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Assessment of Cytological Effects of Food Preservative Potassium Metabisulphite to *Allium cepa*

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Abstract

With the unprecedented development of the food industry, food preservatives have gained a leading role in food processing. In this study, investigations were carried out to assess the cytological effects of potassium metabisulphite (PMB) to onion (*Allium cepa*), one of the most used plants for determining the cytotoxic and genotoxic effects of different chemicals. Meristematic roots of *A. cepa* were treated with PMB solutions in different concentrations, ranging from 10 mg/l to 35 mg/l for 6, 12 and 24 h alongside an untreated control. The quantified parameters for the different concentrations of the PMB were mitotic index (MI %), mitosis phases index (PI %) and total abnormalities index (TAI %) meaning chromosomal aberrations and nuclear abnormalities. The results indicated that PMB reduced MI in *A. cepa* with increasing the concentrations and time exposure as compared with the untreated control. Thus, at concentration of 10 - 35 mg/l PMB, these values were reduced from 8.34% to 4.81% (6 h); 7.18% to 2.35% (12 h) and 4.12 to 1.43 (24 h). Also, the TAI value increased with increasing PMB concentrations and time: 0.51% to 9.98% (6 h), 7.11% to 19.84% (12 h) and 17.79 to 41.21 (24 h). The types of abnormalities induced by PMB in *A. cepa* meristematic cells were micronucleus, C-metaphase, star anaphase, stickiness, laggards, fragments, binucleated cells and pulverised nucleus. These alarming findings indicate the cytotoxic and genotoxic effect of PMB to *A. cepa* and suggest necessity to adopt more natural alternatives for food preservation in the future.

Keywords

Potassium Metabisulphite, Food Preservative, *A. cepa*, Cytotoxicity, Genotoxicity

1. Introduction

For centuries before the medieval period, and for centuries afterward, human beings in all parts of the world used a variety of methods to preserve foods for later consumption [1]. Due to the very high demand for processed food products, the demand for substances to facilitate the processing of the raw material, the preservation over a longer period of the food products has increased. At the beginning of the 19th century, a rapid rise in the use of chemical additives has been observed [2].

Food additives, marked with E letter—due to the alignment with the European Union norms [3] can be identified in most processed food products. They are used in all branches of the food industry. Regulation (EC) No 1169/2011 of the European Parliament and of the Council provide food information that shall pursue a high level of protection of consumers' health and interests by providing a basis for final consumers to make informed choices and to make safe use of food, with particular regard to health, economic, environmental, social and ethical considerations [3].

The usefulness of food additives is that they maintain the quality and safety of the products for a longer period of time, maintain or improve the taste of the products, ensure the control of the acidity and alkalinity of the products, maintain the consistency of the products, the aroma or colour of the food, etc. [4]. Preservatives, one of the categories of food additives, protect food by slowing down the damage caused by bacteria, fungi, yeast and air. A preservative is defined as any substance which is capable of inhibiting, retarding, or arresting, the growth of micro-organisms, of any deterioration of food due to micro-organisms, or of masking the evidence of any such deterioration [5].

Food safety is a global problem, and a large number of consumers worldwide face a variety of food safety risks each year [6]. Scientific food management is an important tool to avoid severe health issues [7] [8] [9]. Food safety affects consumer food choice in ways that are different from other dimensions of quality [10]. The use of chemical preservatives is regulated by maximum permitted levels. These amounts vary between countries. When the food additives are given to organisms in excessive amounts, they may cause toxic reactions [11] [12] [13]. Certain food additives may have harmful effects and may lead to headache, nausea, weakness, difficulty in breathing [14] or gastrointestinal, dermatologic and neurologic adverse reactions [15] [16].

Potassium metabisulphite (PMB) also known as potassium pyrosulphite or potassium disulphite is a white crystalline powder with a sulphur odour and is chemically very similar to sodium metabisulphite, with which it is sometimes used interchangeably. The use of PMB is not universally accepted [17] but is widely used in the wine industry, and in food it is found in many types of biscuits and cakes. It can also be found in fast food and pickles, dried fruits, etc. The maximum daily dose for E 224 (PMB) is 0.7 mg/kg [18].

This study aimed to estimate the cytotoxic and genotoxic potential of PMB

using the *Allium* assay. *Allium cepa* is the most common species used for the assessment of toxicants and their harmful effects on environment as well as human health [19] [20] [21] [22].

Potassium metabisulphite has been found to be mitotoxic as the mitotic index decreases with the increase in the concentration of the food preservative and the treatment period [23].

2. Materials and Methods

2.1. Plant Material and Chemical Food Preservative

For this study, healthy and equal sized bulbs (30 - 35 g weight) of *A. cepa* (2n = 16) were chosen and placed in small glasses with the basal ends dipping in distilled water for 72 hours, time required for the meristematic roots occurrence. A number of 10 bulbs of *A. cepa* were used for each treatment variant alongside an untreated control.

