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Original Research

Effects of Electromagnetic Waves Emitted by Mobile Phones on Germination, Root Growth, and Root Tip Cell Mitotic Division of *Lens culinaris* Medik

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Abstract

In this study, the effects of electromagnetic waves emitted from mobile phones operating at 1800 MHz were investigated on germination, root growth and mitotic division of root tips of *Lens culinaris* Medik. Seeds were split into three groups. The first group was exposed to a mobile phone electromagnetic field for 48 hours at the state of dormancy, and the second group was exposed to the same electromagnetic field at the state of division. The third group, the control group, was not exposed to an electromagnetic field beyond the natural background. The results obtained in the study indicate that electromagnetic waves emitted from mobile phones affect seeds in the state of dormancy more than the state of germination. Germination rate was not affected under the specified exposure conditions, but root growth decreased due to a possible effect of oxidative stress in the state of dormant seeds. There was also a noticeable increment in the c-mitosis rates, especially in the state of dormant seeds. The reason for this increment could be problems in spindle function.

Keywords: mobile phones, electromagnetic waves, biological effects, mitosis, *Lens culinaris* Medik

Introduction

Our environment is subjected to exposure to microwaves and (radio)electromagnetic irradiations as a result of widespread use of wireless telecommunication. This yields a massive increase in electromagnetic pollution [1]. Mobile phones operate on wireless technology using a 900-1800 MHz (GSM) channel and 2200 (UMTS/3G) signals [2]. There is a concern of possible adverse effects of mobile phone radiation as a result of the enormous increase in the use of these phones throughout the world. The potential risks of electromagnetic field (EMF) emitted by mobile phones on living systems has been intensely studied. Most of the studies were focused on human health [3 (a literature review published between 2000-04), 4-8]. Many other studies were conducted on genetic [9-12] and biological [13, 14] effects of mobile phones and the effects of mobile phones on biological systems, including plants [15-20].

Plants are the main producers of organic compounds and oxygen [20]. In the natural environment, they are also exposed to continuous electromagnetic fields. Tomato plants were exposed to a low level of EMF (900 MHz,

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5 V·m^{-1}) for 10 minutes to measure changes in the abundance of three wound-induced transcripts playing a role in the early events of plant responses to stress [17]. They suggested that application of radio frequency fields has a stimulus effect on tomato plants, resulting in the accumulation of stress-related transcripts.

Duckweed (*Lemna minor* L.) was exposed to EMF for two hours to investigate the physiological response of the plant [18]. Oxidative stress was induced, especially at 900 MHz, by exposure of the duckweed to non-thermal exposure to radiofrequency fields, probably due to the effect on antioxidative enzyme activities.

The inhibitory effect of EMF radiation on root growth of mung bean (*Vigna radiate*) has recently been investigated [19]. Significant inhibition of root growth was observed as a result of the application of a cell phone electromagnetic field by inducing reactive oxygen species-generated oxidative stress in a time-dependent manner.

Allium cepa L. seeds were exposed to radio frequency EMFs (400 and 900 MHz, for two hours at different strengths) to investigate the effects of this application on root growth, mitotic activity, and mitotic aberrations of *Allium cepa* L. meristematic cells [20]. It was reported that the root's length and germination rate were not changed depending on the specified application, but mitotic index was increased significantly and mitotic aberrations, such as lagging chromosomes, vagrants, chromosome stickness, and disturbed anaphase were induced. The reason for the increase in the mitotic abnormalities may be explained with the impairment of the mitotic spindle following the application of electromagnetic fields.

In a recent study, cell mitosis was also observed continuously under the microscope on metastatic murine B16F10 melanoma cells that were exposed to modulated EMF similar to mobile phone signals. It was reported that the sequence of mitotic events, the mitosis total duration, and the duration of each of the mitosis phases were not modified by cell exposure to 900 MHz GSM-EMF signal of 2.2 W/kg SAR applied for 1 hour [21].

