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Electromagnetic Waves from GSM Mobile Phone Simulator Increase Germination and Abiotic Stress in *Zea mays* L

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Abstract

Here we investigate the effect of electromagnetic waves with high radio frequency radiation on the antioxidant activities and seed germination of *Zea mays*. Three groups of *Zea mays* seeds were used in this study. The first group was only exposed to RF radiation emitted from the mobile phone simulator. The second group was only exposed to RF radiation emitted from the mobile phone simulator while it was switched off. The third group served as the control. Data showed that the level of germination was significantly higher when seeds were exposed to an electromagnetic field of 900 MHz (EMF), the effects of electromagnetic waves with high frequency (940 MHZ) were studied on lipid peroxidation and proline content in leaves of *Zea mays*. Thirteen-day-old seedlings were subjected to two types of electromagnetic wave treatments for 7 days. The two treatments were indicated according to their duration of exposure to the waves, i.e. (i) exposure for 3 hours a day and (ii) 5 hours a day. After 20 days, the malondealdehyde (MDA) content in the leaves of the treated plants were measured as an indication of lipid peroxidation. We also measured the amino acid proline as a biomarker in abiotic stress. Here the abiotic stress is to be the electromagnetic field with high frequency (940 MHz). Our results show that electromagnetic field causes an increase in MDA content in leaves, compared with the control, sourced from the GSM simulator. Furthermore, catalase enzyme activity increased as a result of exposure to EMF which may be due to the induction of oxidative stress and lipid peroxidation. Proline content increased significantly in plants treated with the electromagnetic field (940 MHz). The increase in proline content is the plant's countermeasure against abiotic stress.

Keywords: *Zea mays* L; Germination; Physiological responses; GSM (900 MHz)

Introduction

Microwaves are electromagnetic waves with frequencies that range from 300 MHz to 300 GHz. Their wave lengths can measure from 1 mm to 1 m. Generally, electromagnetic waves cover a wide range of frequencies between 10-20 Hz. The clinical application of microwaves has numerous examples, including diathermy and thermal rehabilitation. Microwaves serve many industries to the production of paper, plastic, nutrients and wood. Nonetheless, there is a persistent concern that their everyday functions can also pose health risks; their roles in radio and television broadcasting stations, navigation waves, radars, satellites, phones and telegraphs need to be under official supervision for the benefit of public life [1]. Cell phones emit radio waves of 915 MHz, and this could arouse ongoing controversies about the functional disorders that threaten biological systems. Theoretical studies are rather divisive, and in most cases so far, no contextual conclusions have been reached unanimously [2]. Electromagnetic fields (EMF) affect living organisms by causing oxidative stress; they increase the activity, concentration and lifetime of free radicals [3]. Oxidative stress is a function exercised by oxidative metabolites, free radicals and reactive oxygen species (ROS), which are highly reactive and can disrupt normal metabolism and the immune defense [4]. ROS bring changes to enzyme activity and gene expression. They also influence the release of calcium from intracellular storage sites. Oxidative stress also affects membrane structures, cell growth and cell death, thereby contributing to cancer and leukemia [5].

The role of catalase can become an area of discussion for reasons that will be explained further on. Catalase is an enzyme which is mainly present in the peroxisomes of mammalian cells. It is a tetrameric enzyme consisting of four identical subunits of 60 kDa, arranged in a tetrahedral pattern. Each contains an active center, a heme group and NADPH. Catalase has two enzymatic activities depending on the concentration of H_2O_2. If the concentration of H_2O_2 becomes high, then catalase acts catalytically, i.e. removes H_2O_2 by forming H_2O and O_2 - a catalytic reaction. However, at a low concentration of H_2O_2 and in the presence of a suitable hydrogen donor, e.g. ethanol, methanol, phenol and etc., catalase will act to remove H_2O_2, while oxidizing its substrate.

Few studies of this kind have been conducted on plants, and even fewer studies have been directed towards the effects of magnetic or electromagnetic fields on the germination of seeds, plant growth and development [6-9].

