International Journal of Agriculture and Crop Sciences.
Available online at www.ijagcs.com
IJACS/2015/8-4/642-649
ISSN 2227-670X ©2015 IJACS Journal

Effects Of electromagnetic waves with High Frequency(940MHz)Cause increase Catalase and H_2O_2 in Zea mays L.

Habibeh Zare*, Sasan Mohsenzadeh, Ali Moradshahi

Department of Biology, Faculty of Science, Shiraz University, Shiraz 71454, Iran [1]Department of Biology, Faculty of Science, Payame Noor University, Iran

Corresponding author: Habibeh Zare

ABSTRACT: Here we investigate the effect of electromagnetic waves with high radio frequency radiation on the antioxidant enzyme 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 Catalase enzyme activity and H_2O_2 content in leaves of *Zea mays*. 7-days-old seedlings were subjected to two types of electromagnetic wave treatments for 3,5 or7 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 14 days, the Catalase activity in the leaves of the treated plants were measured as an indication of antioxidant enzyme. We also measured the H_2O_2 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 H_2O_2 content in leaves, compared with the control, sourced from the GSM simulator. Furthermore, catalase enzyme activity increased as a result of exposure to EMF.
Keywords: *Zea mays* L., germination, H_2O_2,Catalase and GSM (900 MHz).

INTRODUCTION

Electromagnetic waves have a spectrum that extends from long to short waves. These include radio waves, radar waves, microwaves (short wavelength waves), infrared, ultraviolet, X-rays, and gamma rays. Microwaves are used in sending computer messages, visual and text messages. On average, each optical fiber sends 1000 sound messages with various wavelengths to faraway distances and remote places. Furthermore, these waves are applied in cooking and the waves' energy is absorbed by the food's molecules which results in their intense vibration. In the 21st century, telephones and cell phones have become more prevalent because of the considerable progress in the communications industry (Mousavi et al, 2001).

Microwaves are electromagnetic waves with frequencies that range from 300 MHz to 300 GHz. Their wave lengths can measure from 1mm to 1m. 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 too — 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. (Mortazavi et al,.2009) 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 (Repacho li and Green baum, 1999). Electromagnetic fields (EMF) affect living organisms by causing oxidative stress; they increase the activity, concentration and lifetime of free radicals (Scaiano et al. 1999). 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 (Dat et al. 2001). 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 (Green et al., 1999).

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 (Hirota et al., 1999; Yano et al., 2001, 2002; Rakosy-Tican et al., 2005).

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 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

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 Fig. 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.

Exposure in Simulator GSM

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 ºC for each exposure treatment. Control seeds were handled in the same way and kept in the same growth conditions (23 ± 2 ºC, in darkness).

Seed germination

Before exposure to EMF, the seeds were soaked for 24h 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 ºC 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).

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 25Mm k-phosphate buffer (PH= 6/8), 30 Mm H_2O_2 and diluted enzyme extract in a total volume of 1ml. The decomposition of H_2O_2 was followed by a decline in absorbance at 240 nm (Cakmak and Horst, 1991).

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

H_2O_2 assay

The protocol is as follows: corn leaf were harvested, immediately frozen in liquid nitrogen, ground and powder stored at freezer until H2o2 determination assay.frowsen leaf (150 mg) was directly homogenizedwith 1 ml of solution containing 0.25 ml Trichloroacetic acid(TCA),0.5ml KI(1M) and 0.25 ml potassium phosphate buffer(10mM, pH=7)at 4ºc for 10 min.The homogenate was centrifuged at 12000×g for 15 min at 4ºc. 200 μl of supernatant from each tube were measured absorbtion with spectrophotometer SPEKOL 1500 anlytika jena
At 390 nm.A calibration curve obtained with h2o2 standard solution prepared in 2-10 mM.

.

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.

RESULTS

The effect of GSM on seed germination is presented in figure (1), and the rate of germination among the treated seeds was 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 wherein 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 25 ºC and relative humidity was 31%.