Literature about the biological effects of EMF emitted by mobile phones and their possible effects on plants are still unclear and contradictory. The present study aims to build up the knowledge on the possible genetic effects of mobile phone EMF *in vivo*. *Lens culinaris* Medik (Lentil) was chosen for the current research because of having a relatively small number (2n: 24) of large chromosomes. In addition, lentil seeds are edible and therefore has potential economic use in food industry. Germination root growth and mitotic division of root tips of lentil seeds were analyzed, and aberrations such as c-mitosis, bridges, and laggard or vagrant chromosomes were visibly checked under the microscope.

Materials and Methods

Plant Material and Growth Conditions

Lens culinaris Medic seeds (Lentil) were obtained from the agriculture directorate of Elazig, Turkey. 30 lentil seeds were planted on double-layer filter paper in an 11 mm diameter petri dish as three replicates. Seed samples in the first petri dish were used to apply electromagnetic waves to the seeds in a dormant state for 48 hours, then germinated in a controlled laboratory at 22ºC in natural light. Seed samples in the second petri dish were germinated in a controlled laboratory environment at 22ºC prior to application of electromagnetic waves to the root tips for 48 hours. The third was used as control. Control seeds were not subjected to electromagnetic waves beyond the natural background.

Electromagnetic Field Exposure System

The experiments used mobile phones operating at 1800 MHz with 1 mW antenna output power. An information signal was sent for realization of modulation during communication. The original duration of the information signal was 4.6 ms, but it is compressed to 0.58 ms and transmitted *via* the mobile phones. The Mobile phone and base stations send a pulse whose duration is 0.58 ms every 4.6 ms. In this way energy transfer is also realized.

The first group of seeds were exposed to mobile phone EMF for 48 hours prior to germination (referred to as state of dormancy). EMF was applied to the second group of seeds for 48 hours while germinating (referred to as state of division). The third group of seeds were not subjected to EMF beyond the natural background and used as control. Distance between the petri and mobile phone was 2.2 cm in order to supply 0.76 W/kg SAR. This value has been consciously chosen, because the human brain is subject to the same electromagnetic field strength when a mobile phone is used while talking at a distance of 2.2 cm from the ear. The implemented experiment system is put into a fibreglass box of dimensions 150×120×60 cm. Finally, the box was placed in the incubator in order to keep temperature constant. Signals generated randomly by computer were applied to the voice inputs of each mobile phone to provide data transmission *via* antenna through petries. The system has been shown in Fig. 1.

Cytogenetic Analysis

Seed roots were harvested immediately after exposure. Root tips were cut out and immersed in paradichlorobenzene for 4 h, fixed in acetic acid-alcohol (1:3) for 24 h, then transferred into 70% alcohol and stored in a fridge until use. Root tips were removed from alcohol, washed with tap water, and hydrolized with 1 N HCl at 60ºC for 17 min for mitotic evaluation. They were stained with Feulgen reactive for 1 h. After that the root tips were kept in tap water for 15 min. Finally the very end part of the root tips that stained intensely were cut out and squashed in 45% acetic acid and used for microscopic evaluation.

Results

Possible cytogenetic effects upon EMF emitted by mobile phones were investigated by germination rate, root growth and mitotic division of root tips of *L. culinaris*. The first group of seeds were exposed to mobile phone EMF for

48 hours prior to germination (referred to as state of dormancy). EMF was applied to the second group of seeds for 48 hours while germination (referred to as state of division). The third group of seeds were not subjected to EMF beyond the natural background and used as control.

No significant difference was observed between any investigated groups and control samples in terms of germination rate. 14, 13, and 11 seeds out of 30 were germinated in the state of dormancy, division and control groups, respectively.

Root growth of *L. culinaris* after the determined exposure conditions are presented in Fig. 2. As seen in Fig. 2, significant differences in the root growth were observed among each group. The root growth in the control group averaged 1.73 cm, but the difference was significant between root growth of state of dormancy and state of division, which are 0.70 cm and 2.35 cm in an avarage, respectively.