Maize has more genetic variants compared to other cereals. It is treasured for having the C_4 photosynthetic pathway, for its ease of cultivation, ability of storage and high performance, compared to other plants of its rank. Managing its appropriate density of cultivation is the most important factor in fieldwork. In modified hybrids, being successful in the germination stage can guarantee future survival, stability and favorable yield. Planning the final plant density is achieved with precision when most of the seeds germinate.

This study aims at considering the stimulatory effect of 940 MHz electromagnetic waves on the germination of '524 Maxima' hybrid

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maize seeds exposed to a cell phone simulator device for a duration period of 48 hours. Germination factors and physiological responses were also investigated.

Materials and Methods

Before exposure to EMF, the seeds were soaked for 24 h on moist, sterile filter paper inside petri dishes (90 mm diameter). Seven petri dishes, each bearing 20 seeds, were prepared for each treatment group and their corresponding control group.

Once the Radio High Frequency (RHF) was applied at 900 MHz, the dishes were transferred to the growth chamber in the dark at 23 ± 2°C and were watered regularly. The germination percentage was determined after 3 days.

The effect of exposure time (48h) and field modulation was investigated at 23 Vm (-1). Germination rate and percentage were recorded after 3 days of being in the growth chamber. Seeds that germinated uniformly were selected and cultivated in media containing Perlite and half-strength Hoagland nutrient solution. *Zea mays* seedlings with 13 days of age were hydroponically cultivated and grown in a culture room under standard conditions (30/25°C day/night and 31% relative humidity).

Estimation of lipid peroxidation

The level of lipid peroxidation was measured by estimating the MDA content according to the method proposed by Mortazavi et al., [10]. Each seedling weighed approximately 0.2 gram. Seedlings were homogenized in a cold pestle and mortar with 1.0 ml of 5% trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 12000 rpm for 15 min at room temperature. The supernatant was collected for the estimation of MDA content. The reactive solution contained 1.0 ml of aliquot and 4.0 ml of 0.5% thiobarbituriic acid (TBA) in 20% TCA. The solution was heated at 96°C for 30 min. The reaction was stopped by quickly placing the tubes in ice-chilled water, after being centrifuged at 2000 rpm for 10 min. The solution's light absorbance was taken at 532 nm, while the nonspecific absorbance was taken at 600 nm. Lipid peroxidation was calculated by subtracting the absorption value at 600 nm from the value at 532 nm. The concentration of MDA was calculated by means of the extinction coefficient of 155 $mM^{-1} cm^{-1}$. Results were expressed in the form of πmol/mg protein.

Proline content

Proline was measured according to the method described by Bates et al. [11]. Briefly, one hundred milligrams of frozen plant material was homogenized in 1.5 ml of 3% sulphosalicylic acid and the residue was removed by centrifugation. Then, 100 πL of the extract reacted with 2 ml of glacial acetic acid (GAA) and 2 ml of ninhydrin, since 1.25 g ninhydrin was dissolved in 30 ml GAA, plus 20 ml of 6M phosphoric acid. The dissolving took an hour at 100°C. To the reaction mixture, 1 ml toluene was added and the toluene containing chromophore was heated slightly to reach room temperature. Its absorbance was determined by a spectrophotometer SPEKOL at 520 nm via standard curves.

CAT activity

CAT activity was determined after 19 days following cultivation. Results were interpreted to obtain the mean value from experiments with at least 7 replicates, with 20 seeds in each replicate ± standard error. Data are expressed as percentages, while the control serves as the benchmark.

Activity of catalase (CAT) was measured in a reaction mixture consisting of 25 Mm k-phosphate buffer (pH=6/8), 30 Mm H_2O_2 and diluted enzyme extract in a total volume of 1 ml. The decomposition of H_2O_2 was followed by a decline in absorbance at 240 nm [12].

CAT activity was measured by monitoring the degradation of H_2O_2 at 240 nm, over a time span of 90 minutes against a plant extract, free and blank.

