

RESEARCH PAPERS

Investigating the Effects of 2850 MHz Electromagnetic Field Radiations on the Growth, Germination and Antioxidative Defense System of Chickpea (*Cicer arietinum* L.) Seedlings

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Abstract— The current study was conducted to investigate the effects of EMF-r on germination parameters, stress indices and the antioxidative defense system in seedlings of the chickpea variety PBG 7 upon 0 (control), 1-hour, 2-hours, and 4-hours exposure durations to EMF-r with a frequency of 2850 MHz. Chickpea seeds (0–7 days) were exposed daily to the EMF-r and 4-h exposure showed the most prominent effect. The effect was more pronounced in terms of germination parameters, stress indices and antioxidative status. In terms of germination parameters, vigour index of seedlings was the most affected with respect to control counterparts. An increase in the MDA content indicates the membrane damage, which intensified along with increasing duration of EMF-r exposure. Antioxidative status in terms of SOD and POD was upregulated, especially after 4-h exposure to EMF-r. Also, photosynthetic pigments, i.e. chlorophyll *a*, total chlorophyll content reduced at this duration of exposure. Our findings suggest that 2850 MHz EMF-r affected the germination and growth of chickpea seedlings, and also altered the oxidative metabolism of irradiated samples, especially at 4-h exposure. Our results contribute to research focused on the effect of EMF-r on the entire life cycle and to identify the threshold window limit of EMF-r radiations and to what factors contribute for the plant to be attenuators.

Keywords: Chickpea, EMF radiations (EMF-r), germination parameters, stress indices, antioxidative defense system

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INTRODUCTION

The use of mobile phones and wireless devices has increased tremendously in the past few decades and a large majority of experimental data questions their safety and the deleterious effects of the radiation exposure on the living organisms. A continuous low-power environmental electromagnetic field (EMF) is generated by mobile phones and their wireless communication infrastructure. Although, the local regulatory authority controls the EMF power density in the environment in accordance with the recommendations of the International Commission on Non-Ionizing Radiation Protection [1], there has been a significant increase in the use of EMF devices, which increases the level of electromagnetic radiation in the environment. The present scenario raises concerns for protection against environmental EMF radiation and to

investigate the effects of EMF on living organisms. In 2013, the International Agency for Research on Cancer (IARC) designated EMF radiation as possible human carcinogen emitted by mobile phones [2].

Although many case studies pertaining the effect of EMF have been conducted on the living organisms, plants are still remaining less explored in the terms of effect of EMF radiations (EMF-r). The immobility of the plants renders them to be more susceptible towards the exposure of EMF-r. Moreover, case studies of the effect of EMF-r on plant physiology have been inconclusive. Sensitivity of the plants to EMF exposure has been depicted in terms of upregulation of oxidative stress, i.e. reactive oxygen species (ROS) [3] and even modifications at the gene level [4].

Not only the cellular machinery gets affected, plants also showed phenotypic changes as in *Lens culinaris* Medik, where the germination capacity, seedling length and vigour index (VI) got severely affected on exposure to 1800 MHz for 48 h [5]. Subsequently, EMF-r exposure at seedling stage also negated the

Abbreviations: CRDB—completely randomized block design; EMF-r—electromagnetic field radiations; PV—high proportion variance.

germination parameters in *Phaseolus aureus* L. at a frequency of 900 MHz for 0.5, 1, 2 and 4-h exposure duration [6], *Allium cepa* L. at 400 and 900 MHz for 2 h [7], maize (*Zea mays* L.) at 1 GHz for 1–8 h [8] and in *Rosa hybrida* L. 900 MHz [9]. At the same time, there are examples of an increase in the percentage of germination and plant development for maize at a frequency of 945 MHz for 4 weeks [10], in fenugreek and pea at 850–1850 MHz for 0.5, 1, 2, 4, and 8-h duration of exposure [11], in mung bean at 1805–1850 MHz for 4 and 24 h [12], and in lentil at 1850 MHz for 48-h exposure [5]. Unfortunately, the large disparity in the number of irradiation parameters (i.e., frequency, modulation, time of exposure, power level and distance from EMF source) complicates the studies between these comparative responses and the outcome becomes questionable and contradictory.