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

Table1. Percentage of germination: substantial germination under electromagnetic waves in α probability level equal to P<0.05\

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

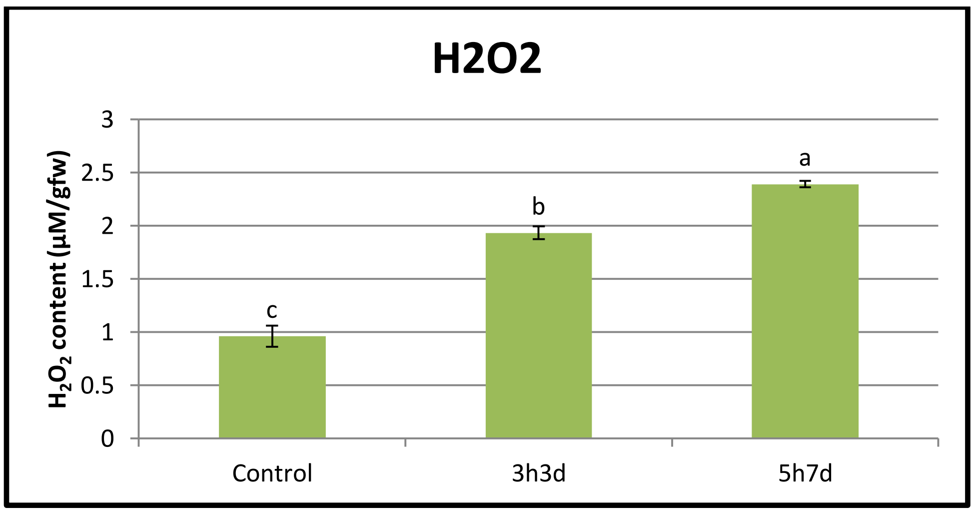

Figure 2. Different durations of EMF exposure affect H_2O_2 content in the leaf. (p <0.05).

Each value represents the mean ± the standard of four replicates.
We also know that the increase in H_2O_2 can disunite membrane integrity.
Hydrogen peroxide (H2O2) is a major reactive oxygen species (ROS) and plays diverse roles in plant development and stress responses(liu et al,. 2014)

Plants, being aerobic organisms, utilize molecular O_2as a terminal electron acceptor. As a reduction, highly reactive intermediates, reactive species (ROS), are produced. ROS such as singlet oxygen (O_2^1), superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) are normal products of metabolism and are produced in all cellular compartments within a variety of processes. In general, they are maintained at constant basal levels in healthy cells. However, they can destroy normal metabolism through oxidative damage of lipids, proteins, and nucleic acids when they are produced in excess as a result of oxidative stress (Gill and Tuteja, 2010). To overcome oxidative stress, together with non-enzymatic antioxidant molecules (ascorbate, glutathione, α-tocopherol etc.), plants detoxify ROS by up-regulating antioxidative enzymes like superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; E.C 1.11.1.6), peroxidase (POX; EC1.11.1.7), ascorbate peroxidase (APX; EC 1.11.1.11) and glutathione reductase (GR; EC 1.6.4.2) (Turkan and Demiral, 2009). SOD provide the first line of defense against the toxic effects of elevated levels of ROS. The SODs converts $O_2^{\cdot-}$to H_2O_2. The hydrogen peroxide produced is then scavenged by catalase and a variety of peroxidases. Catalase dismutates H_2O_2into water and molecular oxygen, whereas POX decomposes $H_2O_2$by oxidation of co-substrates such as phenolic compounds and/or antioxidants. APX is involved in scavenging of H_2O_2in water-water and ASH-GSH cycles and utilizes ASH as the electron donor. GR is a potential enzyme of the ASH-GSH cycle and plays an essential role in defense system against ROS (Gill and Tuteja, 2010; Ahmad et al., 2010).

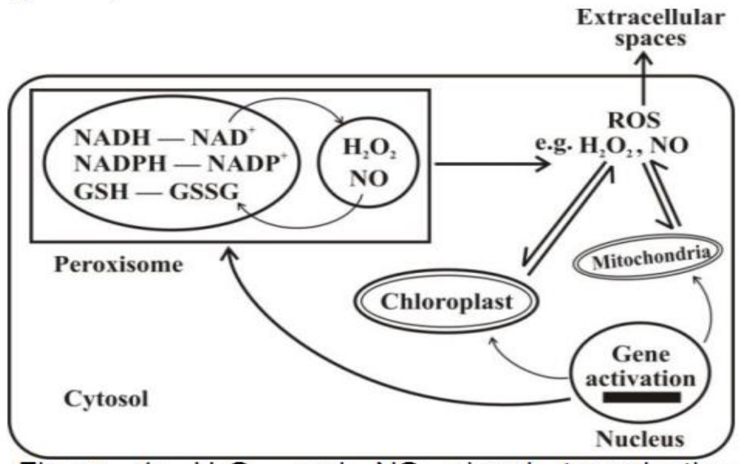

Figure 1: H_2O_2 and NO signal transduction pathway during abiotic stress.