The new emerged roots (10 - 15 mm in length) were treated with PMB (10, 15, 25 and 35 mg/l) diluted with distilled water for 6, 12 and 24 hours at room temperature (24˚C ± 2˚C). *A. cepa* bulbs were purchased from central market of Craiova and PMB was purchased from Merk Romania Company.

The chemical properties of PMB are the following: chemical formula: K2S2O5; molecular weight: 222.33 g/mol; solubility in water (20˚C): 450 g/l; density: 2.34 g/cm^3; CAS no. 16731-55-8; EINECS no. 240-795-3; melting point: 190˚C.

2.2. Microscopic Preparations

After each exposure time, the meristematic roots were cut with sharp blade, fixed in Carnoy fixative (ethyl alcohol:glacial acetic acid 3:1) and hydrolysed in 1N hydrochloric acid (HCl) for 5 min. The roots tips were stained using 5 ml of Schiff Reagent for 45 min at room temperature and then were crushed in drop of 2% acetocarmine. The microscopic preparations were performed by squash technique. For this purpose, the slide was placed and coverslip on a double layer of paper towel, then paper was folded over the coverslip and squash down on the coverslip with a strong vertical pressure, using thumb. The pressure was applied to squash the root tip into a single cell layer. Five replicates were made for each concentration.

The microscopic slides were examined at 1000× magnification.

2.3. Statistical Analyses

Minimum 1000 cells were scored in the roots tip for each concentration. The mitotic index (MI %) was calculated for each treatment as the number of dividing cells/1000 observed cells. MI is used to quantify the differences in cell division when environmental parameters are changed. The phases of mitosis division (prophase, metaphase, anaphase and telophase) were also observed. The phase index (%) was calculated as the number of cells in each phase/the total

number of cells ×100. Total abnormalities index (TAI %) meaning chromosomal aberrations and nuclear anomalies were calculated as the number of aberrant cells/the total number of cells in division ×100.

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%, 0.01% and 0.001% subsequent to the ANOVA analysis.

Light microscopy analyses were used to identify the cytological changes in stages of mitosis as well as the occurrence of some chromosomal aberrations and nuclear abnormalities in *A. cepa* meristematic cells after treatment with PMB. The most representative aberrant cells were photographed with a Canon camera model Prima Super 105X (Canon, Melville, NY, USA).

3. Results

3.1. Mitotic Index and Mitosis Phases Index

Treatment of the meristematic roots of *A. cepa* with PMB showed a strong mitodepressive effect even in lower concentrations (Table 1).

The MI decreased with increase in concentration of PMB and exposure time in every duration treatment. The control value of MI after 6, 12 and 24 hours was 12.39%, 11.21% and 12.92%. This values were dropped from 8.34% to 4.81% (at 6 h after treatment and 10 - 35 mg/l PMB), 7.18% to 2.35% (12 h after treatment and 10 - 35 mg/l PMB) and 4.12 to 1.43 (24 h after treatment and 10 - 35 mg/l PMB), respectively. It was noted that the rate of each one of the mitotic phases was affected by PMB treatments. Thus, PMB increased the percentage of prophase and metaphase when compared with the control after all concentrations and exposure time. Exceptions were only the variant with 35 mg/l PMB and 12 hours of exposure, as well as the variant of 15 mg/l PMB and 24 hours of exposure, where the metaphase index was lower than control. On the other hand, the percentage of anaphase and telophase was decreased when compared with the control after all concentrations and exposure time.

3.2. Cytological Abnormalities

Genotoxic effect of PMB to *A. cepa* was manifested by the appearance of several types of cytological abnormalities (chromosomal aberrations and nuclear anomalies) (Table 2). Their percentages increased as the concentration of the PMB and the exposure time increased when compared with the control. The main types of abnormalities induced by PMB in *A. cepa* meristematic cells were micronucleus, C-metaphase, star anaphase, stickiness, laggards, fragments, binucleated cells and pulverised nucleus, aspects highlighted on the microscopic images through arrows. Thus, changes that occur at the chromosome or nuclear level are indicated (Figure 1). The most common type of abnormality identified was micronucleus followed by stickiness.

Table 1. Mitotic index and mitotic phases index in *A. cepa* meristematic cells after treatment with different concentrations of PMB at different exposure times.

