Investigating the Long–Term Exposure Effects of 2850 MHz EMF-r on Growth, Physiology and Carbohydrate Metabolism of *Triticum aestivum* L.

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Abstract

In recent decades, the widespread adoption of wireless electronic devices, notably cell phones, has led to a considerable rise in environmental electromagnetic field radiations (EMF–r). The EMF-r has potential to interact and interfere with the living organisms. Therefore, it is essential to examine how these radiations affect biological systems, including plants which are immobile in nature and may remain continuously exposed to these radiations. The present study investigated the long-term exposure effects of 2850 MHz EMF–r on 30 and 60–days old wheat (*Triticum aestivam* L.) crop, focusing on their growth, photosynthetic pigments, and carbohydrate metabolism. The findings of the study revealed that the EMF–r exposure leads to a decline in root length, shoot length, and plant biomass along with a marked reduction in photosynthetic pigments. Moreover, a significant reduction in carbohydrate–metabolizing enzyme activity, water–soluble carbohydrate, and reducing sugars content was observed in EMF–r irradiated samples. The study suggests that EMF–r exposure adversely affects the plant growth, photosynthetic pigments, and carbohydrate metabolism, highlighting the need for effective management to prevent productivity losses.

Introduction

Electromagnetism and gravity are two fundamental forces that have played a pivotal role in the emergence of life on Earth, and they are believed to have ongoing effects on all living organisms.[1] Before the 1990s, sources of electromagnetic field radiations were limited, primarily consisting of a few radio and television transmitters. However, the past couple of decades have observed an extraordinary outburst of technological advancements, with global communication in particular experiencing rapid growth.[2] Since the 1990s, the advent of wireless communication has led to a significant surge in the usage of cell phones, resulting in a substantial increase in the presence of electromagnetic pollution.[3]

The electromagnetic spectrum encompasses ionizing radiations, such as gamma and X–rays, which have high frequency and short wavelengths, as well as non–ionizing radiations, like radio and microwaves, which have low frequency and longer wavelengths. Over time, with technological advancements and increased communication sources, the radio frequencies used have also evolved. Initially, mobile phones were analog and operated in the 450–900 MHz range. However, with progress, digital mobile phones with higher frequencies like 1800–1900 MHz (2G), 2100 MHz (3G), 2300–2600 MHz (4G), and more have been developed. The deployment of 5G networks, in particular, has attracted attention and debate. While 5G promises faster data transmission speeds and reduced latency, concerns have been raised regarding its potential health effects due to increased EMF exposure.[4] The World Health Organization (WHO) has documented radio frequency (RF) electromagnetic radiations as possibly carcinogenic in nature.[5]

Electromagnetic field radiations (EMF–r) have been extensively studied for their effects on various living organisms, including animals, humans, birds, micro–organisms, and plants.[6,7,8,9,10] While many scientists worldwide are researching the impact of EMF–r on animals, there has been limited attention given to plants.[11] Plants play a vital role in our environment as primary producers, converting inorganic nutrients into organic compounds and serving as a major source of oxygen. Consequently, it is of paramount importance to evaluate the effects of radio frequency (RF) EMF radiation on plants. Unlike mobile organisms, plants are immobile, which means they are continuously exposed to EMF–r stress, making this research area crucial for understanding the potential impacts on the foundation of our ecosystems.

