



Morphological, biochemical and genotoxic effects of non-ionizing radiation at 1800 MHz and 2400 MHz frequencies in *Allium cepa* L.

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Abstract

The frequent use of electronic devices in daily lives, predominantly reliant on non-ionizing radiation, has increased the prevalence of electromagnetic radiation (EMR) in natural environment. In light of this, effects of EMR at frequencies of 1800 MHz and 2400 MHz characterized by a power of 10.0 dBm (0.01 W), across varying exposure durations of 1 h/day, 2 h/day, 4 h/day, 6 h/day, and 8 h/day for 7 days, in *Allium cepa* L. were studied. The effects of the treatment on the morphological features (root length, fresh weight, and dry weight of roots) and biochemical characteristics (protein content and antioxidative enzymes, namely, ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD), glutathione-S-transferase (GST), guaiacol peroxidase (POD), catalase (CAT), and dehydroascorbate (DHAR)) were studied in roots and bulbs of *Allium cepa* L. Further genotoxicity for different exposure periods at both frequencies was also conducted. Prolonged exposure to electromagnetic radiation (EMR) at both frequencies was found to reduce root length, fresh weight, and dry weight of plant. Furthermore, significant effects were observed on protein content, indicating a reduction with prolonged exposure duration. Investigation into the activities of antioxidative enzymes such as APX, GR, GST, DHAR, CAT, SOD, and POD at a frequency of 1800 MHz and 2400 MHz in roots and bulbs demonstrated a significant enhancement in enzyme activity during 6 h/day and 8 h/day exposure periods. Additional investigation during genotoxicity studies demonstrated the induction of chromosomal aberrations in the root tip cells of the *Allium cepa* L. plant test system. The current study revealed the initiation of oxidative stress and genotoxicity resulting from long-term exposure to electromagnetic radiation in the studied plant test systems.

Keywords Chromosomal aberrations · Antioxidative enzymes · Oxidative stress · Power · Electromagnetic radiation

Introduction

A notable increase has occurred in daily utilization of portable handheld cellular phones with continuous advancement of technology over the past decades. The utilization of these devices has substantially elevated the presence of non-ionizing electromagnetic radiation in the environment, resulting in an increase in the level of exposure of living organisms to electromagnetic field radiation (EMR) (Gautam et al. 2024;

Panda et al. 2024). High doses of ionizing and non-ionizing radiation could coexist and collectively endanger the biota. Human health received a majority of attention when it comes to the negative effects of electromagnetic radiation (Liu et al. 2021; Davis et al. 2023). Nevertheless, research in past years focused on animal systems which yielded the majority of these conclusions, with less focus being placed on the impact of radiation on plants (Vian et al. 2016). However, in comparison to animals, plants serve as excellent models for assessment of radiation effects because of their sessile nature (which consistently maintain a specific orientation of the exposure within the electromagnetic field), sensitivity to changes in the environment, and instantaneous abiotic stress responses. Their distinctive developmental pattern, characterized by a high surface area to volume ratio, uniquely positions them to efficiently intercept EMR (Upadhyaya et al. 2022). Irrespective of the level of exposure, non-ionizing radiation emitted from cell phone towers induced oxidative

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stress and genotoxicity in an *Allium cepa*, particularly at sites with maximum power density (Sharma et al. 2024).

EMR elicited a diverse array of responses in plants. Exposure to EMR influences reactive oxygen species (ROS) metabolism enzymes, a widely used indicator for plant response to various environmental stimuli (Singh et al. 2012). Kumar et al. (2020) recorded the cytogenetic impacts of radiation at 900 MHz and 1800 MHz in *Allium cepa*. According to Adrovic et al. (2021), onion cell chromosomes are well-suited for assessing environmental contaminants due to their limited yet relatively large chromosomes, facilitating the easy detection of chromosomal abnormalities. Furthermore, studies examining the impact of electromagnetic radiation (EMR) on plants have produced inconclusive results and lacked definitive evidence (Kouzmanova et al. 2009; Kaur et al. 2021; Levitt et al. 2022). Only a limited number of studies have shown the detrimental effects of EMR (Sharma et al. 2009; Tkalec et al. 2009; Singh et al. 2012; Qureshi et al. 2017; Sharma et al. 2023a).

In a study that assessed the effects of non-ionizing radiation of 1800 MHz frequency, the overall well-being of the plant was evaluated using the physical indicators of plant height, leaf dimensions, length of the root system, and rate of germination (Upadhyaya et al. 2022). The physiological and biochemical changes in tomato plants subjected to high-frequency radiation were determined. The notable reduction in total phenolic content, flavonoid content, and vitamin C content along with the substantial decline of 59.32% in DPPH indicated that electromagnetic radiation (EMR) had a significant adverse impact on secondary metabolites and ability of tomato plants to generate antioxidants. According to a study by Sharma et al. (2023b), EMR at 900 MHz frequency caused oxidative stress in *Trigonella foenum-graecum* when exposed over extended time. In a study conducted by Gustavino et al. (2016), it was observed that exposure to cell phone radiation within 915 MHz frequency band led to a dose-dependent rise in the formation of micronuclei in secondary root tip cells. Additionally, it was found that radiation impeded spindle formation in broad beans.

Senevirathna et al. (2020) investigated the impact of microwave radiation within the industrial, scientific, and medical (ISM) radio band on *Arabidopsis thaliana* (L.) seeds and seedlings. The exposure involved continuous wave of microwave at a frequency of 2.45 GHz, with a power flux density of $1.0 \pm 0.1 \text{ W/m}^2$, administered over a duration of 48 h. It was observed that peroxidase activity remained constant while microwave irradiation increased the concentrations of hydrogen peroxide, photosynthetic pigments, non-photochemical quenching, and fluorescence in the seedlings. Plants get abiotically stressed in many ways when exposed to EMR. In response to these challenges, plants could create a variety of biological defenses against the strain (Tkalec et al. 2007). During a study to assess the cytogenetic

effects of radiation emitted by a projector on root tip cells of *Allium cepa* (onion) by Alege et al. (2022), it was revealed that radiation exposure resulted in chromosomal abnormalities. Compared to the negative control groups, the irradiated onion root tip cells exhibited a higher mitotic index, which indicated the induction of cell proliferation, and therefore, a significant negative impact of the projector radiation on dividing cells.

Considering the same, the current study was conducted to explore the oxidative stress and genotoxicity induced by electromagnetic radiation at two frequencies, namely 1800 MHz and 2400 MHz, using *Allium cepa* L. plant test system. Both 1800 MHz and 2400 MHz are commonly used frequencies in modern telecommunications including mobile phones and Wi-Fi networks. This makes them highly relevant for studying the potential biological effects of everyday exposure of electromagnetic field (EMF).

Materials and methods

Onion bulbs of uniform size, with an average weight approximately 50–60 g, were purchased and subsequently cleaned by thorough washing under running tap water, followed by rinsing with distilled water. The outer scales and older roots weakly attached to the bulbs were removed with pointed and sharp forceps, exposing the root primordia. Subsequently, multiple bulbs were placed in Couplin jars containing distilled water, maintained at $25 \pm 2^\circ\text{C}$ with relative humidity of 55–60%. A triplicate set of onion bulbs was used for each morphological and biochemical parameter and exposure period. Sets of four onion bulbs each were used for the genotoxicity assay, i.e., one set for the negative control and five sets for the treatments: 1 h/day, 2 h/day, 4 h/day, 6 h/day, and 8 h/day making six sets in total. A total of 78 onion bulbs were used during experimental setup for individual frequency treatment at variable exposure periods such as 1 h/day, 2 h/day, 4 h/day, 6 h/day, and 8 h/day which was comprised of studies on morphological effects (root length, fresh weight, and dry weight of roots), biochemical effects (protein and antioxidant activities), and genotoxic effects.