Time (h)	Concentration (mg/l)	MI (%) ± SD	IP (%)	IM (%)	IA (%)	IT (%)
6	Control	12.39 ± 0.48	42.68 ± 0.12	19.16 ± 0.16	12.56 ± 0.05	25.60 ± 0.16
	10	8.34 ± 0.34	42.96 ± 0.14	24.95 ± 0.10	9.36 ± 1.02	22.73 ± 0.14
	15	8.01 ± 0.85	43.03 ± 1.10	24.98 ± 0.12	9.51 ± 0.09	22.48 ± 0.10
	25	6.08 ± 0.51	44.14 ± 0.09	26.58 ± 0.21	9.21 ± 0.04	20.07 ± 0.12
	35	4.81 ± 0.14*	46.15 ± 1.11	24.38 ± 0.16	9.16 ± 0.07	20.01 ± 0.11
12	Control	11.21 ± 0.56	41.27 ± 1.81	20.22 ± 0.09	14.49 ± 0.14	24.02 ± 0.09
	10	7.18 ± 0.74	41.84 ± 1.04	22.44 ± 0.24	12.20 ± 0.12	23.52 ± 0.16
	15	5.19 ± 0.36	42.65 ± 0.19	22.91 ± 0.09	10.43 ± 0.07	24.01 ± 0.09
	25	2.66 ± 0.22**	44.04 ± 1.22	20.84 ± 0.17	12.37 ± 0.09	22.75 ± 0.16
	35	2.35 ± 0.21**	46.71 ± 1.15	19.86 ± 0.12	6.73 ± 0.19	20.12 ± 0.19
24	Control	12.92 ± 0.91	44.21 ± 1.09	20.11 ± 0.05	12.12 ± 1.01	23.56 ± 0.06
	10	4.12 ± 0.37*	44.75 ± 0.17	23.22 ± 0.09	8.53 ± 0.04	23.50 ± 0.12
	15	3.22 ± 0.42*	50.46 ± 1.26	18.74 ± 0.10	8.79 ± 0.06	22.01 ± 1.02
	25	1.79 ± 0.36***	53.19 ± 1.45	21.710.25	6.64 ± 0.09	18.46 ± 0.11
	35	1.43 ± 0.34***	53.82 ± 1.64	22.62 ± 0.12	1.61 ± 1.24	21.95 ± 0.18

MI = mitotic index; SD = standard deviation; IP = index of prophase; IM = index of metaphase; IA = index of anaphas; IT = index of telophase; significantly different from control: *p < 0.05; **p < 0.01; *** p < 0.001.

Table 2. Mitotic index and mitotic phases index in *A. cepa* meristematic cells after treatment with different concentrations of PMB at different exposure times.

Time (h)	Conc. (mg/l)	CM	MN	S	L	F	SA	BN	PN	TAI
6	Control	-	0.11	-	-	-	-	-	-	0.11
	10	-	0.18	0.33	-	-	-	-	-	0.51
	15	-	-	0.48	-	-	-	-	-	0.48
	25	0.16	1.65	2.42	0.17	-	-	1.43	-	5.83
	35	1.67	1.96	2.74	0.45	0.55	-	2.61	-	9.98
12	Control	-	0.21	0.16	-	-	-	-	-	0.37
	10	-	1.14	1.07	1.06	1.09	-	2.75	-	7.11
	15	1.08	2.01	1.54	1.52	1.33	0.98	2.89	0.74	12.09
	25	1.65	3.74	2.96	1.98	2.04	1.04	2.96	1.08	17.45
	35	1.79	4.02	3.20	2.33	2.65	1.55	3.01	1.29	19.84
24	Control	-	0.38	0.18	0.06	-	-	-	-	0.62
	10	1.84	1.67	3.01	2.39	2.01	1.75	3.08	2.04	17.79
	15	2.32	2.12	3.24	2.64	2.87	2.45	3.21	2.34	21.19
	25	5.84	4.96	4.33	3.33	3.04	3.12	3.42	4.20	32.24
	35	8.34	6.22	5.24	5.62	3.12	3.58	4.21	4.88	41.21

CM = C-mitosis; MN = micronucleus; S = sticky chromosomes; L = laggards chromosomes; F = fragments; SA = star anaphase; BN = binucleated cells; PN = pulverised nucleus; TAI = total abnormalities index.