The current study validates the effect of EMF radiations on germination parameters, stress indices and biochemical constituents in the chickpea *Cicer arietinum* L. variety PBG 7 seedlings exposed to different EMF durations. Earlier studies have been conducted on legumes, *Lens culinaris* and *Phaseolus aureus*, but to our knowledge, no studies have been reported on chickpea regarding the effect of 2850 MHz EMF on the biochemical constituents.

MATERIAL AND METHODS

Seed collection. Seeds of chickpea *Cicer arietinum* L. variety PBG 7 were obtained from the pulses branch of the Department of Plant Breeding and Genetics at PAU Ludhiana.

Dosimetry and experimental exposure. A vector signal generator (SMBV; Rohde and Scharz, Germany) was wired to an amplifier ZHL-2500+ (Minicircuits, USA) to generate homogenous EMF-r of 2850 MHz for the exposure setup. A radiation field meter NMB 550 (Narda Safety Test Solutions GmbH, Germany) was used to record output power densities. At 2850 MHz, the average power density recorded at a distance of 3 cm from the antenna was 0.522 W/m² with a specific absorption rate (SAR) of 4 dW/kg ($\sigma = 2.101$ S/m and $\rho = 1030$ kg/cm³). The SAR value was estimated roughly based on the fact that measuring it directly on exposed plant tissues is challenging [13].

To avoid contamination, seeds of uniform size were surface sterilized with 0.1% mercuric chloride solution for 1 min, then thoroughly washed with distilled water. In sterile 15-cm Petri dishes that were coated with filter paper, moistened with distilled water, and fifteen seeds were planted equidistantly. For 7 days, the Petri plates were watered with 5 mL distilled water on alternate days. Each treatment group included three replicates. Group 1, 2 and 3 were exposed to EMF-r for 1, 2 and 4 h, respectively. However, group 4 was used as a control and was not exposed to any EMF-r. After the exposure, the Petri plates were then placed in a growth

chamber set at 24 ± 2°C, 80 ± 2% relative humidity (RH), and 18/16 h light/dark photoperiod of 240 μmol/(m² s) photon flux density. The germination parameters, plant length, biomass, percent germination was recorded after 7 days of treatment.

Germination parameters. Germination counts were made at 48 h interval till seventh day in control and EMF-r treated seeds. When the radicle reached a length of 2 mm, the seeds were considered to have germinated, and the germination rate was presented as % germination. Seedling root and shoot lengths were measured using a measuring scale on the eighth day after germination and represented in cm. The fresh weight (fr wt) of the roots and shoots was measured. To estimate the dry weight (dry wt), samples were dried in an oven at 70°C for 72 h before being weighing. Both fresh and dry root and shoot weights were expressed in grams (g). The sum of total seedling length and percentage of germinated seeds were used to determine the vigour index (VI) [14] according to the following formula:

$$VI = S \sum (G_t / D_t),$$

where S is seedling length on the seventh day, G_t is number of germinated seeds in the tth day, D_t is number of days from the first day to the tth day.

Estimation of chlorophyll and carotenoids. The photosynthetic pigments were extracted from seedlings by placing 25 mg in 4 mL of dimethylsulfoxide (DMSO) and incubated at 60°C for 1 h [15]. The absorbance values at 663, 645 and 470 nm were used to calculate the concentration of the extracted pigments. Contents of chlorophyll and carotenoid were calculated using the provided equation [16, 17].