A-simulator of cell phone electromagnetic waves

Designing and constructing cell phone wave simulators are one of the most important and sensitive procedures in subsystems. Issues regarding electromagnetic compatibility (EMC) and signal integrity should be considered for the best performance of the device in the process of generating waves. The high frequency system which is designed in this project has several abilities:

- Production of narrow band sine waves with frequencies regulating between 800 and 1000 MHZ.
- Output power exceeding just over 3 watts.
- Modulation of exact output amplitude with the aim of simulating different time frames with regard to a variety of cell phone standards, such as the case in GSM.

Commercial cell phones in modules are not able to control and regulate the properties of the output amplitude in a desired level for exact and standard clinical experiments. Because of this, a cell phone wave simulator system is designed with the aforementioned properties. A complete diagram of the designed system is shown in Figure 1.

In order to generate waves between 800 and 1000 MHz, a crystal voltage controlled oscillator (VCO) was used besides the Pierce Gate architecture. The frequency which is generated by this oscillator is regulated easily by the control voltage which is applied to it in the region of 800 to 1000 MHz. One of the unique properties of oscillators is the linear function in that wave region. Waves are generated by the oscillator and enter a broad band high frequency amplifier including two sequential parts of the Metal Oxide Semiconductor Field Effect Transistor (MOSFET) via a transporting microstrip 50 ohm line. The transporting line is consistent with the output and input impedance. Appropriate ventilation of transistors is an important issue in designing this part which is provided by a suitable heat sink according to the temperature properties of packaging. Finally, a high amplified frequency signal is transported to the SMA transformer and coaxial cable by the transporting line and is then transported to the wave guide system.

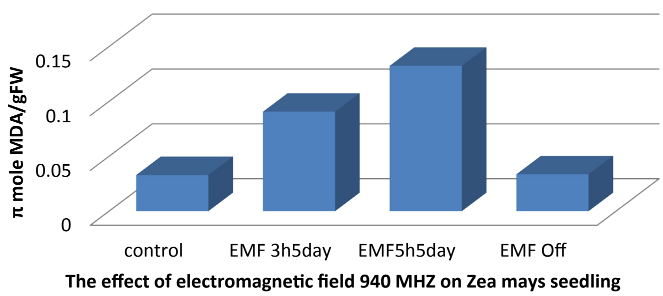

Figure 1: Different durations of EMF exposure affect TBA-MAD content in the leaf. (p□0.05). Each value represents the mean ± the standard of five replicates.

Exposure

The seeds were exposed to a radio frequency (EMF) of 900 MHz for 48h. Three groups of seeds were used in this study. The seeds in the first group were only exposed to RF radiation emitted from a mobile phone simulator. The second group was only exposed to the simulator when it was switched off, and the third group served as the control. The exposure duration for all groups was 48 hours against the simulator system. The temperature inside the simulator system was measured with a thermometer at the beginning and at the end of the exposure. The temperature did not vary more than ± 0.1°C for each exposure treatment. Control seeds were handled in the same way and kept in the same growth conditions (23 ± 2°C, in darkness).

Experimental design and statistical analysis

A factorial design was used in the form of completely randomized blocks. Two factors were considered thereby: (i) cultivars of the *Zea mays* L. and (ii) electromagnetic waves at 940 MHZ which lasted for three different durations. Five replications were used for each treatment. Data were analyzed statistically by the ANOVA, using the SPSS 16 software. The Tukey's test was used for multiple comparisons at the 95% level of significance (Table 1).

Results

The effect of GSM on seed germination is presented in Figure 2 and the rates of germination among the treated seeds were higher than the untreated seeds. In this experiment, seed germination was found to increase significantly (98.8%) when exposed to 940 MHz, sourced from the simulator system. The average of germination was investigated under priming. A stage preceding germination where in the seed imbibes water and synthesizes necessary proteins. The maximum rate of germination was 5% in seeds that had undergone priming and had been radiated with 940 MHz of electromagnetic waves for 48 hours. The maximum rate of seed germination in maize was observed after priming with water, within 48 hours of radiation with electromagnetic waves. Experimental temperature was 25ºC and relative humidity was 31%.

