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ORIGINAL ARTICLE

Evaluation of oxidative stress and genotoxicity of 900 MHz electromagnetic radiations using *Trigonella foenum-graecum* test system

Surbhi Sharma[1] · Shalini Bahel[2] · Jatinder Kaur Katnoria[1]

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Abstract

Unprecedented growth in the communication sector and expanded usage of the number of wireless devices in the past few decades have resulted in a tremendous increase in emissions of non-ionizing electromagnetic radiations (EMRs) in the environment. The widespread EMRs have induced many significant changes in biological systems leading to oxidative stress as well as DNA damage. Considering this, the present study was planned to study the effects of EMRs at 900 MHz frequency with the power density of 10.0 dBm (0.01 W) at variable exposure periods (0.5 h, 1 h, 2 h, 4 h, and 8 h per day for 7 days) on percentage germination, morphological characteristics, protein content, lipid peroxidation in terms of malondialdehyde content (MDA), and antioxidant defense system of *Trigonella foenum-graecum* test system. The genotoxicity was also evaluated using similar conditions. It was observed that EMRs significantly decreased the germination percentage at an exposure time of 4 h and 8 h. Fresh weight and dry weight of root and shoot did not show significant variations, while the root and shoot length have shown significant variations for 4 h and 8 h exposure period. Further, EMRs enhanced MDA indicating lipid peroxidation. In response to exposure of EMRs, there was a significant up-regulation in the activities of enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD), glutathione-S-transferase (GST), guaiacol peroxidase (POD), and glutathione reductase (GR) in the roots and shoots of *Trigonella-foenum graecum*. The genotoxicity study showed the induction of chromosomal aberrations in root tip cells of the *Trigonella foenum-graecum* test system. The present study revealed the induction of oxidative stress and genotoxicity of EMRs exposure in the test system.

Keywords Antioxidant enzymes · Electromagnetic radiations · Chromosomal aberrations

Introduction

Electromagnetic radiations (EMRs) refer to the self-propagating waves which are transverse in nature and can travel through space carrying radiant energy (Corson et al. 1999; Marjanović et al. 2012). Electromagnetic field sources broadly show three frequency ranges viz., static range (0 Hz), extremely low-frequency range (ELF; 0 to 300 Hz), intermediate frequency range (IF; 300 Hz to 10 MHz) and the radiofrequency range (RF and microwaves; 10 MHz-300 GHz) to which people may be exposed (Repacholi 2003). The main sources of EMRs include AM/FM Tower (540 kHz-108 MHz), TV Tower (48 MHz-814 MHz), Wi-Fi (2.4–2.5 GHz), cell Towers (800 MHz, 900 MHz, 1800 MHz, 2100 MHz, and 2300 MHz), and mobile phones (GSM-1800/CDMA GSM-900) (Bhawan and Marg 2014). Electromagnetic radiation can be classified into ionizing radiation and non – ionizing radiation, based on whether it is capable of ionizing atoms and breaking chemical bonds (Mohril et al. 2016; Zeman 2016; Sharma et al. 2017). Non-ionizing radiation is associated with two major potential hazards, electrical and biological (International Commission on Non-Ionizing Radiation Protection 2017). Complex biological effects of weaker non-thermal electromagnetic fields also exist, including weak extremely low frequency magnetic fields, modulated radio frequency, and microwave fields. Living organisms have been constantly in receipt of

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✉ Jatinder Kaur Katnoria
jatinder.botenv@gndu.ac.in; jkat08@yahoo.com

[1] Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar 143005, India

[2] Department of Electronics Technology, Guru Nanak Dev University, Amritsar 143005, India

exposure to varied frequencies of electromagnetic radiations (EMRs) through different natural sources such as the sun, space and the Earth for millennia. However, in the past few decades, exposure to man-made electromagnetic radiation has also been witnessed to increase which undeniably correlates with a rise in emissions of EMRs due to high-speed technical innovations. The WHO has classified mobile phone radiation on the International Agency for Research on Cancer (IARC) scale into Group 2B — possibly carcinogens (Loprieno 1975; Baan et al. 2011). It is documented that there are about 7.377 billion mobile cellular telephone subscriptions globally, with an average of 99.7 subscriptions per 100 inhabitants according to International Telecommunication Union (ITU). With an expansion in the usage of these devices, there has been a terrific augmentation in the non-ionizing radiofrequency electromagnetic fields in the environment which further enhanced the level of exposure of electromagnetic radiations (EMRs) to living beings (Redlarski et al. 2015).

Many studies have been carried out to explore the biological effects of EMRs, and it is documented that exposure to radiations resulted in induction of lesions in vital organs (Sepehrimanesh et al. 2014a), physiological and morphological changes, increased micronuclei formation, altered growth, and adverse cell characteristics such as thinner cell walls and smaller mitochondria (Halgamuge 2017; Kaur et al. 2021; Kumar et al. 2020), alterations in gene and protein expression (Lee et al. 2005; Sepehrimanesh et al. 2014b), inhibitory effects on enzyme activities (Paulraj and Behari 2002), and induction of genotoxicity (Ruediger 2009). The response to power lines or transformers has been widely documented to exhibit a connection with increasing EMRs directly and with an elevating rate of cancer indirectly through different epidemiological studies (Panagopoulos et al. 2013; Chen and Chen 2014). In a study conducted by Alkis et al. (2019), it was concluded that the frontal lobe of rat brain tissues when exposed to 900 MHz, 1800 MHz, and 2100 MHz EMRs generated by mobile phones caused oxidative damage, increase lipid peroxidation, and enhance oxidative DNA damage production. Furthermore, radiofrequency radiations (RFRs) at 2100 MHz tend to create DNA single-strand breaks.

Aside from cancer, the primary relationship between EMR exposure and numerous structures and organs, along with their functions in living organisms and subjective illnesses including weariness, headache, dizziness, nausea, sleep disruption, lack of appetite, and discomfort, has been documented (Chandel et al. 2019). However, holding a mobile phone near the ear for an extended period caused an increase in the temperature of the ear, while absorption of radiofrequency energy by tissues of the ear resulted in hearing loss and also leads to diseases such as Alzheimer's disease, Parkinson's disease, brain tumors, infertility, and other illnesses upon exposure to EMRs (Mudigoudra et al. 2020). Few studies have indicated that radiofrequency radiations (RFRs) led to a change in cell proliferation normal process (Velizarov et al. 1999), disrupt enzymatic activities (Paulraj and Behari 2002; Barteri et al. 2004), and induce genotoxicity (Verschaeve and Maes 1998). Besides this, some observations have also been documented from epidemiological, animal, and in vitro studies. Electromagnetic fields, such as those emitted by cell phones, have been shown to influence normal brain physiology in humans through changes in cortical excitability which modulate neural network activity and contribute to electrical instability (Azmy et al. 2020).

Like other environmental pressures, electromagnetic radiations have also been shown to induce oxidative stress such as an imbalance in the pro-oxidant/antioxidant equilibrium which occurs as a result of an uncontrolled rise in reactive oxygen species (ROS) development and a shift in the enzymatic activity of antioxidant defense proteins such as superoxide dismutase and catalase (Rageh et al. 2012; Lewicka et al. 2015). The overproduction of ROS such as superoxide radicals ($O_2^{\cdot}$), hydroxyl radicals ($OH^{\cdot}$), and hydrogen peroxides (H_2O_2) results in the reaction of these free radicals rapidly with structural and functional organic molecules including proteins, lipids, and nucleic acids which can cause disturbance of cellular metabolism. Plants, being stationary, get exposed to various types of abiotic stresses including exposure to EMRs which can lead to a broad range of biochemical responses to combat the stress (Tkalec et al. 2007). The biological effects of gamma rays in plants have been studied following the interaction with molecules in the cell especially, water which resulted in the production of free radicals. These radicals damage the essential components of plant cells and have been shown to influence the morphology, anatomy, biochemistry, and physiology of plants in multiple ways (Sharma et al. 2017). Furthermore, X-rays, a powerful mutagenic agent, have been reported to induce gene mutations and chromosomal aberrations by either acting directly on the DNA molecule or indirectly through the formation of reactive compounds (Borzouei et al. 2010). Considering the increasing levels of EMRs and their potential health hazards, the present study was planned to explore the oxidative and genotoxicity effects of 900 MHz electromagnetic fields in the *Trigonella foenum-graecum* test system.

Material and methods

Plant material

Seeds of *Trigonella foenum-graecum* were used as an experiment material and were procured from Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu.

Sterilization of seeds

Seeds were soaked in distilled water for 6 h and then sterilized by immersing them in a 4% sodium hypochlorite solution for 10 min with constant stirring. The seeds were then rinsed 3–4 times with distilled water.