Normal mitosis phases have been observed in the 48-hours-old control seedlings that were not exposed to EMF beyond the natural background (Fig. 3).

Experimental results of mitosis in the root tip cells of *L. culinaris* seeds have been given in Table 1. As clearly shown in Table 1, 8.85% and 14.05 of the seeds were germinated at the state of dormancy and state of division seeds, respectively. The percentage of germination in control seeds was 10.35%. This may indicate that electromagnetic waves affect the embryonic cells more than the dividing cells and therefore reduce the rates of germination in these seeds.

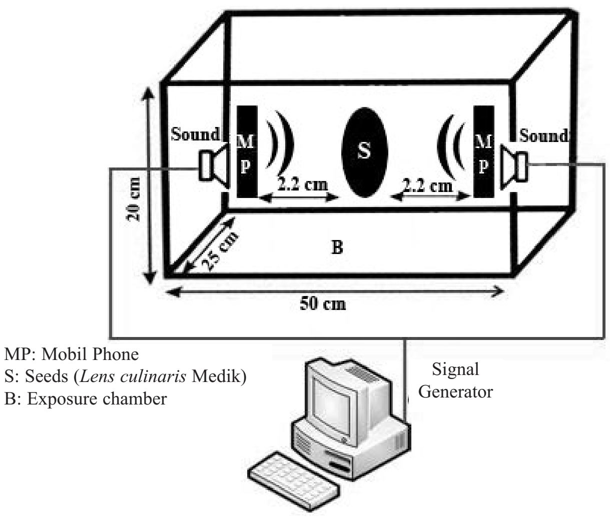


Fig. 1. Electromagnetic field exposure system.

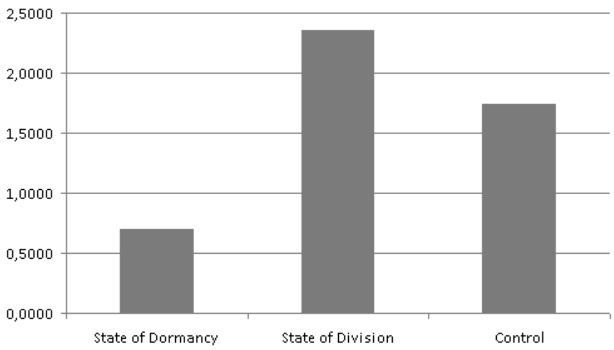


Fig. 2. Root growth of *L. culinaris* after exposure to mobile phone EMF.

Germination was decreased by 12.50% in the state of dormancy seeds compared to the germination rate of the control seeds. In contrast, germination was increased by 35.65% in the state of division seeds compared to the control group as a result of possible thermal effect (Table 1). This observation was also supported by Fig. 2, showing the difference in the germination of each group of seeds. Therefore, it is reasonable to conclude that germination was reduced because of harmful effects of electromagnetic waves on the lentil embrios. Another effect of electromagnetic waves on embrio was observation of abnormal dividing rates in these cells. Application of electromagnetic wave to the seeds at the dormancy state increased abnormal division by 52.38% compared to control cells as shown in Table 1, whereas no reasonable abnormal division effect was observed experimentally on the cells exposed to the electromagnetic waves during germination. Both experimental results are consistent in that embryonic cells were much more affected by the electromagnetic waves than the germinating cells.

Experimental results of abnormal mitosis stages are given in Fig. 4. Numbers and rates of chromosome aberrations in experimental and control groups are presented in Table 2, where the percentage of c-mitosis is 2.65%, 1.71%, and 0.98% in the state of dormancy, state of division, and the control seeds, respectively. Table 2 also shows that increases in the c-mitosis are 185.71% in the state of dormancy seeds and 84.3% in the state of division seeds, compared to the control group. The ratios of other chromosome aberrations were at neglectable levels in different stages of mitosis.

