



Comprehensive analysis of genotoxic effects and antioxidative defence mechanisms in plant test system exposed to 1800 MHz electromagnetic radiations: a root chromosomal aberration and FTIR spectroscopy approach

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Abstract

Objective The proliferation of wireless communication devices has increased the exposure of living organisms to electromagnetic field radiations (EMF-rs), posing potential risks to various biological systems. The present study was planned to explore the genotoxic effects and oxidative stress responses of 1800 MHz electromagnetic radiations in *Trigonella foenum-graecum* L. plant test system. The study also pertained to assess the changes in functional groups using Fourier transform infrared spectroscopy (FTIR).

Methods Twenty seeds of *Trigonella foenum-graecum* L. were placed in Petri plates lined with autoclaved *Whatman No. 1* filter paper. The seeds were evenly distributed and maintained at temperature 25 ± 2 °C and relative humidity 55–60%. The seeds were placed in Petri plates along with the exposure apparatus (antenna) and then enclosed within a chamber consisting of two layers of aluminium sheets. The treatment was administered every day for seven days on various parameters.

Results The investigation showed that increasing the duration of EMR exposure significantly decreased protein content and increased MDA content in seedlings. However, exposure to EMRs for 4 and 8 h per day led to increased activities of different antioxidant enzymes, including guaiacol peroxidase (POD), glutathione-S-transferase (GST), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD). The study also calculated the specific absorption rate using the biological heat transfer equation, which revealed harmful effects of the radiations on the test system by interfering with biochemical processes, leading to genotoxic and oxidative stress.

Conclusion The findings suggest that electromagnetic radiations induced oxidative stress in *T. foenum-graecum* L. and increased activity of antioxidant enzymes as a protective mechanism against cellular damage. The study highlights the potential risks associated with EMF radiations on plant systems and underscores the importance of further research in this field.

Keywords Antioxidant enzymes · Fourier transform infrared spectroscopy · Specific absorption rate · Malondialdehyde

Introduction

Electromagnetic radiations (EMRs) refers to the energy that is propagated through space in the form of oscillating electric and magnetic fields, which are generated by the acceleration of charged particles [1]. In the modern era, a variety of

electrical gadgets produce a wide range of EMRs in the environment. EMRs can be categorized into two groups based on their frequency and wavelength. Ionizing radiation has a shorter wavelength, ranging from 10 to 10^{-4} nm and a higher frequency, from 10^{-16} to 10^{-24} Hz. In contrast, non-ionizing radiation has a longer wavelength, from 1 mm to 100 km and a lower frequency, from 3 kHz to 300 GHz [2, 3]. Various communication devices, including high-voltage lines, mobile phone base stations, radar, radio waves and television transmitters, emit electromagnetic radiations in both residential and occupational settings [4]. The progress of technology in the present world undoubtedly increased the significance of electronic gadgets in our daily lives. However,

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they also leads to a number of health issues [4, 5]. EMRs of different frequencies are used all across the globe in various electronic systems such as global system for mobile communications (GSM) (850 MHz, 900 MHz, 1850 MHz and 1900 MHz), modern cell phone models (1800 MHz to 2200 MHz), laptops (1000 MHz to 3600 MHz) and wireless networks (2.45 GHz) that can pose the adverse health effects [2, 6].

Long-term exposure of EMRs has shown to increase levels of toxic oxygen free radicals in both plants and animals during some studies [7–10]. These free radicals can either obtain electrons from available donors or donate an electron to a suitable acceptor, leading to modifications that generate secondary free radicals [11–13]. This ripple effect has the potential to harm living things. The plasma membrane enzyme NADPH oxidase controls the initial stage of reactive oxygen species (ROS) generation in the occurrence of EMRs. As a result, ROS activate matrix metalloproteases, triggering intracellular signalling cascades that alert the nucleus to the exterior stimulus creating alterations in cell lipids and protein expression [4]. EMRs were also found to disrupt gene expression and enzyme activities following similar phenomenon [14].

Plants, due to their sessile nature, exhibit a wide range of developmental and physiological responses to varied abiotic and biotic stresses. Nevertheless, plants play a vital role in an ecosystem, and hence, it becomes mandatory to evaluate the consequences of non-ionizing EMRs on plants by observing their response to increasing exposure of radiations in their surrounding environment. Although the effects of EMRs on the plant's germination rate, weight and biochemical processes depend upon type of plant species, frequency as well as strength and direction of the field, all plants show sensitivity to this type of abiotic stress [15–18]. In [17], a study was conducted to explore the effects of weak microwave radiation from GSM 900 MHz mobile phone on the growth of soybean seedlings and documented that the exposure of radiations resulted in the reduced growth of soybean seedlings. In another study, the authors reported that radiations led to the significant decline in weight of root and shoot of *Triticum aestivum* [19]. Many studies have reported that EMRs caused change in cell proliferation and induced various types of chromosomal aberrations. Gustavino et al. have documented that exposure to cell phone radiations in the frequency range of 915 MHz resulted in a dose-dependent increase in micronuclei generation in secondary root tips and impaired spindle formation in broad bean [20]. Upadhyaya et al. investigated the effects of 1800 MHz frequency on tomato plant and have shown that EMRs significantly reduced the height, weight, germination and chlorophyll content [21]. However, studies documenting the effects of EMRs on the plant system have majorly focused on the shorter exposure periods with very few studies exploring

the deleterious effects of these non-ionizing radiations on plant seedlings [22].

Apart from plants, studies have also been conducted using animal test system to explore the effects of electromagnetic radiations. Male rabbits exposed to 800 MHz for 8 h per day for 12 weeks have shown a reduction in sperm count [23]. Some epidemiological studies have also shown that radiations from cell phones had a deleterious impacts on the male fertility and spermatozoa [24]. Human spermatozoa exposed to 900 MHz showed morphometry loss in sperms [25]. A study which investigated the connection between mobile phones and thyroid cancer found that prolonged exposure increased the chance of thyroid microcarcinoma (tumour size ≤ 10 mm) [26].

This study aimed to investigate the response of *T. foenum-graecum* L. seedlings to 1800 MHz EMRs in terms of morphological parameters, biochemical activities of different antioxidative enzymes, content of protein and malondialdehyde (MDA). Furthermore, the study pertained to evaluate the potential genotoxic effects of EMRs by assessing chromosomal abnormalities in root tip cells of *T. foenum-graecum* L. FTIR was utilized to investigate changes in the functional groups of active biomolecules when exposed to 1800 MHz EMRs, in comparison with the control condition.