EMR treatment

Onion bulbs were exposed to EMR using experimental setup containing an antenna and radio frequency (RF) signal generator (Agilent N9310A; Keysight Technologies) to produce homogenous EMR at desired frequency and roots exposure at varied duration (1 h/day, 2 h/day, 4 h/day, 6 h/day, 8 h/day) for 7 days. Utilizing a probe connected to RF power meter, the output power at exposure plane in close proximity to the test material was determined. To avoid the absence of exposure to external elements during the entire experimental

setup, the exposure system was enclosed within a chamber completely enveloped by two layers of thick aluminum sheets. The control was kept in the same chamber but without switching on the device. All other conditions for control were kept similar. Upon completion of the experiment, roots were individually separated from bulbs for the examination of morphological parameters. Both roots and bulbs were extracted for determination of protein content and antioxidant enzymes. Assessment of genotoxicity was carried out following *Allium cepa* L. root chromosomal aberration assay.

Morphological parameter

Roots of *Allium cepa* from each treated set (1 h/day, 2 h/day, 4 h/day, 6 h/day, and 8 h/day) and control set were collected after an exposure period of 7 days to electromagnetic radiation (EMR). Ten roots per onion bulb were plucked from the site of origin, fresh weight was measured using a precision balance scale (Model: CY220; Make: Aczet, Mumbai), and the experiment was conducted in triplicate. The root length was presented as the mean of observed root length per exposure period. To obtain the dry weight, root samples were initially dried on filter paper to eliminate excess water and were subsequently placed in an oven for 5 h at 60 °C and the weight of roots was noted using a weighing balance.

Biochemical activities

Preparation of plant extract

To assess antioxidant enzyme activity and protein content, 500 mg of root tissue obtained from each of the three individual *Allium cepa* bulbs per treatment in triplicate was homogenized in potassium phosphate buffer (50 mM; pH 7.0) and then subjected to centrifugation at 8000 rpm for 20 min at 4 °C. Antioxidant enzymes and protein content were assessed using the supernatant obtained from centrifugation.

Protein content

Total protein content was determined using standard protocol given by Lowry et al. (1951). This method quantifies protein concentration by reacting proteins with copper ions and Folin-Ciocalteu reagent in an alkaline environment. This reaction forms a blue complex solution whose absorbance is measured at 660 nm. The intensity of color is directly proportional to the protein concentration in the sample. A standard graph curve was generated using standards of varying concentrations of bovine serum albumin (BSA) solution. This method is highly sensitive and widely applicable across various protein types and sample types, providing precise

and reproducible results essential for biochemical and biomedical research.

Antioxidant activity

Estimation of various antioxidative enzymes in roots and bulbs of *Allium cepa* test system was conducted using standard protocols. Different antioxidants included ascorbate peroxidase (APX), glutathione reductase (GR), glutathione-S-transferase (GST), superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT), and dehydroascorbate reductase (DHAR) for the present study.

Ascorbate peroxidase

The activity was determined according to the protocol provided by Nakano and Asada (1981). The specific activity was expressed as mol UA/mg fresh protein and absorbance was recorded at 290 nm. Reaction mixture was prepared by mixing 220 µl of 50 mM potassium buffer (pH 7), 30 µl of 0.5 mM ascorbate, 10 µl of 1.0 mM H₂O₂, 30 µl of 0.1 Mm EDTA, and 10 µl of extract. The enzyme activity was calculated using extinction coefficient of 2.8 mM⁻¹ cm⁻¹.

Glutathione reductase

The activity of glutathione reductase was assessed through the measurement of NADPH oxidation, recorded at an absorbance of 340 nm. The reaction mixture was prepared by mixing 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. The enzyme activity was calculated utilizing the extinction coefficient of 6.22 mM⁻¹ cm⁻¹ (Carlberg and Mannervik 1975).

Glutathione-S-transferase

Activity of glutathione-S-transferase was assessed using the protocol given by Habig and Jakoby (1981). This method relies on interaction between 1-chloro, 2-dinitrobenzene (CDNB), and reduced glutathione (GSH). The reaction mixture was composed by combining 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. Absorbance was recorded at 340 nm and the activity was computed utilizing extinction coefficient of 9.6×10^{-3} mM⁻¹ cm⁻¹.

Guaiacol peroxidase

Enzymatic activity of guaiacol peroxidase was assessed following the protocol given 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 H₂O₂, and 10 µl of sample extract. The absorbance was recorded at 436 nm and the activity was determined using extinction coefficient of 26.6 mM⁻¹ cm⁻¹.

Superoxide dismutase

Superoxide dismutase (SOD) was assessed for its capacity to impede the photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm. The reaction mixture was prepared by mixing 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. One unit of SOD was defined as the amount of enzyme required to reduce the photoreduction of NBT by 50% at 25 °C (Kono 1978).

Catalase

Catalase activity was assessed by determining the initial rate of H₂O₂ disappearance or the release of O₂, following the protocol of Aebi (1974). The reaction mixture was prepared by mixing 260 µl of 50 mM phosphate buffer (pH 7), 20 µl of 15 mM H₂O₂, and 20 µl of sample extract. The decrease in absorbance at 240 nm, indicative of H₂O₂ decomposition, was used to calculate catalase activity. The change in the extinction coefficient per unit time served as a quantitative measure of catalase activity. The enzyme activity was determined utilizing an extinction coefficient of 39.4 mM⁻¹ cm⁻¹ for H₂O₂.

Dehydroascorbate reductase

The protocol outlined by Dalton et al. (1986) was followed to measure the enzymatic activity of dehydroascorbate reductase (DHAR). DHAR catalyzes the reduction of dehydroascorbate, a process that entails the oxidation of reduced glutathione (GSH) to produce ascorbate and glutathione disulfide (GSSG) as the end products. The reaction mixture was prepared by mixing 200 µl of 50 mM phosphate buffer (pH 7), 30 µl of 1 mM EDTA, 30 µl of 2.5 mM reduced glutathione, 30 µl of 0.2 mM dehydroascorbate, and 10 µl of sample extract. The ultimate activity of DHAR was assessed by measuring the rise in absorbance at 265 nm.

Genotoxicity study

Assessment of genotoxic effects of EMR was carried out using *Allium cepa* root chromosomal assay. Bulbs were peeled off and placed on Couplin jars to initiate root germination at temperature (25 ± 2 °C) with their basal ends immersed in distilled water. Four onion bulbs were used

for each treatment. After the roots attained a size of 1–2 cm after 3 days of exposure to electromagnetic radiation (EMR) at 1800 MHz and 2400 MHz frequencies at varied durations, roots were subsequently excised and fixed in Farmer's fluid (3:1; ethanol:acetic acid glacial) for a duration of 24 h (Kumar and Srivastava 2011). Three root tips were taken from each onion bulb and 12 slides (4 bulbs × 3 roots/bulb) were prepared for each treatment group. Root tips were transferred to solution with 70% alcohol content and preserved until further use. Slides were prepared utilizing the squash method, where root tips were hydrolyzed in solution of 1 N HCl for 1 min followed by transferring them to a watch glass with 9:1 mixture of 2% aceto-orcein stain:1 N HCl for 30 min. After being stained, root tips were squashed in 45% glacial acetic acid after placing on a glass slide and covering it with a coverslip. The slides were then examined under a microscope (Model: MLX-D; Make: Magnus) at a magnification of 1000× to study various types of physiological and clastogenic abnormalities. At least 450–500 total cells with about 150 dividing cells per slide were examined for abnormalities at different stages of the cell cycle in *Allium cepa* root tips. The results of twelve slides were pooled together to estimate genotoxicity in terms of percent abnormal cells (inclusive of physiological and clastogenic aberrations) as given in Eq. (1)

$$\text{Percent aberrant cell (\%)} = \frac{\text{Total number of aberrant cells}}{\text{Total number of dividing cells}} \times 100 \quad (1)$$

Statistical analysis

The mean and corresponding standard error for each outcome in every treatment were calculated based on three replicates. Statistical analysis was conducted using IBM SPSS Statistics 21. Data underwent analysis using ANOVA and the post hoc Dunnett test, maintaining a significance level of $p \leq 0.05$. Additionally, the chi-square test was applied for genotoxicity to discern variations in chromosomal aberrations in *Allium cepa* that occur upon treatment with non-ionizing radiation when compared to the control. Pearson's correlation analysis was applied to check the relationship among different parameters like protein content, APX, GST, GR, DHAR, POD, CAT, and SOD.