RF-EMF–r has been reported to alter the growth, development and the activity of numerous enzymes, including those involved in reactive oxygen species (ROS) metabolism.[12] Decline in germination percentage, seedling vigor index, and germination rate in *Trigonella foenum–graecum* L. upon exposure of 900 MHz EMF–r.[13] Similarly, RF exposure also inhibits and delayed seed germination in *Cicer arietinum* L., and *Phaseolus Aureus* Roxb., respectively.[14,15] In response to 900 MHz EMF-r exposure for 0.5, 1, 2 and h, rhizogenesis is severely impacted in *Vigna radiata* (L.) R. Wilczek along with elevation in the specific activities of stress related enzymes.[3] Further, it was reported that EMF-r exposure at 900 MHz on tomato resulted in transient decrease in the ATP content and adenylate energy charge (AEC) after 30 minutes of electromagnetic treatment.[16] Given the widespread and continuous use of technology that emits EMF–r, it is essential to understand the long–term effects of EMF–r exposure on human health and the environment.[17] However, most of the studies conducted on exposure of EMF-r to plants are of short-term in nature. Moreover, study on EMF-r exposure to wheat crop growth, eco-physiology and carbohydrate metabolism is limited. Being a widely grown staple crop of the country, it is imperative to explore the impact of long-term exposure of EMF-r on the wheat crop growth and carbohydrate metabolism. In the current study, we observed the long-term exposure effects of EMF-r over a period of 30 and 60 days to investigate their impact on the growth, morphology, photosynthesis and carbohydrate metabolism of *Triticum aestivum* L. The hypothesis of the study was that the long-term exposure of EMF-r to wheat crop will reduce the growth by hampering the eco-physiological and metabolic activities. The objectives of the study were to (i) explore the response of crop growth and photosynthetic pigments, and (ii) carbohydrate metabolism of wheat crop exposed to 2850 MHz EMF–r for 30 and 60 days.

Materials and Methods
Plant Material
Seeds of *T. aestivum* variety "SHARBATI–MP" were surface sterilized with sodium hypochlorite solution (NaOCl, 0.1%, w/v) and then rinsed with distilled water. Three replications of each treatment were used, with one seed sown in each pot (~650 grams of sandy loam soil procured from a local nursery, Sector-26A, Chandigarh, India; soil pH= 6.5) in a completely randomized design (CRD). Plant samples from each treatment were stored at –20 ºC. All the chemicals and reagents were purchased from Sigma and Loba Chemie.

EMF–r treatment
A vector signal generator (SMBV100A; R & S, Germany) within frequency range of 9 kHz—3.2 GHz was used to generate the radio frequency (RF) signals. An antenna and a radio frequency power amplifier (ZFL–2500+; Minicircuits, USA)

were equipped with the RF signal generator. Plant samples were subjected to 2850 MHz EMF–r on every alternate day for 30 and 60 days (30 min per day) in an EMF–shielded chamber. A radiation field meter (NBM 550) was used to measure the power flux density.

Plant Morphology and Biomass

After 30 and 60 days of exposure to 2850 MHz EMF–r, plant samples were harvested. Using a measuring ruler, the root and shoot lengths were measured and recorded in centimeters (cm). The biomass of the plant samples was measured with the help of a weighing balance after oven–drying the seedlings at 60 ºC for 72 h and the results were expressed in gram (g).

Total Chlorophyll and Carotenoid Content

The photosynthetic pigment concentration was measured as per Hiscox and Israelstam.[18] Briefly, 25 mg of leaf tissue was added to 4 ml of dimethyl sulfoxide (DMSO) and incubated at 60 ºC for 1 h. Absorbance was read at 470 nm, 645 nm, and 663 nm using a Shimadzu double beam spectrophotometer (UV–1800, 240 V, Shimadzu Corporation, Kyoto, Japan). Following that, the chlorophyll and carotenoid content were determined and represented on a dry weight basis.[19,20,21]

Total Water–Soluble Carbohydrates and Reducing Sugars Content
Extraction

Twenty mg of plant tissue was homogenized in 2 ml of distilled water and centrifuged at 15,000 g for 20 min at 4 ºC using a cold centrifuge (Sigma Inc., USA).