Discussion

The present study was conducted to explore the effects on EMF irradiated by mobile phones on the germination, root growth, and mitotic division of root tips of *Lens culinaris* Medic. The effects of EMF emitted by mobile phones on plant germination is contradictory. *Allium cepa* L. seeds were exposed to 400 and 900 MHz of EMFs for 2 h, and no significant changes were observed in the germination rates of the seeds [20] as in our study, even applying the EMFs for a longer period. In contrast, the effects of radio frequency EMF generated from a radio location station were investigated on needles and cones of pine trees, and low germination of seeds were reported as a result of stress caused by exposure to radiofrequency EMF [16].

A correlation was observed between root growth and the amount of normal dividing cells. Mitotic activity was higher in the state of division and lower in the state of dormancy compared to the control. In parallel to mitotic activity, root growth was also higher in the state of division and lower in the state of dormancy compared to the root growth

Table 1. Experimental results of mitotic division.

Experiment	Cells analyzed	Divided cells		Mitotic index	Division increase compared to control (%)	Abnormal division rate (%)	Abnormal division increase compared to cotrol (%)
		Normal	Abnormal				
Electromagnetic waves applied for 48 hours prior to 48 hours germination (state of dormancy)	2000	177	12	8.85	**-12.50**	6.35	**52.38**
Electromagnetic waves applied for 48 hours while germinating (state of division)	2000	281	12	14.05	**35.65**	4.10	**-1.71**
Control	2000	207	9	10.35	-	4.17	-

of the control seedlings. This supports the idea that root growth can depend on mitotic activity [20]. This positive correlation confirms our findings that mobile phone EMFs may have an effect on the root growth process. Effects of 900 MHz cell phone EMFr were investigated on the rooth growth of *Vigna radiata* (mung bean). It was found that application of cell phone EMFr has an inhibitory effect on the rooth growth of mung bean by inducing reactive oxygen species-generated oxidative stress, despite increased activities of antioxidant enzymes [19]. This may explain our results obtained on the dormancy state seeds that root growth was reduced by 59.55% compared to the control