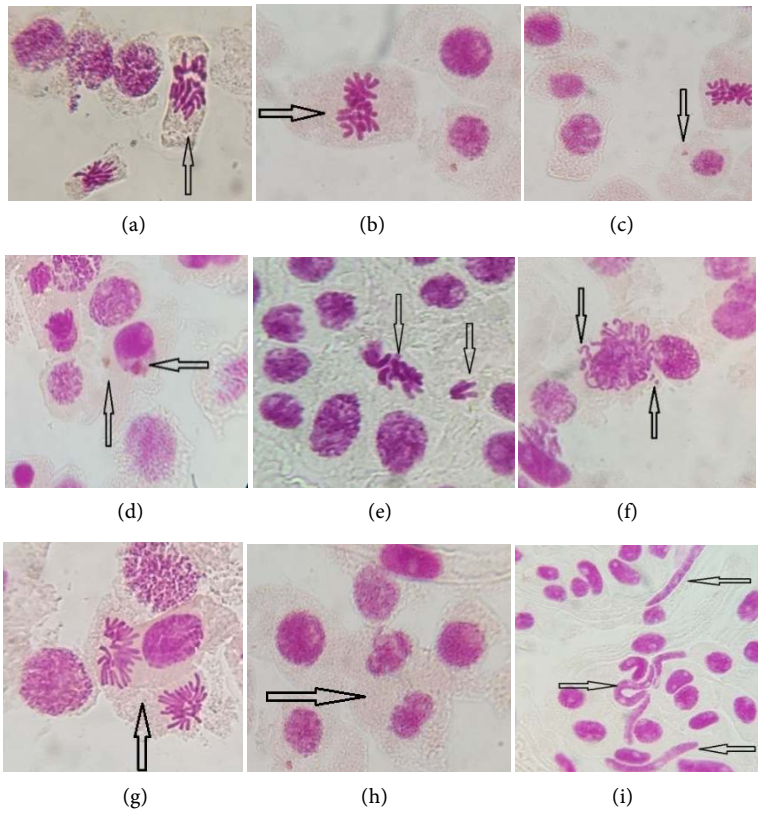

Figure 1. Some cytological abnormalities identified in meristematic cells of *A. cepa* exposed to different concentration of PBM food preservative: cells with C-metaphase indicated by arrow (a) (b); micronucleus indicated by arrows (c) (d); sticky metaphase (bigger arrow) with lost chromosomes (smaller arrow) (e); laggard (arrow above) and fragments (arrow below) in disturbed metaphase (f); cell with star anaphase indicated by arrow (g); binucleated cell indicated by arrow (h) and pulverised nucleus indicated by arrows (i).

Very few cytogenetic abnormalities were detected in the control variants, namely 0.11% micronucleus at 6 h; 0.21% micronucleus and 0.16% sticky chromosomes at 12 h; 0.38% micronucleus, 0.18% sticky chromosomes and 0.06% laggards at 24 h. In contrast, the progressive increase of the micronuclei frequency was observed as the exposure time and PMB food preservative concentration was increased. The highest values from this point of view were recorded at concentrations of 35 mg/l PMB at all three exposure periods, namely: 1.96% at 6 h; 4.02% at 12 h and 6.22% at 24 h exposure time.

TAI (%) meaning chromosomal aberrations and nuclear anomalies has extremely increase, the recorded values being from 0.51% to 9.98% (at 6 h after treatment and 10 - 35 mg/l PMB), 7.11% to 19.84% (12 h after treatment and 10 - 35 mg/l PMB) and 17.79 to 41.21 (24 h after treatment and 10 - 35 mg/l PMB), respectively.

4. Discussion

In the present investigation, we studied the cytotoxic and genotoxic effects of

PMB food preservative in *A. cepa* root-tips using MI (%) and TAI (%) as the toxicological endpoints.

The mitotic index decreased considerably in the different treatments. It showed the mitodepressive activity of PMB. Cytological studies revealed statistically significant ($p < 0.05$) inhibition in mitotic index with an increase in concentration of the PMB when compared with the control. These findings are in agreement with other studies which show the mitodepressive effect of different preservatives in *A. cepa* cells [11] [24]. This could be due to a movement made slower of cells from synthesis phase to mitosis phase of the cell cycle as a result of PMB exposure and its potential to interfere with intracellular components of *A. cepa*. As some authors suggest, decrease in mitotic index might be due to inhibition of DNA synthesis [25] or a blocking in the G2-phase of the cell cycle, preventing the cell from entering mitosis [24].

MI is an acceptable measure of cytotoxicity in all living organisms [26] [27]. The cytotoxicity level can be determined by the decreased rate of MI. In this respect, a decrease of MI below 22% in comparison to controls can have a lethal impact on the organism [28], while a decrease below 50% usually has sublethal effects [29]. In present study, the percentage of anaphase and telophase was decreased when compared with the control groups after the all PMB concentrations and exposure time. This may attribute to the blocking of cell division by the PMB food preservative at the end of the metaphase stage and suggests delaying cell cycle.

Generally speaking, for genotoxicity assessment the magnitude of genetic risk to organisms by environmental agents/ chemicals under a specified level of exposure must be determined [30]. Some authors consider that the plant system can detect the genotoxicity and cytotoxicity more quickly than animal bioassays being optimal to environmental monitoring in general [31].

In our study, cytotoxic and genotoxic effects of PMB on *A. cepa* meristematic roots were indicated at all tested concentrations and all exposure times. All PMB treatments significantly decreased the mitotic activity and increased the frequency of total abnormalities (chromosomal aberrations and nuclear anomalies). In terms of the frequency, the most identified abnormalities were micronuclei in cell interphase. Hamedo and Abdelmigid (2009) considered that micronucleus formation is one of the most economical and most effective ways in determining genotoxicity of different chemicals [32]. Also, the micronucleus assay is recognized as one of the most successful and reliable assays for genotoxic carcinogens that act by causing genetic damage [33] [34] or the most prevalent biomarker of chromosomal defects induced by genotoxic agents [35]. An individual predisposition to diseases is correlated with micronucleus incidence [36] [37]. A variety of genotoxic agents may induce micronucleus formation leading to cell death, genomic instability, or cancer development [35] [38]. A micronucleus may arise from a whole lagging chromosome or an acentric chromosome fragment detaching from a chromosome after breakage which does not integrate in the daughter nuclei [39]. Pollution of the environment with various chemical

agents increases the frequency of micronucleus formation in both plants and animal cells, increasing the ecotoxicological risk [40] [41]. In accordance with Gömürgen (2005), such genetic damages like micronuleus may have clastogenic effect of their inducers [23]. Micronuclei were considered as an indication of a true mutation effect [42] thus, the high percentage of the micronuclei induced by PMB on *A. cepa* cells suggest their mutagenic effect. Therefore, the appearance of micronuclei in meristematic cells of *A. cepa* may suggest caution in the consumption of foods which containing PMG food preservative.