Procedure

Total carbohydrate content was assayed using the Loewus[22] method. To 200 μl of anthrone reagent (0.2 g in 100 ml of H_2SO_4), 50 μl of plant extract was added. The intensity of the greenish–blue color was recorded at 620 nm and represented as mg g^{-1} f. wt. against the standard curve prepared with glucose. The methodology provided by Nelson[23] was employed to determine the reducing sugars content. To 1 ml of reaction mixture (sodium carbonate, sodium sulphate, sodium potassium tartrate, and $CuSO_4.5H_2O$), 100 μl of plant extract, and 40 μl of distilled water were added. Samples were subjected to incubation for 20 min in hot water bath, after which 600 μl of ammonium molybdate and H_2SO_4

were added. Absorbance was measured at 520 nm against standard curve prepared with glucose, and presented as μg mg^{-1} f. wt.

Acid and Alkaline Phosphatase Activity

Twenty mg of plant tissue crushed in 2 ml of 50 mM tris–maleate buffer and 13 mM mercaptoethanol (pH = 7.0), and centrifuged at 15,000 g (20 min, 4 ºC). The methodology described by Malik and Singh[24] was used to assay acid and alkaline phosphatases activity using 0.3 ml of 0.1 M acetate buffer (pH = 5.0) and glycine sodium hydroxide buffer (pH = 10.5), respectively. To 50 μl of 0.2 M para–nitrophenyl phosphate (p–NPP) and 150 μl of enzyme extract were added. Samples were incubated at 37 ºC for 10 min, followed by addition of 2 ml of 0.3 M sodium carbonate solution. The absorbance was measured at 400 nm against the *p*–nitrophenol standard curve, and represented as μg min^{-1} mg^{-1} protein.

Acid and Alkaline Invertase Activity

Twenty mg of plant tissue was crushed in 2 ml of 50 mM tris–maleate buffer containing 20 mM $MgCl_2$ and 1 mM mercaptoethanol (pH = 7.5), and centrifuged at 15,000 g for 20 min at 4 ºC. Acid and alkaline invertase activities were evaluated as per the Nelson[23] using 400 μl of 0.2 M sodium acetate buffer (pH = 4.8) and sodium phosphate buffer (pH = 8.0), respectively. After the addition of 100 μl of enzyme extract, samples were incubated at 45 ºC for 15 min. Absorbance was recorded at 620 nm after addition of 250 μl of Somogyi's reagent and 250 μl of Nelson's reagent against a calibration standard of glucose. The enzyme activities were represented as μg min^{-1} mg^{-1} protein.

α and β–amylase Activity

Twenty mg of plant tissue was crushed in 2 ml of 0.1 M sodium phosphate buffer (pH = 7.0) and centrifuged at 15,000 g (25 min, 4 ºC). α–amylase activity was assayed as per the methodology given by Muentz.[25] To 0.25 ml of enzyme extract, 0.5 ml of substrate solution (starch, potassium dihydrogen phosphate and calcium chloride), 50 μl EDTA and 1.5 ml of iodine solution were added. Absorbance was recorded at 630 nm using starch as standard, and represented as μg min^{-1} mg^{-1} protein. β–amylase activity was examined as per the Bernfeld[26] and later modified by Dure.[27] To 0.25 ml of enzyme extract, 0.35 ml of substrate solution, and 50 μl of

0.1 M EDTA were added. Samples were incubated at 30 ºC for 30 min, followed by the addition of 0.5 ml of dinitrosalicylic acid (DNSA). Absorbance was recorded at 560 nm using maltose as standard and the results were represented as µg min^{-1} mg^{-1} protein.

Statistical Analysis
The student's *t* test was carried out using SPSS (version 16) to determine the significant differences between control and treatment groups at various levels of significance (P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001). The data are presented as mean ± SE (standard error) of three replicates.

Results
EMF–r Impact on the Growth and Photosynthetic Characteristics
The findings of the long-term exposure of EMF–r on root and shoot length, biomass, and photosynthetic properties are summarized in Table 1. A significant (P ≤ 0.05) reduction by ~22 and ~26% in root length was observed in samples irradiated with EMF–r for 30 and 60 days, respectively. Similarly, shoot length exhibited a reduction of ~24 and ~29%, respectively, compared to the control counterparts. The biomass of root and shoot showed a decline of ~33 and ~17% in 30–days old plants and ~40 and ~20% in 60–days old plants, respectively, when exposed to EMF–r. Furthermore, the chlorophyll and carotenoid contents in treated samples exhibited a significant (P ≤ 0.05) reduction of ~24 and ~20% at 30 days, and ~31 and ~34% in 60–days old plants, respectively, compared to their control counterparts.