H2o2 as a signaling molecule

Reactive oxygen species (ROS) accumulate when plants are undervarious biotic (pathogen attack) and abiotic (e.g., high light,drought, heat, salt, and heavy metal) stresses (Apel and Hirt, 2004;Suzuki etal., 2012; Choudhury etal., 2013). On one hand, excessive ROS cause oxidative damage to proteins, DNA, and lipids.On the other hand, ROS also act as signaling molecules to regulate development and stress responses (Apel and Hirt,2004). There are different kinds of ROS in plants, including singlet oxygen(O2),superoxide (O−2, H2O2 , and hydroxyl radical (OH−). Among them, H2O2 is thought to be relatively stable (Bienert etal., 2007)and the most likely signaling ROS to regulate developmental and stress responses (Van Breusegem etal., 2008), and thus one of the most studied ROS species. Hydrogen peroxide could be dete cted quantitatively and quali-tatively. Accurate quantification of H2O2 in plant organs,however,is difficult to achieve owing to the unique properties of H2O2 being highly metabolically active with a half-life of only 1 ms in plants (Reth, 2002;Veljovic-Jovanovic etal., 2002;Petrov and Van reusegem, 2012).

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 (Fig. 3).

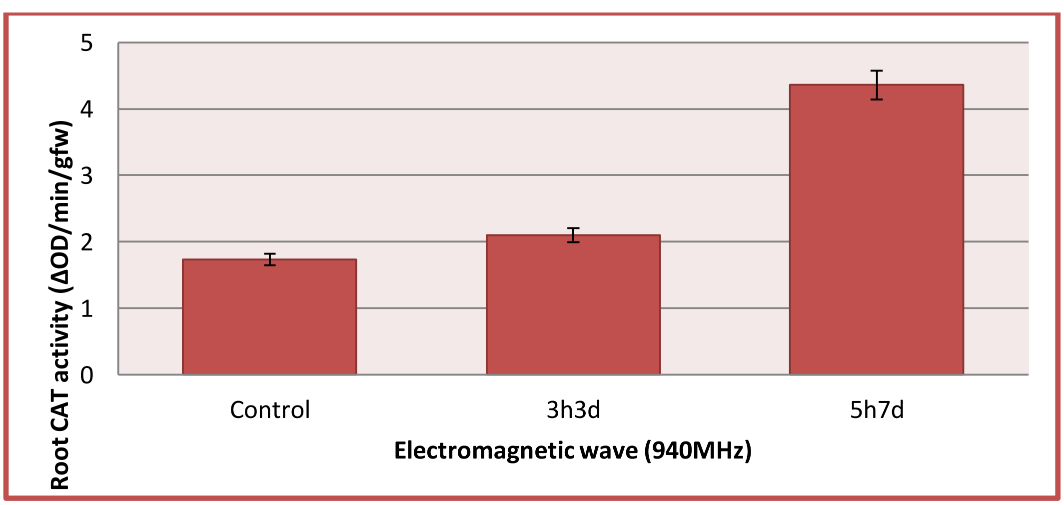

Figure 3. Effect of RF (GSM) and Mobile phone-940 MHz on the activity of catalase in root ($p<0.05$ by Duncan's test).