Material and methods

Plant material

For this study, *T. foenum-graecum* L. seeds from the Fabaceae family were obtained from SKAUST, Jammu, and utilized as the experimental material. The selection of *Trigonella foenum-graecum* L. as a model species is attributed to its widespread cultivation in tropical countries such as India. This plant holds great significance in the local community, as it is commonly consumed due to its numerous beneficial medicinal properties.

Sterilization of seeds

For this study, uniform-sized and healthy seeds of *T. foenum-graecum* L. were selected. Prior to the experiment, the seeds were presoaked in distilled water for six hour. Following this, they were subjected to surface sterilization using a 4% NaClO solution for 10 min.

Experimental design

Twenty seeds of *T. foenum-graecum* L. were first soaked, sterilized and then placed in Petri plates lined with autoclaved *Whatman No. 1* filter paper, and the filter paper was saturated with the help of distilled water. The seeds were

evenly distributed and maintained at temperature 25 ± 2 °C and relative humidity 55–60%. The seeds were placed in Petri plates along with the exposure apparatus (antenna) and then enclosed within a chamber consisting of two layers of aluminium sheets. The treatment was administered every day for seven days. Further, shoots and roots were analysed to study the effects of EMRs on percentage germination, morphological parameters, protein content, MDA and anti-oxidative enzymes activities. The genotoxic effects of EMRs were studied using root tip cells of *T. foenum-graecum* L. [see Supplementary material Fig. 1].

Specific absorption rate (SAR)

Radiofrequency signal generator (make: Key sight Technologies; model: N9310A) was used during the present study to produce electromagnetic radiations of 1800 MHz frequency with power density 10.0 dBm. The value of SAR was calculated using the biological heat transfer equation (1) [18]

$$\text{Specific absorption rate} = C \times \frac{dT}{dt} \quad (1)$$

C is the heat capacity ($\text{J K}^{-1} \text{kg}^{-1}$) [27] and dT (Kelvin) is the increase in the temperature of sample plant corresponding to the elapsed time dt (seconds). In the present study, the calculated value of SAR is $9.8 \times 10^{-2} \text{ W/Kg}$.

The probe connected to RF density metre was used to measure the output density in the plane of exposure close to seedlings of *T. foenum-graecum* L. test system. *T. foenum-graecum* L. seeds were exposed to non-ionizing electromagnetic radiations at varied exposure period (0 h to 8 h per day) using RF signal generator. For control, all other conditions were kept similar, except for switching on the device.

Percentage germination

T. foenum-graecum L. seeds percentage germination was measured after 24, 48 and 72 h. Germination was considered as the appearance of the radicle from the seed. The following equation (2) was used to compute the percentage germination.

$$\text{Percentage germination} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100 \quad (2)$$

Germination index

The germination index of seedlings indicates the rate and extent of seed germination. The germination index of the seedlings was calculated using equation (3).

$$\text{Germination index} = \frac{\% \text{ of germination}_{\text{treatment}}}{\% \text{ of germination}_{\text{control}}} \quad (3)$$

Growth parameters

Root length and shoot length

Following 7 days of exposure to EMRs, seedlings were randomly selected from each treatment group and measured for the length (in cm) of both the root and shoot using a scale and thread.

Seedling vigour index (SVI)

After estimation of root length, shoot length and percentage germination, seedling vigor index was estimated using equation (4).

$$\text{SVI} = (\text{Root length} + \text{Shoot length}) \times \text{Germination percentage} \quad (4)$$

Fresh weight and dry weight

For determining the fresh weight, roots and shoots of *T. foenum-graecum* L. were collected separately from each group after seven days of exposure to non-ionizing EMRs and were weighed using a weighing balance (model: CY 220). For the dry weight, roots and shoots samples were first dried out on filter paper to eliminate extra water and then were kept for 5 h at 60 °C in an oven; after that, weight (roots and shoots) was determined by the use of weighing balance.

Biochemical activities

Preparation of plant extract

The extract of plant tissue was prepared by homogenizing 500 mg roots and shoots in cold potassium phosphate buffer (50 mM; pH 7.0) using a chilled pestle and mortar. The homogenate was subjected to centrifugation (8000 rpm; 20 min; 4° C).

Protein content

Total protein content was measured according to standard protocol given by [28]. Bovine serum albumin (BSA) solutions of varying concentrations were utilized as standards at a wavelength of 660 nm to draw a graph curve for determining protein content.

Antioxidative enzymes

Different protocols were used to examine activities of antioxidative enzymes in *T. foenum-graecum* L. test system roots and shoots with slight modifications. 300 µl of reaction mixture was used for each antioxidant enzyme in an ELISA plate of 96 wells following standard protocols like [29] for APX (EC 1.11.1.11) [30] for GR (EC 1.8.1.7), [31] for GST (EC 2.5.1.18), [32] for POD (EC 1.11.1.7) and [33] for SOD (EC 1.15.1.1) and [34] for CAT. Unit activity and specific activity was measured using equations (5) and (6), respectively.

$$\text{Unit activity} \left(U \min^{-1} g^{-1} \text{ fresh weight} \right) = \frac{\text{Change in absorbance} / \min \times \text{Total volume} (\mu l)}{\text{Extinction coefficient} \times \text{Fresh weight} (g) \times \text{volume of sample extract} (\mu l)} \quad (5)$$

$$\begin{aligned} &\text{Specific activity} (UA \text{ mg}^{-1} \text{ protein}) \\ &= \frac{\text{Unit activity} (\text{units}/\min/g \text{ tissue})}{\text{Protein content} (\text{mg}/g \text{ tissue})} \quad (6) \end{aligned}$$

Malondialdehyde content (MDA)

To prepare the samples for analysis, the plant seedlings were homogenized in a 0.1% trichloroacetic acid (TCA) solution following centrifugation (8,000 rpm; 5 min). The supernatant thus obtained was mixed with 3000 µl of 20% trichloroacetic acid and 0.5% thiobarbituric acid. The supernatant was then subjected to heat treatment at 95 °C in a boiling water bath for a period of 30 min and subsequently chilled rapidly using ice bath. Followed by cooling, the mixture was subjected to centrifugation (8,000 rpm; 10 min) [35].

