

ANALYZES REGARDING THE CYTOTOXICITY OF ZnSO₄ EXCESS ON CELL DIVISION

Sărac Ioan¹, Petrescu Irina¹, Camen Dorin- Dumitru¹, Onisan Emilian¹, Memete Adriana- Ramona², Ganea Mariana^{3*}

1. *Department of Genetic Engineering, University of Life Sciences "King Mihai I" Timișoara, Timișoara, Romania.*
2. *Department of Food Engineering, University of Oradea, Oradea, Romania.*
3. *Faculty of Medicine and Pharmacy, University of Oradea, Oradea, Romania.*

ARTICLE INFO

Received:

26 Feb 2023

Received in revised form:

29 May 2023

Accepted:

04 Jun 2023

Available online:

28 Jun 2023

Keywords: ZnSO₄, Cytotoxicity, Mitotic index, Chromosomal aberration

ABSTRACT

Zinc is one of the essential elements for the development of the body, both plant and human, facilitating the processes of cell division as well as the maintenance of cellular physiological processes in normal parameters. In this work, the effects of zinc were monitored regarding the activity of mitotic division, in different time intervals and with different concentrations. Following the effect of different concentrations of zinc sulfate (ZnSO₄), through the correlative links between the number of cells in division (MI), the mitotic index, the index of chromosomal aberrations (IAC), and the exposure time to the treatment, we managed to monitor the cytotoxic effect of ZnSO₄. From the analysis of the correlations between the percentage of chromosomal aberrations and the different concentrations of ZnSO₄, it was found that there was a strong positive correlation ($r > 0.89$). Therefore, the increased Zn concentration in cells favors the appearance of chromosomal aberrations (IAC). The increase in the exposure time from 24 hours to 72 hours, as well as the increase of the concentration from 10 ppm to 50 ppm, causes a strong negative correlation ($r = -0.84$). From the experiments, we can deduce the importance that zinc has in the cell and how much it can affect if it is present in excess.

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To Cite This Article: Ioan S, Irina P, Dorin-Dumitru C, Emilian O, Adriana- Ramona M, Mariana G. Analyzes Regarding the Cytotoxicity of ZnSO₄ Excess on Cell Division. *Pharmacophore*. 2023;14(3):7-14. <https://doi.org/10.51847/7XJ78ykqic>

Introduction

Zinc is an essential trace element, very present in the human body [1, 2]. Different studies have demonstrated that, through both direct and indirect mechanisms, zinc exhibits a variety of bioactive activities, such as antioxidant, anti-inflammatory, anticancer, and immunomodulatory effects [2-5]. Zinc is also important for plant growth, requiring a balance of all essential nutrients for normal growth and optimal yield. For the human body, plant consumption represents an important source of ZnSO₄ [6, 7].

Zinc is an important component in the structure of a large number of proteins, involved in nucleic acid transcription, due to RNA degradation, decreased RNA polymerase activity, ribosomal deformation, and decreased ribosome number [4, 8, 9]. Zinc is the only metal that is required in all six classes of enzymes (oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases) [2, 10-12].

In plant growth, zinc availability depends on several soil factors, such as concentration in solution, ion speciation, and the interaction of ZnSO₄ with other macronutrients and micronutrients [2, 11, 13].

Zinc deficiency can cause large reductions in the quality and yield of some crops [14, 15]. Also, the deficiency of ZnSO₄ in the soil reduces the concentration and content of ZnSO₄ in the edible parts of basic agricultural plants and diminishes their nutritional quality [3, 6, 13]. Visible symptoms of ZnSO₄ deficiency in various crops usually appear only in cases of relatively severe deficiency [7, 16, 17].

Plant genotypes vary greatly in tolerance to soils with low amounts of plant-available ZnSO₄, both in terms of uptake and utilization [18, 19]. Physiological and molecular mechanisms of tolerance to ZnSO₄ deficiency are only beginning to be

Corresponding Author: Ganea Mariana; Faculty of Medicine and Pharmacy, University of Oradea, Oradea, Romania. E-mail: mganea@uoradea.ro.

understood, and these mechanisms can be used in agricultural crop breeding programs [16, 20-22]. Molecular markers used in the study of micronutrient efficiency (for example, Mn and ZnSO₄) were identified in barley, bread wheat, durum wheat, and maize [1, 23-25].

