CYTOSTATIC EFFECT OF FOOD FLAVORINGS IN THE EXAMPLE TEST-OBJECT *ALLIUM CEPA* L.

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The effect of food flavors on the Allium cepa L. test object was determined. It was estimated the level vigor, germination, mitotic index, aberrations in cells and the delay root growth of onions.

Ana-telofaznyy analysis, chromosomal aberrations, mitotic index, food flavorings.

Real food industry is very diverse. Production of food supplements in the world tends to a continuous quantitative and qualitative growth: Europe - 2% in the US - 4.4%, in Asia - 10-15% [6]. Most synthetic food flavors presented with a set of components that are produced by chemical synthesis, and although their composition and purity provides constant control, they can be hazardous to human health [1]. In addition, the legislation of Ukraine does not have mandatory testing of new food additives mutagen activity, which creates the potential dangers of their use.

The classic method for studying the toxic and mutagenic effects of chemicals on living objects are tested for plant cells, including root meristem A. cepa L. (Allium-test). An important advantage of this method is the close correlation of the results with data obtained in other test systems [2, 3].

The aim of our study was to investigate the potential danger of food flavorings "Hazelnut", "Coffee", "Caramel", their cytostatic and mutagenic action.

Materials and methods.

The paper studied the food flavors, "Hazelnut"; "Coffee"); "Caramel" Daily doses for flavorings "Hazelnut" - 0.5 mg / L, "Coffee" - 0.5 mg / L, "Caramel" - 0.5 mg / l.

For the detection of chromosomal aberrations and cytostatic action of these substances used Allium-test. Seeds of onion (A. cepa L.) sterilize 1% solution of KMnO4, washed in water and germinated for 72 h filter paper in petri dishes at a temperature of 22 ° C. Roots length of 0.5 - 1 cm were fixed 24 hours in a mixture

Clark, washed three times Dist. H_2O and 70% C_2H_5OH and stored in 70% ethanol. Chromosomes were stained with Schiff's reagent (Merk) by cold hydrolysis by Feulgen [5].

As an indicator of cytostatic activity flavorings used mitotic activity index (MI) of root meristem cells. The frequency of aberrant anaphase (CHAA) was calculated as a percentage, the ratio of aberrant ana-telophase and all analyzed according to the formula:

$$CHAA = n*a \cdot 100\% / n,$$

where n*a - number of aberrant ana-telophase,

n - total number of analyzed ana-telophase.

Statistical significance of differences indices MI and CHAA meristem cells was performed by methods № 2. Initial processing of the data was performed to analyze the program package Microsoft Office Excel 2007.

Results.

Sprouting seeds A. cepa revealed a linear relationship between the rate of germination and its concentration of these substances. Established that increasing the concentration of flavors reduces vigor. Was the most toxic flavor "Coffee": the concentration of 1.0 mg / l chemical components that are part of it, completely blocked the seed germination (Table. 1)

1. Effect of food flavorings energy seed germination A. cepa L.,% nutritional

flavorings	flavorings concentration, mg / l					
	Control	0,2	0,4	0,6	0,8	1,0
Hazelnut	27,0 ±1,34	15,0 ± 0,87	7,0 ± 0,57	3,0 ± 0,21	3,0 ± 0,21	1,0 ± 0,05
Coffee	21,0 ± 1,07	11,0 ± 0,62	4,0 ± 0,38	2,0 ± 0,17	1,0 ± 0,05	0
Caramel	23,0 ± 1,13	12,0 ± 0,69	5,0 ± 0,42	2,0 ± 0,17	2,0 ± 0,17	1,0 ± 0,05

It should be noted that the seeds of A. cepa in control had low similarity of 42-54%, but with increasing concentration of food flavors, this figure was significantly decreased significantly (Table. 2).

2. Germination of seeds Allium cepa L.,%

flavorings	flavorings concentration, mg / l					
	Control	0,2	0,4	0,6	0,8	1,0
Hazelnut	54 ± 2,03	30 ± 1,13	14 ± 0,78	6 ± 0,34	6 ± 0,34	2 ± 0,05
Coffee	42 ± 1,45	22 ± 1,08	8 ± 0,43	4 ± 0,12	2 ± 0,05	0
Caramel	46 ± 1,76	24 ± 1,10	10 ± 0,65	4 ± 0,12	4 ± 0,12	2 ± 0,05

When germination of A. cepa bulbs roots in solutions at concentrations flavors 0,2-0,6 mg / l slowed down the growth of roots in 3-4 times, and at concentrations of 0.8-1.0 mg / ml of root growth is almost entirely pryzupynyavsya . Normally, the area's largest mitotic activity of meristem cells located at a distance of 1300-1800 m from the center of cells peace. In the roots, which in solutions proroschuvalysya flavors "Coffee" and "Hazelnut" zone of active cell division decreased 3-6 times (250 - 600 microns).

Cells were for endoderm, after cold hydrolysis showed a positive reaction for DNA magenta-sulfuric acid. Under the influence of synthetic flavoring "coffee" in the cells of the primary cortex roots of onion experienced a rapid destruction of nucleic acids (confirmed Schiff reaction for DNA). Even with short (30-60 min) treatment of roots flavors cell division in the apical meristem almost pryzupynyavsya (Fig. 1).

