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EFFECTS OF MOBILE PHONE RADIATION ON ROOT GROWTH AND MERISTEMATIC CELLS OF *ALLIUM CEPA* L.

REMYA KRISHNAN, G. S. MANOJ, S. R. HARISH, S. S. SUNUKUMAR, L. SHILPA SATHEESH, S. SAJITHA RAJAN and K.MURUGAN*

Plant Biochemistry and Molecular Biology Lab, Department of Botany, University College, Thiruvananthapuram-695034, Kerala, India.

*E-mail: harimurukan@gmail.com

During the last couple of decades there has been a tremendous increase in the use of cell phones. It has significantly added to the rapidly increasing electromagnetic field radiations (EMFr), an unprecedented type of pollution consisting of radiation in the environment, thereby prompting the scientists to study the effects on humans. However, not many studies have been conducted to explore the effects of cell phone EMFr on growth and biochemical changes in plants. The aim of the present study is to investigate whether EMFr from cell phones inhibit root growth by affecting mitotic activity in root meristematic cells of *Allium cepa* L. cv. Srebrnjak Majski. Onion bulbs were exposed for 24, 48, 72, 96 and 120 h to mobile phone radiation of distance 2 cm. The results indicate that EMFs radiations reduced mitotic division in *A. cepa* compared with the respective control. Mitotic index was generally decreased with increased treatment times. The total percentage of aberrations generally increased with duration of treatment. Different abnormal mitotic figures were observed in all mitotic phases. Among these abnormalities anaphase bridges, C-mitosis, micronuclei, lagging, stickiness, breaks and unequal distribution are common. Our results show that exposure to the radiofrequency fields investigated here can induce mitotic aberrations in root meristematic cells of *A. cepa.* The observed effects were markedly dependent on the duration applied. Our findings also indicate that mitotic effects of mobile phone may be due to impairment of the mitotic spindle.

Keywords: *Allium cepa;* Mitotic aberrations; Mitotic index; Mobile phone; Root growth.

Introduction

With the increasing utility and popularity of devices that emit radiofrequency radiation (RFR), such as mobile phones, public attention has been drawn to possible adverse health effects of exposure to this type of radiation. Numerous studies have been documented related to various biological effects of RFR like changes in cell proliferation[1], enzyme activity[2], gene expression[3], cell-membrane permeability ion homeostasis[4], oxidative stress[5] and heat-shock responses[6]. Recently, the possible genotoxic effects of exposure to RF-EMFs have been investigated in a variety of biological systems. The majority of these reports suggest that non-thermal exposure to RFR is not genotoxic and that adverse RFR effects are predominantly the result of hyperthermia[7]. However, some investigations of genetic effects gave positive findings[8], indicating the importance for further laboratory studies.

A number of plant bioassays have been developed for the detection of environmental mutagens, as these assays are relatively easy to perform, inexpensive and they provide a wide range of genetic endpoints[9]. Among them, the *Allium* test is one of the best established test systems; it has been routinely used due to its sensitivity and good correlation with animal test systems. The test is based on assessment of cytotoxic and genotoxic potential by measuring root growth as well as recording mitotic abnormalities and chromosomal aberrations in root tips[10]. The *Allium* test implies germination of onion bulbs, but roots can be obtained for analysis also,by seed germination[11]. Although assessment of the RFR impact on plants is of great importance because plants have an important role in the living world as main primary producers of organic compounds and oxygen, only a few investigations of RFR effects on plants have been reported[12,13]. The only such study on genotoxic effects, a micronucleus assay in *Tradescantia,* showed higher micronucleus frequencies after exposure to EMFs at 10-21 MHz[14]. For this reason, in the present study the effects of exposure to RFR on seed germination and root growth as well as on mitotic activity and induction of

Table1. The effects of EMFr on mitotic and phase indexes in the meristematic cells of *Allium cepa* L. roots.