Malondealdehyde content

Malondealdehyde (MDA) is produced when the polyunsaturated fatty acid of the plasma membrane is peroxidized. It represents the degree of oxidative damage. The electromagnetic field (EMF) with high frequency at 940 MHz caused the MDA to increase, especially when the plants were exposed to the field 5 hours a day, for 5 days. Therefore, *Zea mays* seedlings had a greater degree of lipid peroxidation in the cell membrane. A number of studies have shown that abiotic stress can cause alterations in the structure and composition of lipids in the plasma membrane. An example for such alterations can be the increase in free sterols, which leads to a decrease in the fluidity of the cell membrane [13]. In this study, MDA content increased significantly in plants that were treated for longer durations with EMF (Figure 1).

Treatment	Germination %	Time	Frequency	GSM simulator
Control	2±/ 03	48 h	940 MHz	Out of system
Treatment 1	98/8 ±/04	48 h	940 MHz	Inside system
Treatment 2	3±0/04	48 h	940 MHz	Switch off

Table 1: Percentage of germination: substantial germination under electromagnetic waves in α probability level equal to P<0.05.

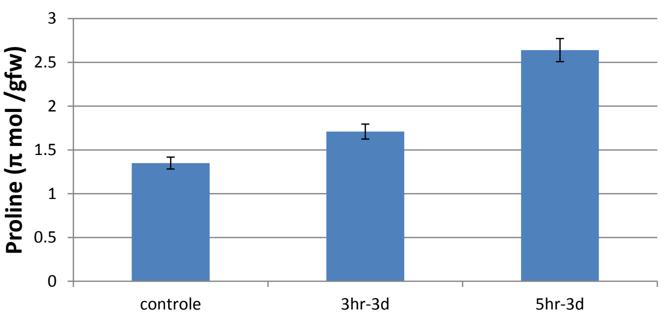

Figure 2: The effect of EMF mobile at 940 MHz on proline content *Zea mays.*

One of the main mechanisms whereby lipid peroxidation happens in the plant is when free oxygen radicals are generated and oxidative stress occurs because the detoxification mechanism is disrupted. Many observations have been reported which indicate the increase in free radicals as a result of lipid peroxidation. MDA is the byproduct of such peroxidation, and it increases in amount too [13]. Our results confirm these observations. One report claims that the increase in MDA content could disrupt the protein-lipid interaction within cell membranes. We also know that the increase in H_2O_2 can disunite membrane integrity.

High frequency mobile waves influence proline amino acid content

Thirteen-day-old maize plants were studied for 3 days-as exposed to EMF for 3 hours during the day and 5 hours during the night. Proline content increased in both treatment groups, but the greater increase was attributed to the second treatment group-5 hours of exposure-whereof proline reaches its highest level.

The average proline content in the control group was 1.29 Mm/g (per unit weight of fresh mature leaf).

The average proline content in the first treatment group was 1.91 Mm/g (per unit weight of fresh mature leaf), which received 940 MHz, 3 hours a day, for 3 days.

The group that received 5 hours of exposure a day, for 3 days, had its proline content 2.61 Mm/g (per unit weight of fresh mature leaf). There was a substantial difference among all three groups (p<0.05) with regard to their proline content.

Electromagnetic waves emitted at 940 MHz from a mobile simulator are referred to as a source of abiotic stress factor affecting plants. In order to verify these contents, complementary experiments were carried out.

CAT activity in seedlings exposed to 940MHz was significantly higher than that of the control group or that of the treatments with GSM simulator in its switched-off mode (Figure 3).

By delving into the interdisciplinary realm of physics and biology, one discovers that epidemiological and experimental data have drawn attention to the biological effects of EMFS (Figure 4).

The primary action of MF in any biological system is the induction of electrical charges and currents [14]. A major molecular effect of Magnetic Fields is the actual influence on nuclear spins of paramagnetic molecules.

This mechanism plays an important role when in the course of chemical reactions, the chemical bond is disrupted and two molecules with unpaired electrons are formed consequently – a radical pair – e.g. oxygen radicals may dominate [15,16] (Figure 5).

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CAT is the key enzyme that exhibits scavenging activity in the form of an antioxidant enzyme against radical oxygen molecules [17-24]. In one relevant study, Fehmiozguner et al. exposed rats to 900 MHz of cell phone microwave for 30 minutes a day, for 10 days, whereof the average power density was $\frac{1}{4}\frac{mw}{cm^2}$ which ultimately led to oxidative stress in the rats.

