IJMBR 4 (2016) 25-34 ISSN 2053-180X

International Journal of Modern Biological Research
www.bluepenjournals.org/ijmbr

Genotoxic effects of some frequencies of low-voltage electrical currents on *Allium cepa* L. meristematic cells

Jasmin Adrovic[1*], Izet Eminovic[2] and Aner Cerkic[2]

[1]University of Tuzla, Faculty of Science, 75 000 Tuzla, Univerzitetska 4, Bosnia and Herzegovina.
[2]University of Sarajevo, Faculty of Science, 33 000 Sarajevo, Zmaja od Bosne 33-35, Bosnia and Herzegovina.

Article History
Received 22 February, 2016
Received in revised form 18 April, 2016
Accepted 25 April, 2016

Keywords:
Genotoxicity, Electrical current, Meristematic cells, Onion.

Article Type:
Full Length Research Article

ABSTRACT

In recent years, the science of bioelectromagnetism, as a modern field of biomedical sciences is becoming relevant in the study of the interactions between external electromagnetic fields and electromagnetic fields created by living cells, tissues or organisms.This article presents the research results of the impact of low-voltage electric current on cell viability of meristematic tissues of onion (*Allium cepa* L.). Chromosomal aberrations occured in the onion root tip cells that were treated with all frequencies of low-voltage electric current. Depending on the frequency of low-voltage electric current, significant differences were observed in the number and type of mitotic and chromosomal aberrations. Based on the results obtained, it can be concluded that some frequencies of low-voltage electric current have recognizable impact on the genotoxic effects of meristematic cells of onion.

INTRODUCTION

Bioelectromagnetism is a modern field of biomedical sciences, primarilly of biology and biophysics. Using a variety of scientific methods and reliable measuring systems of high resolution, today we can study the interactions between external electromagnetic fields and electromagnetic fields created by a living cell, tissue or organism with great success.

The latest scientific research confirms that certain frequencies of electromagnetic fields play an important role in the interaction with electromagnetic fields of biological systems, therefore affecting the levels of biological effects. If biological systems are influenced by an electromagnetic field, the oscillation of free ions with the field frequency and rotation of dipole molecules occur as numerous studies have shown (Adrović, 2006). Electromagnetic fields can cause rotation, deformation, destruction and merging of cells, and disrupt the cell membrane potential (that is, depolarize or hyperpolarize cells), which inevitably leads to serious disruption of the overall energy structure of biological systems (Walleczek and Eichwald, 1995).

The atoms and molecules of various organs of living beings have a whole range of resonating frequencies, where the effect of the resonance can affect the molecular organization of living matter of the exposed organism in various ways. This primarily refers to superposition of electromagnetic waves of the same frequency – the waves from the environment and proper electromagnetic waves of the atoms and molecules of living beings. In the part of the wave field where superposition of waves occurs, there is a superposition of these independent waves at every point of the elastic medium. Resonance appears when a system that can oscillate with its proper frequency is affected by a force whose frequency is exactly equal to the proper frequency of the system. The result of the wave superposition depends on the differences of phases, amplitudes and frequencies of superposing waves (Jovanović et al., 2010).

It is believed that electromagnetic waves produced by induced currents are related to numerous biological effects, such as changes of hydrated ions and protein

*Corresponding author. E-mail: biolog@live.com.

molecules at the cell membranes, changes in the chromosomes, impacts on DNA repair and other genotoxic effects (Jovanović et al., 2010).

Genotoxic effects represent all changes in the structure and the functioning of genetic material caused by genotoxic factors. Under these changes we usually imply mutations, related not only to changes in the genetic material but also to the process that led to these changes. In general, mutations are qualitative or quantitative changes in the hereditary material that result in alteration of specific phenotypic characteristics (Berberović and Hadžiselimović, 1986).

Genotoxic agents are a large group of compounds having endogenous or exogenous origin that cause various damages of hereditary material, that is changes in DNA, single genes or chromosomes and even entire genome of the cell. More specifically, the definition of genotoxic factors is wider - they act not only on the DNA molecule, but also on the cell, mitosis and meiosis, fertility function, etc. (Sofradžija et al., 1989).

Changes and damages of DNA molecules can induce different biochemical processes and mechanisms such as unpredicted DNA synthesis and exchanges of sister chromatid exchange that can be associated with the development of mutations and carcinogenesis. Endogenous genotoxins originate from organisms and are very often responsible for a spontaneous mutation. In addition, serious damage to the DNA molecules may result in a creation of free radicals that are generated in various cellular processes and redox reactions such as electron transfer process in the mitochondria, and the like (Lutz, 1990).

Exogenous genotoxins come from the environment and can cause various changes in the genetic material. They are divided into biological, physical and chemical genotoxic factors. Biological factors are relatively few in number, and the most important of them are the degradation products of some bacteria and viruses, some of which have mutagenic effects. Physical factors are non-ionizing and ionizing radiation and temperature variations in an extreme form. Chemical factors that can cause damage to the hereditary material and their ways of interacting with DNA are numerous and represent the most diverse group of genotoxic agents (Nefic, 2013).

