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Comparative cyto- and genotoxicity of 900 MHz and 1800 MHz electromagnetic field radiations in root meristems of *Allium cepa*

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ABSTRACT

In the last few decades, tremendous increase in the use of wireless electronic gadgets, particularly the cell phones, has significantly enhanced the levels of electromagnetic field radiations (EMF-r) in the environment. Therefore, it is pertinent to study the effect of these radiations on biological systems including plants. We investigated comparative cytotoxic and DNA damaging effects of 900 and 1800 MHz EMF-r in *Allium cepa* (onion) root meristematic cells in terms of mitotic index (MI), chromosomal aberrations (CAs) and single cell gel electrophoresis (comet assay). Onion bulbs were subjected to 900 and 1800 MHz (at power densities 261 ± 8.50 mW m^{-2} and 332 ± 10.36 mW m^{-2}, respectively) of EMF-r for 0.5 h, 1 h, 2 h, and 4 h. Root length declined by 13.2% and 12.3%, whereas root thickness was increased by 46.7% and 48.3% after 4 h exposure to 900 MHz and 1800 MHz, respectively. Cytogenetic studies exhibited clastogenic effect of EMF-r as depicted by increased CAs and MI. MI increased by 36% and 53% after 2 and 4 h exposure to 900 MHz EMF-r, whereas it increased by 41% and 67% in response to 1800 MHz EMF-r. Aberration index was increased by 41%–266% and 14%–257% during 0.5–4 h of exposure to 900 MHz and 1800 MHz, respectively, over the control. EMF-r exposure decreased % head DNA (DNAH) and increased % tail DNA (DNAT) and olive tail moment (OTM) at both 900 and 1800 EMF-r. In 4 h exposure treatments, head DNA (%) declined by 19% and 23% at 900 MHz and 1800 MHz, respectively. DNAT and OTM were increased by 2.3 and 3.7 fold upon exposure to 900 MHz EMF-r over that in the control, whereas 2.8 and 5.8 fold increase was observed in response to 1800 MHz EMF-r exposure for 4 h and the difference was statistically significant. The study concludes that EMF-r in the communication range (900 and 1800 MHz) adversely affect root meristems in plants and induce cytotoxic and DNA damage. EMF-r induced DNA damage was more pronounced at 1800 MHz than that at 900 MHz.

1. Introduction

Electromagnetic field radiations (EMF-r) of 800–2100 MHz range are the most commonly used radiofrequency radiations in wireless communication. Nowadays, there has been an exponential growth in the number of mobile phone users, with over 7.74 billion subscribers worldwide (ITU, 2018). Of late, due to increased awareness about the risks associated with EMF-r, there has been an increase in the studies related to risk assessment of EMF-r in living organisms including plants (Cucurachi et al., 2013 and references therein). Enhanced EMF-r levels in the environment have been regarded as a new contaminant to the list of pollutants (Sage and Carpenter, 2009). The evidences in various areas of investigations, *in vitro* studies, epidemiological studies and animal systems, have shown conflicting reports (none, positive or negative effects) regarding the impact of EMF-r on organisms. For example, studies have demonstrated cytotoxic effects of EMF-r and it has been correlated with tumour development in animals (McCann et al., 1993; Hardell and Sage, 2008; Vijayalaxmi and Prihoda, 2012; Coureau et al., 2014; Park et al., 2018). In contrast, other studies have suggested that EMF-r do not have any cytotoxic or genotoxic effect (Capri et al., 2004; Paparini et al., 2008; Su et al., 2017). The biological effects of EMF-r are dependent on various factors such as frequency, power density, type of tissue, growth stage, and period and levels of exposure (Vian et al., 2016). Nevertheless, the damaging effects of EMF-r should

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not be neglected. Notwithstanding, mobile phones have been designated as "*Possible Human Carcinogen*" (2B) by World Health Organizations' International Agencies for Research on Cancer (IARC).

Plants play an important role in an ecosystem; thus, it is pertinent to investigate how plants respond to increasing EMF-r levels in the environment. We assume that plants capture EMF-r via environmental signals and synchronize their periodical functions. This is possibly the reason for which plants have been less studied as far as the impacts of EMF-r are concerned. Nevertheless, there have been contradictory reports regarding the impacts of EMF-r on plants. Most of the studies have reported reduction in germination and seedling growth upon EMF-r exposure (Halgamuge, 2017). On the other hand, a few studies have reported stimulation in germination and seedling growth upon exposure to EMF-r (Jinapang et al., 2010; Răcuciu and Miclaus, 2007). Studies have revealed that EMF-r caused cell leakage, altered the physiological processes including enzyme activities, and induced oxidative stress in plants (Tkalec et al., 2007, 2009; Sharma et al., 2009, 2010; Jouni et al., 2012). Previously, exposure to 900 MHz EMF-r has been observed to impair mitosis and induce chromosome aberrations and *c*-mitosis (Pesnya and Romanovsky, 2013; Răcuciu, 2009). However, the impact of EMF-r on cell division depends upon the field strength, cell type, and the type of EMF-r (Vian et al., 2016). Tkalec et al. (2009) observed increased mitotic index and chromosomal abnormalities upon exposure to non-thermal EMF-r at 400 and 900 MHz or modulated EMF-r at higher field strengths (41 and 120 $V\,m^{-1}$) for 2 h in *Allium cepa*.