Membrane permeability index. Using a Eutech CON 700 conductometer, the damage in leaves caused by EMF-r stress was quantified in terms of membrane permeability index (MPI) [18]. Leaf discs (0.1 g) were cut, cleaned and immersed in 10 mL of distilled water for 24 h. Conductivity meter was used to test the initial electrical conductivity (EC_i). The same tissue was boiled in a water bath for 30 min, and the final EC (EC_f) was measured after cooling. MPI was calculated according to the following formula:

$$MPI(\%) = [(EC_f - EC_i) / EC_f] \times 100.$$

H₂O₂ content. For the determination of H₂O₂ content, 0.2 g of fresh tissue was homogenized with 20 mL of 0.1% (w/v) trichloroacetic acid (TCA) in a pre-chilled pestle and mortar and the homogenate was centrifuged at 12000 g for 15 min [19]. 0.5 mL supernatant was mixed with 0.5 mL of 10 mM phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide. The absorbance was measured at 390 nm.

Lipid peroxidation. The extract was prepared in the same way as described for the H₂O₂ determination. Then, 0.5 mL of supernatant was mixed with 2 mL of 0.5% (v/v) of thiobarbituric acid (TBA) prepared in

20% TCA. The mixture was heated for 30 min in a water bath at 95°C and then the reaction was terminated in cold bath. The samples were again centrifuged at 10000 g for 10 min. Absorbance of samples was measured using UV 2600 spectrophotometer (Shimadzu, Japan) at 532 nm and 600 nm with TBA reagent as blank. Malondialdehyde (MDA) concentration was calculated with extinction coefficient of 155 mM⁻¹ cm⁻¹ [20].

Total phenolic content. For estimation of total phenolic content [21], extracts were prepared by crushing 100 mg of plant tissue with 10 mL of distilled water. The homogenate was centrifuged at 10000 g for 15 min at 4°C and obtained supernatant was used for further estimation. To 0.5 mL of the enzyme extract was added 0.5 mL of 50% diluted Folin's reagent. After shaking, 0.5 mL of 20% sodium carbonate solution was added and kept for half an hour. Absorbance at 700 nm was measured against a standard (ferulic acid).

Superoxide dismutase (SOD) and peroxidase (POD) activity. SOD and POD activities were extracted from seedlings using 0.1 M potassium phosphate buffer (pH 7.5) containing 1% PVP, 1 mM EDTA and 10 mM β-mercaptoethanol with followed centrifugation at 10000 g for 10 min.

The activity of superoxide dismutase (SOD) was measured in 3 mL of grude extracts mixed with 1.5 mL of 0.1 M Tris-HCl buffer, pH 8.2, 0.5 mL of 6 mM EDTA, 1 mL of 6 mM pyrogallol solution [22]. Absorbance was measured at 420 nm every 30 s for up to 3 min. A unit of enzyme activity is defined as the quantity of enzyme that inhibits auto-oxidation of pyrogallol by 50% as observed in blank. SOD activity was expressed as EU/mg protein.

Peroxidase activity was measured using a reaction mixture containing 6 mL of 0.05 M guaiacol in 0.1 M potassium phosphate buffer (pH 6.5), 0.2 mL of grude extract and 0.2 mL of 0.8 M H₂O₂ [23]. Blank solution contains the reaction mixture without H₂O₂. The reaction was started by adding H₂O₂, and the rate of change in absorbance at 470 nm ($\epsilon = 6740 \text{ M}^{-1} \text{ cm}^{-1}$) was measured every 30 s for 3 min. POD activity was expressed as Kat/s/mg protein.

Protein content. The protein content of the samples was determined using Folin's reagent on reaction with acidic ninhydrin in comparison to standard bovine serum albumin (20–100 g) [24].

Statistical analysis. Each treatment of EMF-r exposure had three replicates and each petriplate acted as an independent replicate. The experimental set up was maintained in a completely randomized block design (CRDB). For biochemical assays, one sample was taken from each replicate. Data was analyzed using SPSS software for one-way analysis of variance (ANOVA), followed by the comparison of mean values using post hoc Tukey's test at $P \leq 0.05$. Principal component analysis and screen plot using SAS soft-

ware was conducted to obtain the degree of contribution of each parameter for assessing the tolerance against EMF-r exposure.