MATERIALS AND METHODS

The main objective of this work was to determine the genotoxic effects of selected frequencies of electromagnetic waves produced by a low-voltage current to the root meristem cells of onion (*A. cepa* L.). In order to achieve this goal, it was necessary to realize the following tasks: performing *Allium* test with certain frequencies, calculation of the mitotic index, analysis of chromosomal aberrations of untreated (control) root meristem cells of onion, analysis of chromosomal aberrations of treated root meristem cells of onion, determination of how impact depends on the frequency value and, finally, performing statistical analysis of the acquired results.

Allium test

Cytogenetic studies on plant species have proven the existence of certain changes in the chromosomes, in the course of action of mutagenic substances. Testing of mutagen eukaryotic nucleus is performed with cytological methods. Mutagens can be detected by cytology, cell inhibition, arresting metaphase, induction of numerical or structural chromosome aberrations, from fragmentation of chromosomes up to disorganization of mitotic fibers, and then through all the other mitotic phases as well. Genotoxicity studies of electromagnetic waves are for the purpose of public health. Analysis of changes in the chromosomes serves as a mutagenicity test and it is one of the few direct methods of measuring damage in an organism's cells exposed to mutagens and carcinogens.

In order to enable assessment of effects or damage that mutagenic agents can cause, it is necessary that the sample (test cell) be in a constant mitotic division so as to identify toxic effects and changes during the cell cycle. In order to do this, the *Allium* test was introduced in practice (Silva et al., 2003). Benefits of plant tests have been often pointed out in recent years. Such benefits include:

- a very good correlation between the plant and mammal systems;
- similar enzyme mechanisms between plant and animal systems;
- high reliability when it comes to negative results at testing for mutagenicity and even carcinogenicity in plants (Fiskesjö, 1993).

Besides, this test is a useful and inexpensive system and the knowledge that is acquired from plant cytogenetic techniques enables its rapid development (Tadesco and Laughinghouse, 2012). Very well clarified cytogenetic characteristics of onion and a small number of chromosomes in the diploid chromosome set (2n=16) are traits that reinforce the position of this test on the top of the scale of genotoxicity tests.

Calculating the mitotic index

Mitotic index of cell population is defined as the ratio of all cells in mitotic phases to the number of analyzed cells. It has long been regarded as a very important criterion for the growth and multiplication of tissue. It is usually measured in fixed and colored specimens. Assuming that

mitosis occurs at random intervals, the mitotic index and the measured duration of mitosis are used to calculate the length of the interphase (Olivo and Delorenzi, 1932).

The value of the mitotic index shows how a certain substance affected the cell division, either proliferative or inhibitory, or had no impact at all. Interpretation of results for the mitotic index is madein relation to the mitotic index of control cells. The value (in percentages) of the mitotic index is calculated as the ratio of the sum of all cells in the cell division to the total number of observed cells, multiplied by 100:

$$\mathrm{M}\,I(\%) = \frac{\sum(P+M+A+T)}{\sum(P+M+A+T+I)} \times 100\%$$

Where:

P - Prophase frequency,
M - Metaphase frequency,
A - Anaphase frequency,
T - Telophase frequency,
I - Interphase frequency.

Analysis of chromosomal aberrations

When observing the preparations, we recorded the number and presence of specific chromosomal aberrations and the irregularities in the development of the cell cycle. The number of repetitions of these abnormalities is recorded on 1000 observed cells per preparation in order to determine the frequency of their occurrence, and thus the effect of test frequencies.

Statistical analyses

For the results obtained after microscopic analysis, by applying adequate mathematical functions from the software GraphPad Prism, arithmetic mean (Xav) and standard deviation (SD) were determined. The method of analysis of variance (ANOVA) was implemented and the significance of differences were tested using GraphPad Prism software, which is specifically designed for experimental biological, medical and pharmaceutical research.

Testing of statistically significant differences between arithmetic means of the mitotic index was performed by analysis of variance (ANOVA) and Newman-Keuls multiple comparison. *T*-test was used to determine whether there was a difference between arithmetic means of the mitotic index of the cells treated with certain frequencies. If the difference was statistically significant, one might conclude that the difference was not accidental, but occured as a result of investigated factors activity. *Z*-test was used to determine whether there was a statistically significant difference in the total number of aberrations of each treatment compared to the control, since large number of samples were being dealt with (n>30) (Petz, 1997).

RESULTS AND DISCUSSION

The average length, that is the growth, of roots is the first indication on the basis of which one can assess the impact of certain frequencies of low-voltage electrical currents to genotoxic effects on meristematic cells of onion.

The lengths of root tips of *A. cepa* L. bulbs after the exposure to electromagnetic waves produced at a voltage of 9V and frequencies of 1 Hz, 17 Hz, 1.77 kHz and 26 kHz are shown in Figures 1, 2, 3 and 4, respectively. The samples were exposed to these frequencies for a period of 7, 15 and 30 min.

In each series of measurements, we observed inhibitory effects of applied frequencies of 1 Hz, 17 Hz and 26 kHz, on the growth of root tips. The inhibitory effects increased with the increase of frequency in a statistically significant manner. Studies have also shown that the growth of the roots depends on the length of the exposure time for samples at specific frequencies. At a frequency of 1 Hz, the growth of roots of the exposed samples was inhomogeneous within the given time intervals of 7, 15 and 30 min. It was clearly visible that the growth of roots at this frequency was the highest for all the applied frequencies. More precisely, we observed the highest growth of the root samples at a frequency of 1 Hz and for an exposure time of 7 min.