Chromosome stickiness may result from entanglement of chromatin fibers, which fall to condense properly in preparation for cell division [43] [44] [45]. Stickiness can cause serious changes in nucleic acids physico-chemical properties or chromatin condensation of the nucleus [46]. Also, sticky chromosomes can cause loss of genetic material and subsequently, the cell-division process occurs irregularly, with some chromosomes not adhering to the assembled chromosomal complex and being lost during the cell cycle. Laggard's chromosomes induction is due to delayed termination of chromosome end or because of failure of chromosomal movement and attributed to the spindle apparatus [46] [47].

A growing number of consumers are aware of the potential negative health effects of chemical preservatives, which has prompted the food industry to find natural products used and developed as alternatives [48]. Concerns about synthetic preservatives have caused increasing interest in natural antioxidants [49]. Some study showed that natural antimicrobial extracts from hot peppers are rich in antioxidant and antiradical compounds [49]. Also, the mixture of natural antibacterial extracts could be used as food treatment to extend the shelf-life of pre-cut carrots by two days without affecting their sensory properties [2].

A. cepa is one of leading vegetable crops in the world and the allelopathic potential and biological characteristics of this species have been deeply investigated. Allelopathy refers to the beneficial or harmful effects of one plant on another plant through releasing of biochemical from plant parts [50] [51] [52] [53]. Plants have the ability to synthesize chemical compounds (active principles), their active properties being correlated with the biochemical mechanisms of human metabolism [54] and this must be taken into account in the future when processing and preserving food. Depending on plant type and bacterial strain, essential oil derivatives could have a high antibacterial activity and they can behave as natural preservatives [2].

The processing of some foods like the fresh-cut fruits includes key preservation techniques, namely the use of packaging with modified atmosphere that can be improved with the addition of antimicrobial and antioxidant agents allowing, in combination with low storage temperature, an extended shelf life, at the same time preserving their nutritional and organoleptic proprieties [55]. From this point of view, the use of edible coatings supplemented with essential oils can constitute in the future a promising approach to improve both preservation and safety of fresh-cut fruits [55].

As some authors stated, if food preservatives is judiciously used, they are an important group of substances that help to raise the keeping quality, the nutritional value and the aesthetic qualities of foods [56] [57]. On the other hand, technological processes that are used for food preservation may affect the functional, nutritional, and biological properties of food proteins [58]. In the future, new preservation technologies will have the purpose not just working to increase longevity; they will work also functioning to sustain the same qualities of the food that make it desirable in the first place [55]. The physical characteristics and chemical composition of the food will no longer be compromised during the preservation process [55].

5. Conclusion

All present results revealed the predominance cytotoxic and genotoxic effect of PMB on *A. cepa*, which were concentrated and time-dependent. Our study about PMB food preservative showed that it cannot assess the actual level of risk for consumer's health. However, disturbed mitosis and the large number of cytological abnormalities identified in meristematic cells of *A. cepa* exposed to action of this food preservative can be a warning sign and suggest necessity to adopt more natural alternatives for food preservation in the future. We intend to test other usual chemical preservatives in terms of their cytological effects on meristematic cells of plants.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Škrinjar, M. and Nemet, T. (2009) Antimicrobial Effect of Spices and Herbs Essential Oils. *Acta Periodica Technologica*, **40**, 195-209. https://doi.org/10.2298/APT0940195S

[2] Ben-Fadhel, Y., Maherani, B., Aragones, M. and Lacroix, M. (2019) Antimicrobial Properties of Encapsulated Antimicrobial Natural Plant Products for Ready-to-Eat Carrots. *Foods*, **8**, 535. https://doi.org/10.3390/foods8110535

[3] Regulation (EU) No. 1169 of the European Parliament and of the Council (2011) https://eur-lex.europa.eu/legal-content

[4] Abdel Ghany, T.M. (2015) Safe Food Additives. A Review. *Journal of Biological and Chemical Research*, **32**, 402-437.

[5] Inetianbor, J.E., Yakubu, J.M. and Ezeonu, S.C. (2015) Effects of Food Additives and Preservatives on Man—A Review. *Asian Journal of Science and Technology*, **6**, 1118-1135.

[6] Sarig, G., Michaeli, Y., Lanir, N., Brenner, B. and Haim, N. (2003) Mechanisms for Acquired Activated Protein C Resistance in Cancer Patients. *Journal of Thrombosis and Haemostasis*, **1**, P0823.

[7] Butnariu, M. and Caunii, A. (2013) Design Management of Functional Foods for Quality of Life Improvement. *Annals of Agricultural and Environmental Medicine*,

20, 736-741.

[8] Bonciu, E. (2017) Food Processing, a Necessity for the Modern World in the Context of Food Safety: A Review. *Annals of the University of Craiova-Agriculture*, *Montanology*, *Cadastre Series*, **47**, 391-398.