Most of the earlier studies have used EMF-r with much higher power density (Gustavino et al., 2016; Tkalec et al., 2009), and the studies investigating the effect of EMF-r at power density comparable to that used by cell phone providers have lesser been explored (Halgamuge, 2017, and references therein). In the present study, we investigated the cyto- and genotoxic potential of EMF-r at 900 and 1800 MHz. Both these frequencies are in the range that is used in telecommunication, thus, analysis of their comparative bio-effects holds importance. Although studies have been conducted to explore the effects of these frequencies on plants, the studies comparing cytogenetic and mutagenic effects of 900 MHz and 1800 MHz (two mostly used communication frequencies) EMF-r are largely missing. We explored cyto- and genotoxicity of 900 MHz (at 261 ± 8.50 $mW\,m^{-2}$) and 1800 MHz (at 332 ± 10.36 $mW\,m^{-2}$) EMF-r on *Allium cepa* (onion) roots under different exposure periods (0.5 h, 1 h, 2 h and 4 h).

Higher plants being static and under a constant cloud of EMF-r, have been documented as an outstanding model for displaying mutagenic and cytogenetic effects of environmental mutagens. As compared to other assay systems, higher plants are highly suitable for testing genetic damage as these produce few false negatives and have high sensitivity. Further, being inexpensive and convenient, plants are perfect model used by scientists for screening the possible damage caused by environmental pollutants (Leme and Marin-Morales, 2009). Therefore, results of assays with higher plants make substantial contributions in shielding the community from genotoxic agents and mutagens. Among higher plants, *A. cepa* (Amaryllidaceae; Onion; $2n = 16$) assay has been considered and accepted as an ideal model for *in-situ* monitoring and detection of genotoxicity and evaluating environmental mutagens (Aksoy et al., 2010; Leme and Marin-Morales, 2009) including radiofrequency radiations (Pesnya and Romanovsky, 2013). It is due to higher percentage of dividing cells, which are relatively large-sized and have fewer number of monocentric chromosomes that stain well (Fiskesjö, 1985). The use of *A. cepa* roots for evaluating chromosomal aberrations is one of the easy, reliable, affordable methods available, and delivers a wide range of genetic endpoints (Grant, 1999; Leme and Marin-Morales, 2009). We investigated the structural and chromosomal aberrations, alterations in mitotic and phase index, and DNA damage in terms of head and tail DNA and olive tail moment upon EMF-r exposure. Screening of cyto- and genotoxic potential gives us an insight into the effects of radiations at chromosomal and DNA level. Chromosomes (and/or DNA *per se*) are an important cellular inclusion and play various vital roles in biological systems, and any alteration in their structure can lead to unfavorable consequences. Such genetic assays have public health relevance as these help in devising protection strategies against mutagenic environmental pollutants, including EMF-r.

2. Material and methods

2.1. Materials

Onion bulbs were purchased from the local market. Bulbs of equal size (average weight: 56.1 ± 0.39 g) were washed with running tap water followed by distilled water. These were scrapped to remove the old and dry scales and roots so that the apices of root primordia were exposed. The bulbs were placed in Coplin jars with their basal end dipped in distilled water and set for rooting in dark at room temperature (25 ± 1 °C). All the chemicals and reagents used for biochemical estimations were of analytical reagent grade and procured from Sisco Research Laboratory Pvt. Ltd., Mumbai, India; Hi-Media, Mumbai, India; and Sigma Co., St. Louis, USA.

2.2. Dosimetry and experimental set-up

The exposure system was kept in an empty separate room coated with special radioshielding paint Yshield® HSF 54. Y-shield attenuates radio frequency (RF) signals regardless of direction of signal polarization and eliminates EMF-r reflection, similar to anechoic material (source: www.lessemf.com/paint.html). Onion bulbs (when root initiation started) were exposed to continuous homogenous wave EMF-r of 900 MHz and 1800 MHz. EMF-r were generated through RF signal generator (Agilent N9310A; Keysight Technologies, USA) operating at frequency range from 9 KHz to 3.0 GHz, supplied with power of 20 dBm (0.1 W); and attached with an RF power amplifier (ZHL-5W-2GX+; Minicircuits, USA) and DC regulated power supply. In all, there were ten groups of onion bulbs with 3 bulbs per group. No exposure was given to group 1 (kept inside the exposure room but without EMF-r; *sham control*); group 2, 3, 4 and 5 were exposed to 900 MHz EMF-r for different exposure periods of 0.5 h, 1 h, 2 h and 4 h; group 6, 7, 8 and 9 were exposed to 1800 MHz EMF-r for 0.5 h, 1 h, 2 h and 4 h; group 10 was treated with 250 μM methyl methane sulfonate (MMS) for 4 h and served as the *positive control*. The three onions in each group were exposed independently and each served as replication. An outline of experimental design has been given in Table 1. For our study, we chose the exposure duration in the range of 0.5–4 h as the average time spent on phone by a mobile phone user lies within this range (Ofcom, 2018). According to communication market report (2018), mobile phone users spend almost 2 h 29 min per day on their mobile phones. Highest usage has been reported among the age group of 15–24, with an average usage of more than 4 h per day (Ofcom, 2018). The output power density at the plane of exposure was measured by ScanEM®-C Probe

Table 1
Experimental design.

Treatment	Duration (h)	Number of Replicates
Negative/Sham control	–	3
Positive control (250 μM MMS)	4	3
900 MHz	0.5	3
	1	3
	2	3
	4	3
1800 MHz	0.5	3
	1	3
	2	3
	4	3