At a frequency of 17 Hz, the growth of roots of the exposed samples was also inhomogeneous within the given time intervals of 7, 15 and 30 min. The growth of the tested roots at this frequency was the highest for an exposure time of 15 min, while the lowest growth of the root samples was recorded for an exposure time of 30 min. The lowest growth of roots of exposed samples was recorded at a frequency of 26 kHz. The growth of the tested roots at this frequency was the highest for an exposure time of 15 min, while the lowest growth of the root samples was recorded for an exposure time of 30 min. The growth effect of onion bulb roots compared with control samples was observed at all applied frequencies. The growth of all control samples was balanced and much higher than that of samples exposed to various frequencies of low-voltage electric current.

The treatment of the root meristem cells of onion (*A. cepa* L.) with different frequencies have resulted in three types of chromosomal aberrations:

1. Aberrations at the level of chromosomes
a) Stray chromosomes

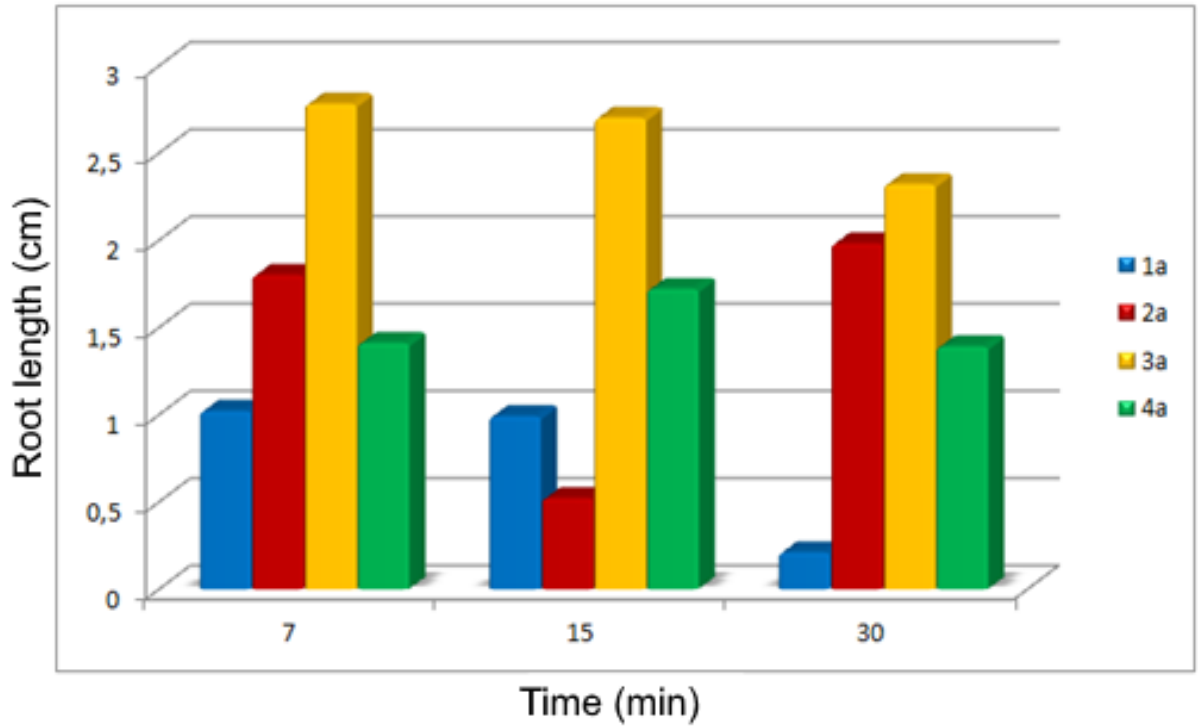

Figure 1. Dependence of roots of *Allium cepa* L. bulbs (1a, 2a, 3a, 4a) growth on the time of exposure to electromagnetic waves at a frequency of 1 Hz and a voltage of 9 V.