[9] Rosculete, E. and Rosculete, C.A. (2017) Organoleptic and Physico-Chemical Analyses on Apple Fruits-Comparative Study. *Annals of the University of Craiova-Agriculture*, *Montanology*, *Cadastre Series*, **47**, 238-243.

[10] Varela, P. and Fiszman, S.M. (2013) Exploring Consumers' Knowledge and Perceptions of Hydrocolloids Used as Food Additives and Ingredients. *Food Hydrocolloids*, **30**, 477-484. https://doi.org/10.1016/j.foodhyd.2012.07.001

[11] Turkoğlu, Ş. (2007) Genotoxicity of Five Food Preservatives Tested on Root Tips of *Allium cepa* L. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis*, **626**, 4-14. https://doi.org/10.1016/j.mrgentox.2006.07.006

[12] Turkoğlu, Ş. (2008) Evaluation of Genotoxic Effects of Sodium Propionate, Calcium Propionate and Potassium Propionate on the Root Meristem Cells of *Allium cepa*. *Food and Chemical Toxicology*, **46**, 2035-2041. https://doi.org/10.1016/j.fct.2008.01.043

[13] Türkoğlu, Ş. (2009) Genotoxic Effects of Mono-, Di-, and Trisodium Phosphate on Mitotic Activity, DNA Content, and Nuclear Volume in *Allium cepa* L. *Caryologia*, **62**, 171-179. https://doi.org/10.1080/00087114.2004.10589683

[14] Sharma, M., Rajput, A., Rathod, C. and Sahu, S. (2018) Food Chemicals Induces Toxic Effect on Health: Overview. *Pharmaceutical and Biosciences Journal*, **6**, 33-37. https://doi.org/10.20510/ukjpb/6/i4/177335

[15] Wilson, B.G. and Bahna, S.L. (2005) Adverse Reactions to Food Additives. *Annals of Allergy*, *Asthma & Immunology*, **95**, 499-507. https://doi.org/10.1016/S1081-1206(10)61010-1

[16] Randhawa, S. and Bahna, S.L. (2009) Hypersensitivity Reactions to Food Additives. *Current Opinion in Allergy and Clinical Immunology*, **9**, 278-283. https://doi.org/10.1097/ACI.0b013e32832b2632

[17] Cauvain, S.P. (2017) Baking Problems Solved. Woodhead Publishing, Sawston, Cambridge.

[18] EFSA Journal. Safety Assessment of the Active Substance Potassium Metabisulfite, for Use in Active Food Contact Materials. https://efsa.onlinelibrary.wiley.com

[19] Mangalampalli, B., Dumala, N. and Grover, P. (2017) *Allium cepa* Root Tip Assay in Assessment of Toxicity of Magnesium Oxide Nanoparticles and Microparticles. *Journal of Environmental Sciences*, **66**, 125-137. https://doi.org/10.1016/j.jes.2017.05.012

[20] Bonciu, E. (2018) Evaluation of Cytotoxicity of the Herbicide Galigan 240 EC to Plants. *Scientific Papers. Series A. Agronomy*, **61**, 175-178.

[21] Bonciu, E., Firbas, P., Fontanetti, C.S., Wusheng, J., Karaismailoğlu, M.C., Liu, D., Menicucci, F., Pesnya, D.S., Popescu, A., Romanovsky, A.V., Schiff, S., Ślusarczyk, J., de Souza, C.P., Srivastava, A., Sutan, A. and Papini, A. (2018) An Evaluation for the Standardization of the *Allium cepa* Test as Cytotoxicity and Genotoxicity Assay. *Caryologia*, **71**, 191-209. https://doi.org/10.1080/00087114.2018.1503496

[22] Rosculete, C.A., Bonciu, E., Rosculete, E. and Olaru, L.A. (2019) Determination of the Environmental Pollution Potential of Some Herbicides by the Assessment of Cytotoxic and Genotoxic Effects on *Allium cepa*. *International Journal of Environmental Research and Public Health*, **16**, 75. https://doi.org/10.3390/ijerph16010075

[23] Gömürgen, A.N. (2005) Cytological Effect of the Potassium Metabisulphite and Po-

tassium Nitrate Food Preservative on Root Tips of *Allium cepa* L. *Cytologia*, **70**, 119-128.

[24] Pandey, H., Kumar, V. and Roy, B.K. (2014) Assessment of Genotoxicity of Some Common Food Preservatives Using *Allium cepa* L. as a Test Plant. *Toxicology Reports*, **1**, 300-308. https://doi.org/10.1016/j.toxrep.2014.06.002

[25] Sudhakar, R., Gowda, N. and Venu, G. (2001) Mitotic Abnormalities Induced by Silk Dyeing Industry Effluents in the Cells of *Allium cepa*. *Cytologia*, **66**, 235-239.