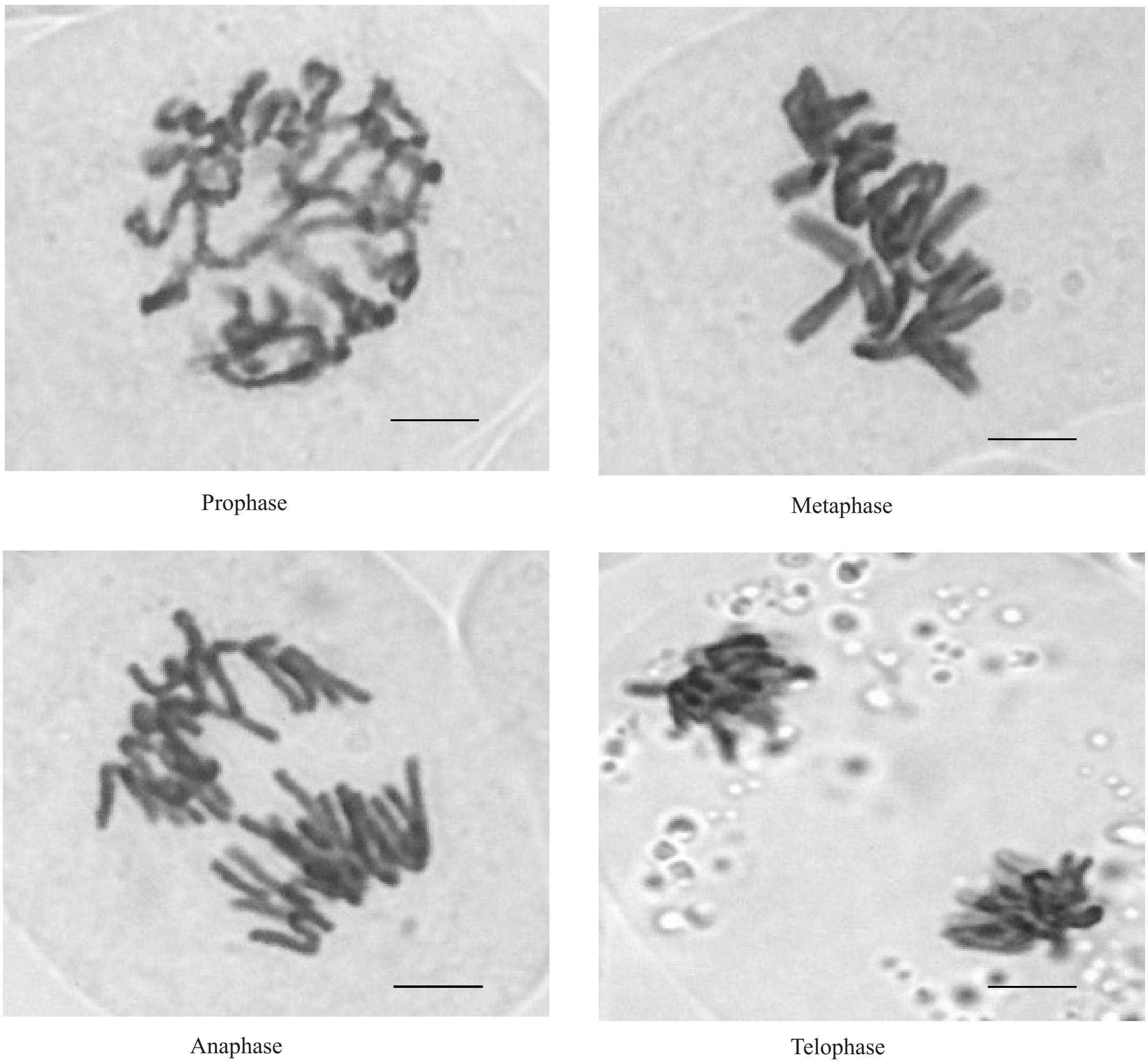


Fig. 3. Normal mitosis stages in the root tip cells of control *L. culinaris* seeds (scale bars: 10 μm).

Table 2. Numbers and rates of chromosome aberrations in experimental and control groups.

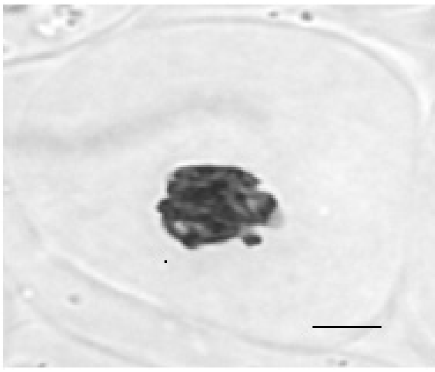
Experiment	c-mitosis		Laggard chromosomes in metaphase		Vagrant chromosomes in anaphase		Bridge in anaphase		Multipolar anaphase		Bridge in telophase		Vagrant chromosome in Telophase		Multipolar telophase	
	Rate (%)	Num.	Rate (%)	Num.	Rate (%)	Num.	Rate (%)	Num.	Rate (%)	Num.	Rate (%)	Num.	Rate (%)	Num.	Rate (%)	Num.
Electromagnetic waves applied for 48 hours prior to 48-hour germination (state of dormancy)	2.65	5	0.01	1	0.01	2	0.01	1	0.01	1	0.00	0	0.01	1	0.01	1
Electromagnetic waves applied for 48 hours during germination (state of germination)	1.71	5	0.01	3	0.01	2	0.00	0	0.00	1	0.00	1	0.00	0	0.00	0
Control	0.93	2	0.01	2	0.01	2	0.00	1	0.00	1	0.00	0	0.00	1	0.00	0

group. Reduced plant growth was also reported as a result of the application of EMF exposure at radio frequencies of 400, 600, and 1900 MHz on *Lemna minor* [22]. The increase in the root growth of state of division seeds could be explained as a result of thermal effect.