Table 1: Effect of 2850 MHz EMF-r on the root and shoot length, biomass, and photosynthetic activity of *T. aestivum* plants harvested after 30 and 60 days.

Parameter studied	Treatment group		*Triticum aestivum*
Root length (cm)	30 days	C	46.43±2.34
		T	36.30±0.78*
	60 days	C	70.57±1.98
		T	52.47±1.52**
Shoot length (cm)	30 days	C	33.50±1.50
		T	25.43±0.75**
	60 days	C	60.43±2.54
		T	42.90±2.07**
Root biomass (g)	30 days	C	0.16±0.00
		T	0.11±0.01**
	60 days	C	0.22±0.02
		T	0.14±0.01*
Shoot biomass (g)	30 days	C	0.45±0.02
		T	0.34±0.02*
	60 days	C	0.78±0.03
		T	0.62±0.02**
Total chlorophyll content (mg g^{-1} d.wt.)	30 days	C	12.04±0.22
		T	9.13±0.60*
	60 days	C	11.96±0.70
		T	8.51±0.91*
Total carotenoid content (mg g^{-1} d.wt.)	30 days	C	3.84±0.10
		T	3.09±0.03**
	60 days	C	3.90±0.20
		T	2.65±0.24*

The data are presented as mean ± SE (standard error) of three replicates. Asterisks indicate difference among treatment groups based on student's t test at different levels of significance (P ≤ 0.05*, P ≤ 0.01** and P ≤ 0.001***). C and T represent control and treatment groups, respectively.

EMF–r Impact on Carbohydrate Metabolism

Upon exposure to EMF-r, *T. aestivum* plant samples exhibited a significant decrease (P ≤ 0.05) in the total carbohydrate and reducing sugars content (Fig. 1). Compared to control, the carbohydrate content of EMF–r exposed shoots exhibited a considerable reduction of ~24% and ~30% in shoots and ~21% and ~44% in the roots of the 30 and 60–days old plants, respectively. Similarly, reducing sugar content significantly declined by ~35 and ~33% in shoots, and ~28% and ~42% in roots, respectively. The acid phosphatase activity exhibited a decline of ~28 and ~42%, and ~38% and ~45% in shoots and roots, respectively. However, alkaline phosphatase activity was found to be reduced by ~38 and ~40%, and ~56% and ~65% after 30- and 60-days exposure to EMF-r in shoots and roots, respectively, compared to their control counterparts (Fig. 2).
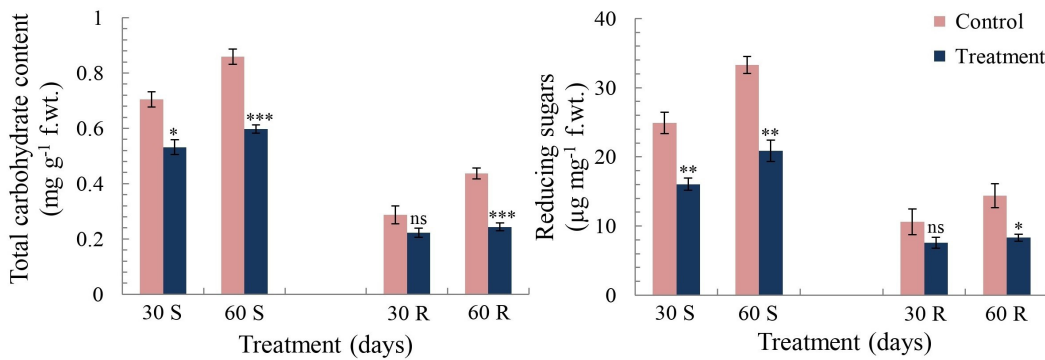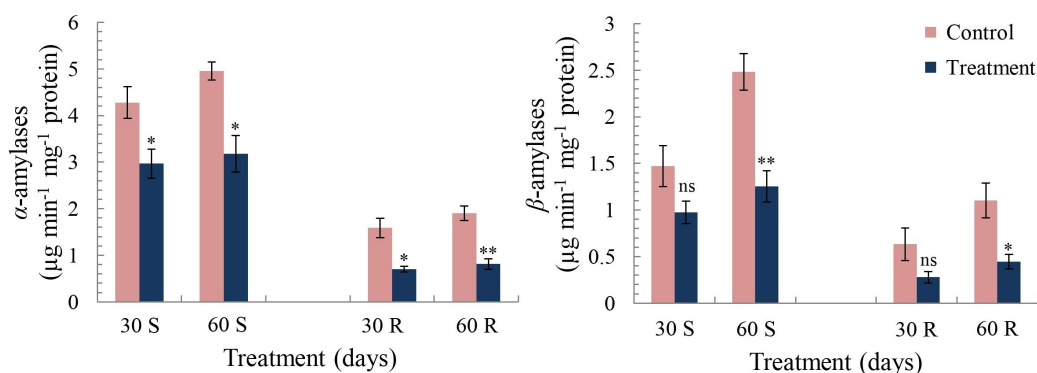