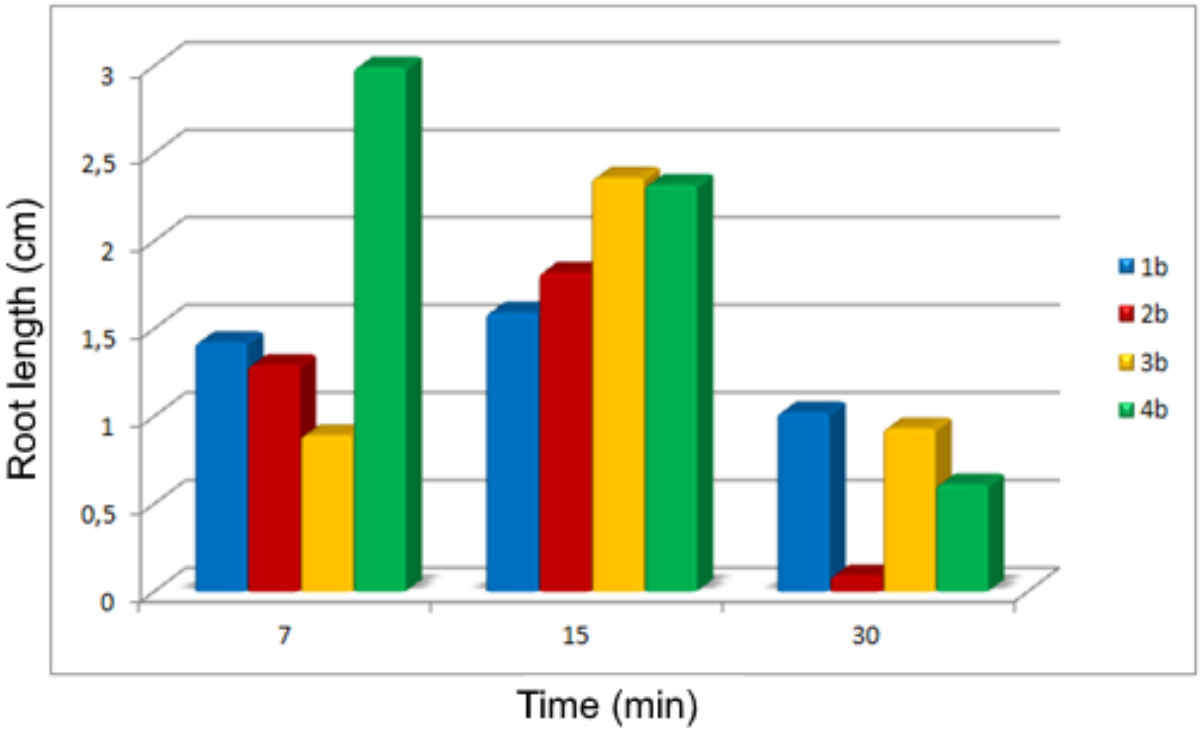

Figure 2. Dependence of roots of *Allium cepa* L. bulbs (1b, 2b, 3b, 4b) growth on the time of exposure to electromagnetic waves at a frequency of 17 Hz and a voltage of 9V.

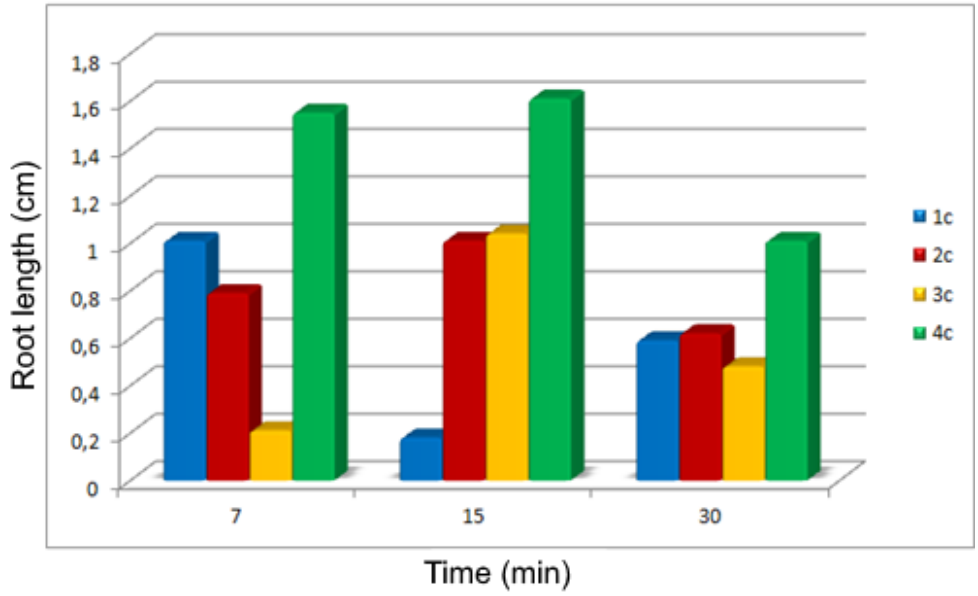

Figure 3. Dependence of roots of *Allium cepa* L. bulbs (1c, 2c, 3c, 4c) growth on the time of exposure to electromagnetic waves at a frequency of 1.77 kHz and a voltage of 9 V.