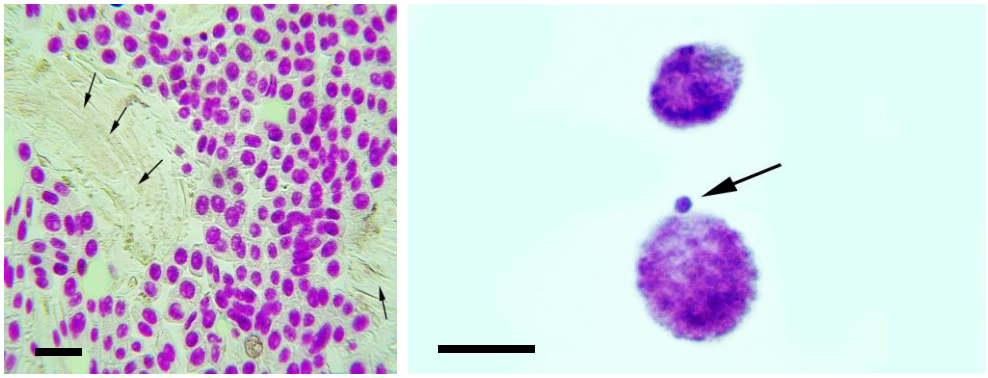

Fig.1. Cytotoxic and mutagenic action of food flavorings "Coffee" in the apical meristem cells of A. cepa (Feulgen reaction for DNA): left - the degradation of nucleic acids and area nekrotyzations cells (range - 30 mm); right - arrow shows the formation of micronuclei (range - 10 microns)

After processing roots synthetic flavors slowed cell division. In terms of cytotoxic effect of the rate of diffusion of active substances and their penetration

through Apoplast testified that the processes of cell division and tension are sensitive to chemicals flavors. Under their influence mitotic index decreased by 10-15 times, from 8.12% to 0.5-1%.

Due to massive violations of cell division, fragmentation, and the backlog of individual chromosomes or fragments formed numerous cells with micronuclei in diameter from 0.8 to 3 microns. Micronuclei in protoplasts aberrant cells are gradually destroyed. Thus, the nucleus in cells that are directly under the influence of synthetic fragrances, gradually acquired traits anomalies lost land or even entire chromosomes. Disruption of the normal mitosis, uneven distribution of genetic material led to the formation of aneuploid and polyploid cells that arise as a result of C-mitosis, cytokinesis without kariokinezu and others. According to our data fragments mitotic chromosomes sometimes united in chromosome aggregates that formed in late telophase some microkernel (1, sometimes 2, 3). Cytogenetic studies have shown (Table. 3), mitotic index figures for flavorings "Hazelnut" and "Caramel" was not significantly different from control. Ander influence flavor "Coffee" mitotic index was significantly lower (χ^2 2 = 10,41, p <0,01).

3. Indicators mitotic index (MI) in A. cepa cells under food flavorings

name of sample	Cells were analyzed for accounting MI	MI ± Sp	importance χ^2
hazelnut	2400	5,08 ± 0,45	0,39
coffee	3900	3,10 ± 0,28*	10,41
caramel	3600	4,89 ± 0,36	0,12
control	2700	4,67 ± 0,41	-

* - Confidence level of p <0,01

Since a significant deviation values MI detected using flavoring "Coffee", we have analyzed the length of the passage of cells of different phases of mitosis. The frequency of anaphase cells aberatnyh A. cepa roots significantly higher than control value only in the sample "Coffee." In samples "Hazelnut" and "Caramel" significant deviations were found (Table. 4).

4. Frequency aberatnyh anaphase (CHAA) root meristem cells A.cepa influenced investigated flavors

Name of the sample	analyzed roots	analyzed anaphase	Revealed aberrant anaphase	CHAA ± Sp	importance χ^2
hazelnut	13	537	19	3,54 ± 0,80	3,28
coffee	12	597	38	6,37 ± 1,00*	15,35
caramel	7	643	17	2,64 ± 0,63	1,02
control	9	555	9	1,62 ± 0,54	-

* - Confidence level of p <0,01

The results confirmed the impact of food flavorings "Coffee" on the ratio of cells in different phases of mitosis, its cytostatic and mutagenic activity.

Conclusions.

Thus, in terms of cytotoxicity investigated flavors can be arranged as follows: "Coffee"> "Hazelnut"> "Caramel". A direct correlation between vigor and seed germination A. cepa concentration and food flavorings.

At concentrations 0,2-0,6 mg / l flavors slowdown root growth 3-4 times, increasing their concentration to 0.8-1.0 mg / ml be suspendedroot growth. Food flavor "Coffee" at a concentration of 0.6 mg / l is a violation of mitosis (asymmetric mitosis, K-mitosis, the formation of micronuclei) and causes partial necrosis of tissue in the apical meristem and root zones stretching cells A. cepa.

REFERENCES

1. Гончаренко Т. П. Харчові добавки як об’єкт моніторингових досліджень / Т. П.Гончаренко, О. Г.Гончаренко // Екологія довкілля та безпека життєдіяльності. – 2008. – №4. – С. 81–84
2. Горовая А. И. Методические аспекты оценки мутагенного фона и генетического риска для человека и биоты от действия мутагенних экологических факторов / А. И. Горовая, Л. Ф. Бобырь ,Т. В. Скворцова // Цитология и генетика. – 1996. – Т. 30, № 6. – С. 78–86.
3. Козовий Р. В. Розробка профілактики негативного впливу мутагенних чинників на спадковий апарат людини / Р. В. Козовий // Вісн. Укр. тов-ва генетиків і селекціонерів. – 2005. – Т.3, №1-2. – С.80–85.
4. Лакин Г. Ф. Биометрия:учебное пособие для вузов / Г. Ф. Лакин. – М: Высшая школа, 1990. – 352 с.

5. Паушева З. П. Практикум по цитологии растений / З.П.Паушева. – М.: Агропромиздат, 1988. – С. 138–170.

6. Смоляр В. І. Токсичні ефекти харчових добавок / В.І.Смоляр // Проблеми харчування. – 2005. – № 1. – С. 5–15.