Results

Effects on morphological parameters

Exposure of *Allium cepa* L. to electromagnetic radiation (EMR) at frequencies of 1800 MHz and 2400 MHz resulted in varied effects on all the parameters under investigation.

Detailed experimental setup including the number of onion bulbs used is shown in Table 1.

As shown in Table 2, the study indicated significant ($p \leq 0.05$) decrease of *Allium cepa* root length under exposure to EMR of 1800 MHz and 2400 MHz frequency exposures for 2 h/day, 4 h/day, 6 h/day, and 8 h/day as compared to the control. Under the exposure to frequency of 1800 MHz, fresh weight exhibited a significant decline in all exposure durations when compared to control ($p \leq 0.05$). Conversely, dry weight showed a significant decrease after 6 h/day and 8 h/day of exposure in comparison to the control. In the case of the 2400 MHz frequency, a significant decrease in fresh weight was observed after 6 h/day and 8 h/day of exposure while dry weight showed a significant

decline after 2 h/day, 4 h/day, 6 h/day, and 8 h/day of exposure per day compared to control.

Effect on protein content

As illustrated in Table 3, exposure of *Allium cepa* to 1800 MHz frequency resulted in a significant ($p \leq 0.05$) decrease in protein content of both roots and bulbs after 6 h/day and 8 h/day of exposure when compared to control. In the case of 2400 MHz frequency, protein content in both roots and bulbs showed a significant decrease after 2 h/day, 4 h/day, 6 h/day, and 8 h/day of exposure when compared to control over 7-day exposure period.

Table 1 Experimental setup for estimation of effects of EMR at 1800 MHz and 2400 MHz frequencies in *Allium cepa* L

Frequency	Exposure period	Total number of onion bulbs used				
		Morphological parameters		Biochemical parameters	Genotoxicity	Total sum
		Root length	Fresh and dry weight	Protein and antioxidants		
1800 MHz	Control	3	3	3	4	78
	1 h	3	3	3	4	
	2 h	3	3	3	4	
	4 h	3	3	3	4	
	6 h	3	3	3	4	
	8 h	3	3	3	4	
	Total	18	18	18	24	
2400 MHz	Control	3	3	3	4	78
	1 h	3	3	3	4	
	2 h	3	3	3	4	
	4 h	3	3	3	4	
	6 h	3	3	3	4	
	8 h	3	3	3	4	
	Total	18	18	18	24	

Table 2 Root length, fresh weight, and dry weight of *Allium cepa* L. roots under exposure to EMR of frequencies 1800 MHz and 2400 MHz for different exposure periods

Treatment (h/day)	1800 MHz frequency			2400 MHz frequency		
	Fresh weight (g) of roots (mean $\pm$ S.E)	Dry weight (g) of roots (mean $\pm$ S.E)	Root length (cm) (mean $\pm$ S.E)	Fresh weight (g) of roots (mean $\pm$ S.E)	Dry weight (g) of roots (mean $\pm$ S.E)	Root length (cm) (mean $\pm$ S.E)
Control	2.07 $\pm$ 0.05	0.08 $\pm$ 0.01	7.20 $\pm$ 0.36	1.60 $\pm$ 0.24	0.08 $\pm$ 0.01	6.33 $\pm$ 0.61
1 h/day	1.47 $\pm$ 0.08*	0.06 $\pm$ 0.01	7.07 $\pm$ 0.37	1.33 $\pm$ 0.02	0.06 $\pm$ 0.01	5.00 $\pm$ 0.60
2 h/day	1.37 $\pm$ 0.07*	0.04 $\pm$ 0.002	5.27 $\pm$ 0.23*	1.22 $\pm$ 0.04	0.04 $\pm$ 0.002*	4.23 $\pm$ 0.43*
4 h/day	1.20 $\pm$ 0.04*	0.04 $\pm$ 0.003	4.80 $\pm$ 0.36*	1.16 $\pm$ 0.03	0.04 $\pm$ 0.003*	4.00 $\pm$ 0.58*
6 h/day	1.18 $\pm$ 0.05*	0.04 $\pm$ 0.01*	4.47 $\pm$ 0.23*	0.85 $\pm$ 0.09*	0.04 $\pm$ 0.01*	3.87 $\pm$ 0.13*
8 h/day	0.92 $\pm$ 0.03*	0.01 $\pm$ 0.01*	2.53 $\pm$ 0.29*	0.64 $\pm$ 0.02*	0.01 $\pm$ 0.01*	2.80 $\pm$ 0.20*

Data presented as mean $\pm$ SE ($n=3$) for each treatment group. Significance was determined using post hoc Dunnett test, comparing control group to others. * $p \leq 0.05$ denotes significance

Table 3 Protein content of roots and bulbs of *Allium cepa* L. under exposure to EMRs of frequencies 1800 MHz and 2400 MHz for different exposure periods

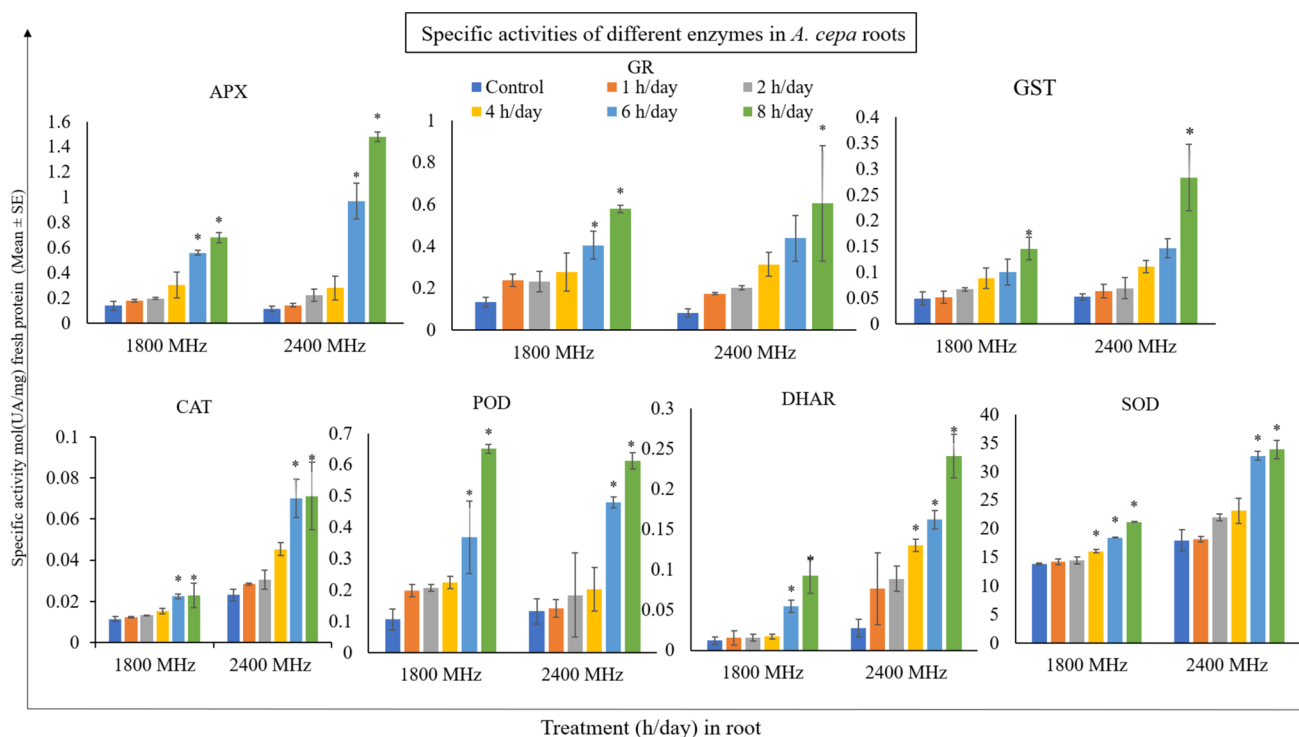
Treatment (h/day)	Protein content (mg/g) (mean $\pm$ S.E)			
	1800 MHz frequency		2400 MHz frequency	
	Root	Bulb	Root	Bulb
Control	2.06 $\pm$ 0.015	2.13 $\pm$ 0.012	1.04 $\pm$ 0.004	1.04 $\pm$ 0.001
1 h/day	2.05 $\pm$ 0.004	2.10 $\pm$ 0.009	0.99 $\pm$ 0.012	1.01 $\pm$ 0.011
2 h/day	2.05 $\pm$ 0.003	2.05 $\pm$ 0.056	0.85 $\pm$ 0.003*	0.99 $\pm$ 0.011*
4 h/day	2.05 $\pm$ 0.001	2.04 $\pm$ 0.001	0.78 $\pm$ 0.018*	0.85 $\pm$ 0.004*
6 h/day	1.91 $\pm$ 0.050*	1.99 $\pm$ 0.003*	0.61 $\pm$ 0.035*	0.78 $\pm$ 0.010*
8 h/day	1.84 $\pm$ 0.011*	1.92 $\pm$ 0.017*	0.57 $\pm$ 0.014*	0.69 $\pm$ 0.003*