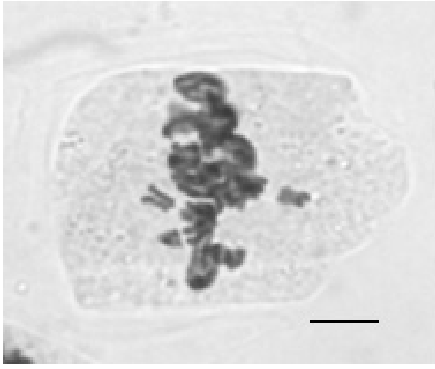
A literature research was performed on the genotoxic effects of radiofrequency electromagnetic fields [12]. Of 101 publications, 49 reported genotoxic effects while 42 did not. Numerical or structural anomalies of metaphase chromosomes were exploited by 21 studies that 9 of them report positive finding and 11 negative [12]. This part of the present study exploits cytogenetic effects of electromagnetic fields emitted by mobile phones on the root meristematic cells of *L. culinaris* seeds. Laggard chromosomes, c-mitosis, vagrant chromosomes, and bridges were investigated parameters (Fig. 4).

The *Allium* test is known to be a standard in monitoring environmental pollution [23]. This test provides information about environmental hazards caused by chemicals, pollutants and contaminants that observation of the adverse effects on chromosomes by this test provides an indication of toxicity [24]. In this present study, c-mitosis, laggard chromosomes in metaphase, multipolar anaphase, vagrant chromosomes in anaphase, bridge in anaphase, multipolar telophase, vagrant chromosomes, and bridge in telophase were observed aberrations (Fig. 4). In a recent study, observation of lagging chromosomes, vagrants, disturbed anaphases and chromosome sticknes in EMF-treated *Allium cepa* root meristematic cells were suggested to be a possible result of exposure to spindle function [20]. In our study, chromosome aberrations except the c-mitosis were found to be similar to the control group. The rate of c-mitosis was 2.64% in state of dormancy seeds, 1.71% in the state of division seeds, and 0.93% in the control group (Table 2). Observation of high rates of c-mitosis in the state of dormancy seeds is consistent with the rate of abnormal division rate results (Table 1). This rate was 4.17% in the control seeds, but 6.35% in the state of dormancy seeds. As suggested by [7, 25], that in the case of c-mitosis, the nuclear spindle is fully inactivated, meaning that no equatorial plate becomes organized and that the centromere division is [25]. Having an increased rate of c-mitosis on both state of division seeds and state of dormancy seeds may support the idea that electromagnetic exposure by mobile phones has a hazardous effect on spindle function of *Lens culinaris* root tip meristematic cells as in *Allium cepa* [20, 25].

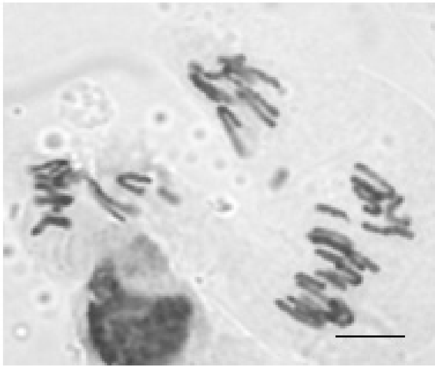
As seen in our general results, dormant seeds exposed to an electromagnetic field emitted by a mobile phone was much more affected than the state of division seeds in terms of root growth and chromosome aberrations. Similar to the results of the dormant seeds in our study, fertile chicken eggs were exposed to a cell phone in the call position over the entire incubation period. A harmful effect was observed on embryo survival upon prolonged and permanent exposure of fertile chicken eggs to cell phone radiation [26]. Detection of negative effects on embryo is consistent with our findings that seeds in the dormant state were more affected.