When ZnSO₄ supply to plants is inadequate, one or more of the important physiological functions that depend on ZnSO₄ are affected and plant growth is negatively influenced [26, 27]. ZnSO₄ deficiency is a severe micronutrient deficiency that threatens world food production [3, 7]. The world's population suffers from micronutrient deficiencies (so-called "hidden hunger"), including ZnSO₄ deficiency (about 40%) [28, 29]. The World Health Organization estimates that ZnSO₄ deficiency affects one-third of the world's population (approximately two billion people), with prevalence rates ranging from 4 to 73% in different regions [3, 6, 13].

A diet with a high proportion of cereal-based foods with low ZnSO₄ content is considered one of the major reasons for the occurrence of ZnSO₄ deficiency in humans, especially in developing countries [3, 7]. Zinc specifically causes cell death in the brain, and zinc accumulation causes cytotoxicity [3, 10, 30, 31]. This study aimed to monitor the genotoxicity and cytotoxicity of different concentrations of ZnSO₄ at different time intervals (24 – 72 h), using the *Allium* test.

Materials and Methods

Garlic bulbs, (*Allium sativum* L.), from the Cenad-Timiș-Romania population, of equal size, were chosen for the experiment. To highlight growth and development, the 4 experimental variants were used: V1- control water (-H₂O), V2- (ZnSO₄- 10 ppm), V3- (ZnSO₄- 20 ppm), V4- (ZnSO₄- 50 ppm).

Garlic bulbs were grown directly in the solutions of the four experimental variants, presented above, at a time interval between 24 and 72 h (hours), following the mitotic activity at the level of the cells in the meristematic tissue.

The tip of the root of the bulbs grown on the four experimental variants was harvested at 24h, 48h, and 72h, performing the following processes: prefixing, fixing, hydrogenation, and coloring of the biological material.

The mitotic index (MI%) and the index of chromosomal aberrations (IAC%) were determined by the *Allium* test to highlight the cytotoxicity and genotoxic effects of ZnSO₄ at the cellular level [32-34].

The sampling of the material from the tip of the root was done in the morning because the rate of mitotic division shows a more intense activity.

Samples harvested from the root tip were treated with fixator Carnoy's for 24 h. After fixation, the roots were hydrolyzed in HCl for 6 min in a water bath, at a temperature of 60°C. Later, the roots were stained with Carr's reagent. The microscopic preparations obtained were analyzed with the Optika microscope [35, 36].

Cytological analyses to determine the mitotic index and the index of chromosomal aberrations, used cells in different phases of division (prophase, metaphase, anaphase, and telophase), as well as the percentage of chromosomal abnormalities (bridge in anaphase, multipolar anaphase, isolated chromosomes, incorrect polarization, polyploid and binuclear cell).

To calculate the results, the following equations were used [35, 37].

$$\text{Mitotic index (MI\%)} = \frac{\text{Number of cells in mitosis}}{\text{Total number of cells}} \times 100 \quad (1)$$

$$\text{Index of chromosomal aberrations (IAC\%)} = \frac{\text{Total number of modified cells}}{\text{Total number of cells}} \times 100 \quad (2)$$

Results and Discussion

The different zinc concentrations affected, depending on the treatment time (24 h, 48 h, 72 h), the mitotic activity at the level of the root growth peak. The toxicity induced by zinc concentrations causes chromosomal aberrations, which can lead to a slowdown in mitotic activity and, implicitly, reduced root growth.

The mitotic index (MI) established based on the calculation equation (1), had different values depending on the exposure time and the concentrations of ZnSO₄ used (**Figure 1**). Thus, at the lowest concentration (V2- 10 ppm) and the time interval 24 h-48h, the mitotic activity, compared to the control variant (V1-H₂O), was similar. Compared to the other variants (20 ppm and 50 ppm) in intervals 24-48 hours after the initiation of the experiment, the mitotic index (MI) showed lower cellular activity.