Data presented as mean $\pm$ SE ($n=3$) for each treatment group, i.e., 500 mg of root tissue was obtained from each of the three individual *Allium cepa* per treatment group. Significance was determined using post hoc Dunnett test, comparing control group to others. * $p \leq 0.05$ denotes significance

Effect on antioxidative activity

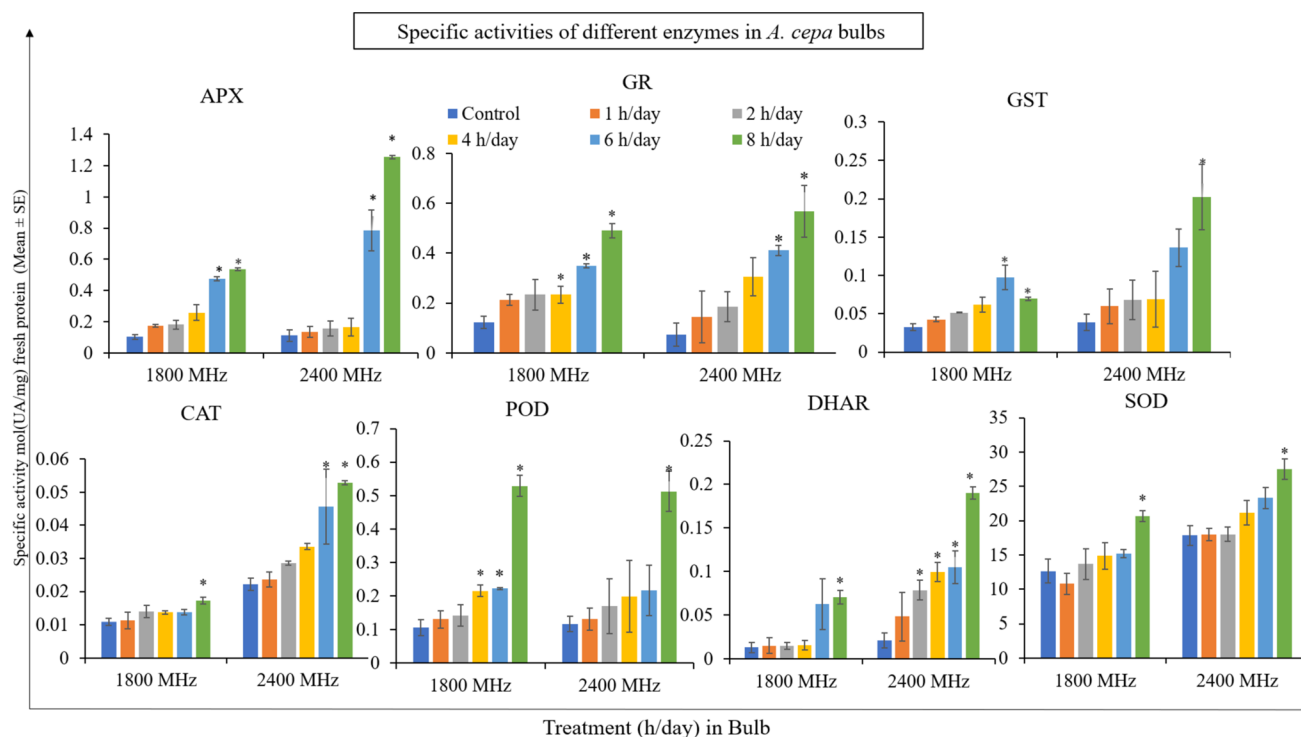
As shown in Figs. 1 and 2, specific activity (mol UA/mg fresh protein) of ascorbate peroxidase (APX) in roots and bulbs of *A. cepa* was found to be increasing upon exposure to EMR of both frequencies such as 1800 MHz and 2400 MHz for all treatment durations (1 h/day, 2 h/day, 4 h/day, 6 h/day, and 8 h/day) when compared to that of control.

The significant variations in root and bulb were seen at treatment durations of 6 h/day and 8 h/day for both frequencies, viz., 1800 MHz and 2400 MHz at $p \leq 0.05$. The specific activity (mol UA/mg fresh protein) of APX enzyme varied between 0.182 (1 h/day) and 0.683 (8 h/day) in roots while 0.103 (1 h/day) and 0.537 (8 h/day) in bulbs as minimum and maximum, respectively for 1800 MHz exposure whereas it varied between 0.145 (1 h/day) and 1.481 (8 h/day) in



APX: ascorbate peroxidase; GR: glutathione reductase; GST: glutathione-S-transferase; CAT: catalase; POD: guaiacol peroxidase; DHAR: dehydroascorbate reductase; SOD: superoxide dismutase

Fig. 1 Antioxidant enzyme activities in roots of *Allium cepa* L. under exposure to EMR of 1800 MHz and 2400 MHz frequencies of exposure for different time periods



APX: ascorbate peroxidase; GR: glutathione reductase; GST: glutathione-S-transferase; CAT: catalase; POD: guaiacol peroxidase; DHAR: dehydroascorbate reductase; SOD: superoxide dismutase

Fig. 2 Antioxidant enzyme activities in bulbs of *Allium cepa* L. under exposure to EMR of 1800 MHz and 2400 MHz frequencies of exposure for different time periods

roots while 0.136 (1 h/day) and 1.256 (8 h/day) in bulbs as minimum and maximum, respectively. The minimum and maximum specific activities (mol UA/mg fresh protein) of APX enzyme for control were obtained as 0.103 and 0.142, respectively, in roots while as 0.112 and 0.117, respectively, in bulbs of *A. cepa*.