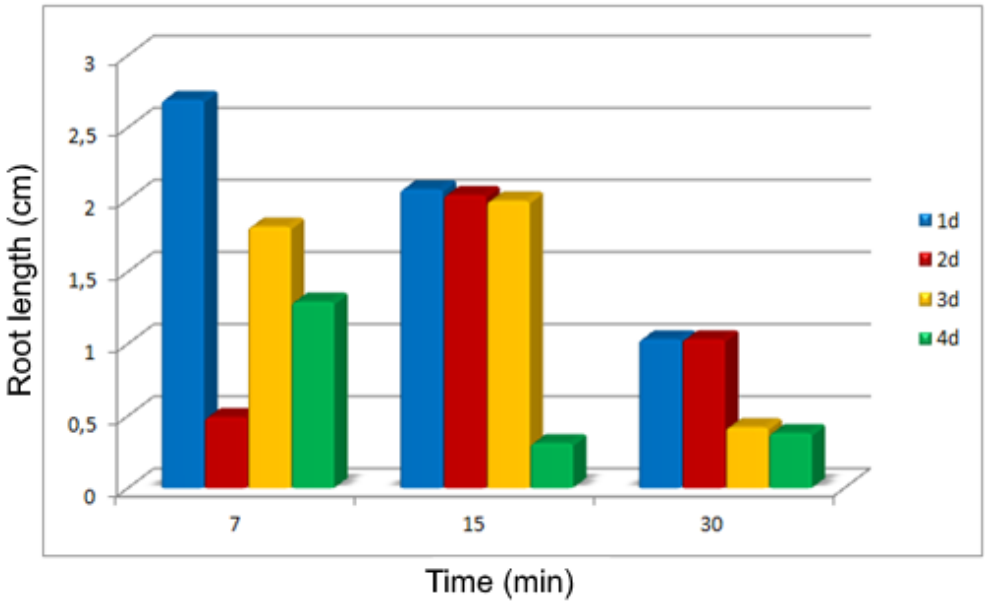

Figure 4. Dependence of roots of *Allium cepa* L. Bulbs (1d, 2d, 3d, 4d) growth on the time of exposure to electromagnetic waves at the frequency of 26 kHz and voltage of 9 V.

b) Micronucleus
2. Aberrations at the level of mitotic spindle
a) C-mitosis
b) Mitotic spindle multipolarity
3. Sub-chromatid aberrations
a) Sticky chromosomes

Cytotoxic activity was investigated by determining the total number of chromosomal aberrations and measuring mitotic activity. Mitotic activity or mitotic index (MI) is expressed as the ratio of number of cells in division to the total number of test cells. In the treatment of samples with different frequencies we observed the different mitotic

Table 1. Mitotic index of meristematic cells of *Allium cepa* L. exposed to different frequencies (Mean ± SD).

Exposure time for frequencies (min)	1 Hz MI	17 Hz MI	1.77 kHz MI	26 kHz MI	Control MI
7	48.19 ± 4.54*	43.82 ± 2.97****	62.85 ± 0.47****	55.08 ± 3.13	56.42 ± 1.04
15	38.18 ± 0.73****	53.43 ± 3.81	65.77 ± 1.59****	59.90 ± 0.33****	54.53 ± 0.28
30	31.29 ± 5.60****	46.84 ± 0.84****	61.96 ± 1.10****	63.28 ± 2.72***	53.87 ± 0.75

SD, standard deviation; MI, mitotic index.
Significant difference compared to the control group, *ρ<0.10; **ρ<0.05; *** ρ<0.01; ****ρ<0.001.

Table 2. Comparative presentation of chromosomal aberrations (Mean ± SD) at frequencies of 1 Hz, 17 Hz, 1.77 kHz and 26 kHz.

Frequency and time of exposure	1 Hz/7 min	1 Hz/15 min	1 Hz/30 min	17 Hz/7 min	17 Hz/15 min	17 Hz/30 min	1.77 kHz/ 7 min	1.77 kHz/15 min	1.77 kHz/30 min	26 kHz/7 min	26 kHz/ 15 min	26 kHz/ 30 min
Micronuclei	0	0	0	0	0	0	1±023	1±0,23	0	0	0	1±0,47
Sticky chromosomes	3	0	3	8±1.64	8±1.64	1±3.25	5±2.35	7±0.94	13±3.30	3±0.70	4±0	5±0.70
Chromosomal bridges	1±0.24	0	1±0.24	0±0.94	2±0.47	2±0.47	3±1.41	0	0	0	1±0.24	1±0.24
Stray chromosomes	10±2.36	8±0.94	2±3.30	12±4.24	3±2.12	3±2.12	8±0.94	9±1.65	3±2.59	10±0.47	16±3.77	6±3.29
Irregular segregation of chromosomes	2±0.47	2±0.47	0	6±8.48	4±9.89	8±7.07	9±0.24	8±0.47	9±0.24	2±4.95	15±4.24	10±0.70
Multipolarity	2±0.70	1	0	5±1.17	4±0.47	1±1.64	0	2±0.70	7±2.82	1±0.24	1±0.24	2±0.47
Apoptosis of cells	0	0	0	0	0	0	0	0	0	0	0	0
Necrosis of cells	0	0	0	0	0	0	0	0	0	0	0	0
Total	18	11	6	31	21	15	26	27	32	16	37	25

activity of meristematic cells (Table 1). Exposure of samples at a frequency of 1 Hz led to a significant decrease in mitotic activity. The most active cell division occured in the treatment at a frequency of 1.77 kHz, at which MI was 63.52%.

Table 2 provides a comparative overview of chromosomal aberrations caused by exposure to electro-magnetic waves at frequencies of 1 Hz, 17 Hz, 1.77 kHz and 26 kHz, and a voltage of 9 V. Figures 5, 6, 7, 8, 9 and 10 show chromosomal aberrations that are observed at various frequencies.