		Phases of Mitosis(as % of MI value)			
Duration of Treatment	MI (%)	PP	MP	AP	TP
Control	12.02±0.76	29.84±2.75	24.84±0.59	19.56±0.55	25.21±0.68
24h	5.11±0.98	35.81±0.70	25.54±1.2	14.56±1.12	21.9±0.28
48h	٦.٨٦±١.6	١٦.٦±١.2	٣٢.٤±١	١٩.٨±٠.٢١	٣١.٢١±٠.٨٢
72h	10.02±1.2 5	30.02±1.2 5	37±0.9	21.6±0.52	35.21±0.7
96h	12.5±1.3	18.5±0.3	38.84±0.78	22.6±0.69	39.21±0.4
120h	6.5±1.9	48.5±0.9	32.84±0.29	16.56±0.59	13.3±1.08

Data are means ±standard errors of ten replicates.
MI: Mitotic Index, PP: Prophase, MP: Metaphase, AP: Anaphase, TP: Telophase.

Table 2. The effects of EMFr on the number of mitotic disturbances in the meristematic cells of *Allium cepa* L. roots.

	Abnormalities %						
Duration of Treatment	C-MP	Chr.Brid	Sticky	Lag	Budding nuc.	Binucl.	Total %
Control	1.02±0.04	0.42±0.02	------	-------	------	------	0.05±0.7
24h	3±0.08	2.81±0.70	------	-------	-------	0.23±0.65	1.23±0.5
48h	34.7±0.6	9.3±0.2	-------	1.8±0.2	0.1±0.12	0.3±0.11	12.9±0.3
72h	11.2±1.2	15.2±1	7±0.5	------	0.21±0.2	0.5±0.32	29.8±0.4
96h	9.5±0.3	10.5±0.3	5.84±0.8	2.6±0.6	0.2±0.4	4.23±0.5	41.23±0.5
120h	28.5±1.4	4.5±0.7	2.84±0.2	2±0.59	1.3±1.08	0.8±0.51	57.3±0.33

Data are means ± standard errors of ten replicates
C- MP: C- metaphase, Chr.Brid: Chromosome bridges, Budding nuc.: Budding nuclei, Binucl.: Binucleate.

chromosome aberrations were investigated in root meristematic cells of *Allium cepa.*

Material and Methods

In this study, the root-tip cells of *A. cepa* (2n = 16) were used as the test system. Bulbs of *A. cepa* were placed in small jars with their basal ends dipping in distilled water and germinating at room temperature (25±2°C). EMF exposure was carried out in a closed shielded chamber designed to act as a Faraday cage on the pattern of MSRC. A thermocol chamber with all its walls completely layered with metal sheets (aluminum; thickness=2 mm) to make the experimental environment free from outside EMF interferences. Two commercially available GSM cell phones (900 MHz band) with modulated voice and low frequency signals of 217 and 8.34 Hz, respectively; were used in the present study. They were placed horizontally inside the chamber (47.5 cm×27 cm×17.5 cm) wall for homogenous exposure at field strength of 5.7 V m^{-1}, and average power density (Pd) of 8.549 μW cm^{-2}. Pd was measured with RF Power Density Meter.During exposure; cell phones were used in silent ringing mode. The phone battery was permanently connected with 12 V DC, 220 V AC adaptor placed 2 m away from the cell phone. Fifty pre-imbibed (for 8 h in distilled water) onion bulbs placed in glass vials were kept between cell phones (at a distance of ~2 cm) for 24, 48, 72, 96 and 120 h. For control, a set of bulbs was kept inside another chamber without cell phones. All other EMFr from sources inside and outside the exposure laboratory were eliminated during exposure treatment. Both the chambers were maintained at a room

temperature of 25°C. After root treatments, the root tips were then fixed in a solution of ethanol (99%) and glacial acetic acid (3:1) for 24 h, washed with distilled water three times, and then dyed with aceto-orcein. Squashes were prepared using 2% aceto-orcein to determine the mitotic index and the presence of chromosomal aberrations.