Genotoxicity studies

T. foenum-graecum L. was used as a plant test system for the estimation of genotoxicity during the present study because of its small chromosomal number, i.e. $2n=16$. For the estimation of genotoxicity of EMRs, 20 seeds were evenly distributed in Petri plates (in triplicate) allowed to germinate for varied treatment durations under exposure to 1800 MHz EMRs. Once the roots had grown to a length of 1 cm within 36–48 h, their tips were excised followed by storage in Farmer's fluid [36]. To prepare the slides, the root tips were subjected to hydrolysis in 1 N HCl for a duration of 1–2 min. The tips of roots were placed on a glass slide and squashed in 45% glacial acetic acid, after staining. The slides were seen under the microscope and cells were scored for various types of abnormalities (model: Magnus MLX-DX). The microscopic investigations for chromosomal abnormalities were performed for control and treatment hour. Total 900 dividing cells from each treatment were scored for chromosomal abnormality as follows in equation (7).

$$\text{Percentage of aberrant cell} = \frac{\text{Total aberrant cells}}{\text{Total dividing cells}} \times 100 \quad (7)$$

Fourier transforms infrared spectrometry (FTIR)

The non-ionizing EMRs induced *T. foenum-graecum* L. seedlings after 8-h exposure duration, were dried and were loaded in FTIR spectroscope in powdered form (make: SHIMADZU, model: IRT racer-100). The study was conducted by selecting the range between 500 and 4000 cm^{-1} and

4 cm^{-1} resolution.

Statistical analysis

Three replications for each treatment were used to express each result's mean along with the corresponding standard error. Each replicate comprised of 20 seeds per Petri plate. To evaluate the association between antioxidant enzymes and root and shoot morphological characteristics, Pearson's correlation coefficient was used. Four slides from each replication were prepared for genotoxicity estimation, and Chi-square test was used to determine a significant difference between different hours. IBM SPSS Statistics 21 was used to conduct the statistical analysis. ANOVA and post hoc Dunnett test were used to analyse the data with the significance level of $p \leq 0.01$ and $p \leq 0.05$.

Results

Effects of EMRs on percentage germination and growth parameters

Exposure of *T. foenum-graecum* L. to 1800 MHz EMRs has resulted in variable effects on all the studied parameters. The percentage germination was found to be significantly decreased during 4 h per day at $p \leq 0.05$ and 8 h per day at $p \leq 0.01$ after 24 h while during 8 h per day after 48 h and 72 h at $p \leq 0.01$ (Table 1). At lower exposure periods, non-significant difference in the length of roots and shoots was observed. Root length significantly decreased at 4 h per day ($p \leq 0.05$) and 8 h per day ($p \leq 0.01$), and shoot length significantly decreased at ($p \leq 0.01$) during 2 h per day, 4 h per day and 8 h per day (Table 1). The seedling vigour index decreased with the increase in the exposure duration when compared to control. Maximum (1003.61) vigour index was

Table 1 Effects of non-ionizing electromagnetic radiations (1800 MHz) on percentage germination, germination index, root length and shoot length (cm) and vigour index of seedling in *T. foenum-graecum* L

Treatment (h per day)	Percentage germination (%)		Germination index 72 h	Length (cm)		Seedling Vigour Index (SVI)	
	24 h	48 h		Seedlings	Root	Shoot	Seedlings
Control	100±0.00	100±0.00	100±0.00	1±0.00	4.82±0.01	5.22±0.02	1003.61
0.5 h	100±0.00	100±0.00	100±0.00	1±0.00	4.70±0.37	4.82±0.08	951.66
1 h	96.67±0.33	100±0.00	100±0.00	0.99±0.01	4.61±0.17	4.22±0.25*	872.55
2 h	93.33±0.33	95±0.58	96.67±0.33	0.95±0.01*	4.24±0.13	3.99±0.09**	782.16
4 h	88.33±0.67*	93.33±0.33	93.33±0.33	0.92±0.02**	3.74±0.15*	3.84±0.08**	695.13
8 h	76.67±0.88**	81.67±0.67**	83.33±0.88**	0.81±0.02**	3.21±0.06**	3.56±0.10**	545.09

The data are expressed as Mean ± SE (n = 3) for each treatment group. Significance determined via Dunnett test, comparing control group to others. * $p \leq 0.05$ and ** $p \leq 0.01$ denote significance

recorded at the control while the minimum (544.61) vigour index was recorded at the maximum exposure period (8 h per day) (Table 1). The germination index was found to be significantly different at 2 h per day ($p \leq 0.05$) and 4 h and 8 h per day ($p \leq 0.01$) (Table 1).

Effects of EMRs on weight (fresh and dry)

Data presented in Table 2 illustrate the impact of EMRs on fresh weight and dry weight. The root and shoot fresh weight showed a significant decrease at longer exposure duration. The fresh weight of the roots exhibited a significant decrease at exposure periods of 4 h per day and 8 h per day ($p \leq 0.05$), whereas the fresh weight of the shoots significantly decreased at 2 h per day ($p \leq 0.05$), 4 h per day and 8 h per day ($p \leq 0.01$). However, non-significant difference was observed in the dry weight of both roots and shoots at different exposure periods.

Effects of EMRs in protein content and MDA content

The total protein content in roots did not show significant decrease in the value at all exposure period, whereas the

protein content of the shoot showed a significant decrease at 8-h per day ($p \leq 0.01$) exposure period when compared to control (Table 3). EMR exposure significantly increased the MDA content in the seedlings at 4 h per day ($p \leq 0.05$) and 8 h per day ($p \leq 0.01$) when compared with control (Table 3).

Effects of EMR antioxidative enzymes activities

In the present study, activities of different antioxidative enzymes have shown increased in their enzymatic activities (Fig. 1). The activity of different enzymes (mol UA/mg fresh protein) in roots was enhanced as 0.03–1.23 for APX, 0.04–0.17 for GR, 0.07–0.13 for GST, 0.02–0.11 for POD and 5.38–11.20 for SOD at 0.5–8-h per day treatment duration, respectively, while in shoots, it varied as 0.02–1.17 for APX, 0.01–0.11 for GR, 0.01–0.12 for GST, 0.02–0.06 for POD and 2.49–11.6 for SOD at 0.5-h-8-h per day treatment duration, respectively, when compared to the control, i.e. 0.03 for APX, 0.02 for GR, 0.06 for GST, 0.02 for POD and 2.92 for SOD in roots, respectively, whereas it varied as 0.01 for APX, 0.03 for GR, 0.01 for GST, 0.02 for POD and 2.32 for SOD at 0.5-h-8-h per day treatment duration, respectively, in shoots. The

Table 2 Effects of non-ionizing electromagnetic radiations (1800 MHz) on fresh weight and dry weight of roots and shoots in *T. foenum-graecum* L seedlings