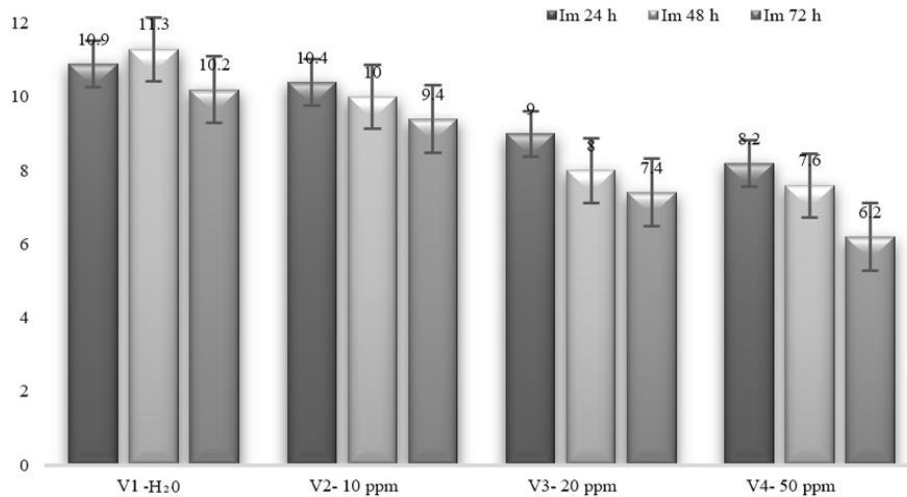


Figure 1. The mitotic index of the *Allium sativum* species on different concentrations of ZnSO₄.

Zinc ions caused an imbalance in the mitotic phases, and the degree of this imbalance is dependent on the variant and the time of exposure. The different concentrations induce a series of chromosomal aberrations in almost all mitotic phases, but with different frequencies as can be seen in **Figure 2**.

The chromosomal aberration index (IAC) increases with increasing ZnSO₄ concentration, according to **Figure 2**. The percentage of chromosomal aberrations shows the highest values at the maximum dose of ZnSO₄, respectively in the experimental variant V4 during the interval 24h-72h.

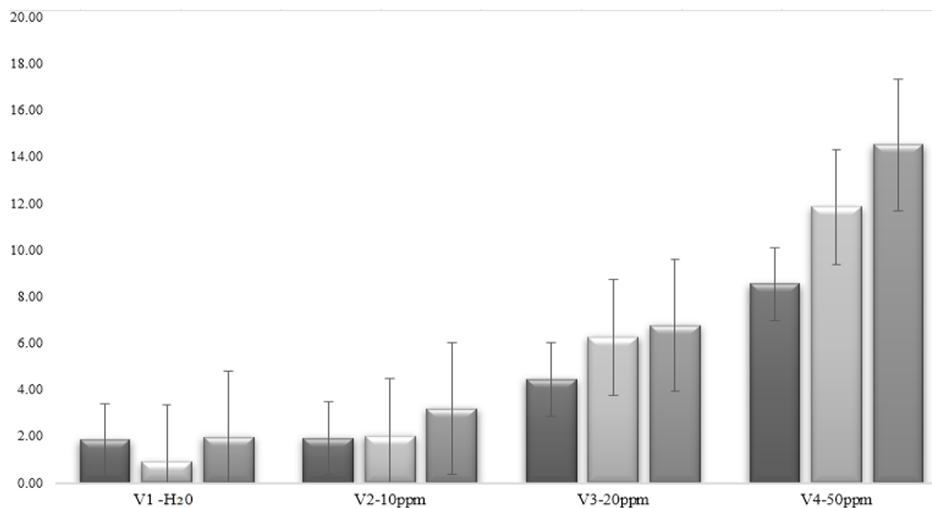


Figure 2. Chromosomal aberration index of *Allium sativum* L. species on different concentrations of ZnSO₄.

Following the calculation of the index of chromosomal aberrations, an increase in abnormalities at the level of mitotic division was observed along with the increase in the concentration and the interval of exposure to ZnSO₄ concentrations. Intermediate levels of the index of chromosomal aberrations were observed in the V3 variant in the interval of 24-72 hours. Exposure for a long time (72h) to ZnSO₄ causes an increase in the aberrations index chromosome (IAC), in all treated variants. This aspect can be observed even at the lowest concentration of V2 (10ppm) where at the short exposure interval (24-48 h) the values of the aberrations index chromosome (IAC), are close to the value of the control variant (V1-H₂O).

In graph a from **Figure 3** there is a close, positive correlation between the number of dividing cells and the mitotic index. These results were to be expected, once the number of cells increases and the mitotic index increases ($r=0.987$).