Exposure treatments

Sterilized seeds were allowed to germinate in Petri plates containing *Whatman No.1* filter paper which was moistened using 5 ml of distilled water. 20 seeds of *Trigonella foenum-graecum* were evenly distributed on filter paper in Petri plates. The exposure system consisted of a radio frequency (RF) signal generator (Agilent N9310A; Key sight Technologies) to generate homogenous EMRs of the desired frequency and an antenna. The output power density at the plane of exposure near the test sample was measured using a probe attached to an RF power density meter. Seeds of *Trigonella foenum-graecum* were irradiated with different doses of non-ionizing electromagnetic radiations for different durations (0 h (control), 0.5 h, 1 h, 2 h, 4 h, and 8 h) from a key sight N9310A RF signal generator at 900 MHz for a power density of 10.0 dBm (0.01 W). The experiment was carried out at a controlled temperature of 25 ± 2 °C and relative humidity 50–60%. The control was kept in the same chamber but without switching on the device. All other conditions were kept similar.

The experiment was designed to study the effects of 900 MHz for the reason that this frequency is used in 2G, 3G, and 4G mobile networks, primarily for two-way communications and TV Towers. Hence, the present study was aimed to explore the harmful effects of EMRs at 900 MHz with variable exposure periods in the *Trigonella foenum-graecum* test system.

The exposure system was placed in a chamber completely covered with two-layered thick aluminium sheets to make sure that the experimental setup was free from exposure to any external source of EMRs. The treatment, on daily basis, was repeated for seven days. At the end of the experimental period, roots and shoots were collected separately for analysis.

Percentage germination

The percentage germination of seeds of *Trigonella foenum-graecum* was measured after 24 h, 48 h, and 72 h. The emergence of the radicle was considered as germination. The percentage germination was calculated using the following equation

$$\text{Percentage germination of seeds} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Morphological parameters

Root length (cm) and shoot length (cm) of the *Trigonella foenum-graecum* were measured with the help of a scale and thread for randomly selected four seedlings after being exposing them to the EMRs for 7 days.

Fresh weight and dry weight

After exposure to non-ionizing EMRs for 7 days, the root and shoot of *Trigonella foenum-graecum* were taken from each treatment group and analyzed for the fresh weight using weighing balance. For the dry weight, samples of root and shoot were kept in an oven at 60 °C for 5 h, and then, the weight of roots and shoots were taken using the weighing balance (Model: CY220).

Relative water content

The relative water content (RWC) was calculated by the formula

$$\text{Relative water content} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Preparation of plant extract for protein content and antioxidant enzymes

Extracts of tissue were prepared immediately after the EMRs exposure. For this, 0.5 g of plant tissues (roots and shoots) were homogenized in cold extraction buffer (50 mM potassium phosphate buffer, pH 7.0) using a pre-chilled mortar and pestle. The homogenate was centrifuged at 4 °C for 20 min at 8000 rpm. The resulting supernatant was used to estimate protein content and antioxidant enzymes.

Estimation of protein content.

Lowry's method was used to estimate the protein content at the absorbance of 660 nm. Different concentrations of Bovine serum albumin solution were used as standards to prepare a graph curve for estimation of protein content (Lowry et al. 1951).

Estimation of antioxidative enzymes

The standard protocols were employed to study different antioxidative enzymes in roots and shoots of the *Trigonella-foenum graecum* test system.

Ascorbate peroxidase

The activity was determined following the protocol given by Nakano and Asada (1981) in which the reaction mixture

was prepared by mixing 220 µl of 50 mM potassium buffer (pH 7.0), 30 µl of 0.5 mM ascorbate, 10 µl of 1.0 mM H_2O_2, 30 µl of 0.1 Mm EDTA, and 10 µl of sample extract in an ELISA plate of 96 wells. The specific activity was expressed as mol UA/mg fresh protein, and the absorbance was noted at 290 nm. The enzyme activity was calculated using the extinction coefficient of 2.8 mM^{-1} cm^{-1}.

Glutathione reductase

The glutathione reductase activity was determined by the oxidation of NADPH at 340 nm absorbance. The reaction mixture contained 200 µl of 50 mM phosphate buffer (pH 7.6), 30 µl of 1 mM oxidized glutathione, 30 µl of 0.5 mM EDTA, 30 µl of 0.1 mM reduced NADPH, and 10 µl of sample extract in an ELISA plate of 96 wells. The enzyme activity was calculated using the extinction coefficient of 6.22 mM^{-1} cm^{-1} (Carlberg and Mannervik 1975).

The glutathione-S-transferase

The glutathione-S-transferase activity was determined following the protocol given by Habig and Jakoby (1981). It is based on the interaction of 1-chloro, 2-dinitrobenzene (CDNB) with reduced glutathione (GSH). An increase in optical density at 340 nm was observed due to the production of CDNB conjugate. The reaction mixture was prepared by mixing 230 µl of 50 mM phosphate buffer (pH 7.4), 30 µl of 2 mM of reduced glutathione, 30 µl of 2 mM CDNB, and 10 µl of sample extract in an ELISA plate of 96 wells. The absorbance was noted at 340 nm. The activity was calculated using the extinction coefficient of 9.6×10^{-3} mM^{-1} cm^{-1}.

Guaiacol peroxidase

The enzymatic activity of guaiacol peroxidase was evaluated using the protocol mentioned by Sanchez et al. (1995). The reaction mixture was prepared by mixing 230 µl of 50 mM phosphate buffer (pH 7.0), 30 µl of 20 mM guaiacol, 30 µl of 12.3 mM H_2O_2, and 10 µl of sample extract in an ELISA plate of 96 wells. The absorbance was noted at 436 nm. The activity was calculated using the extinction coefficient of 26.6 mM^{-1} cm^{-1}.

Superoxide dismutase

Superoxide dismutase (SOD) was assayed for its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm. One unit of SOD was defined as the quantity of enzyme necessary to limit the photoreduction of NBT by 50% at 25 °C (Kono 1978). The reaction mixture consisted of 170 µl of 50 mM sodium carbonate buffer (pH 10.0), 30 µl of 0.1 µm hydroxylamine hydrochloride, 10 µl of 24 µM nitro blue tetrazolium dye (NBT), 10 µl of 0.1 µm EDTA, 30 µl of 0.03% Triton X-100, and 50 µl of enzyme extract in an ELISA plate of 96 wells.

Lipid peroxidation

MDA, a lipid peroxidation by-product that interacts with thiobarbituric acid, was used to indirectly assess the quantity of lipid peroxidation (Heath and Packer 1968). 1 g of plant materials were homogenized in 3 ml of 0.1% trichloroacetic acid (TCA) solution. Following homogenization, the plant samples were centrifuged at 8,000 rpm for 5 min. 3 ml of 0.5% thiobarbituric acid (prepared in 20% trichloroacetic acid) was added to 1 ml of supernatant. After 30 min of heating at 95 °C, the mixture was cooled in an ice bath and centrifuged at 8000 rpm for 10 min. To account for unspecific turbidity, the absorbance of the supernatant was measured at wavelength 532 nm and subtracted from the absorbance at wavelength 600 nm. The extinction coefficient of 155 m M^{-1} cm^{-1} was used to determine the lipid peroxidation.

Trigonella foenum-graecum root chromosomal aberration assay

Trigonella foenum-graecum root chromosomal assay was used to determine the genotoxicity. *Trigonella foenum-graecum* is an annual herbaceous crop, commonly called Methi, belongs to the family Fabaceae, and it has a basic chromosome number ($2n = 16$). The washed and sterilized seeds of *Trigonella foenum-graecum* were placed in Petri plates and were exposed to EMRs. 20 seeds per Petri plate in triplicate were allowed to germinate at the frequency of 900 MHz and power density of 10.0 dBm (0.01 W) for different treatment duration, viz., (0 h, 0.5 h, 1 h, 2 h, 4 h, and 8 h). After different treatment exposure durations (36–48 h), when roots were grown to the size of 0.5–1 cm, the root tips were cut and fixed in Farmer's fluid for 24 h (3:1; ethanol:acetic acid glacial) (Kumar and Srivastava 2011). The root tips were then transferred to 70% alcohol and refrigerated till further usage. Root tips were hydrolyzed in 1 N HCl for 1 min with intermittent warming up of slides. The root tips were then transferred to watch glass containing a mixture of 2% aceto-orecin stain and 1 N HCl in the ratio of 9:1 for 30 min. After staining, root tips were squashed in 45% glacial acetic acid by placing them on the glass slide and covered with a coverslip. The slides were then screened and scored for different types of physiological and clastogenic aberrations under the microscope (Model: Magnus MLX-DX). Microscopic examinations were done for chromosomal aberrations for each set of treatment hours and control. From each slide, a minimum of 900 dividing cells were scored for chromosomal

aberration. The frequency of aberrant cells was calculated using the formula.

$$\text{Percent aberrant cell} = \frac{\text{Total number of aberrant cells}}{\text{Total number of dividing cells}} \times 100$$

Statistical analysis

All results were expressed as mean with the corresponding standard error. We experimented in a completely randomized manner with 3 replicates per treatment. Each replicates comprised of 20 seeds per Petri plate. For genotoxicity estimation, four slides were made from each replicate. Statistical analysis was performed using IBM SPSS Statistics 21. The data were analyzed using one-way analysis of variance (ANOVA) followed by the comparison of mean values of the control with the other treatment groups using the post hoc Dunnett test at $p \leq 0.01$ and $p \leq 0.05$.