Specific activity (mol UA/mg fresh protein) of glutathione reductase (GR) in roots and bulbs of *A. cepa* was found to be increasing upon exposure to EMR of both frequencies such as 1800 MHz and 2400 MHz for all treatment duration when compared to that of control. The significant variations in roots were seen at treatment durations of 6 h/day and 8 h/day and only 8 h/day for both frequencies, viz., 1800 MHz and 2400 MHz respectively at $p \leq 0.05$, while in bulbs, significant variations existed at 4 h/day, 6 h/day, and 8 h/day for 1800 MHz and 6 h/day and 8 h/day for 2400 MHz. The activity of GR enzymes (mol UA/mg fresh protein) in roots varied as 0.238 (1 h/day) to 0.580 (8 h/day) and 0.214 (1 h/day) to 0.490 (8 h/day) in bulbs for 1800 MHz whereas 0.174 (1 h/day) to 0.606 (8 h/day) in roots and 0.144 (1 h/day) to 0.569 (8 h/day) in bulb for 2400 MHz. The minimum and maximum specific activities (mol UA/mg fresh protein) of GR enzyme for control were obtained as 0.082 and 0.133,

respectively, in roots while as 0.073 and 0.121, respectively, in bulbs of *A. cepa*.

Specific activity (mol UA/mg fresh protein) of glutathione-S-transferase (GST) in roots and bulbs of *A. cepa* was found to be increasing upon exposure to EMR of both frequencies such as 1800 MHz and 2400 MHz for all treatment durations when compared to that of control. The significant variations in roots were seen at treatment duration of 8 h/day for both frequencies, viz., 1800 MHz and 2400 MHz at $p \leq 0.05$, while in bulbs, significant variations existed at 6 h/day and 8 h/day for 1800 MHz and 8 h/day for 2400 MHz. The activity of GST enzymes (mol UA/mg fresh protein) was varied as 0.052 (1 h/day)–0.146 (8 h/day) and 0.043 (1 h/day)–0.070 (8 h/day) for 1800 MHz whereas 0.064 (1 h/day)–0.283 (8 h/day) and 0.039 (1 h/day)–0.202 (8 h/day) for 2400 MHz in roots and bulbs, respectively. The minimum and maximum specific activities (mol UA/mg fresh protein) of GST enzyme for control were obtained as 0.049 and 0.052, respectively, in roots while as 0.033 and 0.039, respectively in bulbs of *A. cepa*.

Specific activity (mol UA/mg fresh protein) of guaiacol peroxidase (POD) in roots and bulbs of *A. cepa* was found to be increasing upon exposure to EMR of both frequencies

such as 1800 MHz and 2400 MHz for all treatment duration when compared to that of control. The significant variations in roots were seen at treatment durations of 6 h/day and 8 h/day for both frequencies, viz., 1800 MHz and 2400 MHz at $p \leq 0.05$, while in bulbs, significant variations existed at 4 h/day, 6 h/day, and 8 h/day for 1800 MHz and 8 h/day for 2400 MHz. The activity of POD enzymes (mol UA/mg fresh protein) in roots was in the range of 0.198 (1 h/day)–0.651 (8 h/day) and 0.130 (1 h/day)–0.529 (1 h/day) in bulbs for 1800 MHz whereas 0.141 (1 h/day)–0.612 (8 h/day) in roots and 0.131 (1 h/day)–0.512 (8 h/day) in bulb for 2400 MHz. The minimum and maximum specific activities (mol UA/mg fresh protein) of POD enzyme for control were obtained as 0.106 and 0.132, respectively, in roots while as 0.106 and 0.118, respectively, in bulbs of *A. cepa*.

Specific activity (mol UA/mg fresh protein) of superoxide dismutase (SOD) in roots and bulbs of *A. cepa* was found to be increasing upon exposure to EMR of both frequencies such as 1800 MHz and 2400 MHz for all treatment duration when compared to that of control. The significant variations in roots were seen at treatment durations of 4 h/day, 6 h/day, and 8 h/day of exposure for 1800 MHz frequency and 6 h/day and 8 h/day for 2400 MHz frequency at $p \leq 0.05$, while in bulbs, significant variations existed at 8 h/day for 1800 MHz and 2400 MHz frequencies of exposure. The activity of SOD enzymes (mol UA/mg fresh protein) in roots was enhanced as 14.165 (1 h/day)–21.167 (8 h/day) and 10.80 (1 h/day)–20.663 (8 h/day) in bulbs for 1800 MHz whereas 18.174 (1 h/day)–33.892 (8 h/day) in roots and 18.01 (1 h/day)–27.541 (8 h/day) in bulb for 2400 MHz. The minimum and maximum specific activities (mol UA/mg fresh protein) of SOD enzyme for control were obtained as 13.846 and 17.931, respectively, in roots while as 12.66 and 17.83, respectively, in bulbs of *A. cepa*.

Specific activity (mol UA/mg fresh protein) of catalase (CAT) in roots and bulbs of *A. cepa* was found to be increasing upon exposure to EMR of both frequencies such as 1800 MHz and 2400 MHz for all treatment durations (1 h/day, 2 h/day, 4 h/day, 6 h/day, and 8 h/day) when compared to that of control. The significant variations in roots were seen at treatment durations of 6 h/day and 8 h/day of exposure for both frequencies, viz., 1800 MHz and 2400 MHz at $p \leq 0.05$, while in bulbs, significant variations existed at 8 h/day for 1800 MHz and 6 h/day and 8 h/day for 2400 MHz. The specific activity (mol UA/mg fresh protein) of CAT enzymes was observed to be increasing from 0.012 (1 h/day) to 0.023 (8 h/day) in roots and 0.011 (1 h/day) to 0.017 (8 h/day) in bulbs for 1800 MHz, whereas it ranged from 0.028 (1 h/day) to 0.071 (8 h/day) in roots and 0.024 (1 h/day) to 0.053 (8 h/day) in bulbs for 2400 MHz. The minimum and maximum specific activities (mol UA/mg fresh protein) of CAT enzyme for control were obtained as 0.011 and 0.023, respectively, in roots while as 0.011 and 0.022, respectively, in bulbs of *A. cepa*.

Specific activity (mol UA/mg fresh protein) of dehydroascorbate (DHAR) in roots and bulbs of *A. cepa* was found to be increasing upon exposure to EMR of both frequencies such as 1800 MHz and 2400 MHz for all treatment durations when compared to that of control. The significant variations in roots were seen at treatment durations of 6 h/day and 8 h/day of exposure to 1800 MHz frequency and 4 h/day, 6 h/day, and 8 h/day of exposure to 2400 MHz at $p \leq 0.05$, while in bulbs, significant variations existed at 8 h/day for 1800 MHz and 2 h/day, 4 h/day, 6 h/day, and 8 h/day for 2400 MHz. The activity (mol UA/mg fresh protein) of DHAR enzymes was increased, ranging from 0.015 (1 h/day) to 0.044 (8 h/day) in roots and 0.015 (1 h/day) to 0.041 (8 h/day) in bulbs for 1800 MHz, whereas it ranged from 0.076 (1 h/day) to 0.240 (8 h/day) in roots and 0.021 (1 h/day) to 0.190 (8 h/day) in bulbs for 2400 MHz. The minimum and maximum specific activities (mol UA/mg fresh protein) of DHAR enzyme for control were obtained as 0.012 and 0.028, respectively, in roots while as 0.013 and 0.021, respectively, in bulbs of *A. cepa*.