(CTK015; 3M Technologies, USA) attached to handheld RF power density meter/spectrum analyzer (Spectran HF-4060, range: 100 MHz to 6 GHz; Aaronia AG, Germany). The average power density received at a distance of 3 cm (where onions were placed) from antenna was 261 ± 8.50 mW m^{-2} at 900 MHz and 332 ± 10.36 mW m^{-2} at 1800 MHz with a specific absorption rate (SAR) of 9.02 ± 0.0 × 10^{-2} W kg^{-1} and 1.69 ± 0.0 × 10^{-1} W kg^{-1}, respectively. SAR value was determined roughly as it is relatively difficult to measure it on exposed plant tissues directly (Çenesiz et al., 2011). It was calculated by taking the values of electrical conductivity (σ) and tissue density (ρ) for the dielectric properties of tissue (brain grey matter) at 900 MHz (σ = 0.94 S m^{-1} and ρ = 1030 kg cm^{-3}) and 1800 MHz (σ = 1.70 S m^{-1} and ρ = 1030 kg cm^{-1}) from IFAC (Institute of Applied Physics, Sesto Fiorentino, Italy) database (Andreucceti et al., 1997). The temperature inside the exposure room (near the bulbs) was measured at the start and end of exposure, and a change of +0.1 °C was observed. After exposure, the bulbs were kept inside growth chamber for five days, maintained at 25 ± 1 °C, 78 ± 2% RH, and 18 h/6 h light/dark photoperiod of 240 μ mole s^{-1} m^{-2} photosynthetic flux density.

2.3. Root length and thickness

Five days after exposure to EMF-r, root length and root thickness were measured with a centimeter ruler and Vernier caliper, respectively.

2.4. Cytogenetic studies

Effect of EMF-r on cell division was studied following the squash technique method given by Armbruster et al. (1991) with slight modifications. On fifth day after EMF-r exposure, root tips (~2 mm from the tip; zone of division) were excised and fixed in Farmer's fluid (glacial acetic acid and ethyl alcohol: 1:3, *v/v*) for 24 h. Next day, roots were transferred to 70% ethyl alcohol and stored at 4 °C. Thereafter, the roots were hydrolyzed with 1 N HCl with intermittent heating for 1 min and stained with acetocarmine for 30 min. After staining, two root tips were macerated in a drop of 45% acetic acid on a slide, covered with cover slip and sealed with clear nail polish. For each treatment (exposure period or EMF-r type) three slides (one from each bulb) were prepared, and served as the replications. The slides were observed under a bright field microscope (Nikon eclipse E200, using the 40 × objective lens). Around 800–850 cells (as visible) were screened from each slide and a total of 2500 cells were examined for each treatment. The slides were scored for normal or aberrant cells in different mitotic stages (interphase, prophase, metaphase, anaphase and telophase). Mitotic index (MI) and individual phase index (%) were calculated according to the following formulae:

$$\text{Mitotic Index (\%)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells counted}} \times 100$$

$$\text{Phase Index (\%)} = \frac{\text{Number of cells in particular mitotic phase}}{\text{Total Number of dividing cells}} \times 100$$

In addition, percent of aberration was calculated by counting cells with different types of chromosomal aberrations (CAs) as under (Rank and Nielsen, 1993):

$$\text{Chromosomal Aberration (\%)} = \frac{\text{Number of Aberrant cells}}{\text{Number of dividing cells counted}} \times 100$$

Comet assay (Single Cell Gel Electrophoresis, SCGE).

DNA damage was measured by comet assay performed under alkaline conditions (due to high sensitivity in revealing DNA damage) according to the method described by Gichner et al. (2009). Before the assay, the microscopic slides (with one-fourth frosted ends) were prepared by dipping into 1% normal melting point agarose (NMPA) prepared in phosphate buffer saline (PBS) at 50 °C. These slides served as the agarose base-coated slides. Nuclei were isolated by gently slicing 4–5 root tips with a sharp razor blade in 0.4 M Tris-HCl buffer (pH 7.5) in a watch glass kept over an ice base. The nuclei suspension prepared in Tris-HCl buffer was mixed with 1% low melting point agarose (1:1, *v/v*) in a microcentrifuge tube and pipetted over the agarose base coated slides. The slides were covered with a cover slip and placed on an iced surface for a minimum of 10 min. After 10 min, cover slip was removed and slides immersed in lysis buffer. Thereafter, the slides were placed in a horizontal gel electrophoresis tank containing freshly prepared electrophoresis buffer (1 mM ethylene diamine tetraacetic acid, EDTA, and 300 mM sodium hydroxide, NaOH, pH ≥ 13). The nuclei were incubated at 4–8 °C for 30 min to facilitate DNA unwinding prior to electrophoresis (at 26 V, 300 mA) for 30 min. All the steps of the experiment were conducted under low temperature and dim light. After electrophoresis, slides were rinsed three times with 0.4 M Tris-HCl buffer (pH = 7.5) and air-dried overnight. The electrophoresed slides were stained with ethidium bromide solution (20 µg ml^{-1}) for 5 min in dark, dipped in ice-cold water to remove excess of ethidium bromide, and covered with a cover slip. For each slide, 50 randomly chosen comets were analyzed using a fluorescence microscope (Nikon Eclipse 80*i*) equipped with an excitation filter of 515–560 nm and a barrier filter of 590 nm. A minimum of three roots were taken for each treatment (one root from each bulb) and from each root, two SCGE slides were prepared. In total, 300 nuclei were scored per treatment. A computerized image analysis system (Comet Assay Software; 1.2.3 b comet assay package, CaspLab®) was employed to calculate DNA damage. The DNA damage was assessed in terms of percent DNA in head (%DNAH), percent DNA in tail (%DNAT), and olive tail moment (OTM) (Końca et al., 2003).

Olive Tail Moment (OTM) = %DNAT × (Center of gravity of Tail in *x*-axis direction—Centre of gravity of Head in *x*-axis direction).

2.5. Statistical analyses

The experiments were conducted with three replications; each replication comprised of single onion bulb. For mitotic studies, three independent replicates (as squash; one from each bulb) were prepared and viewed. The comet assay involved three roots per treatment, including control; and a minimum of two gel electrophoresis slides per root. The data were analyzed by one-way analysis of variance (ANOVA) followed by the comparison of mean values using *post hoc* Tukey's test at $p \leq 0.05$. The differences between 900 and 1800 MHz were analyzed using two sample student's *t*-test at $p \leq 0.05$.