RESULTS

Seedlings depicted a negative effect on account of EMF-r exposure (Fig. 1). The inhibitory effect of exposure increased from 1 to 2 h, and the maximum damage was recorded after 4-h of EMF-r exposure. The effect was significant in shoot length that reduced by 7 and 10.96 times with a 4-h duration of EMF-r exposure in comparison to 2-h exposure and control conditions, respectively. After a 4-h exposure to EMF-r, the root length significantly reduced by 5.31 times compared to the control conditions. The effect of EMF-r on fresh and dry weight was more pronounced at 4-h EMF-r exposure duration, i.e. 3.23 and 2.52 times, respectively (Table 1). The vigour index of chickpea seedling declined, as depicted by post hoc analysis test (Fig. 2), by 2.01 times and 6.65 times after 2-h and 4-h EMF-r exposure, respectively. The percent of germination expressed a significant change in chickpea seedlings at a 4-h duration of EMF-r exposure in comparison to control group. Based on the principal component analysis (PCA), PC1 and PC2 contributes a total of 0.689 (68.9%) to explain the seedlings response to EMF-r exposure. However, PC1 contributes the maximum (57.8%) towards PC, the sets of PC1 values were corrected for the diversity of data collected in this principal component.

The damage due to EMF-r exposure was elucidated in terms of photosynthetic pigments (Fig. 3). The maximum significant damage in terms of the content of total chlorophyll (by 1.63 times), chlorophyll *a* (by 1.53 times), chlorophyll *b* (by 2.15 times) was recorded with a 4-h exposure to EMF-r. However, carotenoid did not depict any significant difference upon exposure to EMF-r.

The damage to cellular machinery on account of EMF-r exposure was elucidated in terms of increased electrolyte leakage, MDA, phenolic and H₂O₂ content (Table 2). The significant membrane damage at 4-h exposure to EMF-r is evidenced by a 1.52 times increase in the electrolyte leakage of seedlings in comparison to control samples. Phenolic content did not reflect any significant variation depending on the duration of exposure. The pattern for H₂O₂ content was not steady though. Upon exposure to 2850 MHz EMF-r, the H₂O₂ content increased in 1-h treatment group by a 2.64 times, and then followed by decline after 2 h (by 2.20 times) and 4 h (by 3.60 times) exposure, respectively. The EMF-r exposure upregulated the antioxidative defense system, as evidenced by an increase in the SOD, which has a significant negative correlation with H₂O₂ content ($r = -0.611$).

An increase in MDA levels triggered the antioxidative defense system, significantly upregulating SOD