Genotoxic effects of EMR

As shown in Table 4, exposure to 1800 MHz EMR at different treatment durations like 0 h/day, 1 h/day, 2 h/day, 4 h/day, 6 h/day, and 8 h/day induced percentage chromosomal abnormalities as 0.35%, 1.07%, 2.03%, 4.25%, 9.56%, and 17.63% respectively, while in the case of 2400 MHz frequency of radiation, chromosomal abnormalities were seen to be 0.47%, 1.45%, 2.43%, 4.99%, 11.06%, and 18.97% respectively. The number of dividing cells exhibited variations under different exposure conditions. With an increase in duration of radiation exposure, the number of chromosomal abnormalities also showed enhancement. Following exposure to electromagnetic radiation (EMR), a range of chromosomal aberrations was observed encompassing physiological anomalies such as stickiness (St), abnormal metaphase (Am), delayed anaphase (Da), abnormal anaphase (Aa), laggard (Lg), C-mitosis (Cm), and vagrant (Vg). Additionally, clastogenic aberrations, including ring chromosome (Cr), chromatin bridge (Cg), and chromosomal breaks (Cb), were also observed (Fig. 3).

Pearson's correlation analysis

As shown in Fig. 4, Pearson's correlation analysis was carried out to investigate the correlation among different parameters of protein content, APX, GR, GST, POD, SOD, CAT, and DHAR enzyme activities after 7-day exposure period in both roots and bulbs. In roots, at 1800 MHz, protein content showed a significant negative correlation with r value as -0.971 , -0.955 , -0.902 , -0.957 , -0.968 , and -0.934 at $p \leq 0.05$ for the different antioxidative enzymes like APX,

Table 4 Genotoxic effects of EMRs at 1800 MHz and 2400 MHz frequencies of radiation in *Allium cepa* test for different time periods of exposure

Frequency	Treatment	Genotoxic effects of EMRs in <i>Allium cepa</i>																			
		TC	TDC	Physiological aberrations (PA)								Clastogenic aberrations (CA)					Total aberrations (TA)				
				Cm	St	Vg	Lg	Da	Aa	Am	TPA (Sum)	PA (%)	Cg	Cr	Cb	TCA (Sum)	CA (%)	TAC (Sum)	TAC (%)		
1800 MHz	Control	5900	1692	1	0	1	1	1	1	1	1	1	6	0.35	0	0	0	0	0	6	0.35
	1 h/day	5960	1982	3	0	1	1	4	6	3	3	18	1.07	0	0	0	0	0	18	1.07*	
	2 h/day	5948	1678	6	5	2	2	4	5	8	8	32	1.91	1	0	1	2	0.12	34	2.03*	
	4 h/day	5980	1672	8	9	8	8	7	10	13	63	3.77	3	3	2	8	0.48	71	4.25*		
	6 h/day	5930	1662	21	17	19	11	15	18	10	111	6.67	15	12	21	48	0.48	159	9.56*		
	8 h/day	5900	1656	31	33	30	27	35	27	21	204	12.31	29	29	30	88	5.31	292	17.63*		
2400 MHz	Control	5950	1682	1	1	1	1	1	2	1	8	0.47	0	0	0	0	0	8	0.47		
	1 h/day	5960	1652	4	1	2	2	4	6	3	22	1.33	0	1	1	2	0.12	24	1.45*		
	2 h/day	5938	1648	4	6	4	3	4	8	8	37	2.24	1	1	1	3	0.18	40	2.43*		
	4 h/day	5930	1641	11	10	10	10	10	12	11	74	4.51	3	3	2	8	0.48	82	4.99*		
	6 h/day	5930	1636	24	12	22	12	17	16	23	126	7.70	15	18	22	55	0.61	181	11.06*		
	8 h/day	5980	1634	38	28	36	38	26	29	25	220	13.46	29	31	30	90	5.50	310	18.97*		

The table represents absolute figures based on data obtained from $n = 12$, where n is the number of slides screened

* Significant at $p \leq 0.05$ using chi-square test

TC, total cells; TDC, total dividing cells; PA, physiological aberrations; Cm, C-mitosis; St, stickiness; Vg, vagrant; Lg, laggard; Da, delayed anaphase; Aa, abnormal anaphase; Am, abnormal metaphase; TPA, total physiological aberrations; CA, clastogenic aberrations; Cg, chromatid bridge; Rc, ring chromosome; Cb, chromosome break; TCA, total clastogenic aberrations; TAC, total chromosomal aberrations

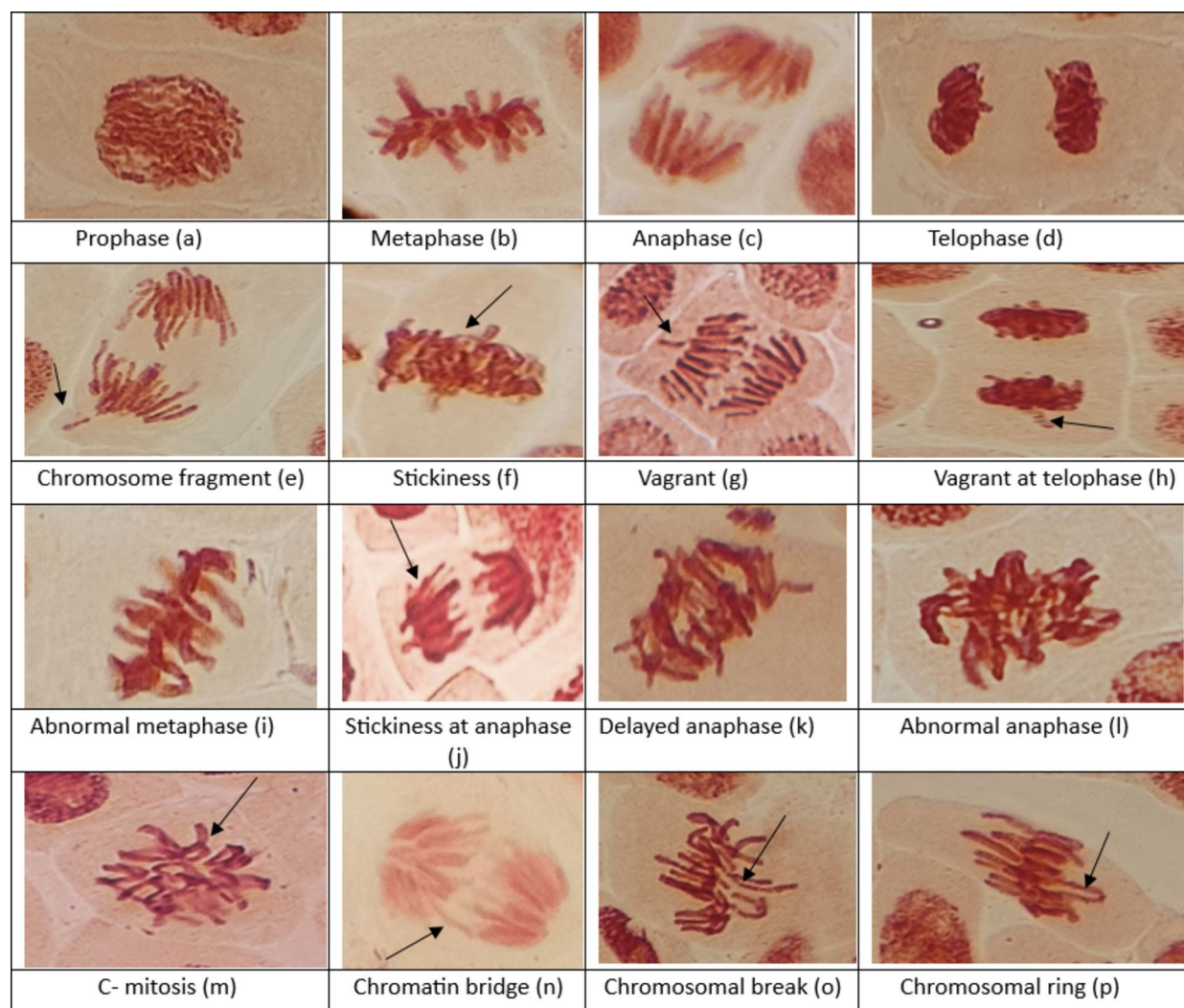


Fig. 3 Different normal stages of mitosis (a–d), physiological chromosomal aberrations (e–m), and clastogenic chromosomal aberrations (n–p) in *Allium cepa* L. root tip cells under exposure to EMR

