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Electromagnetic Irradiation Induced Residual Physiological Effects in Gram Plant

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ABSTRACT:

Plants growing in open environment are exposed to uninterrupted electromagnetic radiation. In general, plant tissues are capable of absorbing significant amount of incident electromagnetic energy because of high permittivity and loss tangent. Over the last decade, electromagnetic energy absorption rates in terms of 'Specific Absorption Rate (SAR)' have been quantified for a number of fruits, flowers and plant models. However, one-time controlled electromagnetic irradiation induced residual physiological plant responses have rarely been reported in literature. Hence, this article aims at investigating the residual physiological effects of one-time (2 hours 30 minutes) controlled electromagnetic radiation at 2.45 GHz on 24 days old gram plant enzyme activities, chlorophyll contents, reactive oxygen species (ROS) generation, lipid peroxidation and cell death. Gram seeds and subsequently germinated plants have been chosen for this investigation because of easy availability and viable germination at any season round the year in Indian climate condition. Targeted gram plants have been exposed to 2 hours 30 minutes long controlled electromagnetic irradiation at 2.45 GHz at the age of 14 days after germination inside an anechoic chamber. Next, those target gram plants have been grown without further electromagnetic exposure for an incubation period of 10 days – in parallel, control gram plants have also been grown in an electromagnetic quiet zone. Obtained results indicate irradiation induced residual physiological stress conditions in gram plant with respect to control samples – cellular damage through reactive oxygen species (ROS) generation, reduced concentrations of chlorophyll a and chlorophyll b along with enhanced plant defense signaling mechanism.

1. Introduction

The amount of electromagnetic radiation that plants and people are exposed to today has multiplied significantly due to the rapid installation of sophisticated wireless communication infrastructure across a variety of frequency bands. It is well known that since the component tissues of both people and plants have high permittivity (ε_r') and conductivity (σ), a considerable portion of incoming electromagnetic energy is absorbed by them [1–10]. Additionally, the "Specific Absorption Rate (SAR)," which is stated in W/kg, has been used to describe the subsequent electromagnetic energy absorption rates in biological things, including people and plants [2&11–12]. Literature has comprehensive mathematical expressions for SAR [13]. It should be mentioned in relation to this work that many studies on the spatial SAR distributions for various fruit, flower, and plant models have been published [2&14–15]. In accordance with national and international electromagnetic regulatory guidelines, a considerable amount of SAR data have been submitted [16–17].

Because they often have a higher surface-to-volume ratio than most other living things, plants are a great choice for studying the biological reactions that electromagnetic radiation causes and the impacts that follow [18,19,20,21]. Studies on the physiological and chemical reactions that plants experience when exposed to electromagnetic radiation on a regular or one-time basis have been conducted throughout the last two decades [22–23]. Because it instantly changes plant gene expressions that are susceptible to stress, controlled

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electromagnetic exposure is thought to be analogous to wounding [18,20, 22&23]. Nevertheless, none of the publications discussed how electromagnetic radiation had lingering physiological impacts in plants. i.e., all published research on the subject has shown instantaneous reactions in plants to either one-time or repeated electromagnetic radiation [18-28]. Furthermore, since target plant specimens were not exposed to regulated electromagnetic fields, a number of earlier investigations were unable to provide definitive findings. Therefore, the purpose of this research is to investigate the physiological effects that remain on 24-day-old gram plants after they are exposed to electromagnetic radiation one time, at the age of 14 days after seed germination. First, the targeted gram seedlings have been grown for 14 days in an electromagnetic quiet zone along with the control gram seedlings. Next, the targeted 14 days old gram seedling have been exposed to 2.45 GHz, non-ionizing controlled electromagnetic radiation for 2 hours and 30 minutes inside an anechoic chamber. Thereafter, this electromagnetic radiation treated gram seedlings have been grown further for additional 10 days. In parallel, the control seedlings have been grown in an electromagnetic quiet environment for the entire duration. This work examines the residual physiological effects of 2.45 GHz non-ionizing controlled electromagnetic irradiation on gram plants. These effects include changes in the activities of the enzymes peroxidase (PO) and phenylalanine ammonialyase (PAL), changes in total phenol content, modifications to the plant defense signaling mechanism, decreases in the concentrations of chlorophyll a and chlorophyll b, generation of reactive oxygen species (ROS), modifications to membrane lipid peroxidation, and plant cell death. To carry out these investigations, a number of sequential electromagnetic exposure and biological investigation protocols have been followed – detailed descriptions have been outlined in the following sections. Obtained results indicate several residual physiological effects in gram plant following above mentioned controlled electromagnetic irradiation.