3. Results

3.1. Root growth

Root length declined ($p \leq 0.05$) after ≥ 2 h of exposure to 900 MHz and 1800 MHz. It was reduced by 11% and 13% after 2 and 4 h exposure to 900 MHz, respectively, whereas 10% and 12% reduction was observed upon exposure to 1800 MHz (Table 2). However, comparing two frequencies, this reduction was observed to be insignificant. In contrast, EMF-r exposure enhanced root thickness in a time-dependent manner. It was increased by 10% and 47% upon exposure to EMF-r of 900 MHz for 2 and 4 h, respectively, whereas 13% and 48% increase in root thickness was observed upon exposure to 1800 MHz, over that in the control (Table 2).

3.2. Mitotic index (MI), aberration percentage and phase index

Exposure to EMF-r (900 MHz and 1800 MHz) for 4 h significantly increased the number of dividing cells over that in the control. MI was increased by ~21, 36 and 53% upon 1, 2, and 4 h of exposure to 900 MHz, whereas in response to 1800 MHz of EMF-r, the increase in

Table 2
Effect of 900 and 1800 MHz EMF-r on root length and thickness in *Allium cepa* roots (n = 3; one onion is equivalent to one replicate).

Treatment/Exposure period (h)	Average root length (cm)	Average root thickness (mm)
0 (Sham control)	11.4 ± 0.21 a	0.60 ± 0.02 b
900 MHz		
0.5	11.1 ± 0.10 ab (−2.6)	0.61 ± 0.03 b (+1.7)
1	10.8 ± 0.20 ab (−5.3)	0.64 ± 0.01 b (+6.7)
2	10.2 ± 0.35 ab (−10.5)	0.66 ± 0.02 b (+10.0)
4	9.9 ± 0.15 b (−13.2)	0.88 ± 0.01 a (+46.7)
1800 MHz		
0.5	11.2 ± 0.30 a (−1.8)	0.61 ± 0.03 b (+1.7)
1	11.2 ± 0 .35 ab (−1.8)	0.63 ± 0.03 b (+5.0)
2	10.3 ± 0 .30 ab (−9.7)	0.68 ± 0.04 b (+13.3)
4	10.0 ± 0.25 ab (−12.3)	0.89 ± 0.03 a (+48.3)

All the values are expressed as mean ± SEM. Different alphabets in a column represent significant difference at $p \leq 0.05$ applying Tukey's test; Numbers in parenthesis represent percent change (+, increase; −, decrease) over the un-exposed (Sham) control.

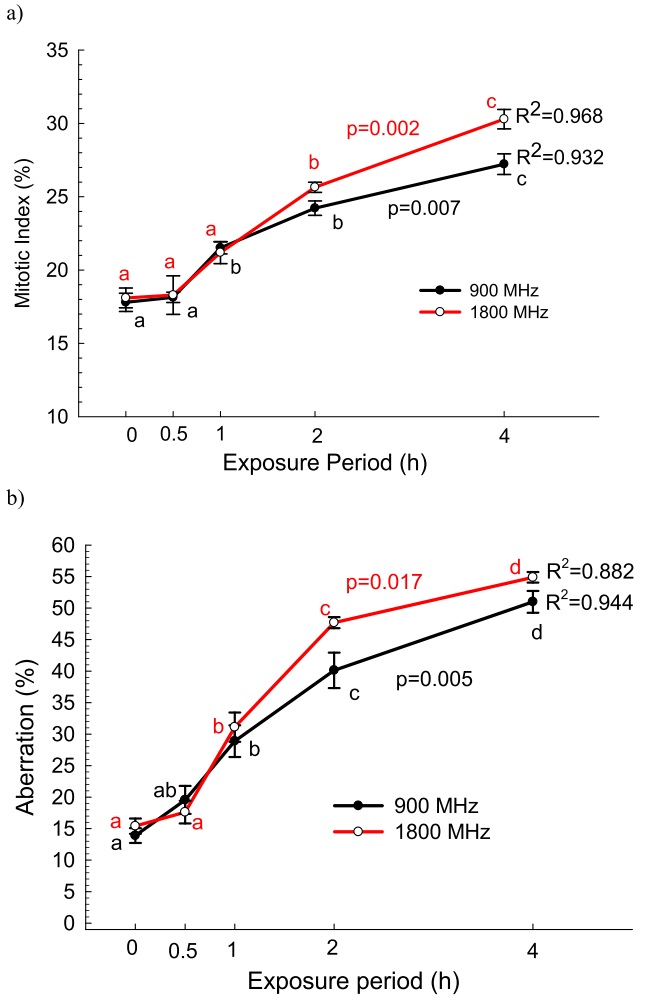


Fig. 1. a) Mitotic index (%) and b) chromosomal aberration (%) in onion root meristematic cells upon exposure to 900 and 1800 MHz EMF-r (n = 3 onions). Different alphabets represent significant difference at $p \leq 0.05$ applying Tukey's test. Percent MI and chromosomal aberration on treatment of 250 μM MMS (positive control) was 15.6 0 ± 0.16 and 62.8 ± 1.79, respectively. R^2 represents Coefficient of determination.

MI was 17%, 41% and 67% ($p \leq 0.05$), over that in the control, respectively (Fig. 1). A significant increase ($p \leq 0.05$) in aberration percentage was observed upon exposure to both 900 and 1800 MHz EMF-r. It increased in the range of 41%–266% and 14%–257% during 0.5–4 h of exposure to 900 MHz and 1800 MHz, respectively, over the control (Fig. 1).

