmoisine solutions, the frequency of chromosomal aberrations was significantly higher than in the control sample (Table 3). Aberrations of the C-mitosis type, lagging and vagrant chromosomes, fragments and cells with micronuclei were found (Fig. 3 and 4).

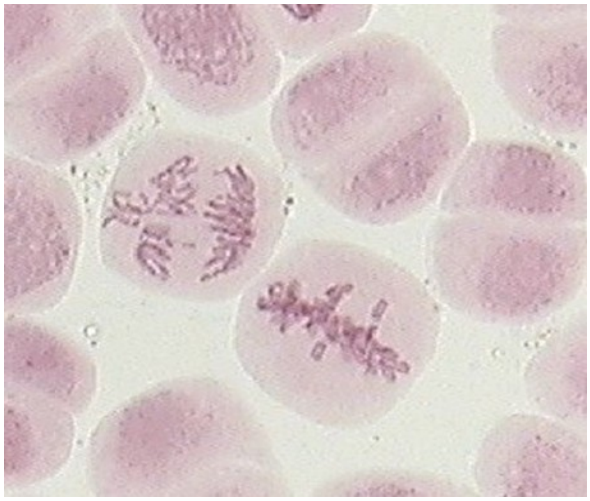

Fig. 3. Lagging chromosomes, vagrant chromosomes and fragments in sample of *A. cepa* treated with carmoisine, magnification x 400.

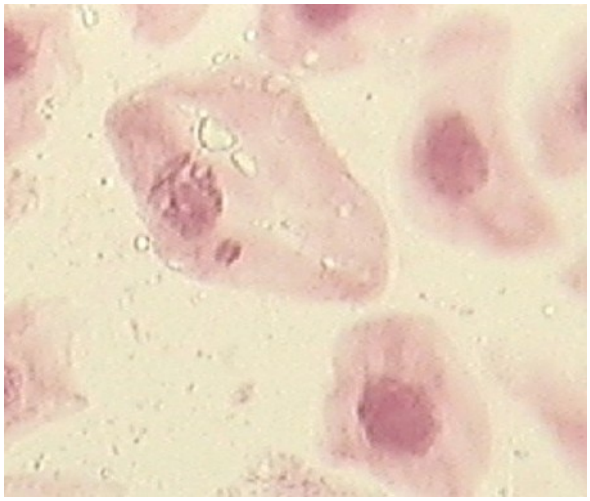

Fig. 4. Micronucleus in sample of *A. cepa* treated with carmoisine, magnification x 400.

In the three lower concentrations of the preservative E211, the frequency of chromosomal aberrations was found to be from 1 to 3.77% in relation to dividing cells and from 0.43 to 1% in relation to the total number of analyzed cells. No dependence of the frequency of aberrations on the concentration of sodium benzoate was established. The frequencies are highest in solutions with a concentration of 50 mg/l, and in the next two concentrations, these percentages decrease.

This unusual trend can be explained by the lower values of the mitotic index and the index of metaphase (sodium benzoate at concentration of 100 mg/l) and the established lack of cells in metaphase, anaphase and telophase (sodium benzoate at concentrations of 200 mg/l and 500 mg/l). At the two highest concentrations of the preservative, dividing cells are detected only in prophase. The registered aberrations are associated with disorders in the formation of the dividing spindle (Fig. 5 and 6) and the micronuclei formation. At the highest concentration of sweetener, the mitotic index is also the lowest and all dividing cells are in prophase. This indicates that sodium benzoate at concentrations above 200 mg/l and aspartame at concentrations above 1000 mg/l block cell division in prophase.