On the other hand, following the correlation analysis between time and mitotic index in the division, there is a very weak negative linear correlation ($r=-0.40$, graph b) for the 24-72 hour interval. Thus, it can be said that time has a negative influence on the mitotic index (MI). The value of the mitotic index calculated in percentage (%) for the variants analyzed in the 24h time interval is superior, and the lowest percentage values are obtained in the 72-hour time interval. The links between the treatments with different concentrations of zinc and the number of cells in division determine a strong negative linear correlation ($r=-0.84$, graph c). Analyzing graph e in **Figure 3** it can be said that zinc has a negative influence on the number of dividing cells and mitotic index.

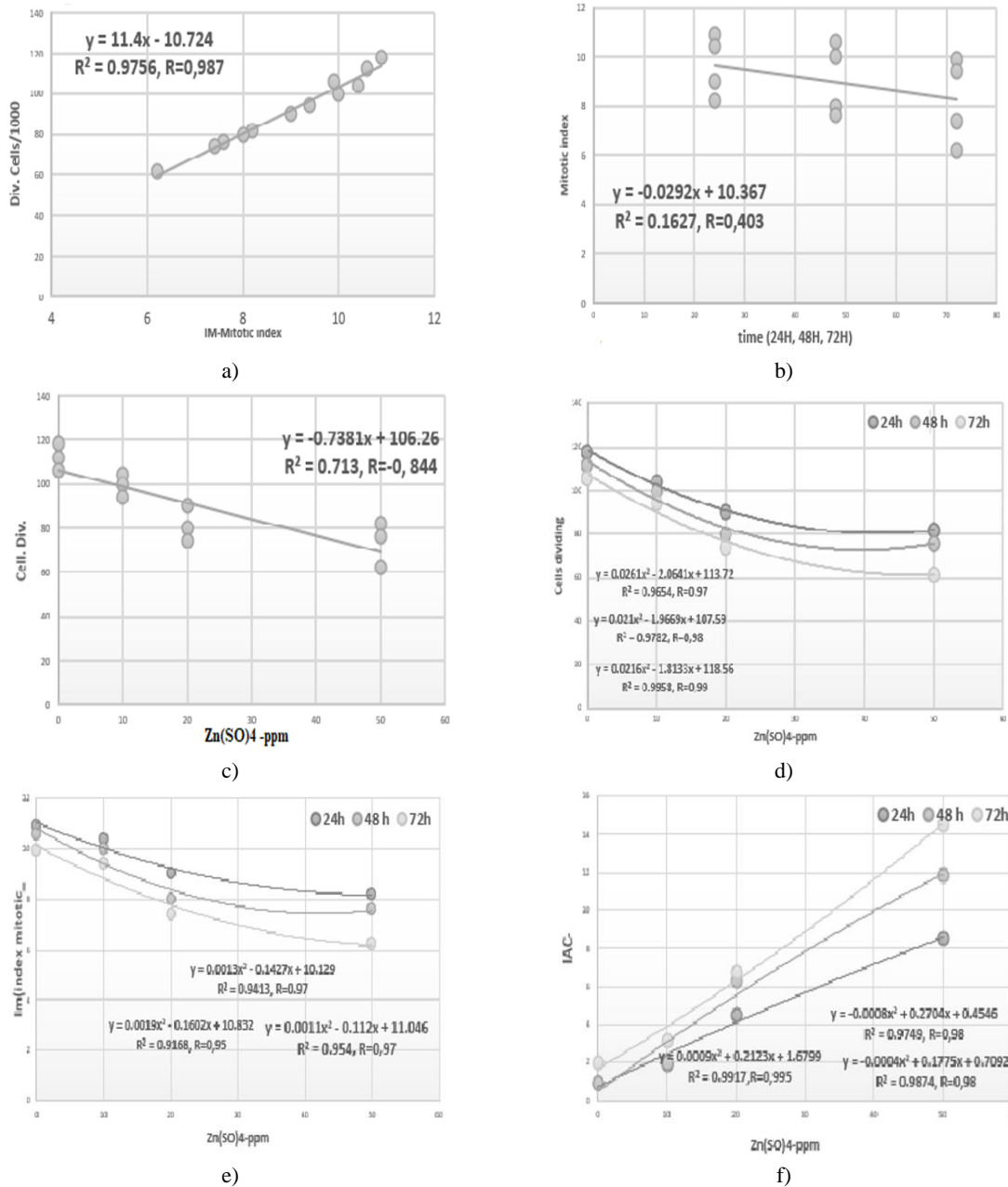


Figure 3. The results of the correlations obtained after the experiment: a) Correlations between cells in the division and the mitotic index. b) Correlations between mitotic index and time. c) Correlations between dividing cells and ZnSO₄. d) Correlations between dividing cells, ZnSO₄ and time. e) Correlations between mitotic index, ZnSO₄, and time. f) Correlations between IAC (index of chromosomal aberrations), ZnSO₄ and time.