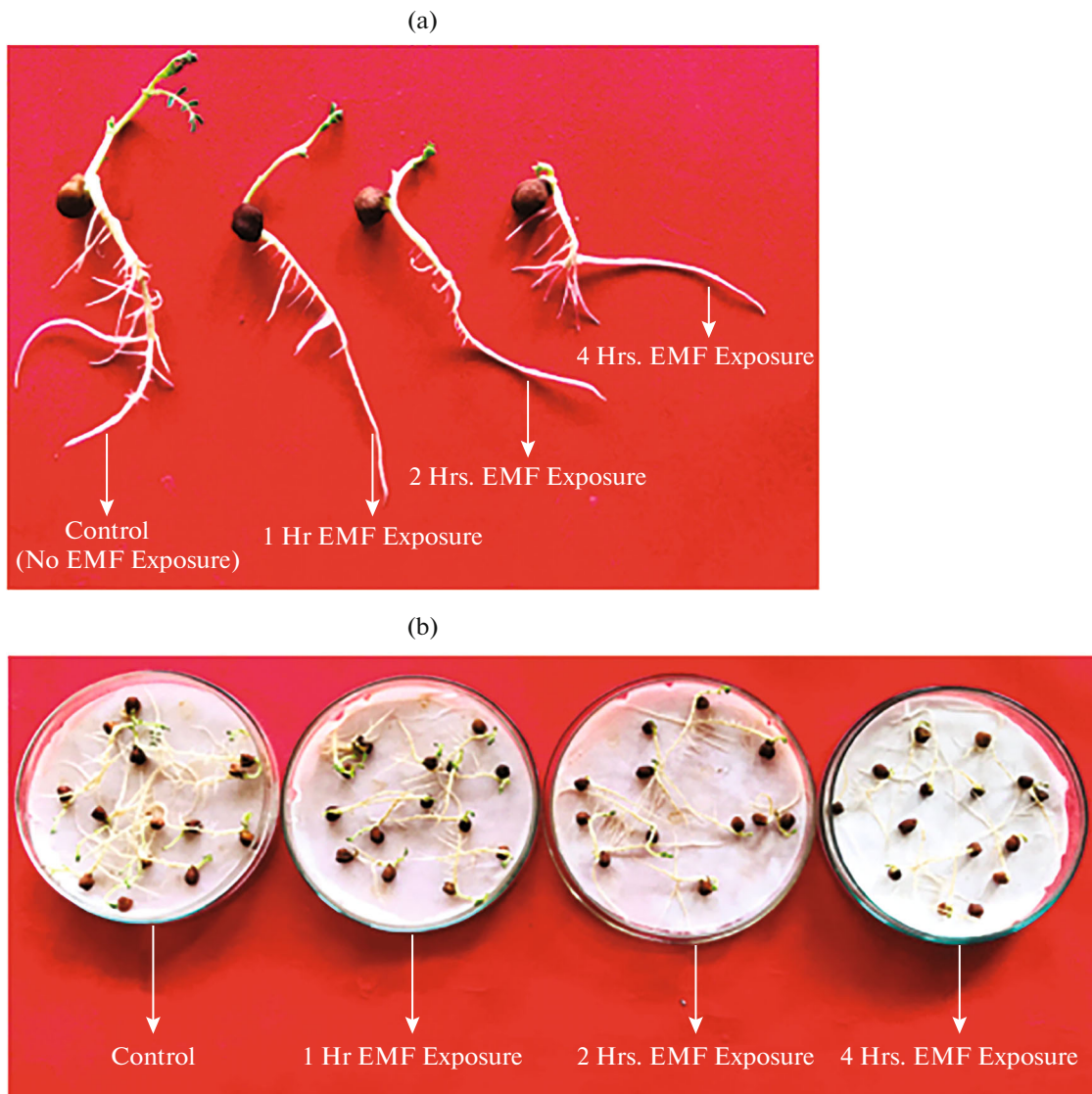


Fig. 1. Phenotype of chickpea *Cicer arietinum* L. seedlings after exposure to EMF-r with a frequency 2850 MHz: (a) image of individual seedling after exposure to EMF-r; (b) general view for 7 day-old seedling represents the effect of EMF-r at 1, 2 and 4 hours exposure.

($r = 0.854$) and POD ($r = 0.734$) activities with the increment in the EMF-r exposure duration (Table 3). The SOD (by 3.28 and 4.82 times) and POD (by 1.16 and 1.40 times) enzymatic activities ameliorated against ROS developed upon irradiation of 2-h and 4-h exposure to EMF-r, respectively.

The protein content did not show any significant difference between the treatment groups (Supplementary Fig. S1).