Results

Effect of EMRs on percentage germination

The data presented in Table 1 and Fig. 1 shows a significant decrease in the percentage germination of the seedlings of *Trigonella foenum-graecum.* The germination was measured after 24 h, 48 h, and 72 h of treatment. The seed germination after 72 h was 100% for the control, 0.5 h and 1 h treated seedlings, whereas it was 96% at 2 h, 95% at 4 h exposure period, and 91% at 8 h.

Effect on root and shoot length

The effect of EMRs on root and shoot length of *Trigonella foenum-graecum* after 7 days of exposure is presented in Table 1 and Fig. 2. No significant difference was observed in the root length and shoot length at 0.5 h, 1 h, 2 h, and 4 h of the exposure period. However, a significant reduction was observed in both root length and shoot length at the 8-h exposure period when compared to the control.

Effect on fresh and dry weight

The effect of EMRs on root and shoot fresh weight and dry weight of *Trigonella foenum-graecum* after 7 days of exposure is presented in Table 2 and Figs. 3 and 4. At all treatment hours, there was a significant difference in the fresh weight of the roots and shoots, but no significant difference in the dry weight of the roots and shoots was observed.

Effect on relative water content

The effect of EMRs on root and shoot RWC is presented in Table 2. No significant difference in the RWC was observed.

Effect on protein content

The protein content in roots was found to be significantly decreased at the 8-h exposure period when compared to the control, whereas in the shoots, the protein content significantly decreased after 2 h, 4 h and 8 h exposure period (Table 3; Fig. 5).

Effect on antioxidative enzymes

The effect of RF-EMRs on the activity of antioxidant enzymes in roots and shoots differed depending on the exposure period as shown in Table 4. At 900 MHz, ascorbate peroxidase (APX) activity in the roots increased significantly (Fig. 6). A significant difference in the roots was found only

Table 1 Percentage germination of seedlings of *Trigonella foenum-graecum* after 24 h, 48 h, and 72 h and mean root length after 7 days at 900 MHz frequency for different exposure periods per day

Treatment (h/day)	Percentage germination			Mean length (cm) of 7-day-old seedlings	
	24 h	48 h	72 h	Root	Shoot
0 h	100 ± 0.00	100 ± 0.0	100 ± 0.00	4.86 ± 0.15	5.24 ± 0.20
0.5 h	100 ± 0.00	100 ± 0.0	100 ± 0.00	4.85 ± 0.22	5.08 ± 0.12
1 h	96.66 ± 0.33	100 ± 0.0	100 ± 0.00	4.70 ± 0.03	4.65 ± 0.22
2 h	91.66 ± 0.33*	95 ± 0.0**	96.66 ± 0.33	4.57 ± 0.36	4.76 ± 0.14
4 h	86.66 ± 0.33**	90.0 ± 0.0**	95.00 ± 0.00*	4.48 ± 0.13	4.35 ± 0.09**
8 h	86.66 ± 0.33**	86.66 ± 0.3**	91.66 ± 0.33**	3.29 ± 0.18**	4.10 ± 0.17*

Data presented as mean ± SE ($n = 3$) for each treatment group. The significance of the data is determined using post hoc Dunnett test by comparing the mean value of the control with other treatment groups *$p \leq 0.05$, **$p \leq 0.01$

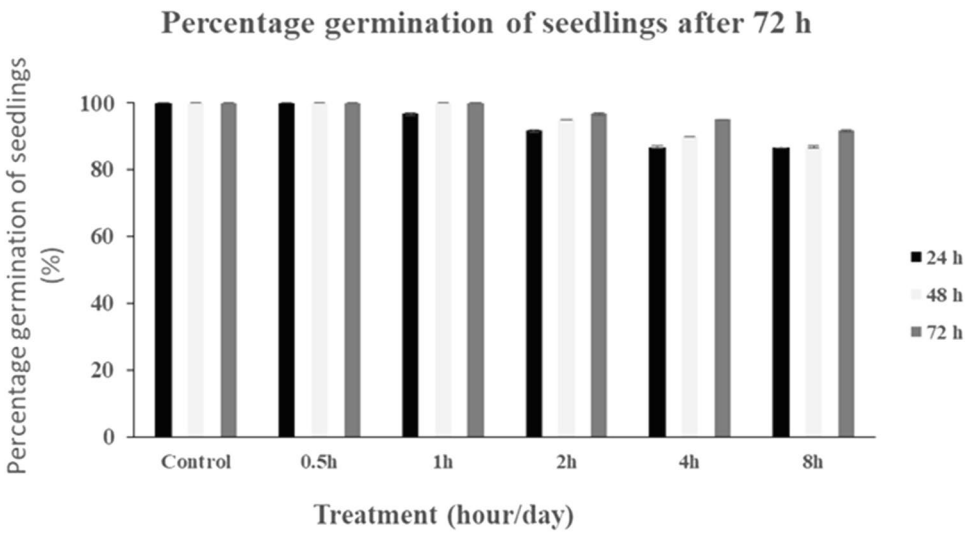


Fig. 1 Effects of EMRs on percentage germination of seedlings at 900 MHz for different exposure periods per day

at 4 h and 8 h of exposure period when compared with the control whereas the ascorbate peroxidase activity in the shoots decreased and the prolonged exposure at 4 h and 8 h failed to induce significant effect (Fig. 6).

Glutathione reductase (GR) and glutathione-S-transferase (GST) activity increased significantly for 8 h exposure period in root and shoot. However, there was no significant difference in the GR and GST enzyme activity at other exposure periods (Figs. 7 and 8). Guaiacol peroxidase (POD) activity in the roots increased significantly at 4 h and 8 h exposure periods, whereas in shoots, the guaiacol peroxidase activity increased significantly only at the 8-h exposure period when compared with the control (Fig. 9).

Superoxide dismutase (SOD) activity increased significantly in root and shoot for the 8-h exposure period, while superoxide dismutase (SOD) activity did not significantly change for other exposure periods (Fig. 10).

Effect on lipid peroxidation and chromosomal aberrations

The level of lipid peroxidation, measured in terms of the MDA content, significantly increased $p \leq 0.01$ after 8 h of exposure, while no significant difference was found in the other exposure periods (0.5 h, 1 h, 2 h, and 4 h) (Table 3; Fig. 11). Total chromosomal aberrations were observed to vary as 0.57% (0 h/day), 3.26% (0.5 h/day), 5.86% (1 h/day), 6.81% (2 h/day), 8.53% (4 h/

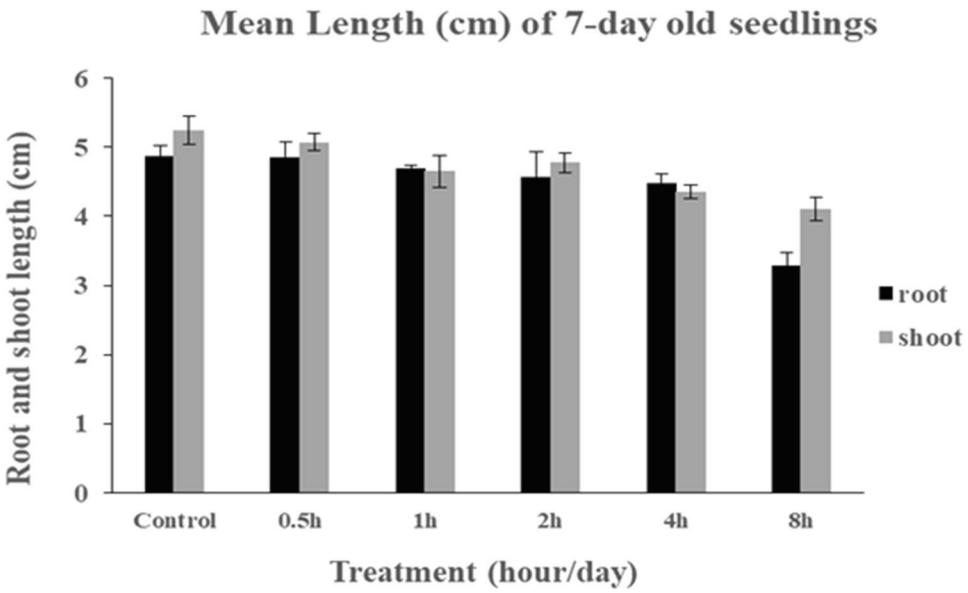


Fig. 2 Effects of EMRs on root and shoot length of *Trigonella foenum-graecum* at 900 MHz frequency for different exposure period per day

