Effect of extremely low frequency electromagnetic fields on antioxidant enzymes in valerian (*Valeriana officinalis* L.)

Sara Farzpourmachiani[1*], Ahmad Majd[2], Sedigheh Arbabian[2], Davoud Dorranian[3] and Mehrdad Hashemi[4]

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Biology, Faculty of Biological Sciences, North-Tehran Branch, Islamic Azad University, Tehran, Iran
3. Plasma Physics Research Center, Science and Research Branch, Islamic Azad University, Tehran, Iran
4. Department of Genetics, Tehran medical Branch, Islamic Azad University, Tehran, Iran.

Abstract

In this research, the effects of electromagnetic fields of various strengths (0, 1 and 2mT) have been investigated on antioxidant enzymes activity in Valerian (*Valeriana officinalis* L.). The dry and wet (soaked for 30 min) Valerian seeds were exposed to electromagnetic fields 30 min per day for 3 days. Each treatment and control groups had 3 replicates and 10 seeds were employed for each replicate. Results showed that electromagnetic field treatment increased significantly root length, fresh and dry weight, ascorbate peroxidase, guaiacol peroxidase, superoxide dismutase, polyphenol oxidase and lipoxygenase activity compared to control for most treatment groups especially in the groups of dry treated seeds. These results showed that electromagnetic fields probably enhanced oxidative stress and treated Valerian seeds probably increased antioxidant enzymes activity to inhibit overproduction of free radicals against electromagnetic fields tension.

Keywords: Valeriana officinalis L.; antioxidant enzymes; electromagnetic field

Abbreviations: APX: ascorbate peroxidase, EMF: electromagnetic field, GPX: guaiacol peroxidase, LOX: lipoxygenase, PPO: polyphenol oxidase, SOD: superoxide dismutase

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Introduction

All organisms live under magnetic and electric fields in their natural environment. However, the artificial fields including high voltage transmission lines and communication devices are more important problems that are widespread in the modern environment. Their interaction with living organisms is not clear but electromagnetic fields (EMFs) probably affect the signal transduction

*Corresponding author
E-mail address:sfarzpourmachiani@yahoo.com
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mechanisms in the cell membrane and the cellular levels of calcium (Lyle, 1997). EMF is a kind of environmental tension that affects the plants at different levels of ontogeny. EMFs have inhibitory or stimulatory effects that depend on plant species, EMF intensity and treatment duration (Majd et al., 2010; Ramezani Vishki et al., 2012). EMFs enhance lipid peroxidation and hydrogen peroxide (Talek et al., 2007), change in Ca^{+2} concentrations, interact with hydrogen ion and cause molecular responses (Roux et al., 2006), affect the membrane structure (Green et al., 1999) and cell division and extension (Dao-liang et al., 2009), and reduce and deform pollen grains (Amjad and Shafighi, 2010). EMFs increase stress-related transcripts (Roux et al., 2006) and decrease ATP and adenylate energy charge and lead to an increase in cellular energy usage (Roux et al., 2006). Singh et al. (2011) expressed that EMF enhances the activities of antioxidant enzymes in *Phaseolus aureus*. EMF treatment changes the configuration of the hypodermal and parenchyma cells (Azharanok et al., 2009) and affects polarity and differentiation (Krizaj et al., 1989). EMF increases survival and crop capacity (Azharanok et al., 2009), accelerates the seedlings growth (Hajnorouzi et al., 2011), induces chromosomal aberrations (Belyavskaya 2004) such as lagging, disturbed anaphases and chromosome stickiness (Tkalec et al., 2009), and oxidative damage (Sharma et al., 2009). Abdolmaleki et al. (2007) reported an increase in cell death, peroxidase activity, and lignifications of cell wall in tobacco cells by static magnetic field. Moreover, Singh et al. (2011) reported that EMFs affect biochemical process and rhizogenesis. Also, in their study Piacentini et al. (2001) reported an increase in seedlings survival.

The roots and rhizomes of *Valeriana officinalis* L. contains valerenic acid, its derivates and valepotriates that are used for solid, oral dosage, tincture, and tea (Bos et al., 1996). The alcoholic extract of *V. officinalis* L. is used to treat anxiety, hysteria, insomnia, nervous tension, and fatigue (Baibado and Cheung, 2011). In this research, we have investigated the effects of electromagnetic fields on antioxidant enzymes activity in Valerian. The goal of this study was to evaluate how Valerian seedlings adapt and respond to electromagnetic fields tension and compare the effects of electromagnetic fields on biochemical processes of wet and dry Valerian seeds. Results could be used in plant physiology and agriculture research to know better about defense mechanisms of plants against electromagnetic fields tension.