DISCUSSION

Among all the germination characteristics that were recorded, vigour index showed the most prominent effect. When subjected to tukey's post hoc test,

each treatment group, i.e. 1, 2 and 4 h of EMF-r exposure revealed a different vigour index when compared to control group. The reduction in root length and the percent of germination of *Vicia faba* L. seedlings on account of EMF-r exposure due to reduced sugar, protein and fat content has been attributed due to downregulation of amylase, protease and lipase enzyme, respectively [25]. However, contrasting results had also been observed in *Oryza sativa* L. [26], where EMF-r exposure increased the average vigour index due to ion activation and protection of meristematic areas of the seedling. The reduction of the VI in seedlings after a 4-h exposure to EMF-r could be attributed to the membrane damage, as evidenced by its negative significant correlation ($r = -0.699$) with

Table 1. Effect of EMF-r exposure at different time intervals on the germination parameters in chickpea (*Cicer arietinum* L.) seedlings

Treatment, h	Germination, %	Shoot length, cm	Root length, cm	Biomass, g fr wt	Biomass, g dry wt
0 (control)	90.00 ± 4.30 ^a	5.60 ± 1.07 ^a	18.59 ± 2.20 ^a	0.72 ± 0.051 ^a	0.38 ± 0.015 ^a
1	88.33 ± 3.19 ^a	4.28 ± 0.81 ^a	9.84 ± 0.98 ^b	0.55 ± 0.050 ^{ab}	0.37 ± 0.028 ^a
2	86.67 ± 2.72 ^a	3.58 ± 1.61 ^{ab}	8.90 ± 0.98 ^b	0.49 ± 0.064 ^{bc}	0.34 ± 0.017 ^a
4	81.67 ± 1.67 ^b	0.51 ± 0.52 ^b	3.50 ± 1.38 ^c	0.22 ± 0.037 ^c	0.15 ± 0.01 ^b

Values represent the mean ± SD (*n* = 3). Data was subjected to tukey’s post hoc test. Different letters represent significant difference at *P* ≤ 0.05 levels.

Table 2. Effect of EMF-r exposure at different time intervals on the electrolyte leakage, phenolic, MDA and H₂O₂ content in chickpea (*Cicer arietinum* L.) seedlings

Treatment, h	Electrolyte leakage, %	Phenolic content, µg/mg fr wt	MDA content, nmol/g fr wt	H ₂ O ₂ content, nmol/g fr wt
0 (control)	55.27 ± 3.23 ^b	5.18 ± 0.28 ^a	82.26 ± 3.42 ^c	4.71 ± 0.29 ^{ab}
1	64.93 ± 0.84 ^a	5.28 ± 0.18 ^a	90.00 ± 2.32 ^{ab}	6.43 ± 0.86 ^a
2	79.54 ± 2.10 ^a	6.22 ± 1.53 ^a	92.90 ± 1.65 ^{ab}	1.79 ± 0.36 ^b
4	84.12 ± 1.33 ^a	6.25 ± 0.94 ^a	113.23 ± 3.55 ^a	2.14 ± 0.03 ^b

Values represent the mean ± SD (*n* = 3). Data was subjected to tukey’s post hoc test. Different letters represent significant difference at *P* ≤ 0.05 levels.

MPI (Supplementary Table S1). The prolonged exposure to EMF-r causes membrane damage, which ultimately leads to MDA accumulation (*r* = 0.649) [3]. MDA, produced on account of peroxidation of membrane phospholipids, is a direct indicator of the membrane damage, where MDA levels increased on account of EMF-r exposure in mung bean seedlings [27, 28].

The content of photosynthetic pigments is directly proportional to the vigour of seedlings that is elucidated in terms of germination parameters. A very strong and significant correlation was observed between the content of Chl *a* and the percent of germination (*r* = 0.74), fresh weight (*r* = 0.73), dry weight (*r* = 0.79) and vigour index (*r* = 0.75) of seedlings. However, prolonged 4-h exposure to EMF-r damages the photosynthetic apparatus and induces stress-like conditions. The negative significant correlation of Chl *a* and total chlorophyll content with MPI (*r* = -0.65; *r* = -0.63 respectively) is a clear evidence of the stress injury. These results correlate with the data reported for *Robinia pseudoacacia* [29], where the reduction in Chl *a* and Chl *b* content was observed after 3–8 h exposure to EMF-r.