Table 2 Fresh and dry weight of root and shoot per seedling of *Trigonella-foenum graecum* after 7 days exposure at 900 MHz frequency for different exposure period per day

Treatment (h/day)	Fresh weight (mg) per seedling after 7 days		Dry weight (mg) per seedling after 7 days		Relative water content (RWC %)	
	Root	Shoot	Root	Shoot	Root	Shoot
0 h	58.81 ± 2.74	84.23 ± 3.04	0.85 ± 0.02	4.2 ± 0.80	98.54 ± 0.08	95.00 ± 1.00
0.5 h	41.15 ± 2.51**	71.58 ± 4.30*	0.85 ± 0.02	4.33 ± 0.69	97.91 ± 0.19	93.97 ± 0.87
1 h	38.4 ± 0.20**	58.53 ± 2.33**	0.83 ± 0.03	4.68 ± 1.34	97.83 ± 0.07	92.14 ± 2.07
2 h	37.56 ± 2.49**	62.89 ± 1.38**	0.78 ± 0.08	4.61 ± 0.81	97.88 ± 0.25	92.64 ± 1.29
4 h	38.26 ± 1.97**	54.59 ± 1.84**	0.76 ± 0.13	4.41 ± 0.31	98.01 ± 0.23	91.87 ± 0.80
8 h	37.52 ± 2.39**	54.06 ± 1.77**	0.68 ± 0.06	4.1 ± 0.49	98.15 ± 0.21	92.40 ± 0.88

Data presented as mean ± SE ($n = 3$) for each treatment group. The significance of the data is determined using post hoc Dunnett test by comparing the mean value of the control with other treatment groups *$p \leq 0.05$, **$p \leq 0.01$

day), and 12.86% (8 h/day) upon exposure (Table 5; Fig. 12). Different types of chromosomal aberrations included physiological aberrations (c-mitosis, delayed anaphases, stickiness, laggards, and vagrants) and clastogenic aberrations (chromosomal breaks and ring chromosomes) were observed during this study.

Discussion

Stress is a negative condition that prevents plants from functioning normally. There are two forms of stress such as biotic and abiotic. Pathogens, insects, and rodents are examples of biological stressors, whereas abiotic stresses include heat, salinity, drought, radiation, and metal toxicity (Qamer et al. 2021; Leaver and George 1998). With the increased use of electronic devices, the concern over the emission of EMRs from the gadgets has increased. The subtle danger of electronic devices lies in their harmful effects on public health due to the release of electromagnetic radiation from them (Liu et al. 2012). The negative consequences of exposure to radiofrequency modulated electromagnetic fields (RF-EMFs) have lately received widespread attention, particularly in light of the significant worldwide growth in cellular phone use (Mohamed et al. 2014). While using a cellular phone, electromagnetic waves are generated in the body, causing health risks, especially in the ear skull area, where they have been shown to affect neurons. Electrical impulses that link two neurons are disrupted by the radiations which lead to deafness and migraines (Tyagi et al. 2011; Bhargavi et al. 2013; Malik 2020). Low blood pressure and other symptoms such as hot ears, burning skin, headaches, dizziness, and tiredness are more common in those who use cell phones for longer period (Goel et al. 2016; Hardell and Koppel 2022). Electromagnetic radiation released by mobile phones might impair human biological functioning (cells, brain, or immune system), increasing the risk of numerous illnesses (Jorgensen and Moscovitch 2011; Kim et al. 2016).

EMRs of over 900 MHz could transfer energy and exert thermal effects on biological matters including different antioxidants enzymes, viz., superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione

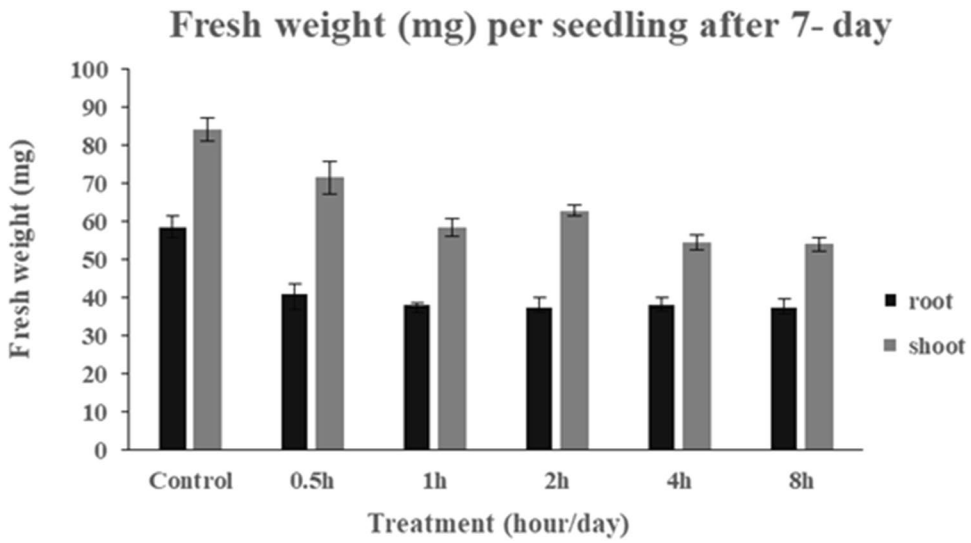


Fig. 3 Effects of EMRs on the fresh weight of root and shoot per seedling of *Trigonella foenum-graecum* at 900 MHz frequency for different exposure period per day

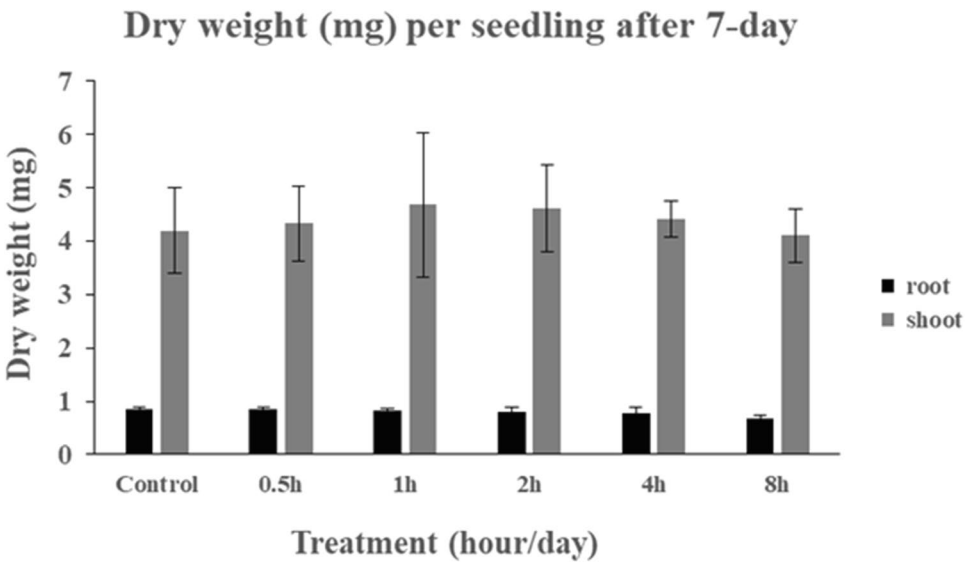


Fig. 4 Effects of EMRs on the dry weight of root and shoot of *Trigonella foenum-graecum* per seedling at 900 MHz frequency for different exposure period per day

peroxidase (GPX), and peroxiredoxin (PrxR) (Caverzan et al. 2012; Wang and Zhang 2017; Wang et al. 2019).