Treatment (h per day)	Fresh weight of 7 day old seedling (mg)		Dry weight of 7 day old seedling (mg)	
	Root	Shoot	Root	Shoot
Control	45.38±1.22	67.18±0.16	1.00±0.02	4.16±0.06
0.5 h	42.92±0.86	67.48±0.51	1.20±0.10	4.10±0.05
1 h	42.67±1.45	67.32±1.71	1.10±0.03	4.31±0.13
2 h	43.36±1.21	58.88±2.66*	1.07±0.06	4.27±0.14
4 h	39.12±0.52*	50.26±1.27**	1.14±0.12	4.21±0.14
8 h	39.71±1.82*	47.45±3.12**	1.14±0.09	4.48±0.27

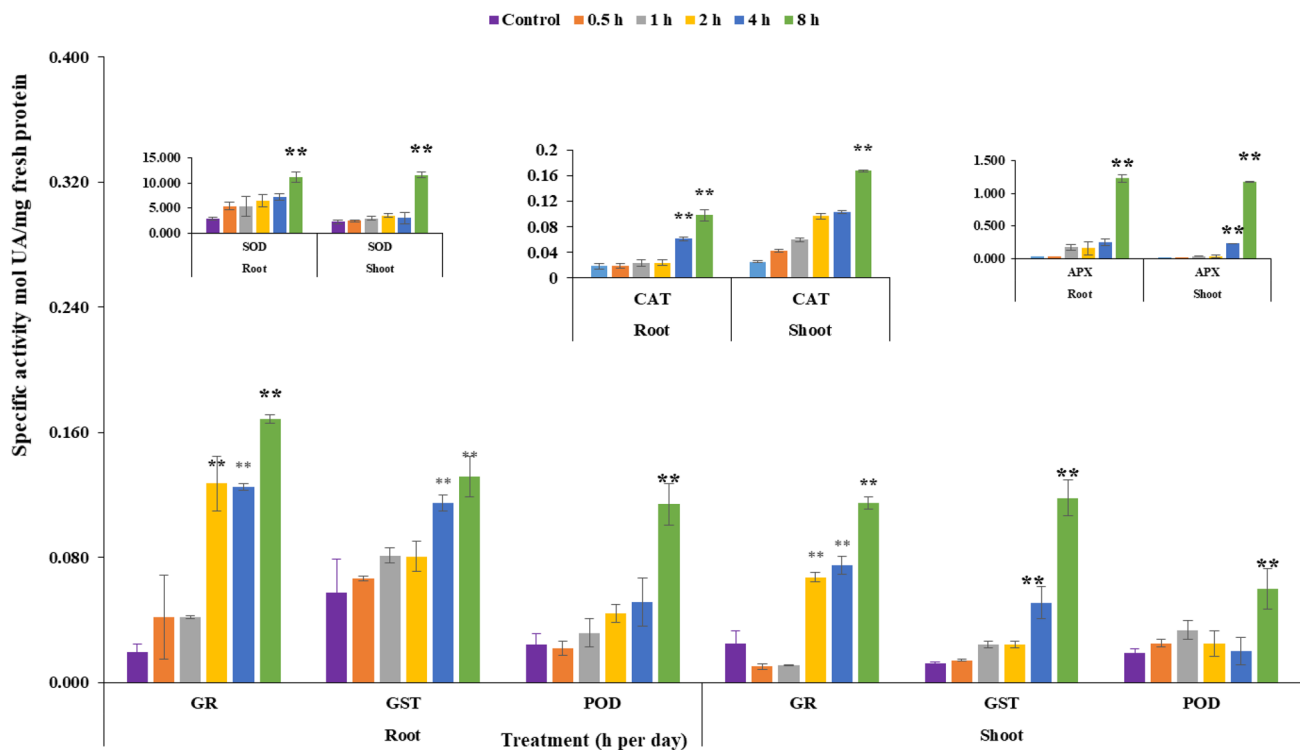
The data are expressed as Mean ± SE (n = 3) for each treatment group. Significance determined via Dunnett test, comparing control group to others. * $p \leq 0.05$ and ** $p \leq 0.01$ denote significance

Table 3 Effects of non-ionizing electromagnetic radiations (1800 MHz) on protein and MDA content of roots and shoots in *T. foenum-graecum L*

Treatment (hour/day)	Total protein content (mg/g) of fresh weight of tissue		MDA content (μmol/g fresh weight of tissue)
	Root	Shoot	
Control	2.40 ± 0.05	3.88 ± 0.39	0.12 ± 0.003
0.5 h	2.35 ± 0.32	3.88 ± 0.39	0.59 ± 0.016
1 h	2.03 ± 0.51	3.37 ± 0.29	0.81 ± 0.01
2 h	1.87 ± 0.63	3.36 ± 0.13	0.97 ± 0.03
4 h	1.65 ± 0.25	3.17 ± 0.15	1.66 ± 0.01*
8 h	1.23 ± 0.12	1.88 ± 0.09**	1.72 ± 0.01**

The data are expressed as Mean ± SE ($n = 3$) for each treatment group. Significance determined via Dunnett test, comparing control group to others. * $p \leq 0.05$ and ** $p \leq 0.01$ denote significance.

Antioxidative enzymes activities at 1800 MHz

**Fig. 1** Effects of non-ionizing electromagnetic radiations (1800 MHz) on antioxidant enzymes activities in roots and shoots of *T. foenum-graecum L*. The data are expressed as Mean ± SE ($n = 3$) for each

treatment group. Significance determined via Dunnett test, comparing control group to others. * $p \leq 0.05$ and ** $p \leq 0.01$ denote significance

EMR exposure for 8 h per day significantly increased the activity of the APX, POD and SOD in roots and shoots ($p \leq 0.01$), GR activity in roots and shoots was increased at 2 h per day, 4 h per day and 8 h per day ($p \leq 0.01$), and GST activity in roots and shoots increased at 4 h per day and 8 h per day ($p \leq 0.01$). There were no notable alterations observed in the functions of antioxidative enzymes

during shorter exposure periods of either 0.5 or 1 h per day (Fig. 1).

Genotoxic effects of EMRs

It was seen that the percentage total chromosomal aberrations varied as 2.8%, 5.49%, 7.93%, 9.16% and 13.93%

Treatment (hour/day)	Total cells	Total dividing cells	Physiological aberrations						Clastogenic aberrations									
			Cm	Da	St	Lg	Vg	Aa	Am	Dm	TPA	TCA		TPA + TCA				
												No	%	No	%			
Control	9130	920																
0.5 h	9235	918	6	1	5	1	0	0	0	8	21	2.28	5	0	5	0.54	26	2.8**
1 h	8555	892	7	0	10	10	10	0	0	3	40	4.48	9	0	9	1.00	49	5.49**
2 h	8312	920	11	11	11	12	8	4	3	4	64	6.95	9	0	7	0.97	73	7.93**
4 h	7070	884	10	9	11	8	8	3	7	6	62	7.01	12	7	19	2.14	81	9.16**
8 h	8230	904	11	14	11	15	14	4	13	19	101	11.17	16	9	25	2.76	126	13.93**

at treatment exposure of 0.5 h to 8 h per day, respectively (Table 4). The number of dividing cells showed significant changes at all exposure periods compared to that of control group. Upon EMR exposure, several chromosomal aberrations including physiological aberrations such as stickiness (St), delayed metaphase (Dm), delayed anaphase (Da), abnormal anaphase (Aa), laggard (Lg), c-mitosis (Cm) and vagrant (Vg) and clastogenic aberrations ring chromosome (Rc) and chromosomal bridge (Cb) were observed (Fig. 2).