Fig. 1: Total carbohydrate content and reducing sugar content of 30 and 60–days old *T. aestivum* shoots (S) and roots (R) upon exposure of 2850 MHz EMF–r.

30 S and 60 S represent shoot samples, 30 R and 60 R represent root samples harvested after 30 and 60 days, respectively. The data are presented as mean ± SE (standard error) of three replicates. Asterisks indicate difference between control and treatment groups based on student's t test at different levels of significance (P ≤ 0.05*, P ≤ 0.01** and P ≤ 0.001***). 'ns' stands for non-significant differences between control and treatment groups.
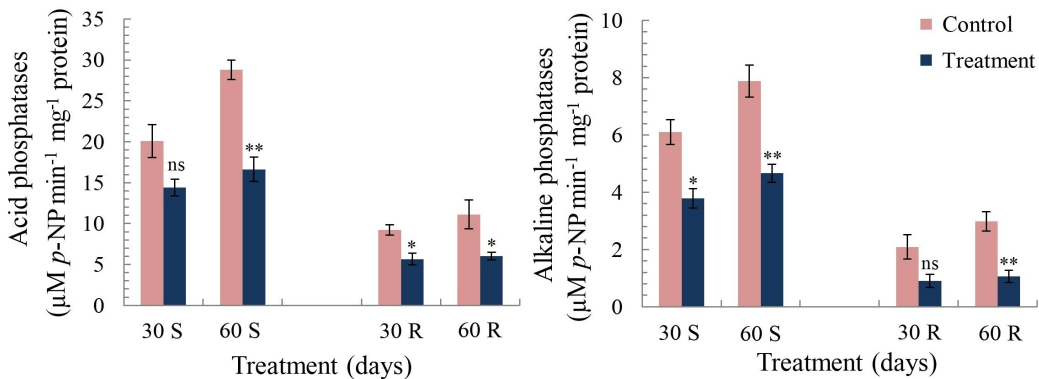
Fig. 2: Acid and alkaline phosphatase activity of 30 and 60–days old *T. aestivum* shoots (S) and roots (R) upon exposure of 2850 MHz EMF–r.