Materials and Methods

Seeds growth condition

Seeds of *V. officinalis* L. were supplied by Pakan Bazr Institute and divided into wet (soaked in distilled water for 30 minutes) and dry seeds groups. Then wet and dry seeds were separately sterilized and cultured on solid MS (Murashige and Skoog, 1962) medium, supplemented with (3 %) sucrose and (0.7 %) agar. Petri dishes containing wet and dry seeds were divided into control and treatment groups. Treatment groups were exposed to electromagnetic fields of 1 and 2 mT intensities. Each treatment and control groups had 3 replicates and 10 seeds were employed for each replicate.

Electromagnetic field exposure

EMFs were generated by a handmade cylindrical-shaped coil that was connected to a 220 V AC power supply (ED-345BM, China), to generate electrical current of 60 HZ. EMF intensities were measured by a B-probe type of Hall sound. Petri dishes containing Valerian seeds were placed in the middle part of coil (Fig. I) and exposed to 1 and 2 mT electromagnetic field strengths for 30 min per day for 3 days. Then they were placed in an incubator under a photoperiod of 16/8 h dark at 23° C and 2 m/mM. Root length and fresh and dry weight were measured on day 40. After 2 months, seedlings were transferred to

Fig I. EMF generation system

pots containing peat and perlite and kept in green house condition for 60 days.

Assay of ascorbate peroxidase activity (EC 1.11.1.11)

The reaction mixture (3 ml) contained 50mM potassium phosphate buffer (pH 7), 0/15 mM hydrogen peroxide, 0/1 M EDTA, 0/5 Mm ascorbate and 50 µl enzyme extraction was prepared and ascorbate peroxidase (APX) activity was determined as the decrease in absorbance at 290 nm for 2min according to Nakano & Asada (1981). One unit of APX was defined as the amount of enzyme that catalyzes the peroxidation of 1 mM of ascorbate per minute.

Assay of guaiacol peroxidase activity (EC 1.11.1.7)

The reaction mixture (3 ml) containing 50 mM potassium phosphate buffer (pH 7), hydrogen peroxide 1%, guaiacol 4% and 30 µl enzyme extraction was prepared. Guaiacol peroxidase (GPX) activity was determined as the increase in absorbance at 470 nm for 3 min due to oxidation of guaiacol (Zhang et al., 2005).

Assay of superoxide dismutase activity (EC 1.15.1.1)

The reaction mixture (3 ml) containing 50 mM potassium phosphate buffer (pH 7,8), 13 mM methionin, 75 mM nitro blue tetrazolium (NBT), 2 µm riboflavin, and 0.2 ml enzyme extraction was prepared and measured as the increase in absorbance at 560 nm according to Gianopolitis and Ries (1977). One unit of superoxide dismutase (SOD) was defined as the amount of enzyme that inhibited NBT photo reduction by 50 % at 25 °C.

Assay of polyphenol oxidase activity (EC 1.14.18.1)

Reaction mixture (6 ml) containing 0.2 M potassium phosphate buffer (pH 6,8) and 0/02 M pirogallul was placed in baine-marrie of 40° C and then 200 µl enzyme extraction was added. Changes in absorption of polyphenol oxidase (PPO) were measured at 430 nm for 4 min according to Raymond et al. (1993).

Assay of lipoxygenase activity (EC 1.13.11.12)

The reaction mixture (3 ml) containing linoleic acid, distilled water, and Tween-20 was prepared. The pH of solution was regulated at 6,5 by adding 0.2 M NaOH and 0.2 M HCL. Then 0.1 M potassium phosphate buffer (pH 6,5) and enzyme extraction was added and read as the increase in absorbance at 234 nm for 4 min according to Dodderer et al. (1993). Lipoxygenase (LOX) activity was defined one unit of substrate that oxidized per minute.

The activity of all antioxidant enzymes were expressed as enzyme units (EU) per milligram of protein per minute.

Statistical analysis

The experimental design was completely randomized with three replications; each replication consisted of 10 plantlets and all of the data were expressed as the mean ± SE. Means were compared using the post hoc Tukey's test at $P<0.05$, level of significance to detect differences between treated and control samples by SPSS 16 software.