ROS generated in the form of hydroxy radical, superoxide ions, singlet oxygen and H₂O₂ content, are accelerated on account of stress conditions [30]. Exposure to 1-h EMF-r increased the production of ROS that is elucidated in terms of H₂O₂ content. However, H₂O₂ content reduced fairly after 2 and 4-h of EMF-r exposure. It is postulated that H₂O₂ at low

endogenous levels acts as signaling molecule [31] that further upregulated the defensive antioxidative system in terms of SOD and POD. A significant increase in the antioxidative defense status, i.e., SOD (*r* = 0.854) and POD (*r* = 0.734) against MDA accumulation was quite evident at a 4-h of exposure to EMF-r. The upregulation in SOD activity further converts the oxygen radicals into H₂O₂ (*r* = -0.611) and then POD acts on the substrate and decomposes into non-lethal water and oxygen bi-products [28].

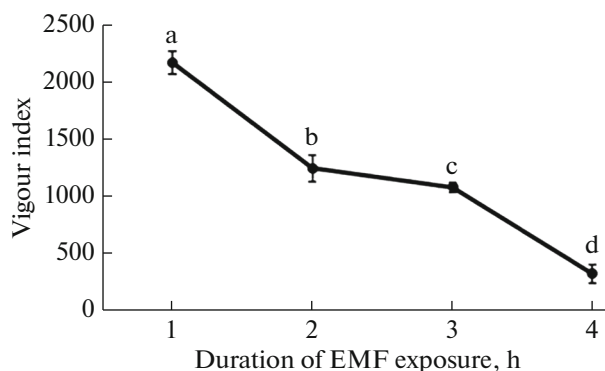


Fig. 2. Effect of 2850 MHz EMF-r exposure on vigour index of chickpea (*Cicer arietinum* L.) seedlings after being exposed for different time durations. Values represent the mean ± SD (*n* = 3). Data was subjected to tukey’s post hoc test. Different letters represent significant difference at *P* ≤ 0.05 levels.

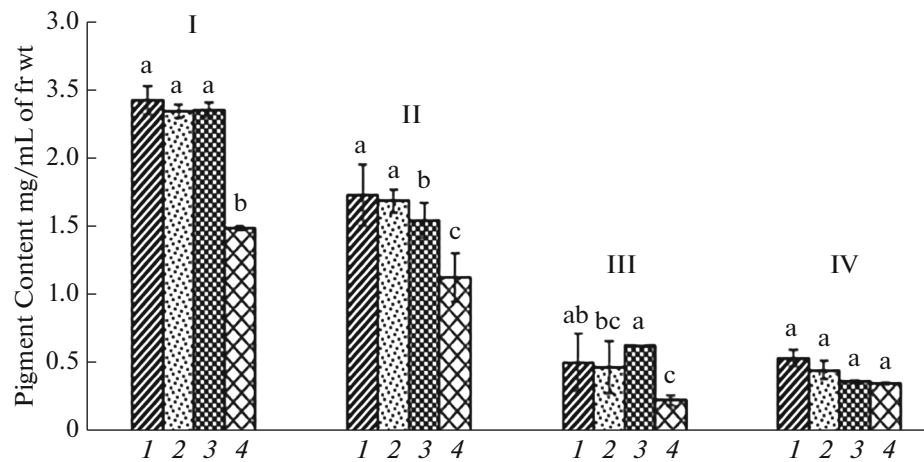


Fig. 3. Effect of 2850 MHz EMF-r exposure of different time durations on the pigment content of chickpea (*Cicer arietinum* L.) seedlings, where (1) 0, (2) 1; (3) 2 and (4) 4 hours of EMF-r exposure; I – total chlorophyll; II – chlorophyll *a*, III – chlorophyll *b* and IV – carotenoids content. Values represent the means \pm SD ($n = 3$). Data was subjected to tukey's post hoc test. Different letters represent significant difference at $P \leq 0.05$ levels.