The Pearson's correlation analysis was carried out to investigate the correlation among roots and shoots different parameters after 7-day exposure period (see supplementary material Tables 1 and 2). In roots, protein content showed a significant negative correlation with APX activity ($r = -0.929$, $p \leq 0.01$), GR activity ($r = -0.935$, $p \leq 0.01$), GST activity ($r = -0.971$, $p \leq 0.01$), POD activity ($r = -0.927$, $p \leq 0.01$) and SOD activity ($r = -0.946$, $p \leq 0.01$). In shoots, protein content also demonstrated a substantial negative relationship with APX activity ($r = -0.935$, $p \leq 0.01$), GR activity ($r = -0.870$, $p \leq 0.05$), GST activity ($r = -0.841$, $p \leq 0.05$) and SOD activity ($r = -0.949$, $p \leq 0.01$). On the other hand, all the antioxidative enzymes showed a positive correlation with each other (Fig. 3 i and ii).

FTIR spectrum was used to ascertain the functional group along with the structure of active biomolecules using the peak value for IR (infrared) band. The output of spectra obtained from FTIR spectra study and the peak values associated with the creation of biomolecules are presented in Fig. 4. FTIR spectroscopy was used to study the powder obtained from exposed seedlings of *T. foenum-graecum* L. The IR spectra of the sample within the range of 500–4000 cm^{-1} were analysed to identify the biomolecules based on their characteristic peak values. During the current study, FTIR spectra of seedlings that had been exposed to EMRs showed a peak (3286.70 cm^{-1}) for the hydroxide group, peak (2924.09 cm^{-1}) for methylene group, peak (1743.65 cm^{-1}) for aldehyde, ester and alkyl carbonate, and peak (1631–1680 cm^{-1}) for ketone and amide group. Epoxy and oxirane rings were seen in an absorption band (1236–1529.55 cm^{-1}) (see supplementary material Table 3). In FTIR spectra, no major differences were observed between the 0-h and 8-h exposure duration.

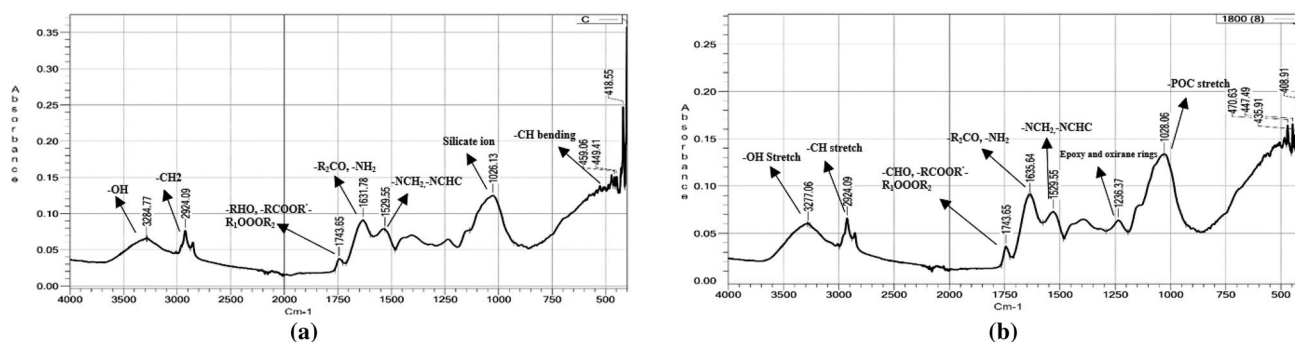


Fig. 2 Representative infrared spectra (4000 to 500 cm^{-1}) of *T. foenum-graecum* L. after exposure to EMRs: (a) control (0 h) and (b) 8-h exposure duration