Three replicates were performed for each treatment and scoring was done from the three roots of each replicate. A minimum of 1000 mitotic cells were counted from each slide. The MI was calculated for each treatment as a number of dividing cells/100 cells. The cytological abnormalities were scored in the mitotic cells and the results are shown in the tables and figures. The most frequent abnormalities are shown in photomicrographs.

Statistical analysis: Analysis of variance of the data was done with the SPSS computer program. For statistical analysis, one-way analysis of variance (ANOVA) was used.

Results and Discussion

The effect of mobile phone radiation on root growth of *Allium cepa* varied with duration of treatment. Morphologically the root growth decreased with increasing duration of the treatment. The inhibitory effect leads to stunted and bended roots. The number of dividing cells in *A. cepa* root meristem reduced in all test materials. The most pronounced effect was noticed at 120 h exposure, while the least at 24 h exposure. After 120 h treatment, the number of prophases increased while that of telophases decreased; in the case of 96 h, the situation was opposite (Table 1). On the other hand, in the 48 h treated roots, the frequency of telophases reduced while that of metaphases increased.

Disturbances of mitosis: In the control no chromosome aberrations were observed (Table 2). 96 h induced mitotic disturbances such as sticky and lagging chromosomes (Fig. 1 and 2). On the other hand, 120 h caused sticky chromosomes, nucleoli partly outside nuclei, "budding" nuclei and micronuclei. The EMFr not only induced mitotic abnormalities but also increased their number compared to the control. The maximum of abnormalities was observed after 120 h treatments (Table 2), mostly of c-metaphases, chromosome bridges, sticky chromosomes, binucleate cells, micronuclei, "budding" nuclei and nucleoli partly outside nuclei (Table 2). In the roots treated with 48 h an increase in the number of abnormalities was mainly the result of c-metaphases and chromosome bridges (Table 2). Contrary to the above-mentioned exposures, in 96 h treatment, c-metaphases and chromosome bridges were less frequent and the number of sticky chromosomes was nearly 40% smaller than that after 120 h treatment. However, binucleate cells were the most numerous in the roots treated with 96h. Moreover, lagging of chromosomes appeared (Table 2). The high frequency of c-metaphases, bridges and lagging chromosomes indicates a colchicine-like action on A. cepa roots by EMFr.

Results concerning biological effects and cytogenetic effects of EMFs published so far are controversial because of many negative as well as positive findings[6,15]. In our study, onion has been used for cytogenetic evaluation of exposure to EMFr since this test system has a long history of use[6] and comparable with other conventional test organisms in terms of sensitivity (lack of false negatives) and predictive value[16]. In addition to commonly used cytogenetic parameters, germination and root growth was also analysed in order to estimate possible effects of EMFs at the macroscopic level, since these parameters have often been used as convenient and sensitive indicators of environmental pollution[16]. Our results show that exposure to EMFs did not significantly change the early root growth in comparison with the control, indicating that exposure to EMFs under the test conditions did not have an inhibitory effect on *A.cepa*. Similarly we could also find reports that radiation effect on seed germination of other plants showed inhibition as well as stimulation of the germination rate[17]. Data from studies investigating the effect of extremely low-frequency EMFs on plant germination and early growth suggest that the EMF effects correlate with ion cyclotron-resonance frequencies for calcium and potassium ions, causing a change in their distribution which consequently influence seed germination and plant growth[17]. In human cells the EMFs has the ability to either increase cellular proliferation[18] or suppress it[6]. These alterations have been associated with increases in gene expression of various cell-cycle regulators such as G2/mitosis-specific cyclin G1, transforming growth factor-b, mitogen-activated protein kinase 3, the c-*myc* proto-oncogene and apoptosis regulator *bax*[19]. In animals, Sykes *et al.*[20] has found an increase in actively dividing cells in the mice after exposure to 105-GHz electromagnetic radiation. In the present study, exposure to EMFs under most of the test conditions induced a significant increase of mitotic activity in root tips of *A. cepa* compared with control. It was somewhat unexpected since the germination rate and root growth did not differ from the control. Since the root growth can depend on both mitotic activity and cell elongation, and these processes could be influenced by EMFr, it is not impossible to notice an exception from commonly expected positive correlation. Moreover, an