Conclusion

Exposure to Radio Frequency at 940 MHz can significantly enhance germination and catalase activity. Since RF-EMFs don't have enough energy to damage DNA directly, the exact mechanism whereby cytogenetic processes change direction can be clarified in future studies [24-28] (Figure 6).

Our results are in good agreement with others who suggested that RFR can cause mitotic incoherence against standard cytogenetic benchmarks. Moreover, it was found that EMF at 915 MHz was able to change chromatin conformation and inhibit the formation or repair of DNA [16,29,30]. In our experiment, the effect on germination was clearly evident after 48 hours of exposure to the mobile simulator at 940 MHz (Figure 7). This suggests that EMFs exert indirect effects on mitosis by altering the conditions in the cytosol; by redefining ionic strength and by providing conditions for reactive oxygen species to occur. Therefore, specific elekctromagnetic conditions can improve germination, and this phenomenon deserves to be studied further on plant species with low seed germination rates.

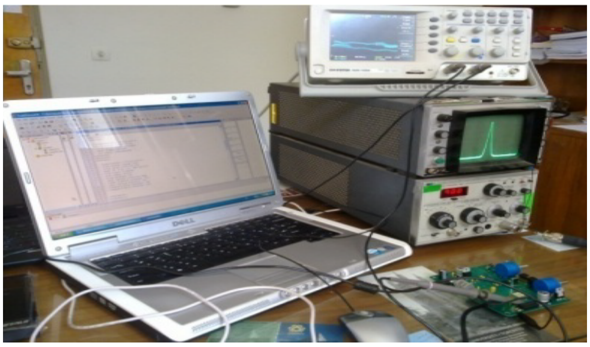

Figure 3: The electromagnetic field device, with high frequency (940 MHz), made in Shiraz University.

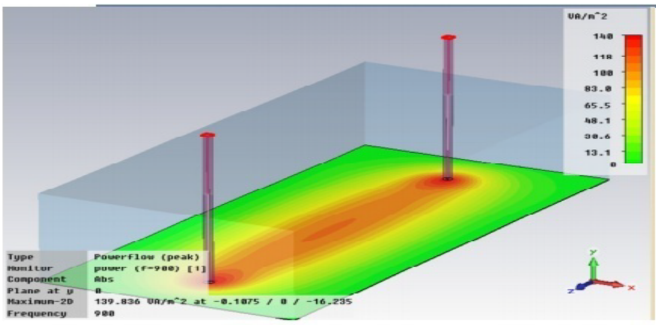

Figure 4: A technical depiction of the electromagnetic field device, emitting high frequency waves at 940 MHz, made in Shiraz University.

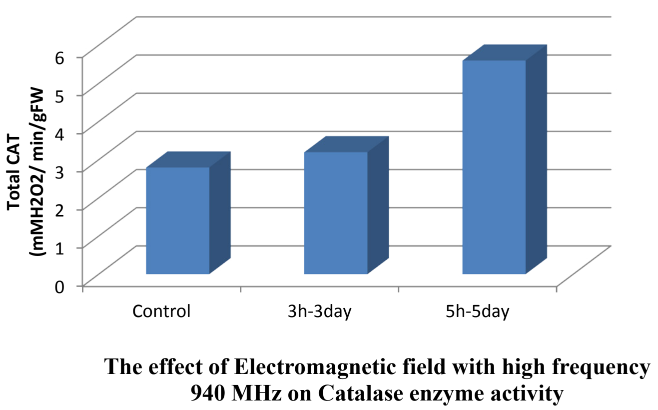

Figure 5: Effect of RF (GSM) and Mobile phone-940 MHz on the activity of catalase (p<0.05 by Duncan's test).

Figure 6: Percentage of germination: considerable germination under electromagnetic waves in α probability level equal to 0.05%, from table 1. The average of comparisons shows that when seed priming in water is combined with electromagnetic waves, the percentage of germination increases significantly, by 98.8%.

Figure 7: A depiction of the GSM mobile simulator system, operating at 940MHz.

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