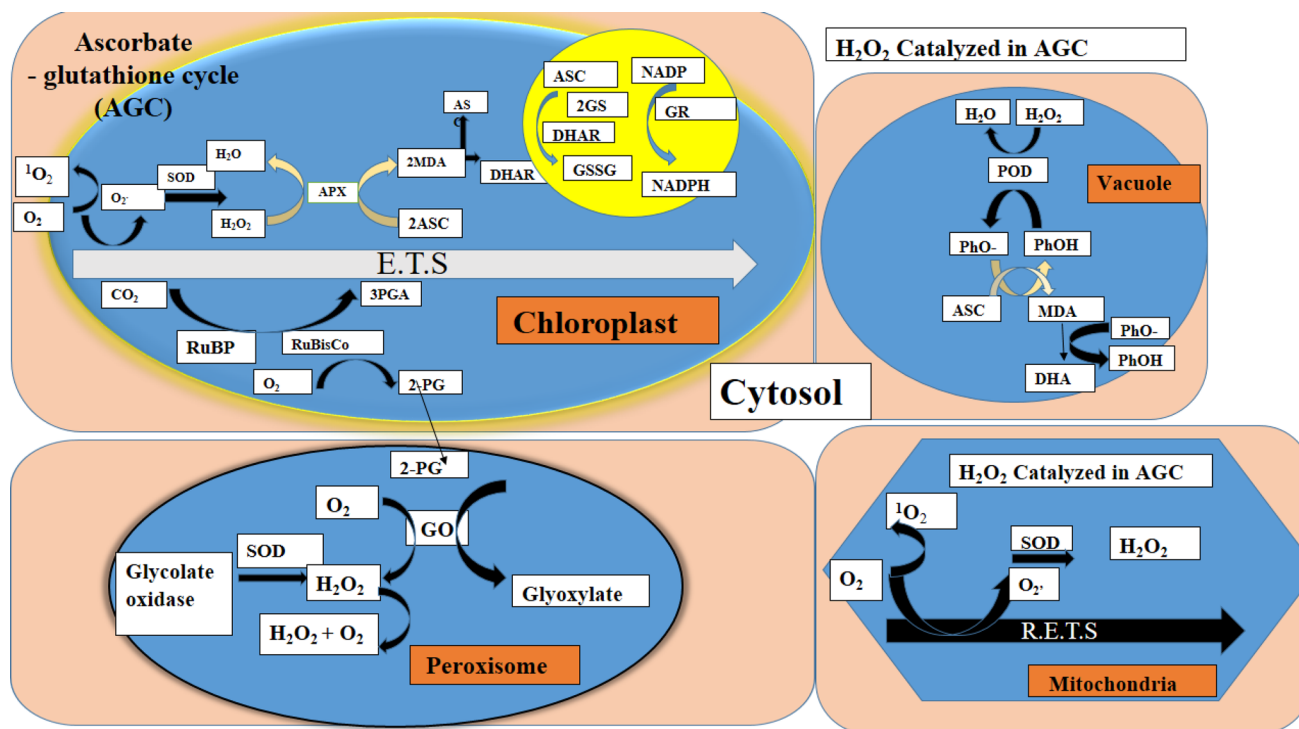


Fig. 3 Localization and detoxification mechanism of ROS by antioxidant enzymes [74–78]. 3-PGA, 3-phosphoglycerate; ETS, electron transport system; DHA, dehydroascorbate; MDAR, MDA reductase; GSSG, oxidized glutathione; PhOH, phenolic compound; RETS, res-

piratory electron transport system; RuBP, ribulose 1,5- biphosphate; RuBisco, ribulose 1,5-biphosphate carboxylase/oxygenase; 2-PG, 2-phosphoglycerate; H_2O_2 , hydrogen peroxide; PhO^- , phenoxyl radical; ASC, ascorbate; GO, glycolate oxidase

Discussion

Electromagnetic radiations (EMRs) at different treatment duration caused morphological, biochemical and genotoxic changes in the *T. foenum-graecum* L. test system. Various physiological features of plants including self-defence, food uptake and adapting to the new environment depend on the capacity to perceive and respond to external mechanical stimuli at different time frames [37].

Plants are endangered by biotic and abiotic stressors in the environment because they are sessile and are prone to the changing surroundings [38–40, 46, 47]. The present work revealed that the EMRs at 4-h and 8-h per day exposure duration significantly reduced the percentage germination. The first step in a plant's response to better growth is seed germination which is most often utilized as plant's growth reactions indicator to any external stimuli. The divergent responses to germination during the EMR exposure study have been documented [23, 41]. During the exposure of

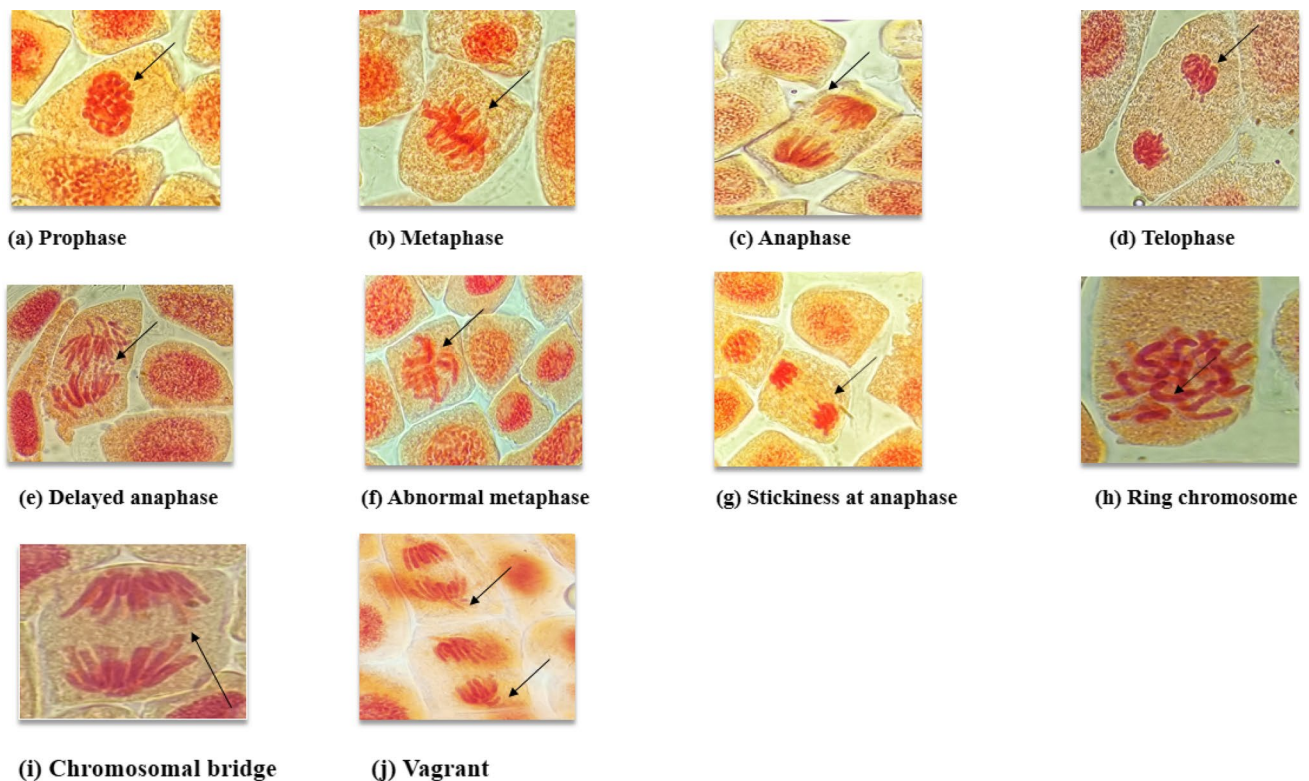


Fig. 4 Cells exhibiting different phases of mitotic division in *T. foenum-graecum* L. Normal cells (a–d) and aberrated cells (e–j)

T. foenum-graecum L. to 1800 MHz EMRs, the root and shoot lengths were significantly lowered at longer periods of exposure. The lengths of the root and shoot are the first biometric indicator in plants produced under stressful conditions. Akbal et al. documented that 1800 MHz exposure to *Lens culinaris* Medik (lentil) led to decreased length of the seedlings attributed to the stressful conditions generated by the EMRs [42].

The root and shoot fresh weight showed a significant decrease at longer exposure periods, with the maximum decrease observed at 4-h and 8-h per day exposure periods. Our findings were parallel with the results obtained by Chen and Chen who reported that the 24-h intermittent exposure period at 1805–1850 MHz had a significant effects on fresh weight of the mung beans [43]. Similar observations were made by Leena and Pooja where radiations from the mobile phone had led to the decrease in length, fresh weight and relative water content in seedlings of *Vigna radiata* (44).