As the dose of zinc increases, the number of dividing cells decreases, the two variables being inversely proportional. Values around 100-120 cells are obtained for the control variant, i.e., for the variant in which zinc was not administered [V1], and the lowest values (60-80 cells) are obtained for the ZnSO₄-50 variant ppm.

Analyzing graph e in **Figure 3**, above, the different concentrations of zinc have a negative influence on the number of cells, i.e., by increasing the dose of zinc, the number of dividing cells decreases. The highest values of 100-129 cells were in the control variant to which zinc was not administered (V1-H₂O). The lowest values (around 60-80 cells) were for the variant V4-ZnSO₄- 50 ppm. The highest number of cells is obtained at the time interval of 24 hours regardless of the dose of zinc, and the lowest at the interval of 72 hours. The number of cells decreases at the dose of 50 ppm compared to the other variants.

From graph f in **Figure 3** above, the results indicate a negative influence of ZnSO₄ on the mitotic index, that is, by increasing the dose of zinc, the mitotic index decreases. The high values (9.9-10.9%) are obtained in the control variant in which zinc was not administered (V1), and the lowest values (6.0-8.9%) are obtained with ZnSO₄ 50 ppm (V4). With the increase in the concentration of ZnSO₄ from 20ppm (V3) to 50ppm (V4) in correlation with the time interval of 24-72 hours, the mitotic index decreases.

The presented results are in accord with the results obtained by other authors, who demonstrated that the cytotoxic effect of

zinc is concentration dependent; Abnormal mitotic phases increased with increasing concentration, and in low concentrations, zinc did not negatively influence the values of the mitotic index [36, 38, 39].

In **Figure 4**, the microscopic images that captured the different mitotic phases in the plant cell are presented.