30 S and 60 S represent shoot samples, 30 R and 60 R represent root samples harvested after 30 and 60 days, respectively. The data are presented as mean ± SE (standard error) of three replicates. Asterisks indicate difference between control and treatment groups based on student's t test at different levels of significance (P ≤ 0.05*, P ≤ 0.01** and P ≤ 0.001***). 'ns' stands for non-significant differences between control and treatment groups.
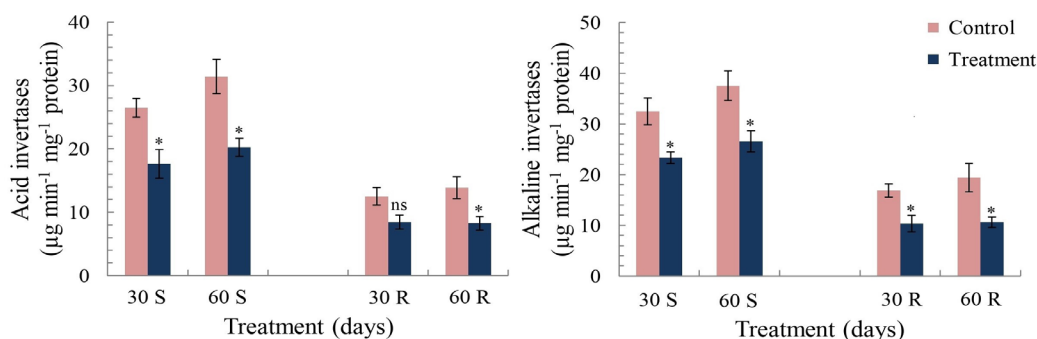
Fig. 3: The specific activity of α–amylase and *β*–amylases enzymes of 30 and 60–days old *T. aestivum* shoots (S) and roots (R) upon exposure of 2850 MHz EMF–r.

30 S and 60 S represent shoot samples, 30 R and 60 R represent root samples harvested after 30 and 60 days, respectively. The data are presented as mean ± SE (standard error) of three replicates. Asterisks indicate difference between control and treatment groups based on student's t test at different levels of significance (P ≤ 0.05*, P ≤ 0.01** and P ≤ 0.001***). 'ns' stands for non-significant differences between control and treatment groups.

The α–amylase activity decreased significantly by ~30 and ~32% in shoots, ~58 and ~53% in roots harvested after 30 and 60 days, respectively. However, β–amylase activity declined by ~34 and ~49% in shoots, and ~57 and ~50% in roots compared to the control (Fig. 3). Furthermore, the activity of acid invertases significantly decreased by ~33 and ~35% in shoots, and ~32 and ~40% in roots, while alkaline invertases activity declined by ~28 and ~25% in shoots, and ~38 and ~42% in roots after 30 and 60 days of irradiation, respectively (Fig. 4).

Fig. 4: The specific activity of acid and alkaline invertases enzymes of 30 and 60–days old *T. aestivum* shoots (S) and roots (R) upon exposure of 2850 MHz EMF–r.

30 S and 60 S represent shoot samples, 30 R and 60 R represent root samples harvested after 30 and 60 days, respectively. The data are presented as mean ± SE (standard error) of three replicates. Asterisks indicate difference between control and treatment groups based on student's t test at different levels of significance (P ≤ 0.05*, P ≤ 0.01** and P ≤ 0.001***). 'ns' stands for non-significant differences between control and treatment groups.

Discussion

The findings of the current study revealed that exposure to EMF–r adversely affected both the root and shoot lengths of *T. aestivum*, especially when subjected to longer duration of 60 days, resulting in a hindrance to plant growth. This decline in root and shoot length of EMF–r irradiated samples aligns with previous research findings.[3,14,28] In general, the plant's response to EMF–r varies considerably and lacks consistency. It depends on factors such as exposure type, its intensity, duration, plant's genotype and many more.[29] The decrease in photosynthetic pigments observed following EMF–r exposure indicates that EMF–r may disrupt the photosynthetic machinery.[30,31]