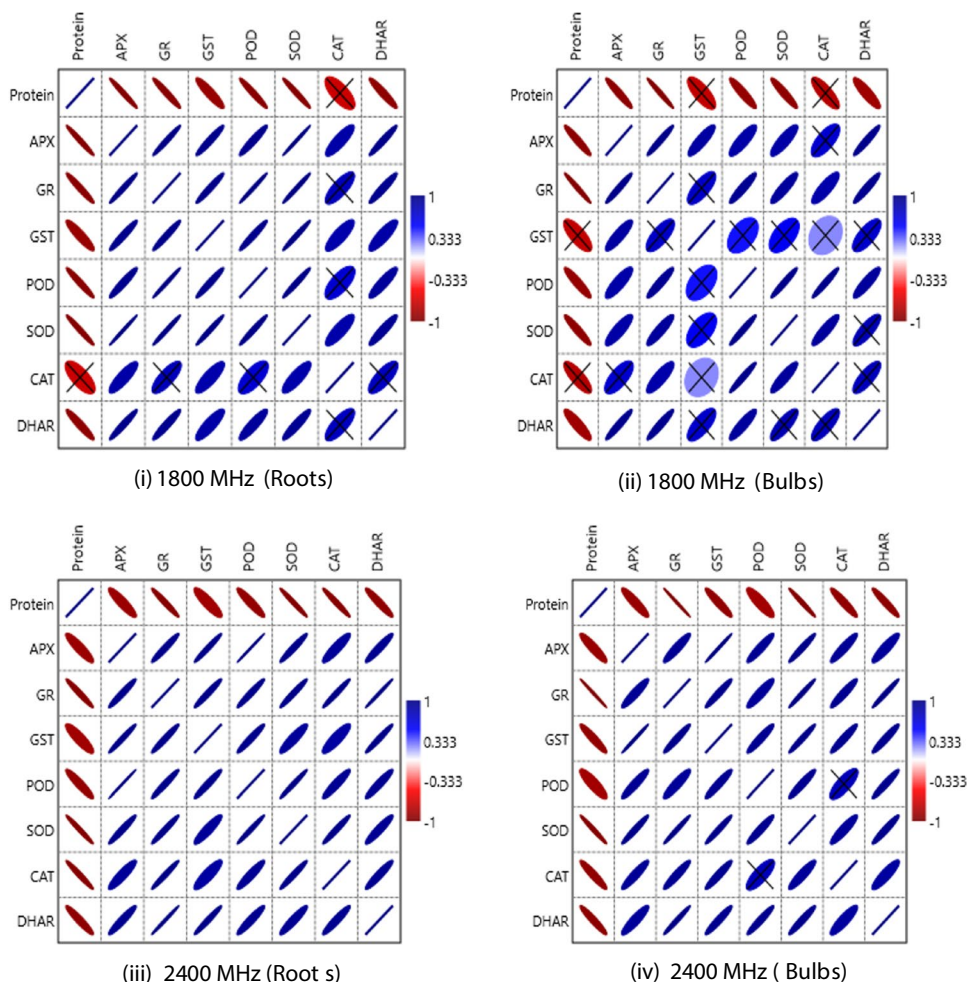
GR, GST, POD, SOD, and DHAR, respectively, while it showed no significant correlation with CAT. Protein content in bulbs was significantly negatively correlated with r values as -0.938 , -0.974 , -0.913 , -0.929 , and -0.872 at $p \leq 0.05$ for APX, GR, POD, SOD, and DHAR, respectively, and it showed no significant correlation with GST and CAT. In the case of 2400 MHz frequency, protein content in roots and bulbs showed a significant negative correlation with different antioxidative enzymes APX, GR, GST, POD, SOD, CAT, and DHAR activity in roots ($r = -0.889$, -0.962 , -0.865 , -0.914 , -0.975 , -0.965 , and -0.932 at $p \leq 0.05$ respectively) and in bulbs ($r = -0.899$, -0.992 , -0.925 , -0.857 , -0.977 , -0.925 , and -0.939 at $p \leq 0.05$ respectively). On the other hand, all the antioxidative enzymes showed a positive correlation with each other in both 1800 MHz and 2400 MHz frequencies of radiation.

Discussion

The pivotal role of plants in ecosystems necessitates a thorough examination of their responses to the increasing levels of environmental electromagnetic radiation (Halgamuge 2017). The present study demonstrated exposure period-dependent responses for morphological, biochemical, and genotoxic effects with root of onions.

As shown in Table 2, the morphological parameter of root length exhibited a decrease with extended exposure periods for both 1800 MHz and 2400 MHz frequencies of radiation. A study by Akbal et al. (2012) observed that exposure of 2 h/day, 4 h/day, 6 h/day, and 8 h/day showed that electromagnetic waves emitted from mobile phones operating at 1800 MHz resulted in a decrease in root growth of *Lens culinaris* Medik. In the case of 1800 MHz and 2400 MHz frequencies of radiation, significant ($p \leq 0.05$) reduction in root

Fig. 4 Representation of Pearson's correlation comparisons between different enzymes (APX, GR, GST, POD, SOD, CAT, DHAR) and protein content of *Allium cepa* L. roots and bulbs, respectively, after exposure to EMR of 1800 MHz and 2400 MHz (i–iv). Narrow, elongated ellipses indicate stronger correlations, with blue ovals representing positive correlations and red ovals representing negative correlations. The wider ellipses suggest weaker correlations. Ovals without crosses are significant at $p \leq 0.05$ level. The values on the right-hand axis represent the Pearson correlation coefficients, which are dimensionless. They range from -1 to 1 , where 1 indicates a perfect positive correlation, -1 indicates a perfect negative correlation, 0.333 represents a moderate positive, and -0.333 moderate negative correlation



length was observed in 2 h/day, 4 h/day, 6 h/day, and 8 h/day when compared to control. In the present study, a decrease in root length when exposed to radiation was observed, which is in agreement with previous research findings related to cellphone radiation (Singh et al. 2012; Kumar et al. 2016).

As presented in Table 2, fresh weight showed significant ($p \leq 0.05$) decrease at all time periods of exposure for 1800 MHz frequency of radiations whereas for dry weight significant decrease was observed at 6 h/day and 8 h/day when compared to control. In 2400 MHz frequency of exposure, significant ($p \leq 0.05$) decrease was observed at 6 h/day and 8 h/day for fresh weight and 2 h/day, 4 h/day, 6 h/day, and 8 h/day for dry weight when compared to the control. Our observations corroborated the results obtained by Chen and Chen. (2014) where 24-h intermittent exposure period to 1805–1850 MHz significantly affected fresh weight of mung beans.

As presented in Table 3, protein content showed significant variation in roots and bulbs at 6 h/day and 8 h/day of exposure to 1800 MHz and 2 h/day, 4 h/day, 6 h/day, and 8 h/day of exposure to 2400 MHz. The variation in protein content in *Allium cepa* roots and bulbs after radiation exposure

can be attributed to oxidative stress, as EMR exposure generates reactive oxygen species (ROS) that cause oxidative protein damage. Additionally, EMR interfered with ribosomal function and alters gene expression related to protein synthesis and stress response, disrupting protein synthesis. This exposure also activated proteolytic pathways, increasing the degradation of damaged proteins (Sharma et al. 2010).