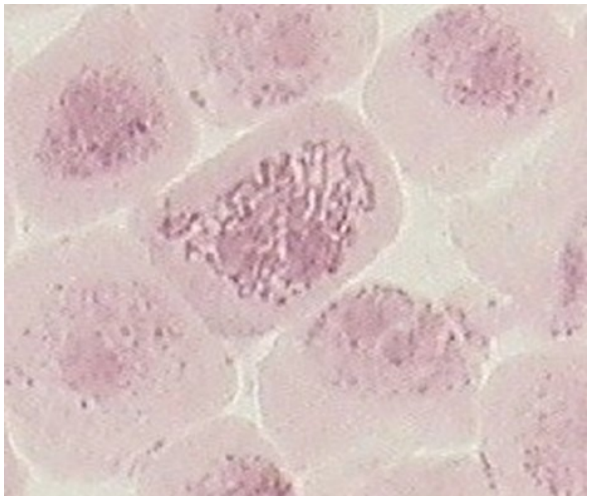

Fig. 5. Disturbed spiralization in cells of *A. cepa*, treated with sodium benzoate, magnification x 400.

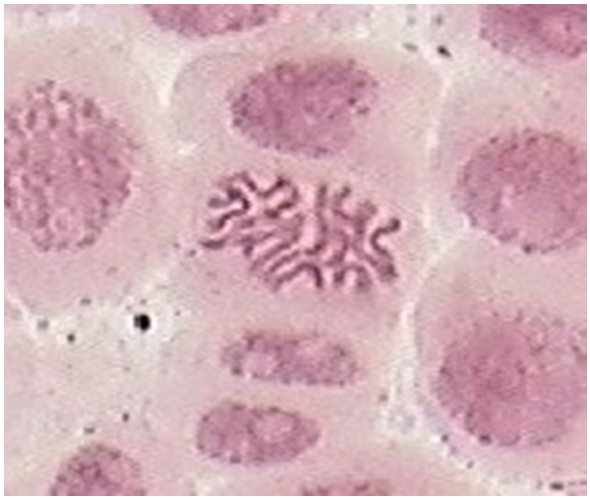

Fig. 6. C-mitosis in cells of *A. cepa*, treated with sodium benzoate, magnification x 400.

Zabka et al. (2012) found that treatment with certain chemicals disrupts the proper course of the cell cycle and activates the mechanisms of control and retention of cell division known as 'reference points'. When cells are exposed to adverse conditions, for example under the influence of a toxic agent, this could disrupt division and lead to abnormal mitosis (Rybaczek et al. 2007).

In addition to the chromosomal aberrations: C-mitosis, lagging and vagrant chromosomes, fragments and cells with micronuclei, found in the samples treated with the colouring agent carmoisine, diagonal anaphases and anaphase bridges were found in the meristem cells of onions under the influence of sodium benzoate (Fig. 7 and 8).

The spectrum of structural mutations in chromosomes and mitotic disorders provoked by the various tested concentrations of aspartame is the widest. The studied sweetener additionally causes deviations of the type: multipolar metaphases and anaphases and pulverized chromosomes (Fig. 9-12).

C-mitosis occurs as a result of improper organization of the microtubules that make up the filaments or the dividing spindle itself (Fiskesjo, 1988; Haliem 1990). The most likely cause of anaphase bridges is adhesion as a result of chromatid separation errors (Gaulden, 1987), caused by

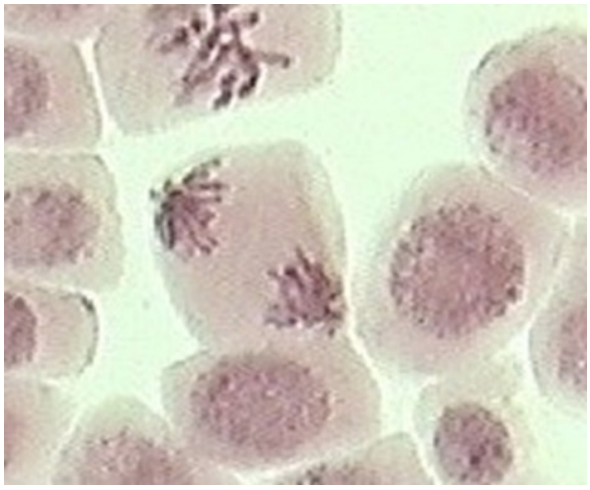

Fig. 7. Diagonal anaphase in cells of *A. cepa*, treated with sodium benzoate, magnification x 400.