In the current investigation, we observed a significant decrease in the total carbohydrate levels during the 30 and 60 days of exposure duration. It is worth noting that no prior reports are available that have documented the long-term exposure effects of EMF–r on carbohydrate metabolism. Nevertheless, in a similar study, an initial increase followed by decrease in carbohydrate content at 2 and 4 h of EMF–r exposure duration was observed.[32] A reduction in the carbohydrate content in *V. radiata* after one week of EMF–r exposure was observed.[11] On the contrary, elevation in total soluble sugar and carbohydrate levels of *Zea mays* L. and *T. aestivum* was reported upon exposure of 945 MHz and 50 Hz EMF–r, respectively.[33,34] The carbohydrate content in plants can vary significantly due to the intricate interplay between the plant and its surrounding environment. To adapt to adverse abiotic conditions, plants store various metabolites, and the specific carbohydrates that can vary depending on the stress conditions.[35] However, when stress levels become more intense, as observed in the current study, there tends to be a decline in carbohydrate content.[36]

The reduction in carbohydrate content could potentially be attributed to increased activity levels of enzymes responsible for breaking down starch i.e., α–amylases and β–amylases, or a decline in photosynthetic pigments, leading to the absence of newly synthesized carbohydrates due to damage in photosynthetic machinery, as observed in the current investigation. Starch, produced during the photosynthesis process, serves as a temporary reserve of fixed carbon, while sucrose is the primary source of energy. Within plant cells, starch is enzymatically broken down by α– and β–amylases, resulting in the formation of glucose and maltose, either individually or in combination, which are subsequently utilized in the glycolytic pathway or for sucrose synthesis.[37,38]

A significant decline in the α– and β–amylases activities was observed upon exposure of 2850 MHz EMF–r in both 30 and 60–days old plant samples. Our findings corroborate with Mahajan *et al.*[36] where the authors observed reduction in the activity of amylases in *Z. mays* roots. The possible reason for decline in amylase activity could be due to cellular damage and oxidative stress in exposed samples in response to EMF–r. Enzymes, including amylases, may become damaged or inactivated by reactive oxygen species generated during stress responses.[39] Invertases play a key role in regulating the distribution of sucrose to various cellular compartments, by breaking down sucrose into glucose and fructose. They also play a significant role in adapting to environmental stress conditions, such as maintaining osmotic pressure, contributing to defense responses, and participating in cell and development.[40] The reduced activity of acid and alkaline invertases implies interference with the allocation of carbon resources, as well as the regulation of growth and development.*41* In the present study, a reduction in invertases activity was observed in plants exposed to long-term EMF–r exposure. This subsequently restricts the availability of sucrose within the cells. These findings corroborate with the results obtained by Sidhu *et al.*[42] who demonstrated a decline in the production of invertases in *Coronopus didymus* L. when exposed to different lead (Pb) concentrations.

Acid and alkaline phosphatases, play a key role in managing the distribution of inorganic phosphorus (Pi) to various parts of developing plants, to maintain the pace of growth and the efficiency of physiological processes.[43] In our current investigation, a significant decline in the activity of both acid and alkaline phosphatases with longer exposure periods was observed. The reduction in phosphatase activity during prolonged exposure results in reduced Pi availability, thereby leading to diminished growth.[44]

Conclusions

The present study offers concrete evidence on the impact of prolonged exposure to 2850 MHz EMF–r on *T. aestivum* at varying durations. The findings suggest that EMF–r negatively affects plant physiology, leading to disruptions in normal processes during different growth stages. Notably, EMF–r exposure impedes growth by disrupting photosynthetic pigments and carbohydrate metabolism. These effects are directly linked to the duration of exposure, with more significant damage occurring during longer exposure periods, emphasizing the influence of EMF intensity on biological outcomes. The increasing frequency and exposure duration may hamper the crop metabolism and nutritional value in the coming time which needs attention.

Conflict of Interest

Authors declare that they do not have any conflict of interest.

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' Contribution

AP: Experimentation, Data collection, Calculation, Analysis, Graph Preparation, Initial Drafting of manuscript; DRB: Conception, Research Design, Review and finalization, Supervision; SK: Research Design, Data curation, Statistical analysis, Review and finalization; RS: Statistical analysis, Review and finalization.

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