[26] Smaka-Kinel, V., Stegnar, P., Lovaka, M. and Toman, J. (1996) The Evaluation of Waste, Surface and Ground Water Quality Using the *Allium* Test Procedure. *Mutation Research*, **368**, 171-179. https://doi.org/10.1016/S0165-1218(96)90059-2

[27] Prajitha, V. and Thoppil, J.E. (2016) Genotoxic and Antigenotoxic Potential of the Aqueous Leaf Extracts of *Amaranthus spinosus* Linn. Using *Allium cepa* Assay. *South African Journal of Botany*, **102**, 18-25. https://doi.org/10.1016/j.sajb.2015.06.018

[28] Antonsiewicz, D. (1990) Analysis of the Cell Cycle in the Root Meristem of *Allium cepa* under the Influence of Ledakrin. *Folia Histochemica et Cytobiologica*, **28**, 79-95.

[29] Panda, B.B. and Sahu, U.K. (1985) Induction of Abnormal Spindle Function and Cytokinesis Inhibition in Mitotic Cells of *Allium cepa* by the Organophosphorus Insecticide Fensulfothion. *Cytobios*, **42**, 147-155.

[30] Khanna, N. and Sharma, S. (2013) *Allium Cepa* Root Chromosomal Aberration Assay: A Review. *Indian Journal of Pharmaceutical and Biological Research*, **1**, 105-119.

[31] Sobieh, S.S. (2019) Investigation of Mitodepressive and Clastogenic Potentials of Diatomaceous Earth Based on Plant Bioassay. *The Egyptian Journal of Botany*, **59**, 1-14.

[32] Hamedo, H.A. and Abdelmigid, H.M. (2009) Use of Antimicrobial and Genotoxicity Potentiality for Evaluation of Essential Oils as Food Preservatives. *The Open Biotechnology Journal*, **3**, 50-56. https://doi.org/10.2174/1874070700903010050

[33] Lorge, E., Hayashi, M., Albertini, S. and Kirkland, D. (2008) Comparison of Different Methods for an Accurate Assessment of Cytotoxicity in the *in Vitro* Micronucleus Test. Theoretical Aspects. *Mutation Research*, **655**, 1-3. https://doi.org/10.1016/j.mrgentox.2008.06.003

[34] Akremi, N., Cappoen, D., Anthonissen, R., Bouraoui, A. and Verschaeve, L. (2016) Evaluation of the Genotoxicity and Cytotoxicity of Semipurified Fractions from the Mediterranean Brown Algae, *Dictyopteris membranacea*. *Pharmacognosy Magazine*, **12**, 395-399. https://doi.org/10.4103/0973-1296.188318

[35] Luzhna, L., Kathiria, P. and Kovalchuk, O. (2013) Micronuclei in Genotoxicity Assessment: From Genetics to Epigenetics and Beyond. *Frontiers in Genetics*, **4**, 131. https://doi.org/10.3389/fgene.2013.00131

[36] Andreassi, M.G., Botto, N., Cocci, F., Battaglia, D., Antonioli, E., Masetti, S., Manfredi, S., Colombo, M.G., Biagini, A. and Clerico, A. (2003) Methylenetetrahydrofolate Reductase Gene C677T Polymorphism, Homocysteine, Vitamin B12, and DNA Damage in Coronary Artery Disease. *Human Genetics*, **112**, 171-177. https://doi.org/10.1007/s00439-002-0859-3

[37] Speit, G. and Trenz, K. (2004) Chromosomal Mutagen Sensitivity Associated with Mutations in BRCA Genes. *Cytogenetic and Genome Research*, **104**, 325-332. https://doi.org/10.1159/000077511

[38] Kirsch-Volders, M., Vanhauwaert, A., Eichenlaub-Ritter, U. and Decordier, I.

(2003) Indirect Mechanisms of Genotoxicity. *Toxicology Letters*, **140**-**141**, 63-74. https://doi.org/10.1016/S0378-4274(02)00498-8

[39] Terradas, M., Martin, M., Tusell, L. and Genesca, A. (2010) Genetic Activities in Micronuclei: Is the DNA Entrapped in Micronuclei Lost for the Cell? *Mutation Research*, **705**, 60-67. https://doi.org/10.1016/j.mrrev.2010.03.004

[40] Barbosa, J.S., Cabral, T.M., Ferreira, D.N., Agnez-Lima, L.F. and de Medeiros, S.R. (2010) Genotoxicity Assessment in Aquatic Environment Impacted by the Presence of Heavy Metals. *Ecotoxicology and Environmental Safety*, **73**, 320-325. https://doi.org/10.1016/j.ecoenv.2009.10.008

[41] Dourado, P.L.R., Rocha, M.P., Roveda, L.M., Raposo Junior, J.L., Cândido, L.S., Cardoso, C.A.L., Morales, M.A.M., Oliveira, K.M.P. and Grisolia, A.B. (2017) Genotoxic and Mutagenic Effects of Polluted Surface Water in the Midwestern Region of Brazil Using Animal and Plant Bioassays. *Genetics Molecular Biology*, **40**, 123-133. https://doi.org/10.1590/1678-4685-gmb-2015-0223