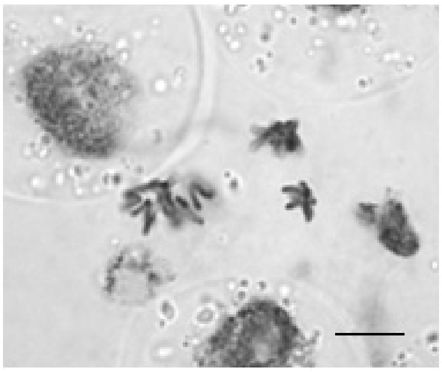
c-mitosis



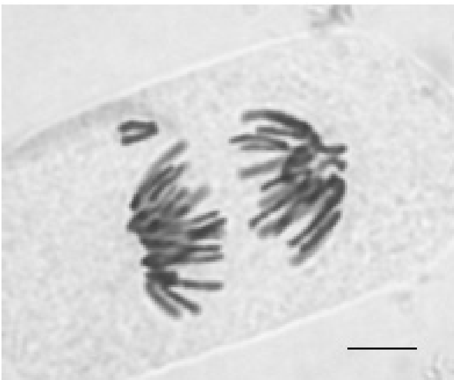
Laggard chromosomes in Metaphase



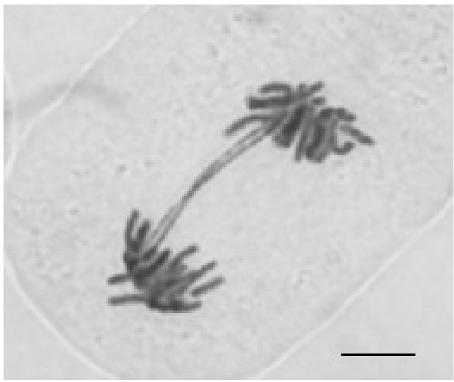
Multipolar Anaphase



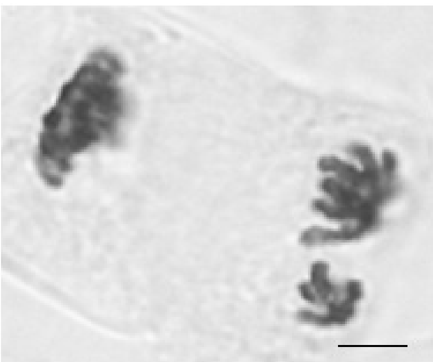
Multipolar Anaphase



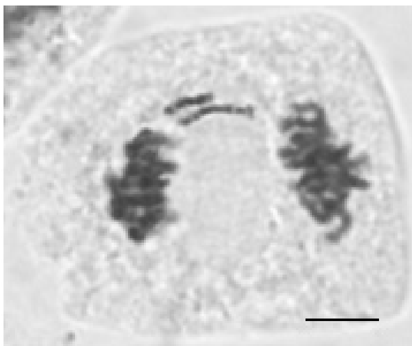
Vagrant chromosomes in Anaphase



Bridge in Anaphase



Multipolar Telophase



Vagrant chromosomes and bridge in Telophase

Fig. 4. Chromosome aberrations in the root tip cells of *Lens culinaris* Medic seeds caused by electromagnetic waves emitted from mobile phones (scale bars: 10 µm).

Conclusions

The results obtained in the study indicate that electromagnetic waves emitted from mobile phones operating at 1800 MHz affect seeds in the state of dormancy rather than the state of germination. Germination was not affected under the specified exposure conditions, but root growth was decrased due to a possible effect of oxidative stress [18] in the dormant seeds. There was a noticeable observation in the c-mitosis rates, especially in the dormant seeds. None of the exposed seedlings had a greater frequency of other chromosome aberrations such as laggard chromosomes, vagrant chromosomes, and formation of bridge.

As our environment is highly exposed to electromagnetic waves by many sources, including mobile devices, these negative affects of electromagnetic fields emitted by mobile phones on any living cells and also edible plants should not be ignored. All these results may indicate that these effects should be further evaluated and investigated for other organism cells, especially for human cells.

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