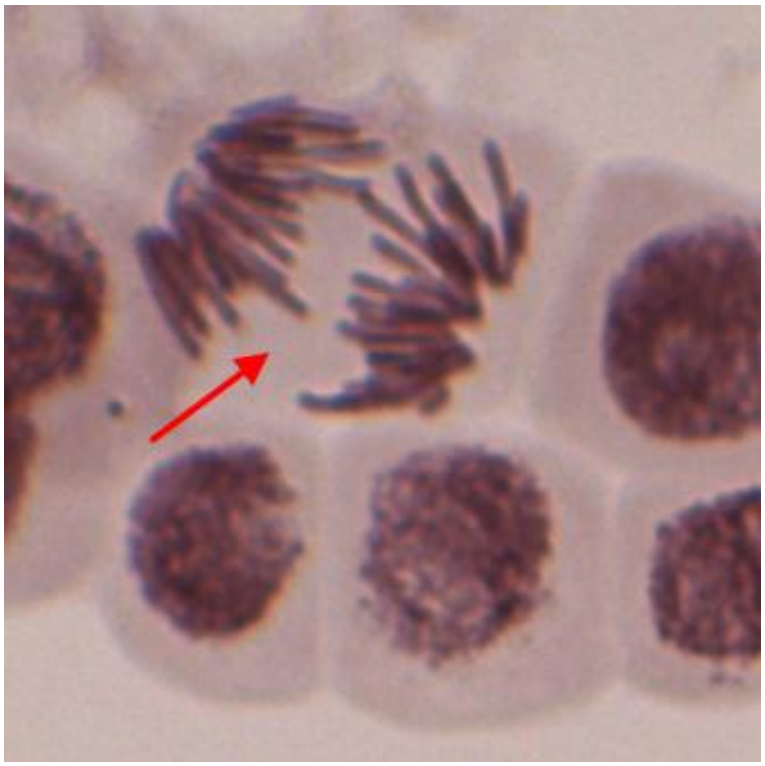

Figure 5. Multipolar anaphase (exposure at a frequency of 17 Hz).

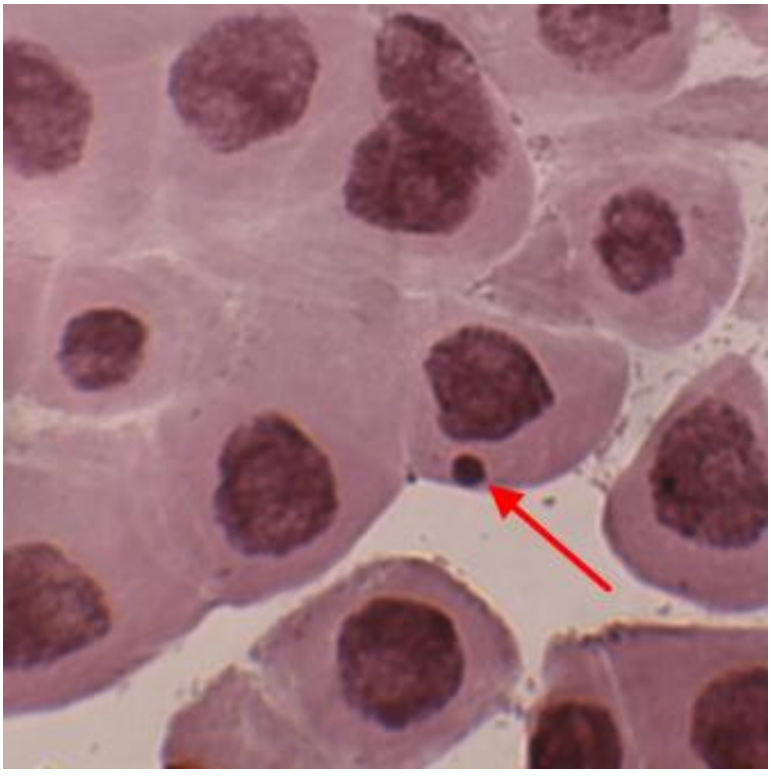

Figure 6. Micronucleus (exposure at a frequency of 1.77 kHz).

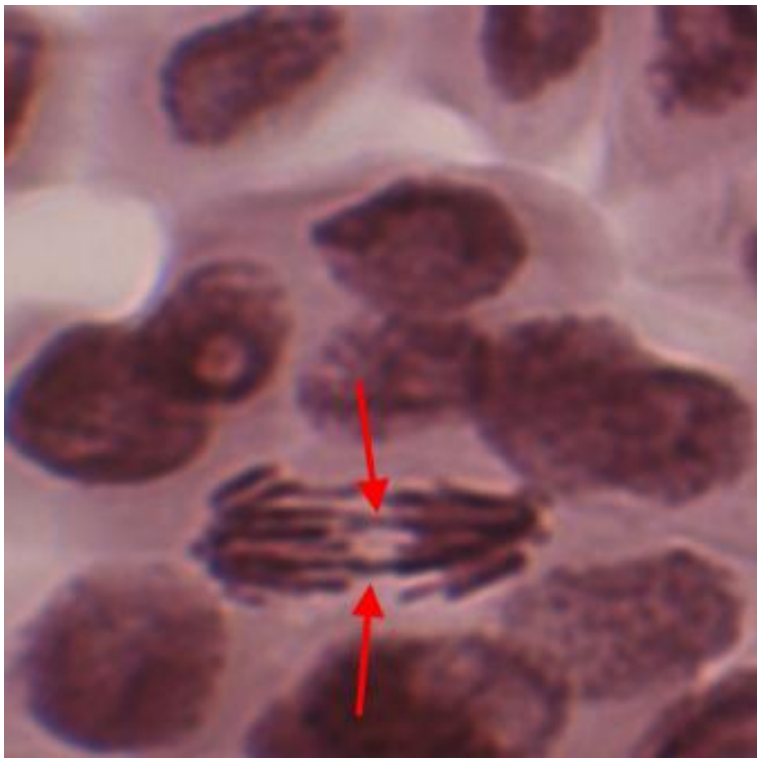

Figure 7. Chromosomal bridges (exposure at a frequency of 1.77 kHz).