In the present study, roots and shoots of *Trigonella foenum-graecum* have been exposed to non-ionizing EMRs at a frequency 900 MHz and a constant power density of 10.0 dBm (0.01 W) to evaluate the ability of EMRs to induce antioxidative stress and genotoxicity in *Trigonella foenum-graecum*. Our data demonstrated that EMRs led to a statistically significant decrease in the percentage germination (72 h) in the seeds of *Trigonella foenum-graecum* ($p \leq 0.05$) for 4 h and ($p \leq 0.01$) for the 8-h exposure period to EMRs. The results obtained by Cammaerts and Johansson (2015) and Halgamuge et al. (2015) were similar to our findings to explore the germination rate of *Lepidium satvium* which showed that exposure to 900 MHz and 1800 MHz field with an intensity of 70–100 W/m^2 for 10 days has reduced percentage germination. The effects of EMRs on root length and shoot length of *Trigonella foenum-graecum* after 7 days of exposure have been studied. No difference in the root length was observed at the lower exposure period while at the 8-h exposure period a statistically significant difference ($p \leq 0.01$) in the root length was observed when compared with the control. A significant reduction in the shoot length was observed at 4 h ($p \leq 0.01$) and 8 h ($p \leq 0.05$) exposure periods. Such a reduction in length on exposure to radiations is corroborated by similar findings made earlier with cell phone radiations (Kumar et al. 2016; Singh et al. 2012), radio-frequency EMF-r (Akbal et al. 2012), and magnetic fields (Penuelas et al. 2004). In a recent study, the root length of the *Allium cepa* has shown a significant decline after 2 h and 4 h exposure period at 900 MHz and the decrease was 11% and 13%, respectively, and EMR-induced inhibition in root growth was found to be due to consequences of decreased cell elongation (Kumar et al. 2020). In this study, the results of fresh weight of root and shoot were found to be statistically significant at all treatment hours. The fresh weight of root was significant at 0.5 h, 1 h, 2 h, 4 h, and 8 h ($p \leq 0.01$), while the fresh weight of shoot was significant at 0.5 h ($p \leq 0.05$), 1 h, 2 h, 4 h, and 8 h ($p \leq 0.01$). This finding is related to the study of the effects of radiation on different species of beans, where 24 h intermittent radiation exposure significantly affected the weight of Mung beans (Chen and Chen 2014). Our results are also in line with the findings obtained from a study conducted by Borzouei et al. (2010) where radiations imposed a significant effect on the weight of root and shoot. The dry weight of the root and shoot was found to be statistically insignificant. Radiofrequency exposure from mobile phones 900 MHz and 1800 MHz for 0.5 h, 1 h, 2 h, 4, and 8 h affected both the biochemical and morphological processes in *Pisum sativum* and *Trigonella foenum-graecum* (Sharma and Parihar 2014). In *Rosa-Hybrid*,

Table 3 Protein content and MDA content of *Trigonella foenum-graecum* at 900 MHz frequency for different exposure period per day

Treatment (h/day)	Total protein content in (mg/g) of fresh weight of tissue		MDA content (μmol/g fresh weight)
	Root	Shoot	Seedlings
0 h	2.54 ± 0.280	5.33 ± 0.110	0.15 ± 0.003
0.5 h	2.88 ± 0.048	4.92 ± 0.411	1.60 ± 0.016
1 h	2.74 ± 0.007	4.29 ± 0.245	2.80 ± 0.111
2 h	2.47 ± 0.026	4.04 ± 0.057*	3.67 ± 0.086
4 h	2.02 ± 0.300	3.46 ± 0.070**	4.50 ± 0.063
8 h	1.12 ± 0.098**	2.11 ± 0.126**	13.1 ± 0.087**

Data presented as mean ± SE ($n = 3$) for each treatment group. The significance of the data is determined using post hoc Dunnett test by comparing the mean value of the control with other treatment groups *$p \leq 0.05$, **$p \leq 0.01$

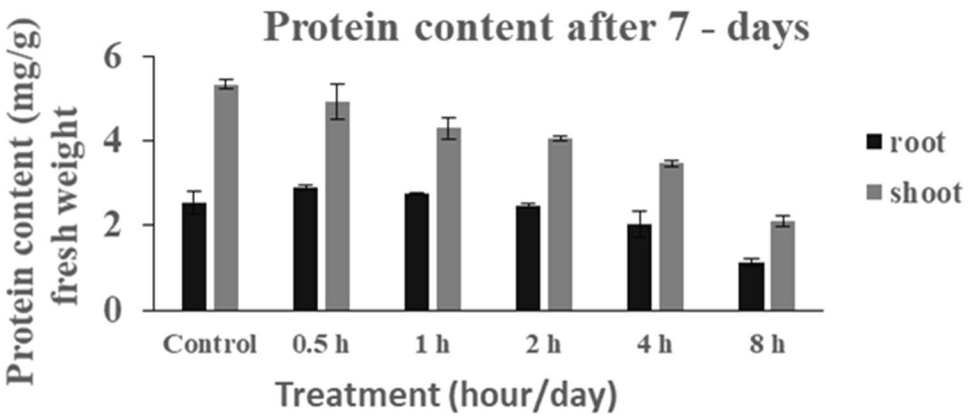


Fig. 5 Effects EMRs on the protein content of roots and shoots of *Trigonella foenum-graecum* at frequency 900 MHz for different exposure period per day

Table 4 Activity of different antioxidant enzymes in roots and shoots of *Trigonella-foenum graecum* upon exposure to EMRs at 900 MHz frequency for different exposure period per day

Plant material	Treatment (h/day)	The specific activity of antioxidant enzymes in roots and shoots of *Trigonella foenum-graecum* (mol UA/mg fresh protein (mean ± S.E)				
		Ascorbate peroxidase	Glutathione reductase	Glutathione-S-transferase	Guaiacol peroxidase	Superoxide dismutase
Roots	0 h	0.04 ± 0.01	2.22 ± 0.75	1.20 ± 0.38	0.43 ± 0.17	3.73 ± 0.70
	0.5 h	0.30 ± 0.02	1.48 ± 0.82	1.12 ± 0.41	0.48 ± 0.17	4.53 ± 0.41
	1 h	0.29 ± 0.01	1.74 ± 0.87	1.17 ± 0.44	0.50 ± 0.18	4.35 ± 1.06
	2 h	0.28 ± 0.05	2.09 ± 0.89	1.23 ± 0.49	0.57 ± 0.18	4.92 ± 1.03
	4 h	0.48 ± 0.10*	2.60 ± 0.90	1.41 ± 0.53	0.74 ± 0.18*	5.58 ± 0.59
	8 h	0.99 ± 0.12**	5.38 ± 0.27**	2.76 ± 0.41**	1.26 ± 0.37**	13.59 ± 1.90**
Shoots	0 h	0.16 ± 0.02	1.10 ± 0.49	0.67 ± 0.19	0.26 ± 0.95	2.29 ± 0.22
	0.5 h	0.10 ± 0.03	1.06 ± 0.50	0.68 ± 0.20	0.34 ± 0.093	2.48 ± 0.42
	1 h	0.07 ± 0.04	1.70 ± 0.47	0.69 ± 0.22	0.45 ± 0.101	2.45 ± 0.41
	2 h	0.09 ± 0.04	1.59 ± 0.52	0.77 ± 0.23	0.33 ± 0.118	2.68 ± 0.29
	4 h	0.15 ± 0.01	1.96 ± 0.50	0.76 ± 0.26	0.36 ± 0.129	2.68 ± 0.46
	8 h	0.45 ± 0.15	3.5 ± 0.03**	1.53 ± 0.16**	0.68 ± 0.112**	9.91 ± 0.36**

Data presented as mean ± SE ($n = 3$) for each treatment group. The significance of the data is determined using post hoc Dunnett test by comparing the mean value of the control with other treatment groups *$p \leq 0.05$, **$p < 0.01$