2. Investigation Setup and Exposure Protocol

Gram seeds and subsequent plants were used in this experiment because of their rapid cultivated rate in Indian environment, which makes them commonly farmed. One hundred such healthy, fresh gram seeds were selected, and the seeds were split evenly between two clay pots with soil that had been fertilized. Subsequently, the gram plant seedlings were allowed to germinate and develop in an electromagnetic quiet zone for a period of 14 days. Figure 1 shows the experimental setup for the exposure purpose, which includes a power meter, an RF power source, and a computer for data recording. Figure 2 shows the image of the gram plant sapling.

Figure 1. Experimental Setup.

Figure 2 Gram Plant saplings.

Next, at the age of 14 calendar days, targeted gram saplings have been taken inside an anechoic chamber and exposed to 2.45 GHz controlled one-time electromagnetic radiation for 2 hours and 30 minutes – whereas, the control gram saplings have been kept in electromagnetic quiet zone under similar conditions except radiation treatment at 2.45 GHz. The targeted gram saplings have been exposed to 2.45 GHz electromagnetic radiation using a portable radio frequency (RF) signal generator (12.08 dBm output power at 2.45 GHz), computer, RF cable (2.08 dB insertion loss) and standardized transverse electromagnetic (TEM) horn antenna (12 dB gain at 2.45 GHz). The targeted 14 days old gram saplings have been placed in far-field of the horn antenna (1.5 m apart)

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inside the anechoic chamber. Figure 3 illustrates targeted gram saplings exposure setup inside anechoic chamber.
Once the 14 days old gram saplings have been irradiated for two hours and 30 minutes, both target and control gram plants have further been grown for 10 days incubation period in an electromagnetic quiet zone. Twenty-four (24) days old electromagnetic radiation exposed (right side) and normally grown (left side) gram plant saplings are illustrated in Figure. 4.

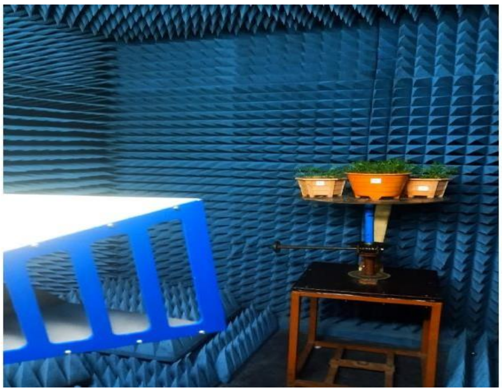

Figure 3. Targeted gram saplings exposed to one-time electromagnetic radiation at 2.45 GHz inside an anechoic chamber.

Figure 4. Gram saplings are normally grown on the left side whereas the 2.4 GHz radiation exposed gram saplings are on the right side at 24 days age (i.e. 10 days post one-time electromagnetic irradiation at 2.45 GHz)

3. Plant specimen preparations for estimating physiological responses

This section describes different specimen preparations and subsequent methodologies for estimating above mentioned 2.45 GHz one-time electromagnetic irradiation induced physiological effects in 24 days old gram saplings. Biochemical protocols for estimating peroxidase (PO) and phenylalanine ammonialyase (PAL) activities, total phenol content, chlorophyll contents, reactive oxygen species (ROS) generation, lipid peroxidation and cell death have been listed in this section.

3.1 Estimation of peroxidase (PO) and phenylalanine ammonialyase (PAL) enzyme activities

Leaf samples of both control and radiation treated plant specimens (24 days old) have been collected measure PO and PAL enzyme activities. 0.1 M phosphate buffer (pH 7.0) and 0.1 M borate buffer (pH 8.7) have been used to crush the leaf samples for estimating PO and PAL activities. PO enzyme activity has been measured following the protocol reported by Hemeda and Klein [29]. Increased absorbance has been monitored at 470 nm. Activity of PO enzyme has been expressed in micromole/min/mg protein. PAL activity has been

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measured as described by Dickerson *et al.* [30] where 12 mM L-phenylalanine, 0.1 M borate buffer and enzyme extract have been used to prepare the reaction mixture. OD value has been taken at 290 nm. The enzymatic activity has been expressed in nmol of trans cinnamic acid/min/g protein.