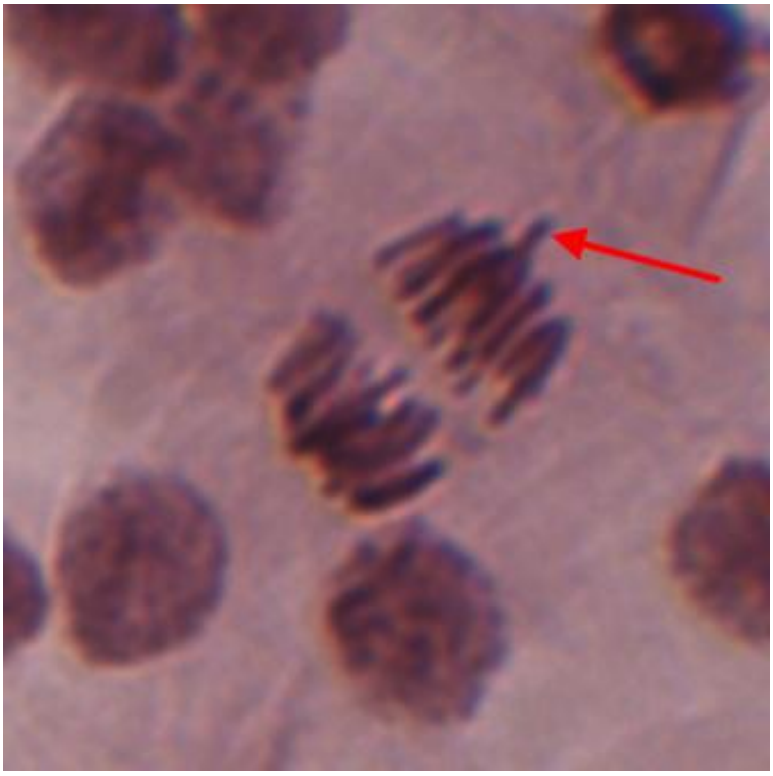

Figure 8. Stray chromosomes (exposure at a frequency of 17 Hz).

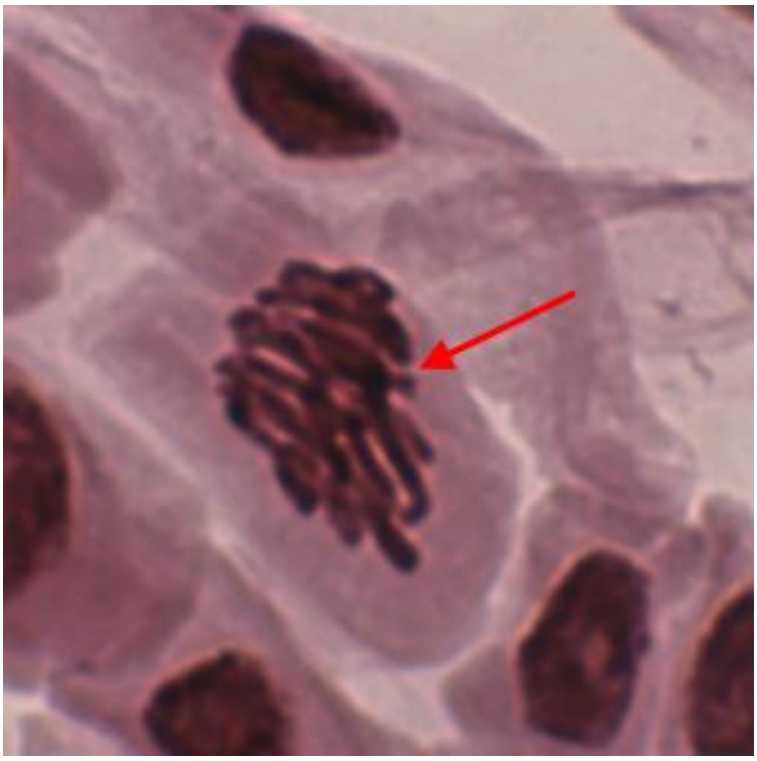

Figure 9. Irregular segregation of chromosomes (exposure at a frequency of 26 kHz).