EMF-r (both 900 and 1800 MHz) induced chromosomal aberrations (CAs) in root tip cells of *A. cepa* (Fig. 2). In response to 900 MHz EMF-r, the most frequent aberrations were bi-nucleated cells in early prophase, followed by *c*-mitosis, swollen cells, elongated cells, and laggards (Table 3). Likewise, upon exposure to 1800 MHz EMF-r, the most frequent aberrations recorded were bi-nucleated cells in early prophase, followed by *c*-mitosis, sticky chromosomes, elongated cells, vagrant chromosomes and laggards. In addition, chromosomal bridges and polyploidy were also observed (Table 3). Exposure to EMF-r (900 and 1800 MHz) caused a significant decline in the prophase index (%), whereas metaphase, anaphase and telophase indices were enhanced over that in the control (Table 4). It was in sharp contrast to the observations made with MMS treatment, which enhanced prophase and telophase index, but declined metaphase and anaphase index (Table 4). The changes in phase index were more pronounced in response to 1800 MHz EMF-r than in 900 MHz treatments, particularly at 2 and 4 h. Prophase index declined over the control by 16–53% on exposure to 900 MHz EMF-r for 1–4 h, whereas the reduction was in the range of 4–42% in response to 1800 MHz EMF-r exposure for similar duration (though statistically insignificant from that of 900 MHz) (Table 4). Metaphase index did not change significantly upon EMF-r exposure except at 2 h of exposure to 1800 MHz EMF-r (Table 4). EMF-r exposure significantly enhanced anaphase index by 15–31% and 11–40% at 900 MHz and 1800 MHz EMF-r for 1–4 h, respectively, and the difference between values at two frequencies was statistically significant at 2 and 4 h. In contrast, a significant change in telophase index was observed at 2 and 4 h upon 900 MHz and at 0.5 h upon1800 MHz exposure over that in the control and the difference between two frequencies was significant except at 2 h (Table 4). In general, reduction in metaphase and telophase and enhancement in anaphase was statistically more pronounced (applying *t*-test, $p \leq 0.05$) at 1800 compared to 900 MHz (Table 4).

3.3. DNA damage

At shorter (≤2 h) exposure, no significant change was noticed in DNA migration. However, exposure to both 900 and 1800 MHz EMF-r for 4 h resulted in an appreciable migration of DNA from nucleus, thereby forming a comet tail. It was evident from a significant decrease in DNAH, and a significant increase in DNAT and OTM values upon EMF-r exposure, and the values are parallel to the positive control MMS (Fig. 3; Table 5). DNAH declined by 19% and 23% upon 4 h exposure to 900 MHz and 1800 MHz EMF-r, respectively, in contrast to the control, whereas MMS treatment reduced DNAH by 16%. Compared to control, DNAT was increased by 2.3, 2.8 and 2.0 folds upon exposure to 900 MHz, 1800 MHz and MMS, whereas OTM was increased by 3.7, 5.8 and 1.2 folds, respectively (Table 5). In general, DNA damage upon 4 h of exposure to EMF-r was significantly greater than that observed in response to MMS used as positive control and 900 MHz (applying *t*-test, $p \leq 0.05$) (Table 5).

4. Discussion

The present study demonstrated that continuous EMF-r of 900 and 1800 MHz induced cytotoxic and genotoxic damage in the root meristems of onion in an exposure period-dependent manner. In general, EMF-r induced CAs and DNA damage was more pronounced at 1800 MHz than that at 900 MHz. Further, genotoxic damage (at 4 h of EMF-r exposure) was significantly greater than that observed with MMS (250 μM) used as the positive control. Increase in MI, CAs and DNA damage observed on exposure to 1800 MHz EMF-r could be due to greater incident power density (332 ± 10.36 mW m^{-2}) at the surface