3.2 Estimation of total phenol content

Total phenol has been measured following the method reported by Zieslin and Ben Zaken [31]. Leaf tissue has been crushed in 80% methanol and kept at 65°C for 15 minutes. After centrifugation, the supernatant has been mixed with distilled water and 1 N Folin Ciocalteu reagent. After incubation, absorbance has been measured at 725 nm. Measured phenol content has been expressed in µg gallic acid/ g fresh tissue.

3.3 Estimation of chlorophyll contents

Chlorophyll extraction has been performed using 80% acetone as per the protocol reported by Arnon*et al.* [32]. After centrifugation (8000× g for 10 minutes), absorbance of supernatant has been measured at 645 nm and 663 nm. Chlorophyll a and chlorophyll b contents have been expressed in mg/ L.

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3.4 Visualization of reactive oxygen species (ROS) generation, lipid peroxidation and celldeath

ROS has been visualized using DCFDA (6.25 µM) staining from leaf samples – as described by Watkins *et al.* [33]; images have been taken in a Floid Cell Imaging Station microscope (Life Technologies) under green filter. Qualitative assay of lipid peroxidation has been performed using Schiff's Reagent. Histochemical detection of cell death has been performed using the protocol reported by Sarkar *et al.* [34]. Furthermore, Trypan blue staining has been performed to check the extent of cell death.

4 Result and Discussions

In this study, 24-day-old gram plants were exposed to 2.45 GHz electromagnetic irradiation to investigate any lingering effects on various enzyme activities, chlorophyll levels, ROS production, lipid peroxidation, and cell death. Different enzyme activities and gene expression were changed by electromagnetic radiation therapy (Kouzmanova et al. 2009; Vian et al. 2006; Jangid et al. 2010) [1921]. To the best of our knowledge, this is the first publication describing the biological impacts of electromagnetic radiation that persist in gram plants even after a 10-day incubation period. Plant samples was taken for a specific study from one gram plant specimen that had been exposed to electromagnetic radiation in pairs with a randomly chosen control gram plant specimen at each time point. Every single research has been carried out three times (n=3) under precisely the identical circumstances. The gram plant specimens included in these studies were slaughtered as soon as the samples were collected. The data show the mean ± standard error of the mean (SEM) from three independent experiments performed for replication.

4.1 Effect of electromagnetic radiation on peroxidase (PO) enzyme activity

In this investigation, 24-day-old gram plants treated with 2.45 GHz radiation showed decreased defense enzyme and antioxidant activity. After 10 days of incubation after a single electromagnetic irradiation, gram plants treated with electromagnetic radiation showed a nearly 10% decrease in PO activity compared to the control plants. For further information, please see Table 1. According to Sarkar et al. (2021) [34], PO enzyme is engaged in both plant defense and ROS scavenging. According to previous reports, 30 minutes of UV radiation increased the PO activity in soft wheat seeds by 25.9%; however, longer UV treatment decreased PO activity (Sharlaeva and Chirkova 2021) [24], suggesting a strengthening of the plant's defensive system under stress. It seems that 2.45 GHz electromagnetic radiation is seen by gram plants as an abiotic stress factor.

Table 1. Effect of electromagnetic radiation in treated plants over control plants

Studied Parameters	**Control**	**Treatment**
PO (micromol/ min/mg protein)	22.55 ± 0.543	20.16±0.699
PAL (nmol of trans-cinnamicacid/min/g protein)	220.69 ± 1.91	231.45 ± 1.65

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Total Phenol (µg gallic acid/ g freshtis-sue)	450.13 ± 10.95	584.75 ± 12.96
Chlorophyll a (mg/ L)	25.18 ± 0.369	22.73 ± 0.646
Chlorophyll b (mg/ L)	12.08 ± 0.236	7.82 ± 0.270

4.2 Effect of electromagnetic radiation on enylalanine ammonialyase (PAL) enzymeactivity and total phenol content

In comparison to the PO activity, another key defense enzyme in phenyl propanoid pathway namely PAL has shown induced activity among 2.45 GHz electromagnetic radiation treated plants over the control plant specimens. Additionally, compared to the control specimen, the aforementioned radiation treatment produced a 1.3-fold increase in total phenol content (please see Table 1). It is well recognized that phenolics play a role in plant defense; PAL, the primary enzyme in the phenyl propanoid pathway, is also engaged in a number of other plant biosynthetic activities (Sarkar et al., 2021), [34]. Hence, long-lasting increment in PAL and phenolics following one-time electromagnetic irradiation (2.45 GHz) indicates towards strengthening plant defense mechanism under stress condition. Thus, it seems that gram plant perceives 2.45 GHz electromagnetic radiation as an abiotic stress factor.