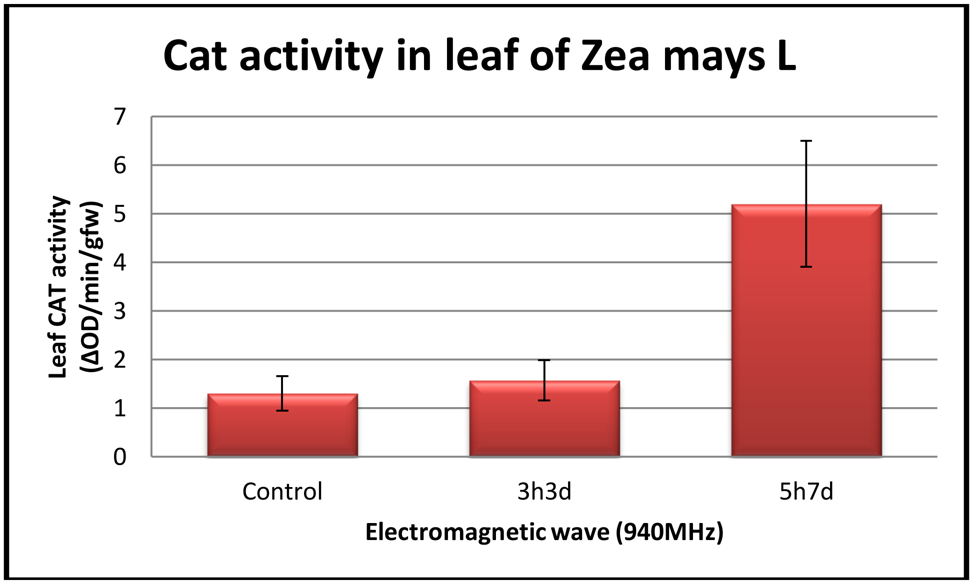

Figure 4. Effect of RF (GSM) and Mobile phone-940 MHz on the activity of catalase in leaf ($p<0.05$ by Duncan's test).

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.

The primary action of MF in any biological system is the induction of electrical charges and currents (ROY and Repacholi; 2005). 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 (Kula et al., 2002).

CAT is the key enzyme that exhibits scavenging activity in the form of an antioxidant enzyme against radical oxygen molecules.

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.

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 (Mortazavi et al., 2011). In our experiment, the effect on germination was clearly evident after 48 hours of exposure to the mobile simulator at 940 MHz. 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 electromagnetic conditions can improve germination, and this phenomenon deserves to be studied further on plant species with low seed germination rates.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.

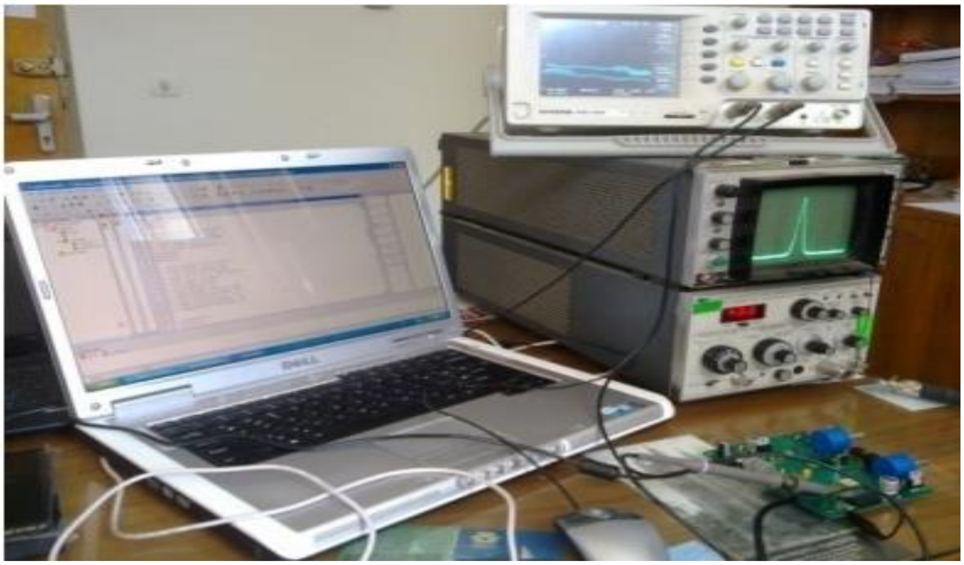

Figure 5. The electromagnetic field device, with high frequency (940 MHz), made in Shiraz University of Iran 2011.