The activities of antioxidative enzymes were significantly increased compared to the control group, in the present study. The changes in response to EMR exposure were reported by [47] including biochemical processes that leads to the generation of ROS, increased in MDA content of variety of cellular membranes, protein oxidation, damage of DNA and antioxidative metabolism. An upsurge in free radicals or a decrease in antioxidative potential might alter the

equilibrium between oxidants and antioxidants [48]. Antioxidant enzymes and certain plant metabolites are crucial for preventing oxidative damage, which help plants adapt and ultimately survive stressful situations [49]. Plants have been documented to increase the activity of antioxidative enzymes to combat oxidative stress [50].

Several previous studies have demonstrated the ability of cells to prevent intracellular damage caused by free radicals through intracellular antioxidant system [51, 52]. SOD is a crucial element of the plant's antioxidant defence system, as it is responsible for dismutating two superoxide radicals ($O_2^{\cdot-}$) into H_2O and O_2 [53]. During the current investigation, APX and SOD have shown an increased activity at 4-h and 8-h per day exposure period under EMR stress, demonstrating that both of these enzymes acted simultaneously to remove hydrogen peroxide radical (H_2O_2) and prevented the generation of severely toxic hydroxyl radical ($OH^{\cdot}$). In the defence mechanism of plant, glutathione (GSH) acts as an endogenous antioxidant (54). GSH prevents hydrogen peroxide from entering the cell, which is essential for the detoxification and smooth operations of GR, GST and POD [55]. These enzymes can inactivate a wide range of hazardous chemicals, scavenge lipid peroxidation metabolites, detoxify ROS in cells and decrease DNA damage [56, 57]. The activity of GR and GST significantly increased for 4-h and 8-h per day exposure period in *T. foenum-graecum* L.

roots and shoots. The increased activities of GR and GST at 4-h and 8-h per day exposure duration suggested that these enzymes act as crucial cellular defence against oxidative damage caused by EMRs. GST recovered their detoxifying and antioxidant capacities and played a vital role in scavenging, leading in the elimination of ROS and a decrease in DNA damage induced by EMRs [57–59]. The role of glutathione reductase in the plant's response to oxidative stress is significant, as it helps to maintain the intracellular glutathione pool in its reduced state, i.e. GSH. GSH, in turn, acts as an antioxidant and aids in scavenging ROS, such as H_2O_2 and $\text{O}_2^{\cdot-}$. The pathway of ROS detoxification in

different cell organelles by antioxidant enzymes is shown in Fig. 5.

Peroxidases are enzymes that have a wide distribution and serve various roles in the growth, differentiation and development of plants. POD is an antioxidant enzyme that uses H_2O_2 as an electron acceptor to catalyse the breakdown of other molecules and transforms H_2O_2 to H_2O [60]. The enhanced activity of POD indicates that this enzyme plays an innate defensive role in *T. foenum-graecum* L. against oxidative damage caused by exposure to EMRs. Similar increased level of POD enzyme has been reported that tomato plant under stress of EMRs leads to rise in POD activity could be correlated with the stress response in the

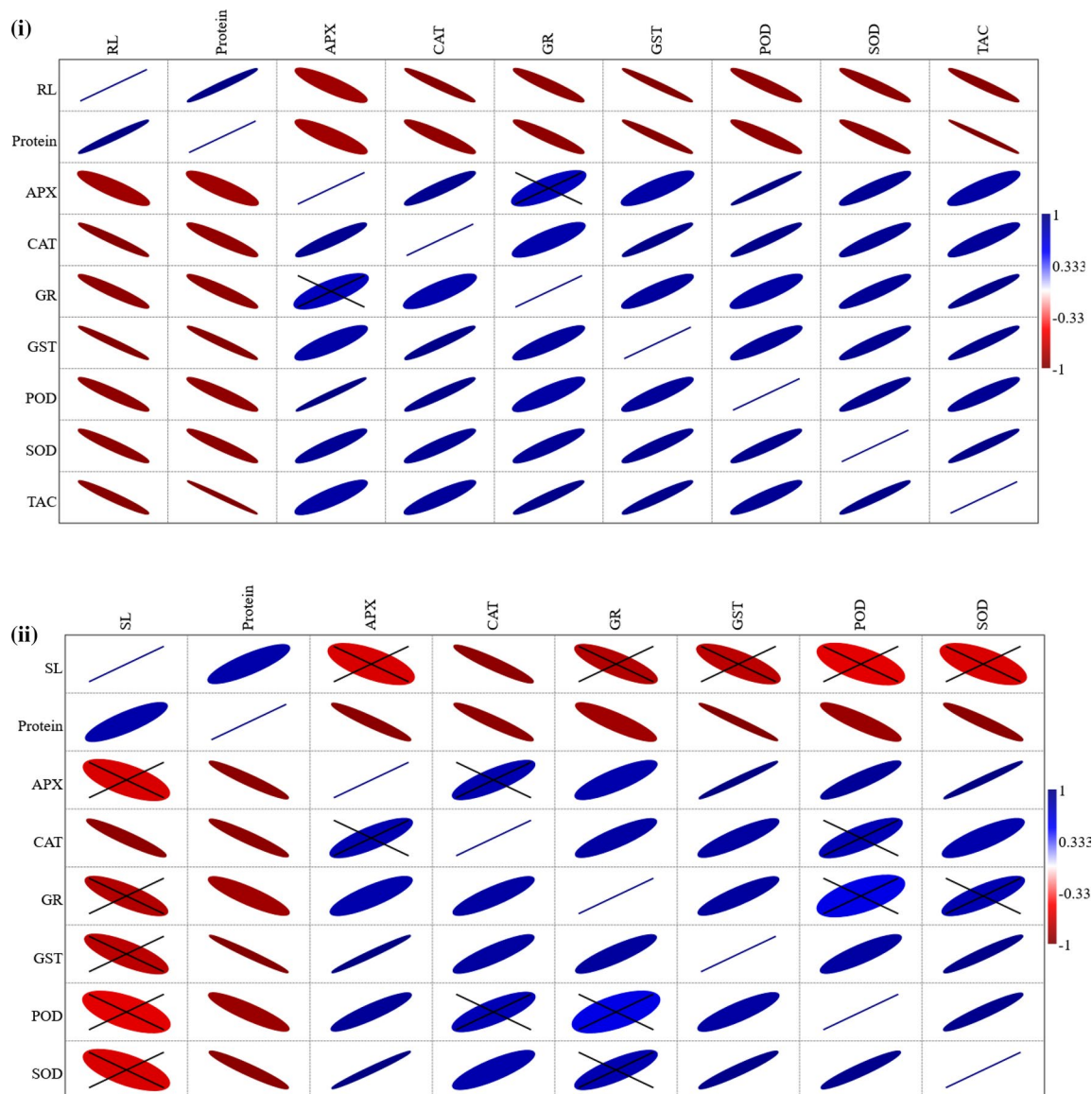


Fig. 5 (i-ii). Representation of Pearson's correlation comparisons between enzymes and different parameters of *Trigonella foenum-graecum* L. roots and shoots, respectively, after exposure of EMRs at