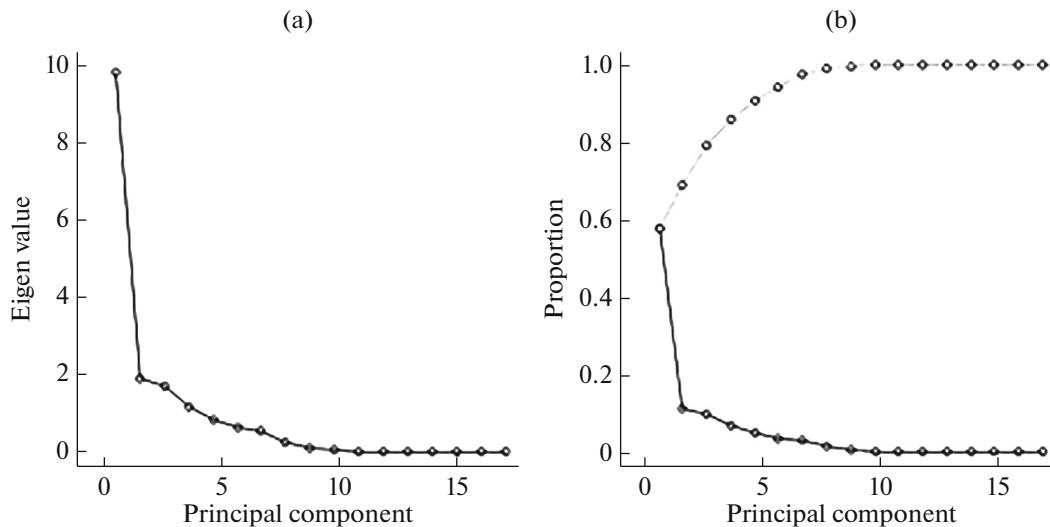


Fig. 4. Screen plot analysis of the principal component with the eigenvalues (a) and proportion (b) on account of EMF-r exposure in chickpea (*Cicer arietinum* L.) seedlings. Dotted lines indicate cumulative proportion of eigenvalues.

Table 3. Effect of EMF-r exposure at different time intervals on the SOD and POD activity in chickpea *Cicer arietinum* L. seedlings

Treatment, h	SOD EU/mg protein	POD Kat/s/mg protein
0 (control)	34.60 \pm 3.42 ^d	106.06 \pm 5.50 ^c
1	67.66 \pm 6.37 ^c	102.27 \pm 3.35 ^c
2	113.53 \pm 6.94 ^b	123.39 \pm 5.45 ^{ab}
4	166.82 \pm 2.26 ^a	148.21 \pm 5.51 ^a

Values represent the mean \pm SD ($n = 3$). Data was subjected to tukey's post hoc test. Different letters represent significant difference at $P \leq 0.05$ levels.

The variables in each group were clustered in the form of a selection index via principal component analysis as depicted in Supplementary Fig. S1 and Table S2. Based on the PCA, PC1 in all character groups had a high proportion variance (PV) value, i.e., above 57.8% (0.578). The optimization in the representative PC assessment is based on the cumulative proportion which reaches up to 0.8 [32–34]. Analysis of the values of PC1 (PV vs CP standard) the use of PC1 was considered as selection index to sufficiently represent the variance of tolerance properties against EMF-r exposure (Fig. 4). The eigen vector values on PC1 were also need to be corrected by multiplying the

eigenvector for each PC1 to the PV value (0.578) as mentioned in Supplementary Table S2.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at <https://doi.org/10.1134/S1021443722060310>.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. This article does not contain any studies involving animals or human participants performed by any of the authors.

AUTHOR CONTRIBUTIONS

Dr. D. Batish provided the lab facility, chemicals and designed the experiments. Dr. N. Johal and A. Pal helped in the execution of experiment, biochemical analysis and collection of data. Dr. S. Chandel and M. Pal helped in writing the manuscript, statistical analysis and proofreading of the manuscript.

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