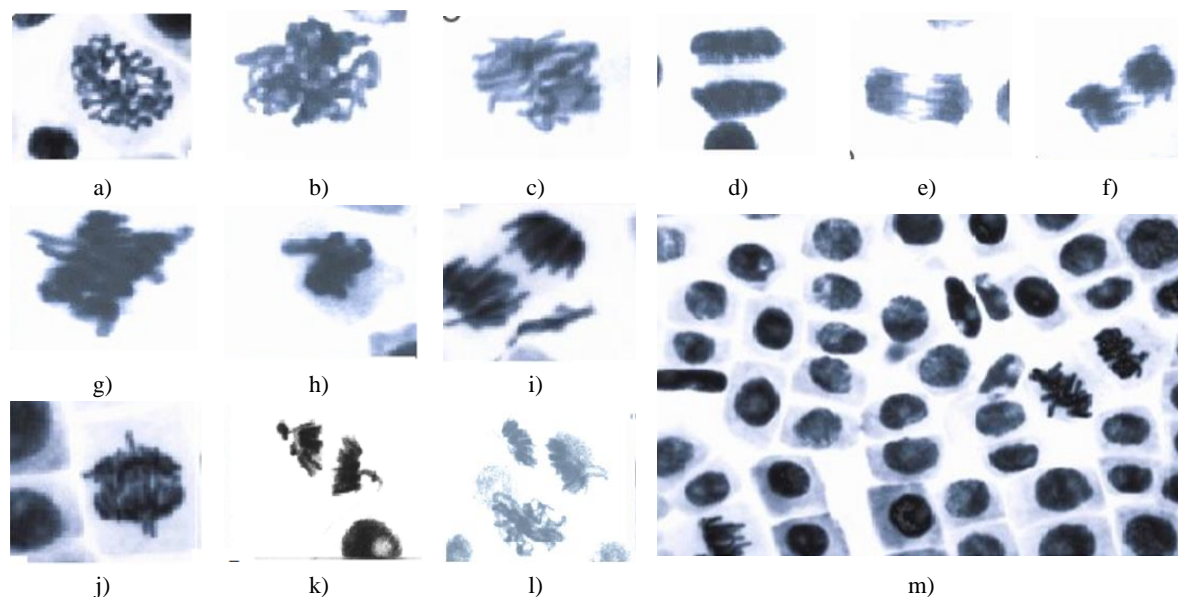


Figure 4. Images regarding the different mitotic phases observed in the plant cell (a & b) Normal prophase, c) Normal prometaphase, d) Normal telophase) under the influence of different concentrations of $ZnSO_4$ (e & f) Abnormal anaphase by the formation of the chromosomal bridge at concentrations d 50 ppm, g) Chromosomes advanced in metaphase at concentrations of 20 ppm, (h & i) Chromosomes advanced in metaphase and anaphase 20 ppm, j) Anaphase with a ball appearance and advanced chromosomes -50 ppm, (k & l) Anaphase with advanced chromosomes, m) Microscopic field image).

The results regarding the index of chromosomal aberrations (IAC%) indicate a level of close positive correlation in relation to the zinc concentration used and the exposure period from 24h to 72h. There is a directly proportional correlation between the increase in the index of chromosomal aberrations in relation to the increase in concentration and exposure period, obtaining a correlation value of $r > 0.9$. This cytotoxicity is justified by the low rate of the mitotic index. Since the mitotic activity is very high in children, the cytotoxicity effect may cause a reduced mitotic index. The mitotic index decreases with increasing $ZnSO_4$ content. Therefore, we can expect that a possible increase in the amount of zinc in the human body in newborns will facilitate the inhibition of growth and development.

Research on zinc uptake by plants has shown a rate of about 31% [40]. According to research reports, the amount of zinc in the human body shows normal values of 1.5 g in women and 2.5 g in men [41], the highest amount of zinc is distributed in the tissues of support. Bone and muscle tissues are the most important sources from which the human body can ensure its zinc content. The second important source of zinc for the human body is represented by plant tissue, respectively the plants consumed (cereals) with the highest zinc values, followed by other foods of plant origin [5, 41-43].

Also, various studies have shown that crop nutrition, for enzymatic, oxidative, and metabolic processes, depends on zinc [42, 44, 45], and zinc deficiency in the soil, recorded at the global level, represents a major problem, both for plant production and for human health [44, 46, 47]. Consuming foods containing high concentrations of Zn also has an immediate positive impact on human nutrition and, consequently, on human health [47, 48]; Thus, several studies have demonstrated the antioxidant effect of zinc on the human body, as well as the anti-inflammatory, anti-cancer and immunomodulatory effect [2-5].

Graham and Welch 1996 estimated that there is a zinc deficiency of 50% in soils used for grain production worldwide [49]. All crops worldwide suffering from zinc deficiency lead to severe yield losses and nutritional deficiencies [44, 50].

Conclusion

The *Allium* test was performed to monitor the genotoxicity and cytotoxicity of different concentrations with $ZnSO_4$. The results indicate a cytotoxic effect depending on the time (24 h, 48 h, 72 h) of the treatment. The toxicity induced by zinc concentrations causes chromosomal aberrations, which can lead to a slowdown in mitotic activity and implicitly a reduction in the rate of root growth. This could also be due to the toxicity of the metal ion disrupting the physiological processes, through the fixation of the ions by the plant tissue. The levels of close correlation ($r > 0.9$) were observed by the values of the mitotic division depending on the exposure time and the concentrations of $ZnSO_4$ taken in the study, which indicates the cytotoxic effect of $ZnSO_4$.

The recommendations following the cellular analyzes regarding the direct supplementation of plants, respectively the increase of the zinc content during the periods of plant development for the supplementation of nutrients in human bodies can be made up to doses of 10 ppm of zinc. However, increasing the concentration of zinc in plant development with a content of over

20ppm, for a higher supplement for the body, is not recommended because decreasing MI and increasing IAC will decrease the final production of the plant. Thus, the increased storage of zinc in plant cells, respectively the plant test or plants is included in well-defined intervals. Of course, during the ripening periods of the plants, one can opt for an increase in the concentration of zinc, since it no longer affects the mitotic indices therefore increasing the concentration of zinc in the ripening periods, can have an indirect effect on reducing nutritional deficiencies on humans and avoiding the fortification or addition of zinc surplus to balance the nutritional balance at the population level.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

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