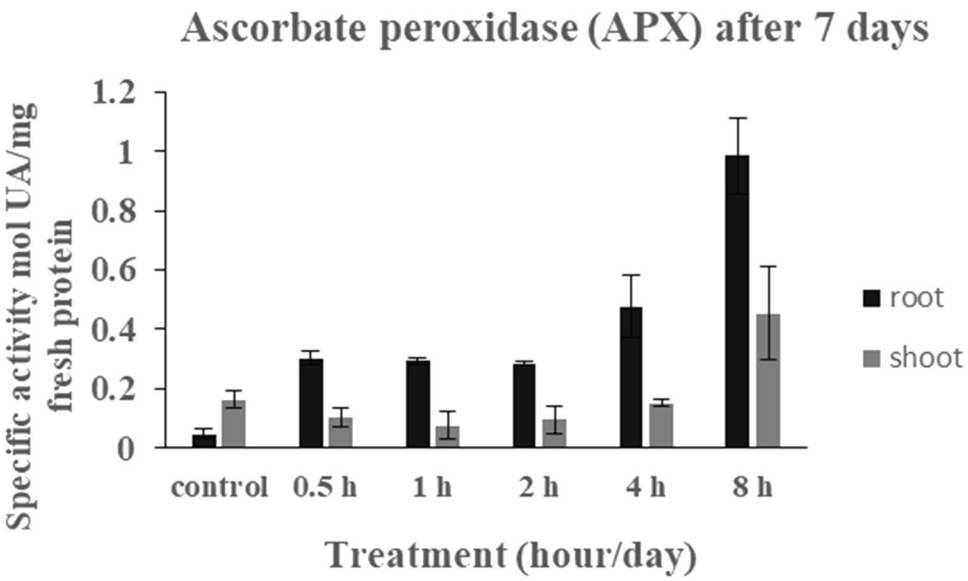


Fig. 6 Effects of EMRs on the activity of ascorbate peroxidase in root and shoot of *Trigonella foenum- graecum* at 900 MHz for different exposure period per day

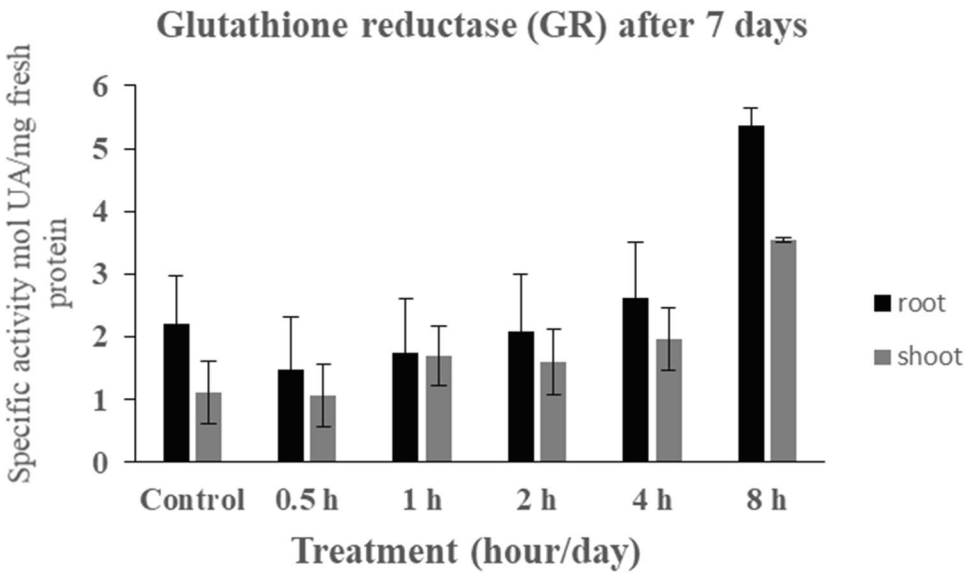


Fig. 7 Effects of EMRs on the activity of glutathione reductase (GR) in root and shoot of *Trigonella foenum-graecum* at 900 MHz for different exposure period per day

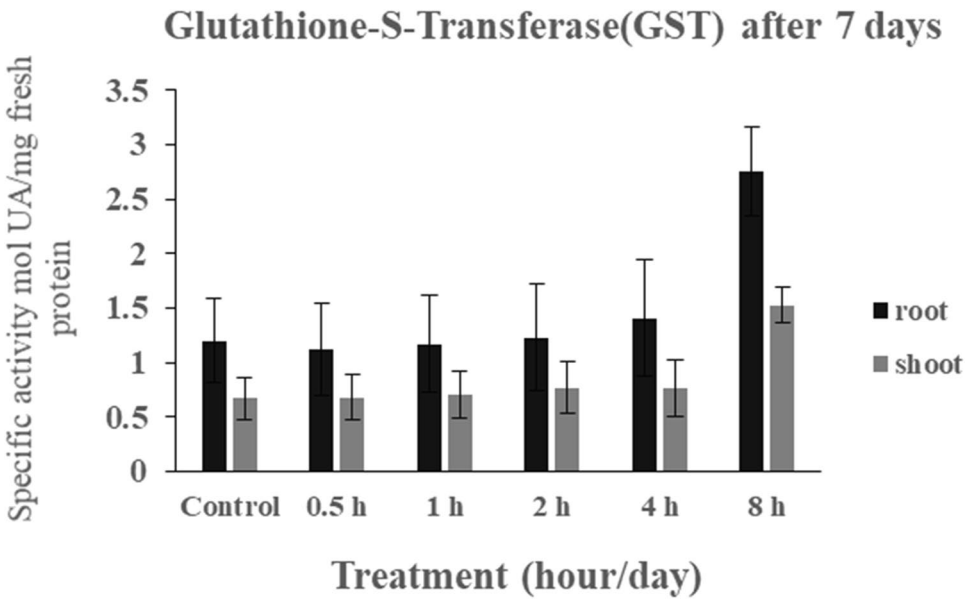


Fig. 8 Effects of EMRs on the activity of glutathione-S-transferase (GST) in root and shoot of *Trigonella foenum-graecum* at 900 MHz for different exposure period per day

the effect of low amplitude and high frequency 900 MHz electromagnetic-field exposure on plant growth in post-formed organs was examined. The results obtained from the study depicted that it caused delayed and decreased plant development in post-formed organs (Grémiaux et al. 2016). The RWC of the root and shoot after EMR exposure was found to be insignificant at all treatment hours.

EMR exposure at a frequency of 900 MHz enhanced the activity of antioxidative enzymes ascorbate peroxidase, glutathione reductase, glutathione-s-transferase, guaiacol peroxidase, and superoxide dismutase in roots and shoots of *Trigonella foenum-graecum*. The ascorbate–glutathione pathway requires ascorbate peroxidase (APX) and glutathione reductase to scavenge H_2O_2 generated mostly in chloroplasts and other cell organelles and to maintain the cell's redox state (Verma and Dubey 2003). The activity of APX in roots was significant at 4 h ($p \leq 0.05$) and 8 h ($p \leq 0.01$). The activity of GR and GST were significant in both roots and shoots at 8 h ($p \leq 0.01$). The activity of POD in root was significant at 4 h ($p \leq 0.05$) and 8 h ($p \leq 0.01$), whereas in shoots, the activity was found to be significant at 8 h ($p \leq 0.01$). For SOD, the enzymatic activity was found significant both in roots and shoots at 8 h ($p \leq 0.01$). Similar responses were seen in earlier studies in *Vigna radiata* upon exposure to EMRs at a frequency of 900 MHz. The induction of oxidative stress occurred which causes a significant increase in the MDA content, SOD, APX, GR, POD, and CAT activities (Singh et al. 2012). Three *Trigonella* species,

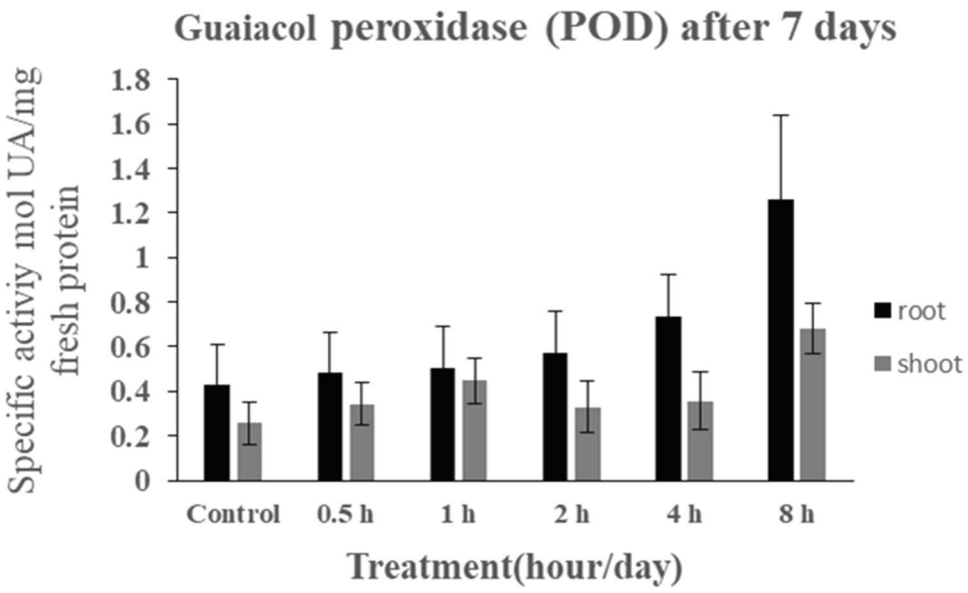


Fig. 9 Effects of EMRs on the activity of guaiacol peroxidase in root and shoot of *Trigonella foenum-graecum* at 900 MHz for different exposure period per day