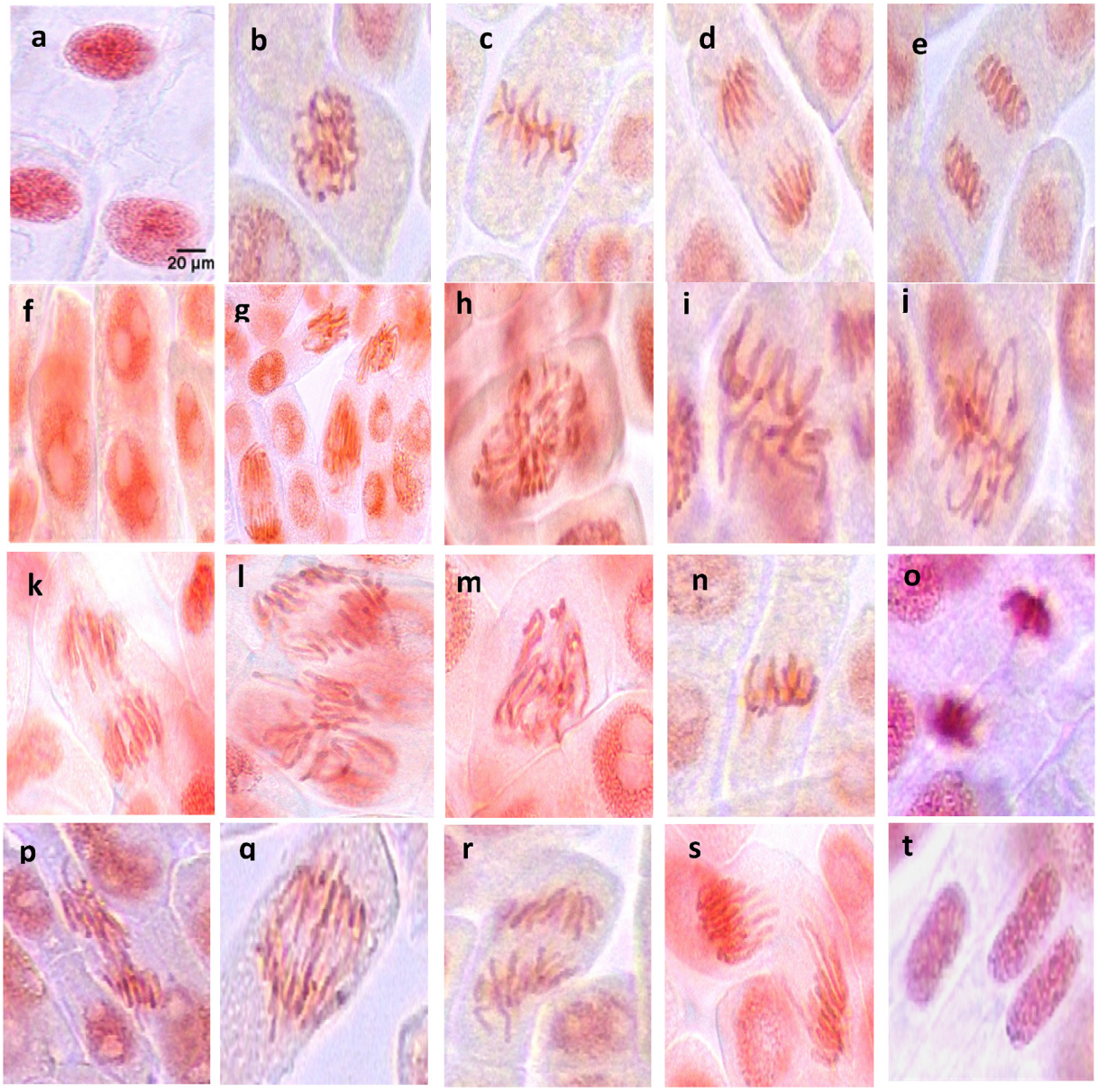


Fig. 2. Normal stages of mitotic division: (a) Interphase, (b) Prophase, (c) Metaphase, (d) Anaphase, and (e) Telophase. Different types of chromosomal aberrations induced by EMF-r: (f) Binucleated cells, (g,h,i,j) *c*-mitosis, (k,l) Spindle abnormalities in anaphase, (m,n) Sticky chromosomes, (o) Bridge in telophase, (p,q,r) Vagrant chromosome in anaphase, (s) Diagonal anaphase, and (t) Elongated cell. All micrographs were taken using a 10× objective and have the same magnification (bar = 20 μm).

of experimental set up than at 900 MHz. Since the effective resistance of cell membrane has been inversely correlated to frequency (Lvovich, 2012), therefore, the greater damage to cells and tissues was observed at 1800 MHz than at 900 MHz. Previously, studies demonstrated that EMF-r and cell phone radiations inhibit root growth, alter biochemical processes, and induce free radical-mediated oxidative damage (Tkalec et al., 2005, 2009; Sharma et al., 2009; Singh et al., 2012; Kumar et al., 2016; Chandel et al., 2017). In our study, we observed that roots were thickened upon exposure to EMF-r. The increase in root thickness suggests enhanced lignification and thickening of cell wall under EMF-r induced stress, thereby, resulting in reduced root growth (Neves et al., 2010). Although, we did not explore the biological mechanism of declined root length and increased root diameter under the influence of EMF-r. Nevertheless, EMF-r induced inhibition in root growth could be the consequence of decreased cell elongation.

The observed increase in MI and CAs on exposure to 900 and 1800 MHz EMF-r is corroborated by earlier studies of Tkalec et al. (2009) and Pesnya and Romanovsky (2013). Tkalec et al. (2009) observed lagging chromosomes, vagrants, disturbed anaphases and chromosome stickiness on exposure to 400 and 900 MHz of EMF-r. Pesnya and Romanovsky (2013) demonstrated that modulated EMF-r of 900 MHz (mobile phone) for 3 and 9 h induced aneugenic and clastogenic effects and considerably enhanced the mitotic index in *A. cepa*. A variety of abnormalities, e.g. polyploid cells, and three groups – metaphases, amitosis, and nuclear budding – were obtained upon irradiation. These also resulted in an increase in CAs and frequency of micronuclei in *A. cepa*. These researchers reported that the effect was dependent on modulation, frequency and strength of EMF-r. The observed interference of EMF-r with mitosis could be due to spindle impairment (Tkalec et al., 2009). EMF-r impairs mitotic activity and increase the frequency of CAs by stimulating the cellular proliferation in *Helianthus annuus* (Shestopalova and Baeva, 2007). Previously, 915 MHz of EMF-r (at power density = 25–50 W m^{-2}) was observed to increase micronuclei frequency in *Vicia faba* root tips after 72 h