Results

Growth characteristics

EMFs caused an increase in the root length for most treatment groups especially in wet ones. Wet treated seeds had the longest roots compare to dry seeds. The longest and lowest root lengths were observed in the wet and dry seeds pretreated with 1 mT intensities, respectively. There was a significant difference between these groups (Fig. II).

EMFs increased dry and wet weight of Valerian seedlings. The pretreated seeds upon dry condition with 1 mT had the lowest freshe weight. There was a significant difference between these seeds and the wet treated seeds with 1 mT. The wet control and the wet treated seeds with 1 mT had the lowest fresh weight compared to the others (Fig. III). The maximum and minimum dry weights were observed in the dry treated seeds

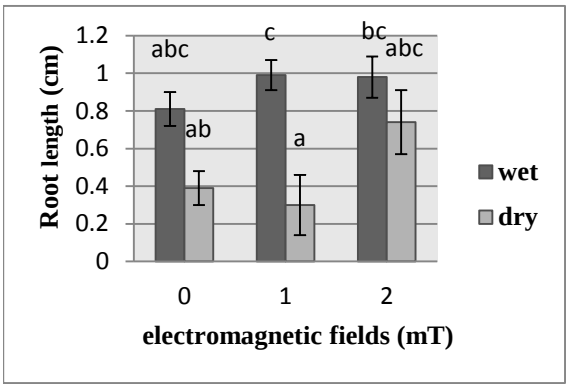

Fig. II. Effect of the electromagnetic fields on root length in Valerian (*V. officinalis* L.).Data are presented as the means ± SE. Means followed by different letters represent significant difference at P<0.05 according to post hoc Tukey's test.

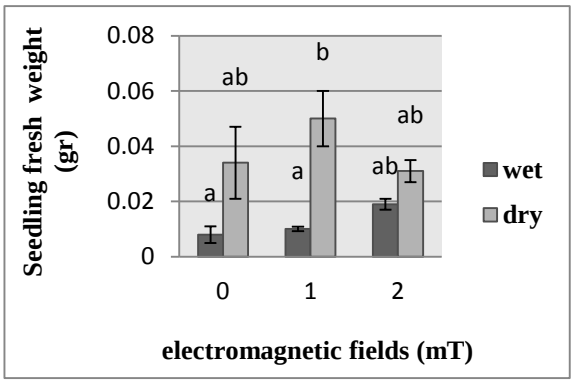

Fig. III. Effect of the electromagnetic fields on fresh weight in Valerian (*V. officinalis* L.). Data are presented as the means ± SE. Means followed by different letters represent significant difference at P<0.05 according to post hoc Tukey's test.

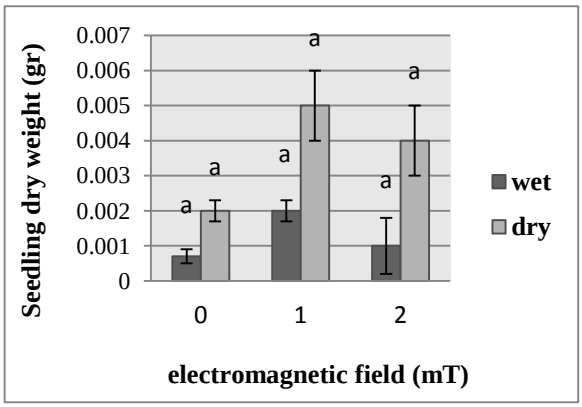

Fig. IV. Effect of the electromagnetic fields on dry weight in Valerian (*V. officinalis* L.). Data are presented as the means ± SE. Means followed by different letters represent significant difference at P<0.05 according to post hoc Tukey's test.

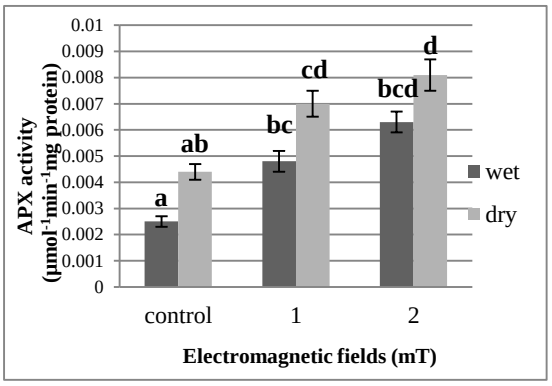

Fig. V. Effect of the electromagnetic fields on APX activity in Valerian (*V. officinalis* L.). APX activity exposure to electromagnetic fields. Data are presented as the means ± SE. Means followed by different letters represent significant difference at P<0.05 according to post hoc Tukey's test.