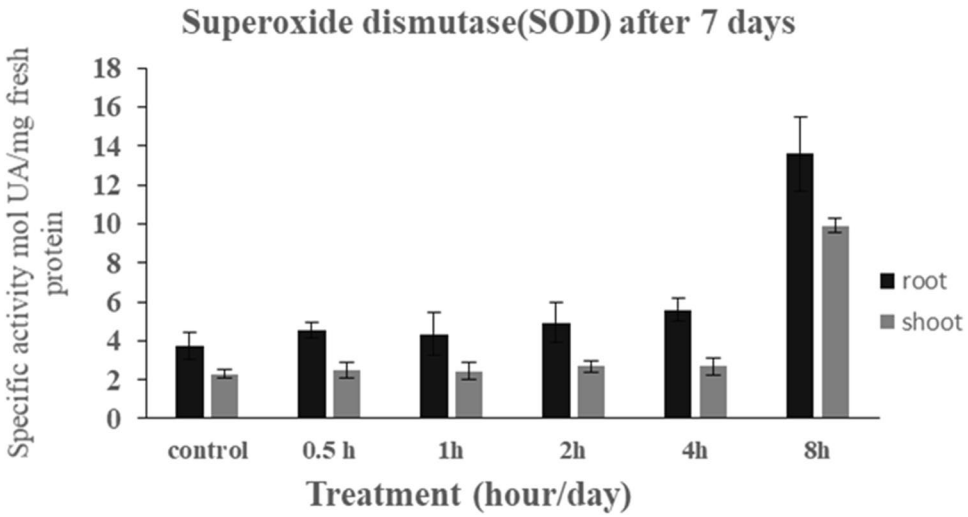


Fig. 10 Effects of EMRs on the activity of superoxide dismutase (SOD) in root and shoot of *Trigonella foenum-graecum* at 900 MHz for different exposure period per day

viz., *T. hamosa*, *T. stellata*, *and T. anguina*, were subjected to ionizing gamma radiations, and a rise in SOD, APX, and GR activities were seen in the roots and shoots of all *Trigonella* species analyzed (Al-Rumaih and Al-Rumaih 2008).

Cells have an innate method to deal with stress caused by excessive ROS generation and one of such techniques is the upregulation/increased synthesis of antioxidant enzymes that scavenge free radicals (Gill and Tuteja 2010; Sepehrimanesh et al. 2016). Lipid peroxidation research was used to measure the EMR-induced membrane damage. In the present study, when seeds were exposed to 900 MHz EMRs at different treatment hours, MDA content increased which is a marker of lipid peroxidation and the MDA content was found to be significant at 8 h ($p \leq 0.01$). MDA is a free radical produced as a consequence of lipid peroxidation and elevated levels of MDA after exposure supports the theory that EMRs promoted lipid peroxidation caused membrane damage and induced oxidative stress in *Trigonella foenum-graecum* seedlings.

Chromosomal abnormalities assess the exposure of different species to various physical and chemical substances that harm human health and are also thought to be the outcome of these agents' genotoxic effects (De Souza et al. 2013). When *Trigonella foenum-graecum* root tip cells were exposed to EMRs for 4 or 8 h, different chromosomal aberrations were observed (Fig. 13). Exposure to EMRs resulted in chromosomal abnormalities such as physiological aberrations (stickiness, c-mitosis, delayed anaphase, and aberrant metaphase) ascribed to spindle abnormalities and clastogenic aberrations (chromosomal bridge and ring chromosome). The

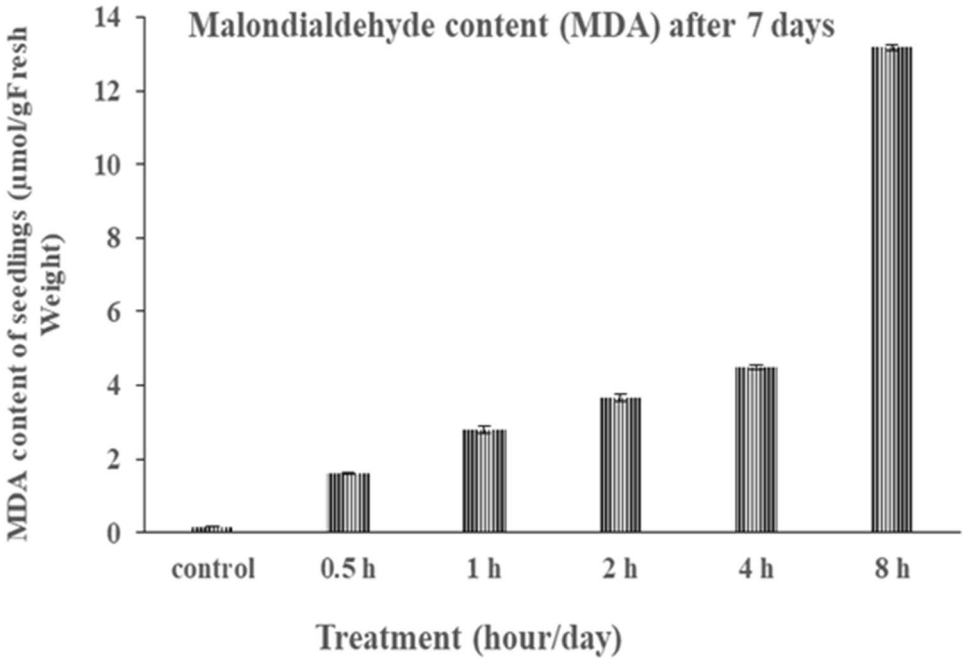


Fig. 11 Effect of EMRs on lipid peroxidation (measured as MDA content) in seedlings of *Trigonella foenum-graecum* at 900 MHz frequency for different exposure period per day

Table 5 Genotoxic effects of electromagnetic radiations at 900 MHz after 72 h with different exposure period/day in the root tip cells of *Trigonella foenum-graecum*

Type of chromosomal aberration	Number of cells with aberrations at 900 MHz					
	0 h/day	0.5 h/day	1 h/day	2 h/day	4 h/day	8 h/day
Physiological aberrations (PA)						
c-Mitosis	1	7	7	11	12	9
Delayed anaphase	2	0	0	11	9	11
Laggard/s	1	1	10	12	7	15
Stickiness	2	9	12	11	11	11
Abnormal metaphase	0	0	0	0	7	13
Abnormal anaphase	0	0	0	0	3	3
Delayed metaphase	0	8	3	0	4	19
Vagrant/s	0	0	10	8	8	14
Total PA	6	25	42	53	61	95
Clastogenic aberrations (CA)						
Chromatin bridge/s	0	5	11	9	11	16
Ring chromosome	0	0	0	0	6	6
Total CA	0	5	11	9	17	22
Total aberrant cells (PA + CA)	**6**	**30**	**53**	**62**	**78**	**117**
Total dividing cells	1060	920	904	910	914	910
Total cells	8440	8915	7365	7755	7606	7730
Total aberration (%)	**0.57**	**3.26****	**5.86****	**6.81****	**8.53****	**12.86****

Significance level was determined using a chi-square test, $^{**}p \leq 0.01$

most prevalent ones found in EMRs-exposed cells included delayed metaphase, c-mitosis, and aberrant metaphase. The 8-h exposed cell had the most abnormalities, followed by the 4-h exposure period. The occurrence of chromosomal stickiness may be due to denatured nucleoproteins or degraded/depolymerized DNA caused by EMRs treatment, which resulted in chromosome clumping (Kumar et al. 2003; Choudhary et al. 2012; Chandel et al. 2019). The current findings are similar to prior studies that demonstrated greater mitotic index and chromosomal aberrations in *A. cepa* root meristems after 400 MHz (Tkalec et al. 2009) and 900 MHz EMF-r exposure (Pesnya and Romanovsky 2013). This aberrant mitotic activity might be attributed to spindle damage following EMF-r therapy (Prokhorova et al. 2008) or represent an after effect of