4.3 Effect of electromagnetic radiation on chlorophyll content

Chlorophyll, an important photosynthetic pigment, has shown significant reduction in 2.45 GHz electromagnetic radiation treated gram plants. Concentrations of both chlorophyll a and chlorophyll b have been reduced following the one-time electromagnetic radiation treatment – even after 10 days incubation period. In contrast to 10% reduction in concentration of chlorophyll a, chlorophyll b has shown a significant reduction of 35% among electromagnetic radiation treated gram plants (with respect to control plant specimens) – please, refer to Table 1 and Figure 5a. & 5b. The decrease in chlorophyll levels in gram plants caused by electromagnetic irradiation is consistent with other research that has been published in the literature (Salama et al. 2011) [25]. In this regard, it should be mentioned that plant chlorophyll a, chlorophyll b, and total chlorophyll concentrations are reduced even in the presence of ultra violet (UV) radiation (Salama et al. 2011) [25]. Thus, electromagnetic radiation seems to have detrimental effects on chlorophyll contents in gram plants.

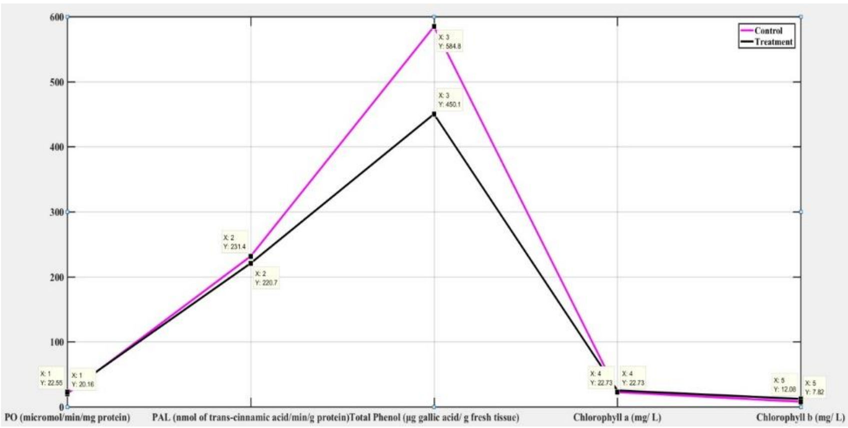

Figure 5a. Graph showing difference in concentration of chlorophyll a, chlorophyll b amongelectromagnetic radiation treated gram plants with respect to control plant specimens

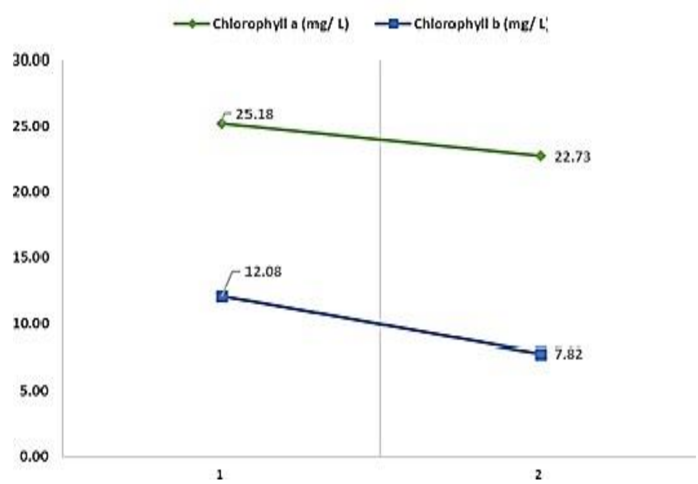

Figure 5b. Comparison between the Chlorophyll Content

4.4 Effect of electromagnetic radiation treatment on reactive oxygen species (ROS)generation

ROS production generally contributes to the signaling cascade that plants utilize to defend themselves, but too much ROS production may harm cells. In order to verify ROS formation, DCFDA staining was used in this experiment. The leaves of gram plants exposed with 2.45 GHz electromagnetic radiation exhibited more ROS specific fluorescence in comparison to the leaves of control plants (Figure 6). According to research reviewed by Tan et al. (2023) [26], radiation therapy has also been shown to change the ROS concentration of plants. According to observations, plants see electromagnetic radiation at 2.45 GHz as an external stressor and produce more ROS to bolster their defensive signaling mechanisms.