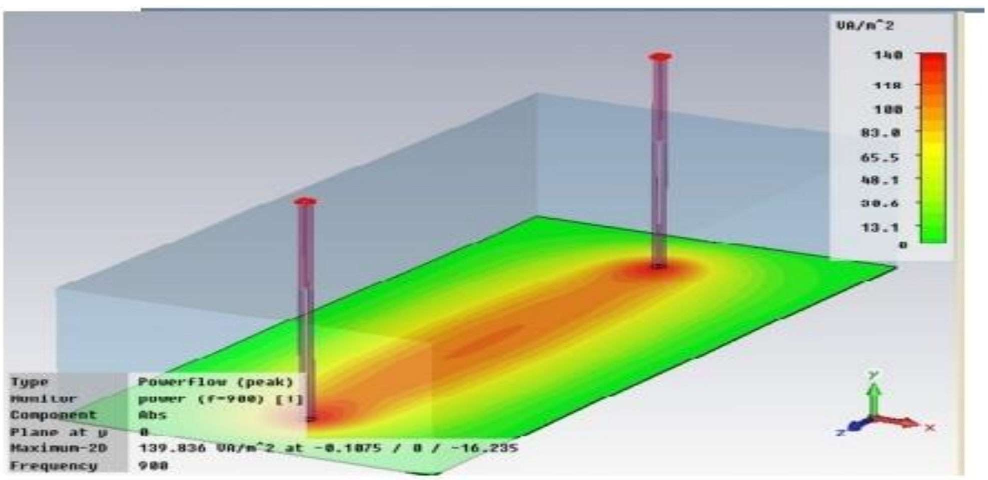

Figure 6. A technical depiction of the electromagnetic field device, emitting high frequency waves at 940 MHz, made in Shiraz University of Iran 2011.

Figure 7. Percentage of germination: substantial germination under electromagnetic waves in α probability level equal to $P<0.05$

REFERENCES

Cakmak I, Horst WJ. 1991. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (Glycine max). Plant Physiol 83:463–468

Dat JF, Van Montagu M, Inze D, Van Breusegem F. 2001. Catalase-deficient tobacco plants: Tools for in planta studies on the role of hydrogen peroxide. Redox Rep 6:37–42.

Green LM, Miller AB, Agnew DA, Greenberg ML, Li J, Villeneuve JP, Tibshirani R. 1999. Childhood leukemia and personal monitoring of residential exposures to electric and magnetic fields in Ontario. Can Cancer Causes Control 10:233– 243.

Hirota N, Nakagawa J, Koichi K. 1999. Effects of a magnetic field on the germination of plants. J Appl Phys 85:5717– 5719.

Hung SH, Yu CW, Lin CH. 2005. Hydrogen peroxide functions as a stress signal in plants. Botanical studies Bull. Academic journal. 46: 1-10.

Kula A, Sobczak A, Kuska R. 2002. Effects of electromagnetic field on free-radical processes in steelworkers. Part I: Magnetic field influence on the antioxidant activity in red blood cells and plasma. J Occup Health 44:226–229.

Loreto F, Velikova V. 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiology. 127: 1781-1787.

Mortazavi SMJ, Mosleh-Shirazi M, Tavassoli A, Taheri M, Bagheri Z, Ghalandari R, Bonyadi S, Shafie M, Haghani M.2011. A comparative study on the increased radio-resistance to lethal doses of gamma rays after exposure to microwave radiation and oral intake of flaxseed oil. Iranian Journal of Radiation Research 2011, 9(1):9-14.

Mortazavi SMJ, Ahmadi J, Shariati M.2007. Prevalence of subjective poor health symptoms associated with exposure to electromagnetic fields among University students. Bioelectromagnetics 2007, 28(4):326-330.

Neil SJ, Desikan R, Clarke A, Hurs RD, Hancock JT. 2002. Hydrogen peroxide and nitric oxide as signaling molecules in plants. Journal of Experimental Botany. 53: 1237-1247.

Rakosy-Tican L, Aurori CM, Morariu VV.2005.Influence of near null magnetic field on in vitro growth of potato and wild Solanum species. Bioelectromagnetics 26(7): 548-57.

Repacholi MH, Greenebaum B. 1999. Interaction of static and extremely low frequency electric and magnetic fields with living systems: Health effects and research needs. Bioelectromagnetics 20:133–160.

Roy C, Repacholi M. 2005. Electromagnetic field and Health, AWHO perspective, World Health Organization, Geneva,Switzerland, Available from: http://www.world-aluminiu-m.org/news/montreal/roy.htm.

Scaiano JC, Cozens FL, Mclean J. 1994. Model for the rationalization of magnetic field in vivo. Application of the radical-pair mechanism to biological systems. Photochem Photobiol 59:585–589.

Yano A, Hidaka E, Fujiwara K, Iimoto M. 2001. Induction of primary root curvature in radish seedlings in a static magnetic field. Bioelectromagnetics 22:194–199.

Yano A, Ohashi Y, Hirasaki T, Fujiwara K. 2004. Effects of a 60 Hz magnetic field on photosynthetic CO2 uptake and early growth of radish seedlings. Bioelectromagnetics 25:572–581.