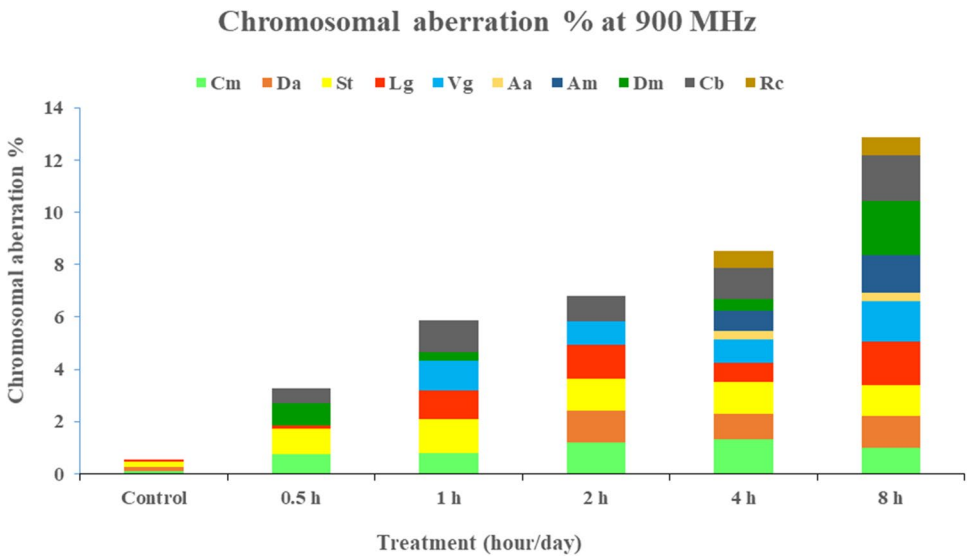


Fig. 12 Spectrum of different types of chromosomal aberrations induced in root tip cells of *Trigonella foenum-graecum* following treatment with EMRs at 900 MHz after 72 h with different exposure periods per day

delayed mitosis (Tkalec et al. 2009). In onion meristematic cells, EMF-r exposure caused chromosomal abnormalities such as vagrant and lagging chromosomes, c-mitosis, mitotic bridge, chromosome stickiness, fragmentation, and spindle disruptions. Stickiness and c-mitosis were the most common ones identified in EMF-exposed cells. The presence of chromosome stickiness might be attributed to denatured nucleoproteins or degraded/depolymerized DNA after EMF-r therapy, which results in chromosome clumping (Kumar et al. 2003; Choudhary et al. 2012). Similarly, c-mitosis may be caused by microtubule disintegration (Rieder and Cole 2000). Thus, the development

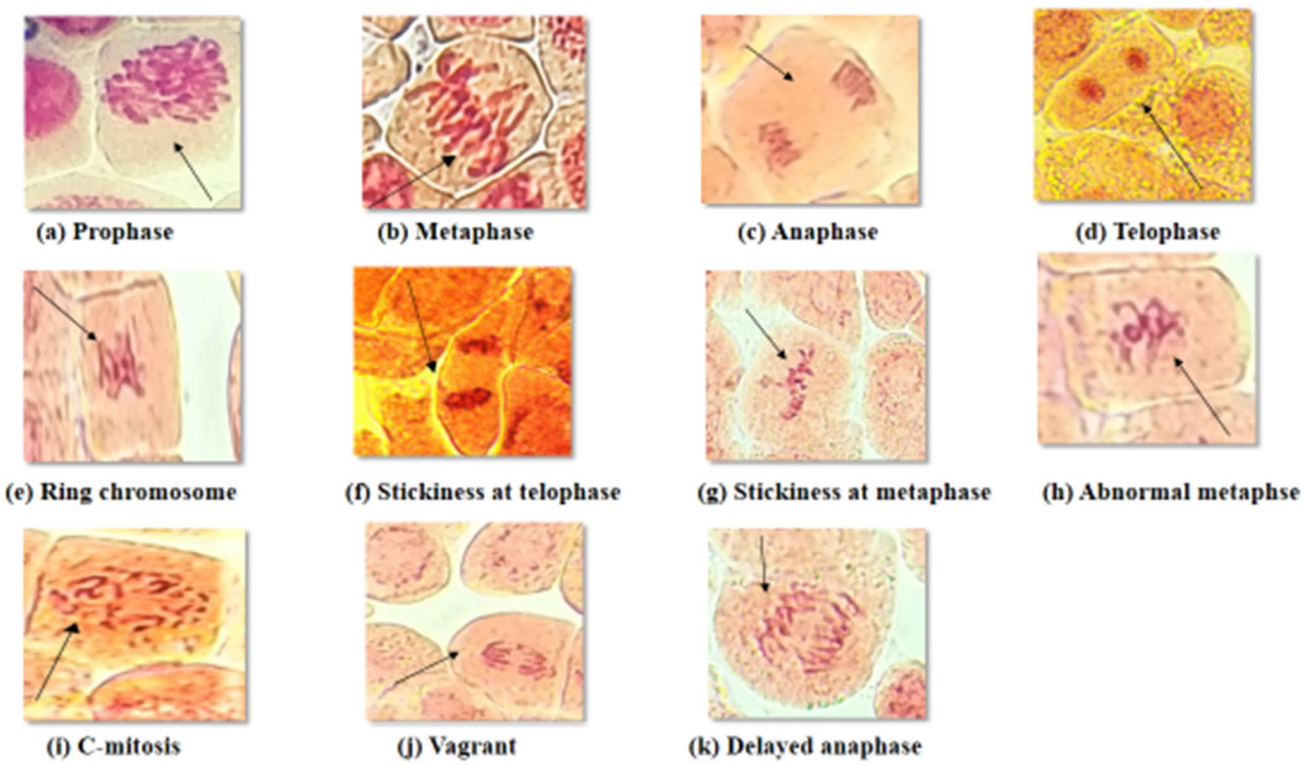


Fig. 13 Normal cells showing different phases of mitotic division (**a**–**d**) and cells with chromosomal aberrations (**e**–**k**) in root tip cells of *Trigonella foenum-graecum*

of various chromosomal abnormalities might be linked to spindle disruptions caused by EMF-r exposure (Pesnya and Romanovsky 2013).

EMRs lack the energy to directly break a chemical bond (Vian et al. 2016; Chandel et al. 2019). EMF-r has also been shown to enhance micronuclei frequencies in *Vicia faba* (Gustavino et al. 2016) and *Tradescantia* (Haider et al. 1994). The use of a transverse electromagnetic wave mode (TEM) cell to expose *Vicia faba* to EMRs at 915 MHz for 72 h has shown genotoxic effects (Gustavino et al. 2016). Radiofrequency radiations at 2100 MHz were shown to have cytotoxic and genotoxic effects on *A.cepa* root meristems, and the biological effects were time-dependent, with the largest alterations happening after 4 h of exposure (Chandel et al. 2017). Similarly, exposure to mobile phone radiations at 2350 MHz induced cytotoxic and genotoxic effects on *A.cepa* root meristematic cell. Cytotoxicity was measured in this work by analyzing parameters such as mitotic index (Chandel et al. 2019). The 4G uses 700 MHz, 850 MHz, 900 MHz, and 1800 MHz for the transmission, while the 5G uses 6–100 GHz frequency which is a matter of concern for the plants as well as animals and the problems caused by the EMRs depend on the power density and frequency (Malka N Halgamuge 2017). The biological effects of EMF-r have piqued the interest of scientists, including environmentalists and biologists. Yet there are gaps in the existing literature regarding the effects of EMF-r on plants and associated changes at various levels (genetic, molecular, biochemical, cellular, and morphological) and stages of growth and development from seed germination to flowering stage (Kaur et al. 2021).

Conclusions

The current study indicated that EMRs at 900 MHz caused oxidative stress as well as genotoxic consequences in *Trigonella foenum-graecum*. The observed biological impacts were time-dependent with the greatest changes occurring after 4 and 8 h of treatment. As the concentration of EMRs emitted by man-made devices such as mobile phones is rapidly rising in the environment these days, it has become imperative to consider the biological impacts of these radiations and also some studies has already reported that the EMRs released by mobile phones might impair human biological functioning (cells, brain or immune system) which might leads to the increased risk of numerous illnesses. The present research is of significant importance because rapidly emerging EMRs in the environment have the potential for inducing oxidative stress and genetic damage in living beings. As a result, the current study argues for a correct risk assessment in terms of the effects of EMRs on the environment, as well as the development of solutions, to limit the effects of EMRs on the environment. However, more studies are compulsory to explore the mechanisms of action of these radiations at the molecular level. Accordingly, advance research is required in this field for a better understanding and formulation of effective protective measures for mitigation of the damaging effects of these radiations.

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Declarations

Ethics approval. The ethical approval was not needed.

Consent for publication. The authors declare consent for publication in Protoplasma.

Conflict of interest The authors declare no competing interests.

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