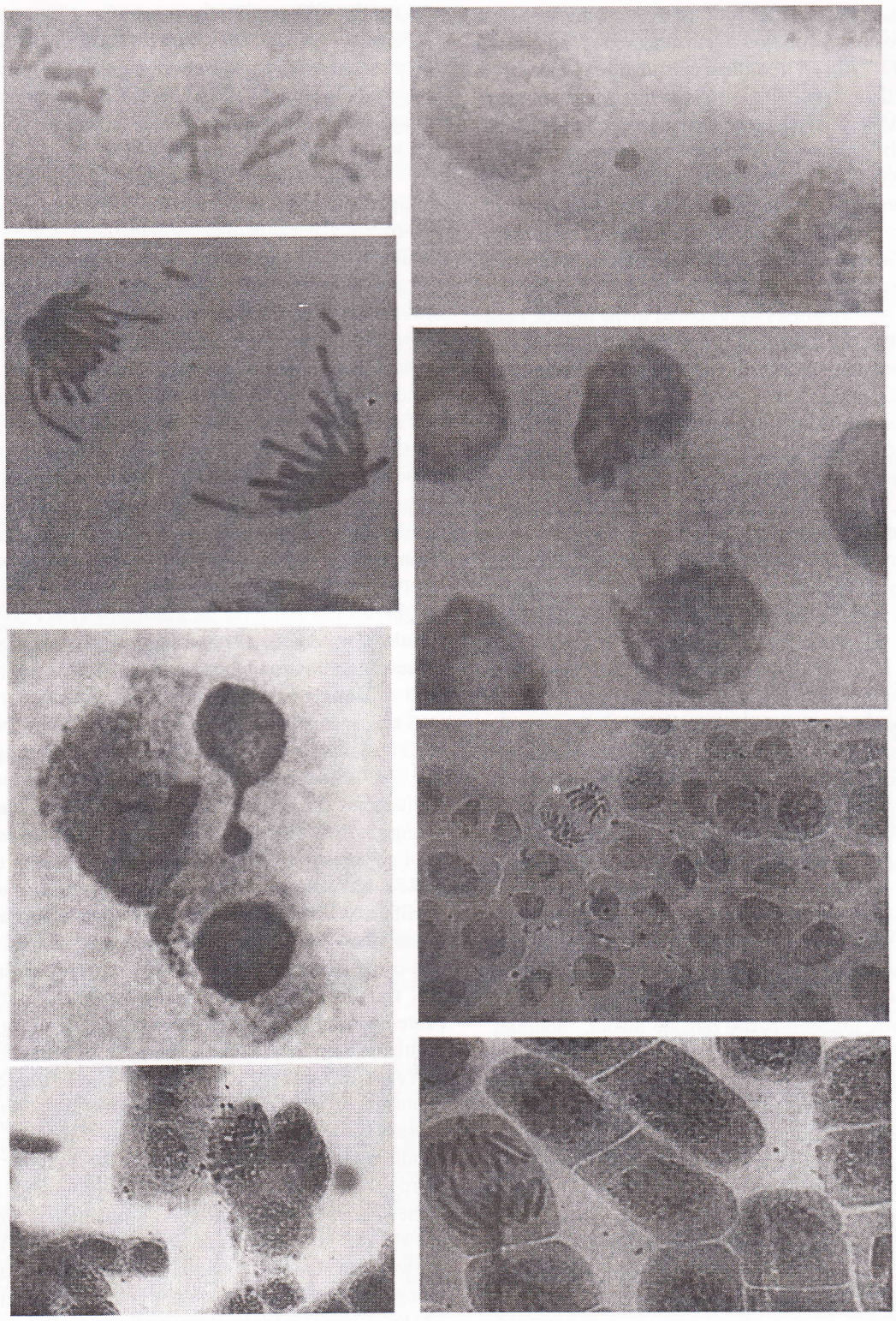

Fig. 1. Chromosome abnormalities

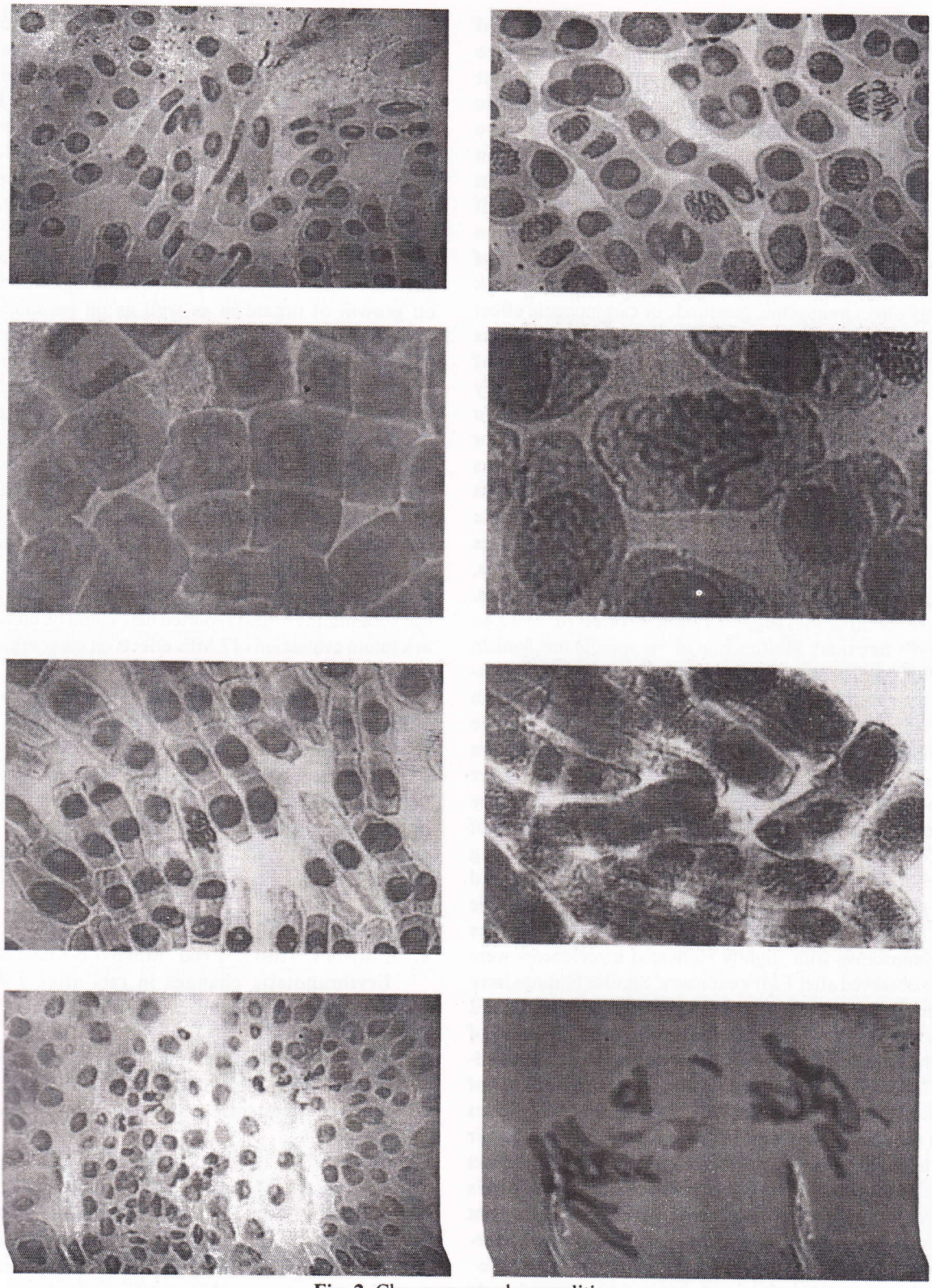

Fig. 2. Chromosome abnormalities

increased mitotic index seems not to be related to increased cell proliferative activity, but it could be a consequence of delayed mitosis. The appearance of vagrant chromosomes after exposure to EMFr implies mitotic spindle disturbances, an aberration that could cause delayed prophase and/or metaphase and lead to an increased mitotic index[21] as observed in our study. In barley, exposure to EMF at 61.5GHz also affected cell division. It increased the degree of cell-division synchronization[22], which could also be a consequence of mitotic delay[21]. A number of studies demonstrated the lack of any direct mutagenic, genotoxic or carcinogenic effect of MPR at intensities[23]. At the same time, genotoxic effects were reported-mainly as increased frequency of micronuclei[12] and single-strand DNA breaks[1]. The only genotoxic effect on plants was studied in *Tradescantia* after exposure to short-wave EMFs (10-21 MHz, used for broadcasting) which caused a higher micronucleus frequency[2]. In our study, EMFr under specified conditions of exposure induced a significant increase in mitotic abnormalities in root tips of *A. cepa.* Major abnormalities found after EMFr exposures were lagging chromosomes, vagrants, disturbed anaphases and chromosome stickiness. These abnormalities suggest a possible effect of EMFs on spindle function[1]. Malfunction of the spindle mechanism could be connected with the effect of EMFr on calcium-ion homeostasis in