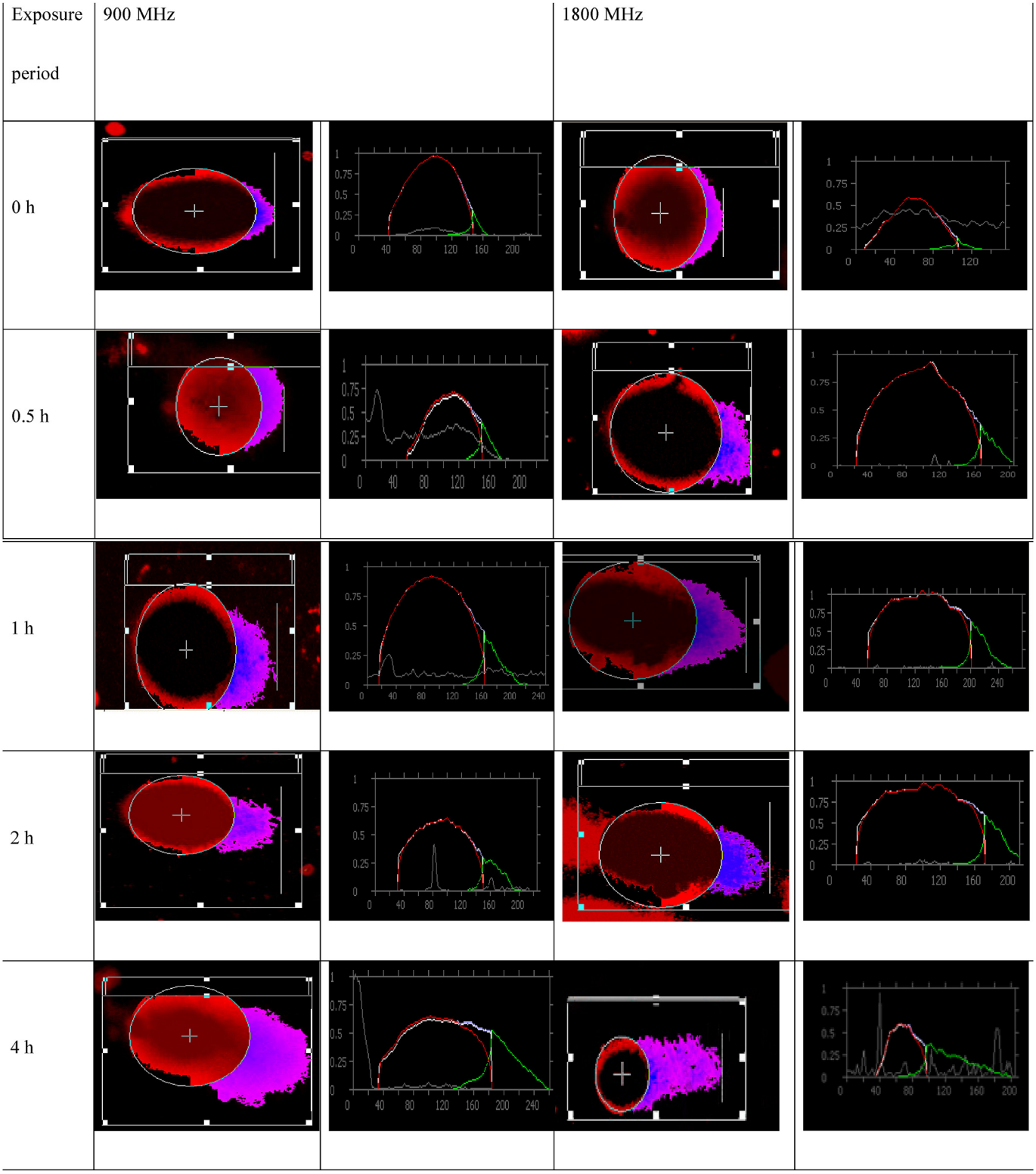


Fig. 3. EMF-r induced DNA damage in *Allium cepa* root meristematic cells at 900 MHz or 1800 MHz. Individual comet images were automatically generated by image analysis software (CaspLab®). Under each frequency range, in the left panel comet images with measurement frame, head and tail are presented; whereas in the right panel, intensity profiles are plotted with length (μm = pixels × 0.64) on *x*-axis and %DNA (× 10^2) on *y*-axis.

exposure (Gustavino et al., 2016). However, the power density of EMF-r used in the present study (0.26 W m^{-2} and 0.332 W m^{-2} at 900 and 1800 MHz) is ~100 fold less. The chromosome stickiness suggests toxic influence of EMF-r on chromosome, which could be due to either degradation of chromosomal DNA or depolymerization of nucleic acids or dissociation of nucleoproteins (Kumar et al., 2003). The condensation and stickiness of chromosomes, and disruption in mitotic spindle result in metaphasic disturbances. Haider et al. (1994) reported the clastogenic effects of EMF-r and increased micronuclei formation in *Tradescantia* due to short wave electromagnetic fields (10–21 MHz).

Table 3
Structural and chromosomal aberrations and mitotic disturbances in onion root meristematic cells upon exposure to 900 and 1800 MHz EMF-r for varying time period or MMS (250 μM; positive control). A total of around 2500 cells were scored for each exposure period/treatment.

Aberration Type	Treatment										
	900 MHz					1800 MHz					MMS
	Exposure Period (h)										
	0 h	**0.5 h**	**1 h**	**2 h**	**4 h**	**0 h**	**0.5 h**	**1 h**	**2 h**	**4 h**	
Asymmetric Cell	–	2	2	6	2	–	–	4	–	–	8
Elongated Cell	3	9	13	7	10	6	12	10	10	10	19
Binucleated cells	11	19	37	51	43	9	22	34	44	30	18
Swollen Cell	–	4	8	4	17	–	6	16	10	8	11
Polyploidy	–	–	–	–	–	–	–	–	–	2	22
C-mitosis	3	5	9	11	17	6	10	16	20	28	34
Laggard Chromosomes	–	–	–	3	7	–	–	–	–	6	19
Vagrant Chromosomes	–	–	2	1	3	–	–	–	–	12	26
Chromosomal Bridge	–	–	1	5	2	–	–	2	4	4	31
Sticky Chromosomes	1	2	1	3	3	2	6	10	10	10	28
Diagonal Spindle	–	1	–	2	–	–	–	–	4	–	5
Spindle Disturbance in Prophase	1	–	–	3	1	2	2	2	4	6	6
Spindle Disturbance in Metaphase	1	–	1	2	5	–	–	2	4	8	7

Table 4
Index rates of mitotic phases in *Allium cepa* root tip cells after exposure to 900 MHz and 1800 MHz EMF-r (n = 3; one onion is equivalent to one replicate).