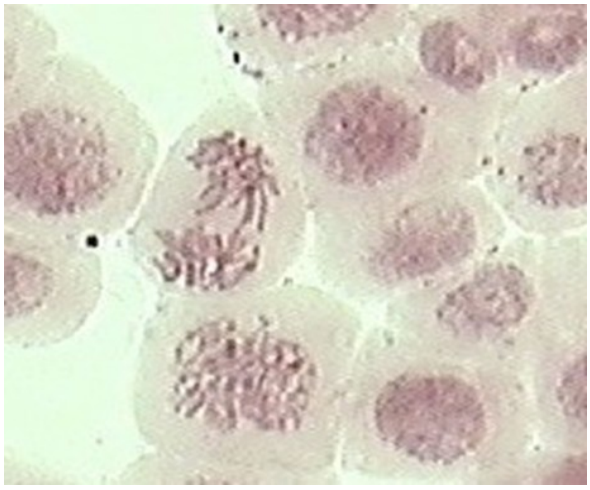

Fig. 8. Anaphase bridge in cells of *A. cepa*, treated with sodium benzoate, magnification x 400.

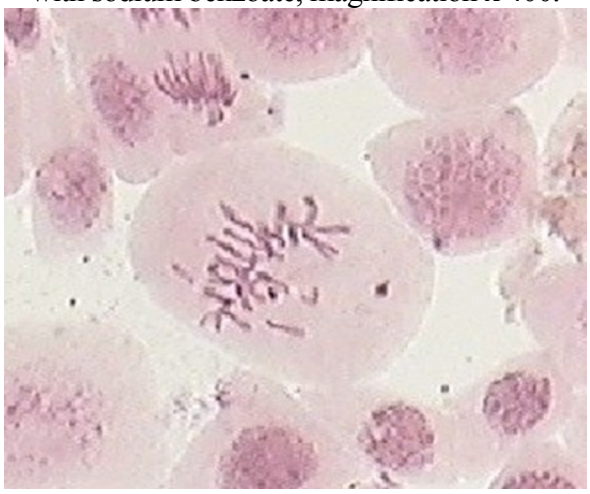

Fig. 9. Lagging chromosomes and fragments in metaphase in cells of *A. cepa* treated with aspartame, magnification x 400.

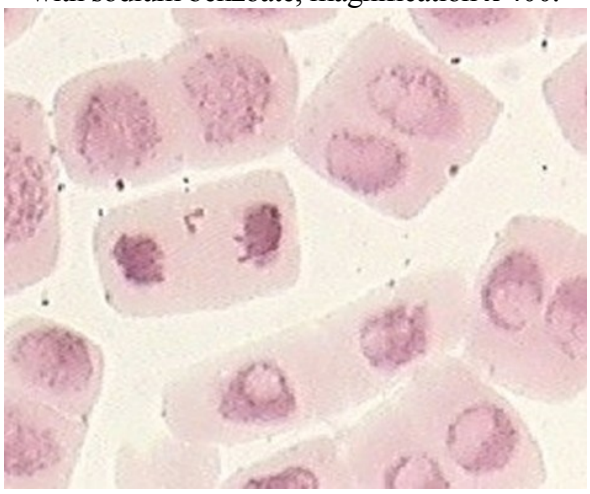

Fig. 10. Acentric fragment in telophase in in cells of *A. cepa* treated with aspartame, magnification x 400.