1800 MHz (i-ii). The figure with red ovals indicates negative correlation, whereas ovals with blue colour indicates positive correlation. Oval without cross are significant at ($p \leq 0.05$) level

plant system [21]. Several environmental stresses, viz. ultra-violet radiation, ozone, salinity, drought, osmotic stress, heavy metals and cold, increased the activity of peroxidase enzyme, and thus, it could be utilized as a stress indicator in plants [61, 62]. However, peroxidases are widely accepted as ‘stress enzyme’. Various studies investigating the effects of EMRs on the activities of various antioxidative enzymes in plants have reported an increase in enzymatic activity with an increase in exposure duration. This finding was reported in studies conducted by [59, 63].

Lipid peroxidation, a process by which unsaturated fatty acid-containing components of phospholipids oxidize, causes fast destruction of cell membranes. During lipid peroxidation, polyunsaturated fatty acids are converted into physiologically active compounds that accumulate in the membrane. This process can cause disruptions in membrane transport, alterations in membrane structure, changes in cell membrane fluidity and modifications to the function of cell membrane enzymes. These alterations can lead to oxidative damage, making lipid peroxidation a biochemical marker for free radical-mediated injury. In the present work, the content of malondialdehyde (MDA), a by-product of lipid peroxidation, was observed to increase with an increasing duration of exposure to electromagnetic radiations in *T. foenum-graecum* L. This result is consistent with previous studies which reported that EMRs induced oxidative stress in other plant species, such as *Vigna radiata* and *Triticum aestivum* [64]. Parallel to our findings, higher content of malondialdehyde MDA (lipid peroxidation) has been reported under heavy metal stress in two species of mangrove plants, i.e. *Bruguiera gymnorhiza* and *Kandelia candel* [65]. These findings provide additional evidence that lipid peroxidation is a common response to various stressors in plants, including EMRs and heavy metals.

The current study demonstrated that exposure to non-ionizing EMRs of 1800 MHz resulted in genotoxic damage to the root meristems of *T. foenum-graecum* L. test system, which was found to be dependent on the duration of exposure. According to the present investigation, total chromosomal aberrations (TCA) were more pronounced and significantly higher at 4-h per day (9.16%) and 8-h per day (13.93%) exposure period, respectively (Table 4). Plants upon exposure to EMRs showed adverse effects in cellular organelles, particularly chromosomes. The primary event caused by radiation was a chromatid or chromosome break [3].

Different chromosomal aberrations observed during the present study indicate the DNA damaging effects of EMRs. The order of physiological aberrations obtained during current study is St, Cm, Lg, Dm, Vg, Da, Am and Aa while the order of clastogenic aberrations obtained is Cb and Rc (Table 4 and Fig. 2). The total physiological aberration (TPA) and total clastogenic aberration (TCA) at 8-h per

day exposure period were found to be 11.17% and 2.76% (Table 4), respectively. Our results were in conformity with the investigations of earlier studies who reported that EMRs led to severe chromosomal aberrations in the studied plant systems [9, 66]. Cytotoxic and genotoxic effects of electromagnetic radiations at two different frequencies (900 and 1800 MHz) were studied in *Allium cepa* root meristems and it was found that the radiations induced DNA damage was more deleterious at 1800 MHz frequency [9]. The primary cause of the chromosomal aberrations could be related to increased levels of oxidative stress indicators and decreased levels of antioxidative metabolic enzymes.

The study conducted by Dogan et al. utilized Fourier transform infrared spectroscopy to investigate the molecular changes induced by radiation in the macromolecular components of plants [67]. The spectral region between 3360 and 3549 cm^{-1} exhibited peaks caused by stretching vibrations of OH groups present in various compounds such as water, alcohols, phenols, polysaccharides, peroxides and amides [68, 69]. Additionally, distinctive hydrogen peroxide absorption bands were identified in the spectral region between 3750 and 3150 cm^{-1} . The amide II and III regions were observed between 1580 and 1480 cm^{-1} and 1300–1230 cm^{-1} , respectively, and were associated with the C-N stretching and N-H bending vibrations of the peptide group, as reported in previous studies [70–72]. The FTIR spectra also showed that the vibrational range of carbohydrates was between 854.25 and 758.03 cm^{-1} , with starch components exhibiting a vibrational frequency at 584.52 to 532.96 cm^{-1} [68, 73, 74].

Conclusion

The current study revealed that exposure to non-ionizing electromagnetic radiations at 1800 MHz caused genotoxic effects and oxidative stress in *Trigonella foenum-graecum* L. plant system. The outcomes of the present work indicated that exposure to EMRs of 1800 MHz led to reduced root and shoot length, as well as decreased fresh weight and protein content. The elevated levels of MDA content, a marker of oxidative stress, indicated that EMRs have induced oxidative damage in the plant test system. Furthermore, the present investigation has further shown that the plant has responded to the stress caused by EMRs by increasing their antioxidant defences. Hence, it can be revealed that EMRs at 1800 MHz can cause harmful effects in the living system, and therefore, it is mandatory to reduce the exposure risks in living beings to these non-ionizing radiations in order to avoid the ill health effects. This may include limiting the usage of electronic devices that emit EMRs, such as cell phones and Wi-Fi routers, and taking measures to reduce exposure to

other sources of EMRs. By taking such precautions, it is possible to reduce the potential health risks allied with exposure to non-ionizing EMRs.

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Author contribution JKK and SB conceptualized the idea, designed the experiment and reviewed the overall manuscript. SS performed the whole experiment, wrote the original draft, compiled the overall manuscript and analysed the findings. PS and JS assisted in the analysis work related to FTIR.

Data availability The corresponding author can be contacted to request access to the data, which will be made available.

Declarations

Conflict of interest On behalf of all authors Surbhi Sharma, Priyanka Sharma, Shalini Bahel, Joat Singh, and Jatinder Kaur Katnoria the corresponding author states that there is no conflict of interest.

Consent to publish There is no need for consent to publish this paper.

Ethics approval and consent to participate No need.

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