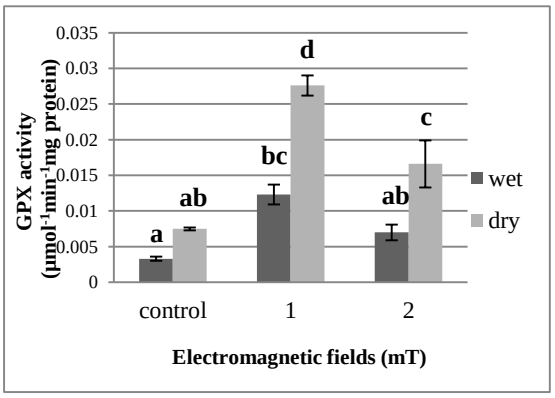

Fig. VI. Effect of the electromagnetic fields on GPX activity in Valerian (*V. officinalis* L.). GPX activity exposure to electromagnetic fields. Data are presented as the means ± SE. Means followed by different letters represent significant difference at P<0.05 according to post hoc Tukey's test.

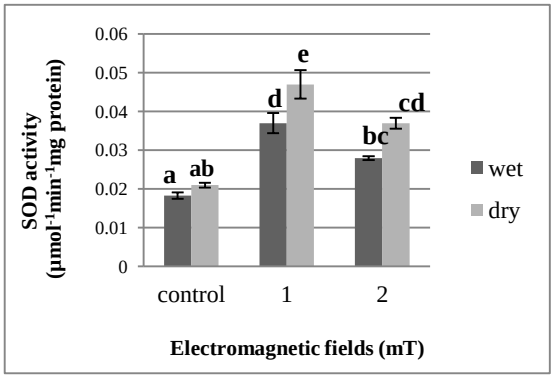

Fig. VII. Effect of the electromagnetic fields on SOD activity in Valerian (*V. officinalis* L.). SOD activity exposure to electromagnetic fields. Data are presented as the means ± SE. Means followed by different letters represent significant difference at P<0.05 according to post hoc Tukey's test.

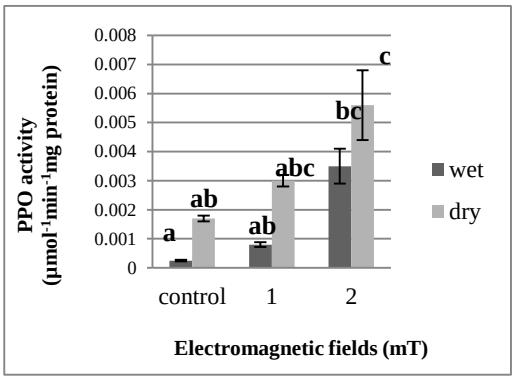

Fig. VIII. Effect of the electromagnetic fields on PPO activity in Valerian (*V. officinalis* L.). PPO activity exposure to electromagnetic fields. Data are presented as the means ± SE. Means followed by different letters represent significant difference at P<0.05 according to post hoc Tukey's test.

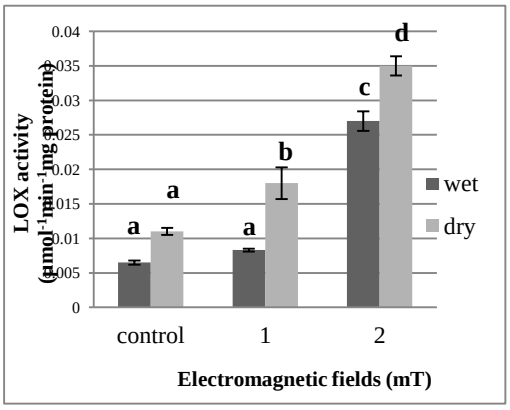

Fig.IX. Effect of the electromagnetic fields on LOX activity in Valerian *(V. officinalis* L.). LOX activity exposure to electromagnetic fields. Data are presented as the means ± SE. Means followed by different letters represent significant difference at P<0.05 according to post hoc Tukey's test

with 1 mT and the wet treated seeds with 2 mT, respectively. There was not any significant difference between these seeds, controls, and the other treatment groups (Fig. IV). The activity of scavenging enzymes (APX, GPX, SOD, PPO, and LOX) increased significantly upon exposure to electromagnetic fields. The activity of APX, PPO, and LOX enhanced significantly by increasing the EMF intensities. The pretreated seeds upon dry condition had more APX activity than wet ones. The exposure samples upon 2 mT strength had the highest APX activity compared to control and the other experimental groups (Fig. V). According to Figs. VI and VII, the GPX and SOD activity increased significantly in treated samples compared to control; however, the level of increase was less in the treated seeds with 2 mT strength. Dry treated seeds had more antioxidant enzymes activity than wet ones.