Treatment/Exposure period (h)	Prophase Index (%)	Metaphase index (%)	Anaphase index (%)	Telophase index (%)
0 (Sham control)	24.4 ± 0.26 b	31.7 ± 0.50 c	25.4 ± 0.48 f	18.5 ± 0.50 bc
900 MHz				
0.5	20.5 ± 0.36 c	32.3 ± 0.64 bc	29.1 ± 0.56 e	18.1 ± 0.30 bc
1	17.0 ± 0.43 d	32.9 ± 0.71 abc	30.1 ± 2.1 de	20.0 ± 0.52 ab
2	14.2 ± 0.30 e	33.0 ± 0.45 abc	32.0 ± 0.20 cd	20.7 ± 0.36 a
4	11.4 ± 0.30 f	34.5 ± 0.0.35 ab	33.2 ± 0.46 bc	20.8 ± 0.46 a
1800 MHz				
0.5	23.5 ± 0.45 b	33.7 ± 0.57 abc	28.1 ± 0.29 e	15.3 ± 0.42 d[a]
1	20.5 ± 0.30 c	34.6 ± 0.26 a	28.8 ± 0.52 e	16.7 ± 0.49cd[a]
2	17.4 ± 0.21 d	29.3 ± 0.21 d[a]	35.2 ± 0.43 ab[a]	19.4 ± 0.30 ab
4	14.2 ± 0.32 e	33.1 ± 0.22 abc	35.6 ± 0.47 a[a]	18.5 ± 0.21 bc[a]
MMS (Positive control)	28.5 ± 0.67 a	27.8 ± 0.30 d	23.7 ± 0.43 f	20.0 ± 0.38 ab

Data represented as mean ± SEM. Different alphabets in a column represent significant difference at $p \leq 0.05$ applying Tukey's test.
[a] Represents the significant difference between 900 and 1800 MHz applying two sample *t*-test at $p \leq 0.05$.

Table 5
Effect of 900 and 1800 MHz EMF-r exposure on Head DNA (%), Tail DNA (%) and Olive tail moment (μm) in nuclei isolated from root tips of *Allium cepa* (n = 3; one onion is equivalent to one replicate).

Treatment/Exposure period (h)	Head DNA (%)	Tail DNA (%)	Olive Tail moment (μm)
0 (Sham control)	92.4 ± 0.23 a	7.6 ± 0.23f	3.1 ± 0.0.26 f
900 MHz			
0.5	89.9 ± 0.27 b	10.1 ± 0.27 e	5.3 ± 0.32 ef
1	88.9 ± 0.15 bc	11.1 ± 0.53 de	5.9 ± 0.40 def
2	87.5 ± 0.52 cd	12.5 ± 0.52 d	9.1 ± 0.65 cd
4	74.6 ± 0.38 f	25.4 ± 0.38 b	14.5 ± 1.41 b
1800 MHz			
0.5	89.9 ± 0.32 b	10.1 ± 0.37 e	6.1 ± 0.26 def
1	87.3 ± 0.17 cd	12.7 ± 0.17 d	9.1 ± 0.70 cd
2	87.1 ± 0.30 ad	12.9 ± 0.30 d	10.9 ± 0.96 c
4	70.8 ± 0.62 g[a]	29.2 ± 0.60 a[a]	21.1 ± 0.59 a[a]
MMS (Positive control)	77.9 ± 0.32 e	22.1 ± 0.37 c	6.7 ± 0.36 de

Data represented as mean ± SEM. Different alphabets in a column represent significant difference at $p \leq 0.05$ applying Tukey's test.
[a] Represents the significant difference between 900 and 1800 MHz applying two sample *t*-test at $p \leq 0.05$.

Exposure to EMF-r also resulted in formation of chromosome fragments which may be attributed to the inversion in acentric chromosome or the negative effects of reactive oxygen species generated by EMF-r (Tkalec et al., 2007; Gustavino et al., 2016). Goodman et al. (1995) revealed that the electrical component of EMF-r affects ionic pumps, enzymes, nuclear material, and nucleotide molecules within the cells. In the present study, we used alkaline comet assay to quantify the DNA damage on exposure to EMF-r in nuclei isolated from root meristems of onion. We observed a significant increase in the %DNAT and OTM over the control at longer exposure time to 900 and 1800 MHz. These findings are corroborated by the studies of Tkalec et al. (2013) who reported DNA damage in *Eisenia fetida* coelomocytes upon exposure to 900 MHz. EMF-r generate free radicals that cause damage to nucleic acids and macromolecules (Sharma et al., 2009). The genotoxicity could be due to: (a) interaction of EMF-r with electrons within bases of DNA, resulting in non-uniform charge flow, and consequently leading to bending of DNA helix and initiation of transcription (Blank and Goodman, 2011), or (b) the interaction with DNA-repair mechanisms (Ruediger, 2009), or (c) conformational changes of DNA and disaggregation (Marianna et al., 2013). However, we did not explore such a mechanism in the present study and further research is required in this direction.

5. Conclusions

On the basis of the above observations and discussion, the study concludes that EMF-r within the cell-phone frequency range (900 and 1800 MHz) and at intensity much lower than the adopted Indian ICNIRP (International Commission on Non-Ionizing Radiation

Protection) limits of 450 mW m^{-2} and 900 mW m^{-2} for 900 MHz and 1800 MHz, respectively, adversely affect root meristems in plants, and induce cytotoxic and DNA damage. EMF-r induced DNA damage was more pronounced at 1800 MHz than that at 900 MHz. Genotoxic damage (at 4 h of EMF-r exposure) was also significantly greater than that observed with MMS used as the positive control in the study. However, it is not certain whether under similar exposure conditions, these effects would appear in nature. The present study hold significance since the used specific absorption rate (SAR) are lower than the averaged SAR value adopted as safety standards in India, and the increased safety concerns and risk assessment studies linked to the rapidly increasing EMF-r in the natural environment.

Declaration of competing interest

Authors declare that they have no conflict of interest.

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