Overproduction of reactive oxygen species causes stress in cells but cells have built-in mechanisms to combat it. One such mechanism is the upregulation or increased production of antioxidant enzymes, which scavenge free radicals (Gill and Tuteja 2010; Sepehrmanesh et al. 2016).

As shown in Figs. 1 and 2, the present study indicated that activity of different antioxidants increased significantly when compared to control following exposure to both 1800 MHz and 2400 MHz frequencies of radiation. The present study has shown the increased activity of antioxidant enzymes and decreased content of protein in root and bulb under exposure to EMR compared to control group at both frequencies of EMR. The findings indicated that enzymes played a protective role in mitigating cellular damage caused by exposure

to non-ionizing radiation. Upon being subjected to exposure, the quantities of antioxidant enzymes like APX, GR, GST, POD, SOD, CAT, and DHAR in roots and bulbs of *Allium cepa* exhibited an increase over time when compared to that of the control. APX showed a significant increase in the activity at 6 h/day and 8 h/day periods of exposure for both roots and bulbs in 1800 MHz and 2400 MHz frequencies of radiation. APX catalyzes the conversion of hydrogen peroxide (H_2O_2) into water using ascorbate as a substrate. This reaction is crucial for maintaining cellular redox balance and preventing oxidative damage. Under electromagnetic exposure, the increased generation of ROS can overwhelm the capacity of APX, leading to oxidative damage and decreased protein content (Schuermann and Mevisen 2021).

Similarly, glutathione-S-transferase showed increased activity of antioxidative enzyme at 8 h/day for root and 6 h/day and 8 h/day for bulb, and in the case of 2400 MHz frequency of radiation, roots and bulb showed enhancement in activity at 8 h/day period of exposure of radiations. Glutathione-S-transferase (GST) detoxified reactive electrophiles by conjugating them with GSH, aiding in the detoxification of ROS. Under prolonged radiation exposure, GST activity might increase initially to cope with oxidative stress, but sustained exposure could lead to enzyme inhibition and decreased detoxification efficiency (Li et al. 2023). Several prior investigations have demonstrated the capacity of cells to safeguard against intracellular damage induced by free radicals via their intracellular antioxidant system. The assessment of peroxidase enzyme activity served as a notable test to evaluate the antioxidant potential of plants, as highlighted by Hassanpour et al. (2021). Activity of POD and GR in roots increased at 6 h/day and 8 h/day and bulbs at 4 h/day, 6 h/day, and 8 h/day for 1800 MHz frequency of exposure of radiations and in 2400 MHz frequency showed enhancement in activity of GR at 8 h/day and POD at 6 h/day and 8 h/day in roots. For bulbs, GR showed increase at 6 h/day and 8 h/day while POD at 8 h/day period of exposure. Our study is in line with the study of Upadhyaya et al. (2022) that showed the short-term elevation in enzymatic antioxidants POD activity occurred within the initial 24–48 h of exposure. This was subsequently succeeded by a consistent decrease over time, indicating a deterioration in the defense system and overall plant health. POD is involved in the removal of H_2O_2 and played a role in lignin biosynthesis and stress responses. EMR exposure induced POD activity as part of the oxidative stress response, but prolonged exposure could result in enzyme inactivation and reduced effectiveness in ROS detoxification (Xiang et al. 2024). Sharma et al. (2023a) reported similar results in *Trigonella foenum-graecum* L. which showed increase in antioxidant enzyme activity where exposure for 1800 MHz frequency radiation demonstrated an elevation in the activity of the antioxidant GR after 4/day and 8 h/day of daily exposure over 7 days.

GR is involved in the regeneration of reduced glutathione (GSH) from its oxidized form (GSSG). GSH is a vital antioxidant that protects cells from oxidative damage. EMF exposure can deplete GSH levels due to increased oxidative stress, reducing the overall effectiveness of the antioxidant defense system and leading to protein degradation (Nuhu et al. 2020). SOD functions as the primary line of defense for plants against heightened levels of ROS, working to alleviate the excessive generation of superoxide. The increased activities of SOD were linked to the heightened generation of reactive oxygen species (ROS) following exposure to electromagnetic radiation (Tkalec et al. 2007; Sepehrimanesh et al. 2016). In our current study, activity of SOD enzyme for 1800 MHz frequency of exposure showed enhancement in roots at 4 h/day, 6 h/day, and 8 h/day and bulb at 8 h/day period of exposure. In 2400 MHz, activity enhanced at 6 h/day and 8 h/day for roots and 8 h/day for bulb. SOD catalyzes the dismutation of superoxide radicals (O_2^-) into oxygen and H_2O_2 , which is further detoxified by other antioxidants (Batool et al. 2022). Similarly, activity of enzymes CAT and DHAR increased, consistent with the finding of Sharma et al. (2023b) which showed that extended exposure in *Trigonella foenum-graecum* L. resulted in increased antioxidant activity. CAT decomposes H_2O_2 into water and oxygen, providing a crucial defense against oxidative stress (Anwar et al. 2024).

DHAR regenerated ascorbate from its oxidized form (dehydroascorbate), maintaining ascorbate levels for antioxidant defense. Under EMR stress, the increased demand for ascorbate could deplete DHAR activity, impairing the regeneration of ascorbate and weakening the antioxidant defense (Ding et al. 2020).

As shown in Table 4 in the current study, the exposure to 1800 MHz and 2400 MHz induced chromosomal aberration and results in increase of percent aberrant cells. Increase in chromosomal aberrations resulting from exposure to non-ionizing electromagnetic fields was consistent with findings from previous studies (Tkalec et al. 2009; Pesnya and Romanovsky 2013). A study by Kumar et al. (2020) showed that 900 MHz and 1800 MHz electromagnetic field radiations induced genotoxicity in root meristems of *Allium cepa*. The researchers noted that the impact of EMR was contingent upon modulation of their field strength, frequency, and intensity (Tkalec et al. 2009; Kumar et al. 2020). Tkalec et al.'s (2009) research demonstrated that non-thermal exposure to radiofrequency fields under investigation may cause mitotic abnormalities in *Allium cepa* root meristematic cells. The applied field frequencies, in addition to field strength and modulation, had a major impact on reported effects.

As Fig. 3 illustrates, exposure to 1800 MHz and 2400 MHz frequencies induced different types of chromosomal abnormalities such as physiological aberrations

(stickiness, C-mitosis, delayed anaphase, laggard, vagrant, abnormal metaphase, and abnormal anaphase) ascribed to spindle abnormalities and clastogenic aberrations (chromatin bridge, ring chromosome, and chromosomal break) were observed. EMR has also been proven to elevate the occurrence of micronuclei in *Vicia faba*. Employing a transverse electromagnetic wave mode (TEM) cell to subject *Vicia faba* to electromagnetic radiation (EMR) at a frequency of 915 MHz for a duration of 72 h has revealed discernible genotoxic effects (Gustavino et al. 2016).

Conclusion

The exposure to non-ionizing electromagnetic radiation (EMR) at 1800 MHz and 2400 MHz frequencies induced morphological changes like decrease in root length, fresh weight, and dry weight of the roots when compared to control. The induction of oxidative stress in terms of increased activities of enzymes such as ascorbate peroxidase, glutathione reductase, glutathione-S-transferase, superoxide dismutase, guaiacol peroxidase, catalase, and dehydroascorbate reductase as well as genotoxic effects like chromosomal aberrations in root tip cells of *Allium cepa* indicated the deleterious effects of EMR at both frequencies. The harmful effects of exposure to non-ionizing radiations were also evident from decrease in the protein content. The present study is in agreement with the latest studies and provides the evidence for the hypothesis that EMR causes deteriorating effects in living systems. Thus, by generating information about factors influencing plant responses to EMR, efficacious strategies can be designed to alleviate its detrimental impacts.

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Data availability Data will be made available on reasonable request.

Declarations

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