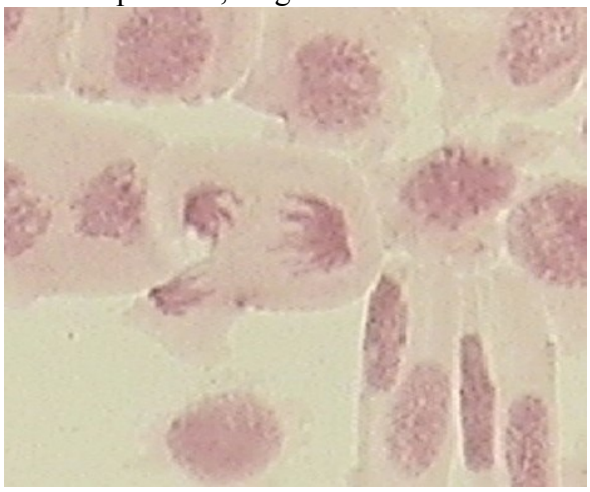

Fig. 11. Multipolar anaphase in cells of *A. cepa*, treated with aspartame, magnification x 400.

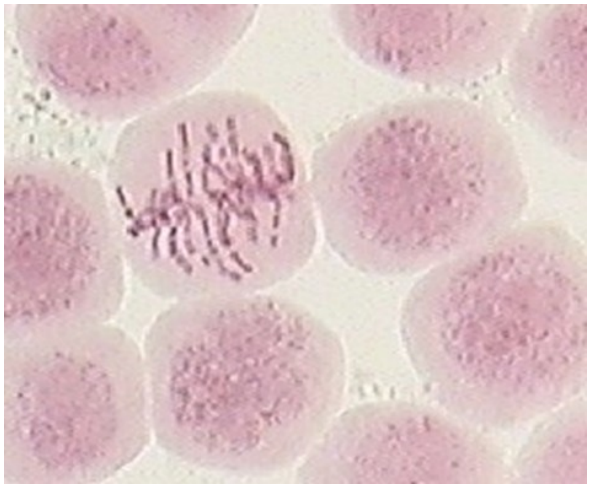

Fig. 12. Pulverized chromosomes in cells of *A. cepa*, treated with aspartame, magnification x 400.

The mutagenic effect of carmoisine has been found in human, murine and bovine cell lines (Zhang & Ma, 2013a Amin et al., 2010; Arvin et al., 2013; Datta et al., 2013).

A study by Macioszek & Kononowicz (2004) showed that some colorants and food additives can cause genotoxic effects in human lymphocyte cells and in the root meristem of *Vicia faba in vivo*. Using the *Allium cepa* – test system, Mahfoz et al. (2010) showed that carmoisine induces a wide range of chromosomal abnormalities in dividing cells. Tripathy & Rao (2015) found decondensation of chromosome arms in *Allium cepa*, treated with the colorant.

Sodium benzoate has a clastogenic and mutagenic effect, demonstrated by applying the Drosophila SMART test, *Allium* – test and in human lymphocyte cell lines (Nair, 2001; Yilmaz et al. 2008; 2009; Zengin et al. 2011). At concentrations up to 4.5x10-5 mol/L, sodium benzoate is intercalated with DNA in the nucleus of thymus cells in cattle (Zhang & Ma 2013b).

In the cells of the root meristem of onion De la Cruz et al. (2013) found a genotoxic effect of aspartame in concentrations of 100, 300 and 400 mg/l. The genotoxicity of increasing aspartame concentrations was observed as polyploidization and hyperamplification in Oreochromis niloticus and *Allium cepa* cells. The elimination of this additional DNA is the probable cause of the formation of nuclear buds, which subsequently turn into micronuclei (Fernandes et al., 2007; Prieto et al., 2008).

Conclusions

As a result of the study conducted and analysis of the results obtained, it can be concluded that carmoisine, sodium benzoate and aspartame used in food production have a cytotoxic effect on the cells of the root meristem of *Allium cepa*. It has been expressed in the suppression of cell division and the reduction of the mitotic index. The studied food additives have a specific genotoxic effect. It has been established by anaphase method and micronucleus mutagenicity test that all three substances cause chromosomal aberrations of the C-mitosis type, 'vagrant' and lagging chromosomes, fragments and micronuclei. Sodium benzoate and aspartame provoke the formation of chromosomal bridges and diagonal anaphases, and only the sweetener aspartame causes the formation of pulverized chromosomes and multipolar metaphases and anaphases.

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