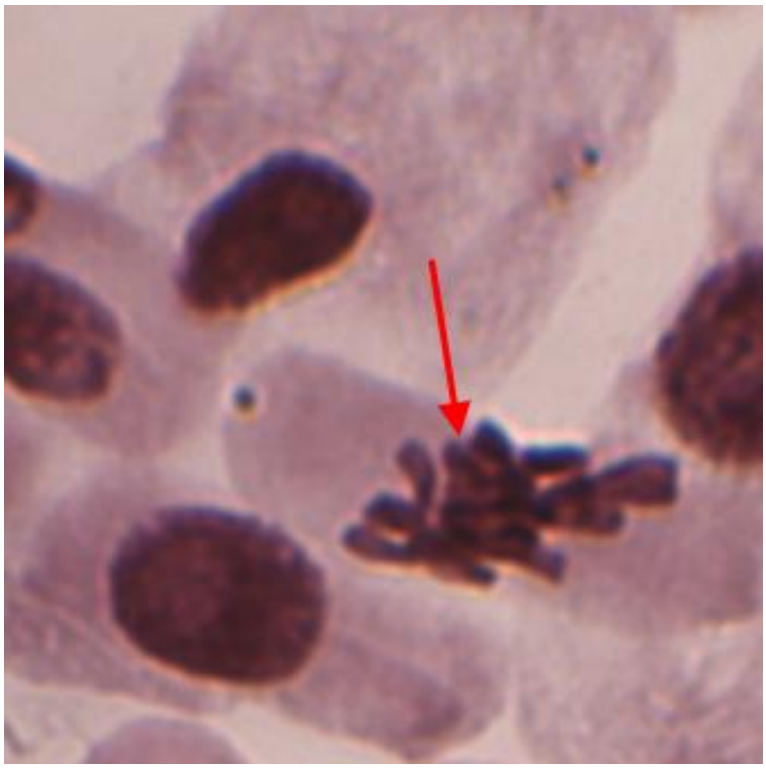

Figure 10. Sticky chromosomes (exposure at a frequency of 17 Hz).

Conclusion

Electromagnetic fields of a wide range of frequencies are increasingly present in the environment. Despite numerous studies around the world in recent years, it is still not clear how biological systems correspond-react and/or adapt to changes caused by the influence of external electromagnetic fields. We have shown that the growth of root tips of onion *Allium cepa* L. may be influenced by electromagnetic waves and that the growth effect depends on the exposure time. We observed slow growth of onion root tips, after treatments performed at frequencies of 1 Hz, 17 Hz and 26 kHz of a low-voltage electric current.

The growth of onion root tips at a frequency of 1.77 kHz and for a 15 min exposure time was the largest of all the applied frequencies and exposure times. Cytogenetic analysis performed on the onion roots indicated a reduced mitotic activity depending on the applied frequency. Conducted *Allium* tests pointed to geno- and cyto-toxicity of sample treatments at specific frequencies of low-voltage electric currents. Chromosomal aberrations appeared in the cells of onion roots treated at all frequencies. Depending on the frequency, we noted significant differences in the number and type of mitotic and chromosomal aberrations.

At a frequency of 1 Hz, the most common forms of aberration were stray chromosomes and improper segregation of chromosomes. At a frequency of 17 Hz, we recorded the highest number of sticky chromosomes, stray chromosomes and abnormal segregations of chromosomes. At a frequency of 1.77 kHz, the most common forms of aberration were stray chromosomes, sticky chromosomes and improper segregation of chromosomes. At this frequency we also observed micronuclei, multipolarity and chromosomal bridges. At a frequency of 26 kHz, the most common forms of aberration were stray chromosomes, improper segregation of chromosomes and sticky chromosomes. Chromosomal bridges, micronuclei and multipolarity also appeared at this frequency. Based on the results obtained and their discussion it can be concluded that some frequencies of low-voltage electrical currents have a distinctive influence on the genotoxic effects in meristem cells of onion.

REFERENCES

Adrović F. (2006). Fizika – Odabrana poglavlja iz optike, atomske i nuklearne fizike, Univerzitetski udžbenik, Copygraf Tuzla, Tuzla, ISNB 9958-9593-5-6.

Berberović L. J. & Hadžiselimović R. (1986). Rječnik genetike. Svjetlost, Sarajevo, ISBN 86-01-00723-6.

Fiskesjö G. (1993). The *Allium* test – A potential standard and assessment. Environ. Toxicol. Risk Assess. 1216(2).

Jovanović J., Đinđić B., Sokolović D., Krstić D., Petković D., Babović P. & Marković I. (2010). The damaging effects of exposure to extremely low frequencies of electromagnetic fields. Acta Medica Medianae. 49(1):54-58.

Lutz (1990). Endogenous genotoxic agents and processes as a basis of spontaneous carcinogenesis. Mutat. Res. 238:287-295.

Nefic H. (2013). Chromosomal and nuclear alterations in root tip cells of *Allium cepa* L. induced by Alprazolam. Med. Arch. 67:388-392.

Olivo O. M. & Delorenzi E. (1932). Archve of experiments. Zelforsch. 13:221.

Petz B. (1997). Osnovne statističke metode za nematematičare. Naklada slap, Zagreb.

Silva J., Erdtmann B. & Henriques J. A. P. (2003). Genetica toxicological Alcance Porto Alegre. Pp. 70-74.

Sofradžija A., Hadžiselimović R. & Maslić E. (1989). Genotoksičnost pesticida Svjetlost, Sarajevo.

Tadesco S. B. & Laughinghouse H. D. (2012). Bioindicator of genotoxicity: The *Allium cepa* test. In: Srivastava J. (ed) Environmental contamination, In Tech. Pp. 137-157.

Walleczek J. & Eichwald C. (1995). Model based on combined self-organized macroscopic and microscopic mechanisms for EMF effets in biological systems. Project Abstracts, pg. A-24, the Annual DOE/EPRI Review on Bioeffects of Electric and Magnetic Fields." Palm Springs CA.