The activity of PPO and LOX were enhanced by increasing in EMF intensities especially in dry condition. The exposure samples upon 2 mT strength in dry condition had the highest PPO activity compared to control and the other experimental groups and there was a significant difference between these samples and the other groups (Fig. VIII). Treatment with the EMFs caused significant changes in the activity of LOX. Treated samples with 1 mT in wet condition had lower LOX activity than the other treatment groups but there was not any significant difference. The highest LOX activity was observed in 2 mT strength in dry condition (Fig. IX).

Discussion

Electromagnetic fields such as power lines are the kind of abiotic stresses that affect the plants at various levels of ontogeny, anatomical structure, and biochemical reactions. Environmental stresses such as electromagnetic fields can induce oxidative stress in plants and lead to disruptions in normal mechanisms of cellular signaling such as disturbance in defensive processes and change in cell function and structure, and even lead to cell death (Hajnorouzi et al., 2011). The plants have oxidative defense system that protects them against ROS such as superoxide, hydroxyl, and hydrogen peroxide. Superoxide oxidizes amino acids and switches CAT and POX activity. Hydrogen peroxide initiates Haber-Weiss reaction and leads to generation of hydrogen peroxide. Hydroxyl radicals cause peroxidation of the membrane lipids. The reactive oxygen species are produced in mitochondria, chloroplast, and peroxisome as by-product of metabolic reactions of photosynthesis and respiration. Some of them may change genes and defense proteins activity. But they can be harmful at high concentrations through oxidation of proteins, lipids and nucleic acids, cellular structure changes, degeneration of polysaccharides, and mutation (Phillips et al., 1986).

Oxidative defense system consists of antioxidants and free-radical-scavenging enzymes such as peroxidases, superoxide dismutase, and

catalase with detoxification ability of active oxygen forms in plants exposed to environmental stresses (Ali et al., 2003). Studies have suggested that POXs involve different roles in plants such as auxin catabolism, senescence, lignifications, and suberization (Atak, 2003; Piacentini, 2001). They play a role in root formation and defend plants against stresses such as chemical and EMFs stress and pathogens as well (Singh et al., 2012; Atak et al., 2003). SOD protects plants against free radicals by dismutation of superoxide to oxygen and hydrogen peroxide. An increase in SOD activity causes an overproduction in H_2O_2. APX converts hydrogen peroxide to oxygen and water by ascorbate-glutathione cycle and protects plants against ROS damage. CAT acts this reaction in chloroplast. SOD and CAT combination acts under tensional conditions and leads to protect against and and delays in plant senescence (Piacentini, 2001). PPOs have a role in phenolics oxidation and root formation.

Results obtained in this study showed an increase in root length, fresh and dry weight, and antioxidant enzymes activity especially in dry condition compared to wet seeds even in control samples. EMFs probably increase auxin rate and produce growth proteins in nucleus. They can be effective on genes regulators like cytokinin and increase mitosis divisions in shoot and root meristems (Lyndon, 1997).

The activity of GPX and SOD increased in the most treatment groups especially upon exposure to 1 mT strength and dry condition. However, the activity of APX, PPO, and LOX was enhanced by increasing in EMF intensities. EMFs probably increased free radicals in Valerian so that the plant enhanced the antioxidant enzymes activity to protect itself against oxidative damages and cellular injury. Promotion of peroxidases activity can extend the cell wall integrity and lead to root cells growth. LOX activity increased in EMF treatment groups especially in samples of dry condition. EMFs probably peroxide lipids of cell membrane by increasing LOX activity so ROS amounts probably increased in treated samples of dry condition compare to wet ones. They increased more antioxidant enzymes activity than wet seeds to scavenge free radicals and protect themselves against oxidative injury.

These results are in agreement with the other reports. Ramezani Vishki et al. (2012) reported that extremely low frequency EMF increased root length in *Satureja bachtiarica* L. Piacentini et al. (2001) reported that magnetic field increased SOD, GR, and CAT activity in *Cucumis*, enhanced their survival, and delayed signs of senescence. Sharma et al. (2009) reported an increase in SOD, APX, GPX and GR activity in *Vigna radiate* L. upon cell phone EMF exposure. Singh et al. reported (2011) that POXs and PPOs activity enhanced by cell phone EMFr.

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