[42] El-Shahaby, O.A., Abdel Migid, H.M., Soliman, M.I. and Mashaly, I.A. (2003) Genotoxicity Screening of Industrial Wastewater Using the *Allium cepa* Chromosome Aberration Assay. *Pakistan Journal of Biological Sciences*, **6**, 23-28. https://doi.org/10.3923/pjbs.2003.23.28

[43] Joti, Y., Hikima, T., Nishino, Y., Kamada, F., Hihara, S., Takata, H., Ishikawa, T. and Maeshima, K. (2012) Chromosomes without a 30-nm Chromatin Fiber. *Nucleus*, **3**, 404-410. https://doi.org/10.4161/nucl.21222

[44] El-Ashry, Z.M. (2003) Effects of Vitamin A on Phosphamidon-Induced Mutagenesis in *Vicia faba* L. *Egyptian Journal of Genetics And Cytology*, **32**, 303-316.

[45] Moustafa, M.F., Alrumman, S.A., Zaki, H., Loutfy, N. and Hashem, M. (2016) Cyto-Physiological Effects of Aqueous Extracts of Some Weeds and Clove on the Growth of Chinese Faba Bean (*Vicia faba* L.). *Journal of Advances in Biology & Biotechnology*, **9**, 1-8. https://doi.org/10.9734/JABB/2016/27964

[46] El-Ghamery, A.A. and Mousa, M.A. (2017) Investigation on the Effect of Benzyladenine on the Germination, Radicle Growth and Meristematic Cells of *Nigella sativa* L. and *Allium cepa* L. *Annals of Agricultural Sciences*, **62**, 11-21. https://doi.org/10.1016/j.aoas.2016.11.002

[47] Adam, F.I.M. and El-Ashry, Z.M. (2010) Evaluation of Genotoxicity of 4-n-Nonylphenol Using *Vicia faba* L. *Journal of Biological Sciences*, **10**, 368-372. https://doi.org/10.3923/jbs.2010.368.372

[48] Mei, J., Ma, X. and Xie, J. (2019) Review on Natural Preservatives for Extending Fish Shelf Life. *Foods*, **8**, 490. https://doi.org/10.3390/foods8100490

[49] Yeon, S.J., Kim, J.H., Cho, W.Y., Kim, S.K., Seo, H.G. and Lee, C.H. (2019) *In Vitro* Studies of Fermented Korean Chung-Yang Hot Pepper Phenolics as Inhibitors of Key Enzymes Relevant to Hypertension and Diabetes. *Foods*, **8**, 498. https://doi.org/10.3390/foods8100498

[50] Butnariu, M. (2012) An Analysis of Sorghum Halepense's Behavior in Presence of Tropane Alkaloids from *Datura stramonium* Extracts. *Chemistry Central Journal*, **6**, 75. https://doi.org/10.1186/1752-153X-6-75

[51] Bostan, C., Butnariu, M., Butu, M., Ortan, A., Butu, A., Rodino, S. and Parvu, C. (2013) Allelopathic Effect of *Festuca rubra* on Perennial Grasses. *Romanian Biotechnological Letters*, **18**, 8190-8196.

[52] Putnoky, S., Caunii, A. and Butnariu, M. (2013) Study on the Stability and Antioxidant Effect of the *Allium ursinum* Watery Extract. *Chemistry Central Journal*, **7**, 21. https://doi.org/10.1186/1752-153X-7-21

[53] Abou El-Ghit, H.M. (2016) Physiological Allelopathic Effect of Aqueous Extracts of Cucumber, Carrot, Onion, and Garlic Seeds on Germination and Growth of Pea. *Journal of Pharmaceutical, Chemical and Biological Sciences*, **4**, 13-19.

[54] Butnariu, M. (2014) Detection of the Polyphenolic Components in *Ribes nigrum* L. *Annals of Agricultural and Environmental Medicine*, **21**, 11-14.

[55] Vieira, A.I., Guerreiro, A., Antunes, M.D., Miguel, M.G. and Faleiro, M.L. (2019) Edible Coatings Enriched with Essential Oils on Apples Impair the Survival of Bacterial Pathogens through a Simulated Gastrointestinal System. *Foods*, **8**, 57. https://doi.org/10.3390/foods8020057

[56] Shim, S.M., Seo, S.H., Lee, Y., Moon, G.I., Kim, M.S. and Park, J.H. (2011) Consumers' Knowledge and Safety Perceptions of Food Additives: Evaluation on the Effectiveness of Transmitting Information on Preservatives. *Food Control*, **22**, 1054-1060. https://doi.org/10.1016/j.foodcont.2011.01.001

[57] Kaptan, B. and Kayısoglu, S. (2015) Consumers' Attitude towards Food Additives. *American Journal of Food Science and Nutrition Research*, **2**, 21-25.

[58] Esteghlal, S., Gahruie, H.H., Niakousari, M., Barba, F.J., Bekhit, A.D., Mallikarjunan, K. and Roohinejad, S. (2019) Bridging the Knowledge Gap for the Impact of Non-Thermal Processing on Proteins and Amino Acids. *Foods*, **8**, 262. https://doi.org/10.3390/foods8070262