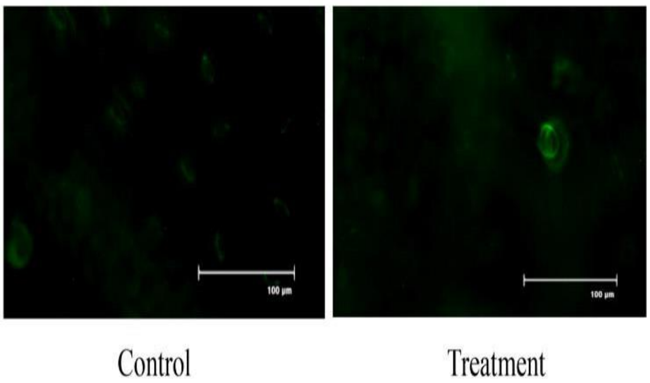

Figure 6. Effect of 2.45 GHz electromagnetic radiation treatment on reactive oxygen species (ROS) generation.

4.5 Effect of electromagnetic radiation treatment on lipid peroxidation

Membrane lipid peroxidation indicates the degree of membrane damage and is directly associated with excessive ROS production. According to the overproduction of ROS, lipid peroxidation has been shown to be more prominent in plants exposed with 2.45 GHz electromagnetic radiation than in control specimens. Figure 7 illustrates

the higher lipid peroxidation specific coloring in the radiation-treated plants indicated above, as determined by qualitative lipid peroxidation quantification using Schiff's reagent. higher ROS generation has previously been linked to increased lipid peroxidation (Sarkar et al., 2021) [34]. Results from observations show that 2.45

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GHz Damage to membranes by electromagnetic radiation is harmful to plants' health.

Figure 7. Effect of 2.45 GHz electromagnetic radiation treatment on lipid peroxidation

4.6 Effect of electromagnetic radiation treatment on cell death

It is known that higher ROS generation is involved to cellular deterioration. Trypan blue staining of cell death has shown more cellular damage specific blue coloration in 2.45 GHz electromagnetic radiation treated gram plant leaves with respect to control plants (Figure 8).

Figure 8. Effect of 2.45 GHz electromagnetic radiation treatment on lipid peroxidation

5. Conclusions

To the best of our knowledge, one-time hours-long 2.45 GHz electromagnetic irradiation induced residual physiological effects in 24-day-old gram plants have been reported for the first time in this article. Reported residual plant physiological effects in 24-day-old gram plants have been reported at an irradiation level (2.45 GHz). Cellular damage through reactive oxygen species (ROS) generation, membrane damage linked to peroxidation of membrane lipids, reduced chlorophyll a and chlorophyll b concentrations, increased plant defense signaling mechanism (increment in PAL enzyme activity and total phenol content) along with increased plant cell death due to one-time 2.45 GHz, electromagnetic irradiation clearly indicate that plants in general sense electromagnetic radiation as an abiotic stress factor [27]. Long-lasting degradation in plant photosynthetic pigment concentrations (chlorophyll a and chlorophyll b) marks the photosynthetic impairment following one-time electromagnetic irradiation at 2.45 GHz. Current findings suggest that exposure to moderate or even weak electromagnetic radiation induces residual physiological stress in 24-day-old gram plants. Reported residual physiological effects in 2.45 GHz electromagnetic radiation treated 24- day-old gram plant also endorse past reported molecular effects in rice plants due to one-time or periodic irradiation [22]. It should be noted that residual physiological or molecular plant responses under electromagnetic irradiation at multiple frequencies can be more prominent – however, the same needs to be tested on a larger scale. Only then, physiological and molecular effects in plants due to different electromagnetic exposure levels (frequency, radiated power density, time duration and so on) can better be understood. Altogether, findings in this article demand for re-evaluating the current global and national electromagnetic exposure guidelines to better protect plants from detrimental effects of electromagnetic radiation.

Statements and Declarations

The authors do not have any competing interest and all of them have agreed to submit them manuscript for publication.

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