cells. Calcium ions in excess can disturb polymerisation of microtubules, thereby affecting spindle formation[4]. Other possibilities for spindle malfunction could be changes in the cytoskeleton proteins after exposure or reorganization of cytoskeleton due to the electric field[5]. Recently published results on the effects of 935-MHz continuous-wave frequency field on lung fibroblasts of the Chinese hamster (V79 cells) reported alterations in microtubule proteins-the proteins responsible for spindle assembly[24]. In some cases metaphase chromosomes with slightly elongated centromeres were also observed after EMFr exposure. Similar findings have been reported in *A. cepa* root-tip cells cultivated for 2 days at various distances from video-display units of computers or TV sets and also in *Allium* treated with oil-field wastewater containing small amounts of radionuclides[25]. However, this cytogenetic change does not necessarily represent biological damage. Since EMFr do have enough energy to directly damage DNA the exact mechanism of cytogenetic effect due to exposure to EMFs is yet to be clarified. Our results are in good agreement with those of other authors who suggested that EMFr-induced micronuclei could be the outcome of spindle disturbance or DNA damage[24]. Moreover, it was found that EMF at 915 MHz induced changes in chromatin conformation and inhibited formation of DNA-repair foci in human lymphocytes *in vitro.* In our experiment the effect on the spindle was noticed 48 h after exposure to EMFr. Such a delayed effect suggests an indirect effect on mitosis through changes of conditions in cytosol (for example ionic strength and/or induction of reactive oxygen species), which could influence condensation and replication of genetic material.

This is consistent with previous findings that exposure to most EMFs at 900 MHz had a significant effect on growth of organism as well as on parameters of oxidative stress, while at 400 MHz the effect was observed only at the highest field strength and with field modulation. The results from animal and cell studies showing that the effects of radiofrequency fields are associated with certain frequency, field intensity windows and field modulation has already been reported. In our study, we found a possible cytogenetic effect of EMFr of certain field strengths on plants that are important components of the environment. However, since the original procedure of the *Allium* test has been standardized[26] and there has been good correlation between effect on onion-root cells and animal test systems, the results reported here could be considered in a future evaluation of EMFs effects on other organisms, even on human health.

Evidence suggests that cell processes can be influenced by weak electromagnetic fields (EMFs). EMFs appear to represent a global interference or stress to which a cell can adapt without catastrophic consequences. The impaired root growth and mitotic abnormalities observed in the present study in onion from mobile phone EMFs may be due to oxidative stress induced by the reactive oxygen species such as superoxide anion, H_2O_2 like free radicals.

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