Study of Effects of Extremely Low Frequency Electromagnetic Radiation on Biochemical Changes In Satureja Bachtiarica L

Ramezani Vishki F., Majd A., Nejadsattari T., Arbabian S.

Absract— Plants are organisms that are exposed to various abiotic and biotic environmental impacts. Plants are able to recognize and respond to their surrounding environment with high specificity. Electromagnetic field radiation is an important effective stress factor on growth and development of plants. Our research were focused on plants grown from wet pretreated seeds with low frequency electromagnetic field exposure and compared with the control. Three replicates were used in the experiment with 30 seeds in each samples. The wet seeds treatment, were spread on the moist filter papers in Petri dishes and then were placed in the middle parallel coils of electromagnetic radiation generator and were exposed by a magnitude of 1mT, to 2hr. Untreated seeds were used as control under similar condition. It means they were placed in the similar coil but not connected to the power. Study of morphological and growth of seedlings, showed that in treatment samples, in comparison to control, the percentage of seed germination and average of root length increased, but different of root length was not significantly. A significant decrease in mean of shoot length, rate of Leaf area, fresh and dry weight was observed, Also caused significant increase in activity of non-enzymatic antioxidant content in treatment samples in comparison of control.

Index Term— electromagnetic field, growth, non-enzymatic antioxidant, Satureja bachtiarica

Nomenclature— EMFr: electromagnetic field radiation ; ROS: reactive oxygen species

1. INTRODUCTION

Plants are able to recognize and respond to their surrounding environmental stresses. When plants are subjected to environmental stress condition, the balance between the production of reactive oxygen species (ROS) and the quenching activity of antioxidants is upset, often resulting in oxidative damage [5]. ROS are produced within cells as a consequence of normal metabolic processes, but the production of ROS often increases when cells are under stress [37]. ROS participate in signal transduction, but also modify cellular components and cause damage. Abiotic stress results in the formation of ROS in plants which creates a condition called oxidative stress that can damage cellular components [5]. Oxidative stress occure when there is a serious imbalance between the production of ROS and antioxidative defence.

- Fariba Ramezani Vishki, PhD Student of department of biological Science, Islamic Azad university, Science and Research branch, Tehran, Iran.
  E-mail: Ramezanivishki@yahoo.com
- Factually of department of biological Science, Islamic Azad university, Tehran North branch,Tehran, Iran
- Factually of department of biological Science, Islamic Azad university, Science and Research branch, Tehran, Iran

It is well documented that abiotic stresses exert at least in part of their effects by causing oxidative damage. Plants have developed efficient antioxidant system that can protect plants from this disaster. In plants affected by stress, a response is induced by changes in the plant metabolism, growth and general development [28]. The production of ROS are inevitable under stress, Hence, plants are equipped with an array of enzymatic and non-enzymatic antioxidant molecules to alleviate cellular damage caused by ROS[8]; [14], [5]. In fact a potential link between abiotic stress such as electromagnetic field radiation (EMFr) and its effects on living organisms is the fact that EMFr cause an oxidative stress that is, increase in the activity, concentration and lifetime of free radicals [28], [2]. Exposure to electromagnetic field can lead to cell death as a result of increase in free oxygen radicals and DNA damage [23]. Several studies have been conducted to find out the effect of EMFr on the growth and physiology of the plants [3], [44]. such as studying effects of EMFr on seeds germination and seedlings growth and seed vigor [7],[29],[31]. Plants produce a high diversity of secondary metabolites and antioxidant defence with a prominent function in the protection against stresses on the basis of their defense reactions. secondary metabolites are to be involved in plant chemical defense systems. High concentrations of secondary metabolites for example phenols and flavonoids, might result in a more resistant plant [28]. Electromagnetic radiation stress to induce proline accumulation in plants [22], [43]. proline accumulation is believed to be very important as part of the physiological adaptation of plants to stress [12], [1], [34], [16], [35]. Our study predicate to the effects of low frequency

electromagnetic radiation as abiotic stress on parameters growth and activity of defence mechanisms of Satureja plant (*Satureja bachtiarica L*.). This would help us to improve general knowledge about mechanisms of the response of higher plants to EMF.

2. MATERIALS AND METHODS

2.1 Electromagnetic field exposure

Exposure to EMF was performed using a locally designed EMF generator. The magnetic field was provided by a parallel pair of identical circular coils spaced one radius apart and wound so that the current electrical flow through both coils in the same direction. magnetic field exposure arrangement is produced the low frequency uniform and homogeneous form experiments over a known strength volume. This system consisted of one handmade coil, cylindrical in form, made of 21cm in diameter and 100 roll of winding. To ward production of field with intensity of 1 mT, was transmitted 1.16 amper electrical flow between the coils. The coil was not shielded for electrical field and the seeds were exposed to both magnetic and electric fields generated by the coils. The winding results in a very uniform magnetic field between the coils with the primary component parallel to the axes of the two coils (Fig. I). The samples placed in the middle of a horizontally fixed coil and were exposed .The temperature was measured with a thermometer to be 22+1°C.

Fig. 1 electromagnetic field exposure arrangement

2.2 Experimental design

Seeds of *S.bachtiarica L*. were obtained from seed and plant improvement agriculture institute, Karaj, Iran, which were selected for a uniform size and shape .Three replicates were used in the experiment with 30 seeds in each treatment. In case of wet seeds treatment, the seeds were spread on the moist filter papers in Petri dishes and then placed in the middle of a horizontally fixed coil and were exposed to EMF by a magnitude of 1 mT, to 2hr in the EMF generator apparatus. Untreated seeds were used as control under similar condition. It means they were placed in the similar coil but not connected to the power. Then difference among the seedlings grown from treated seeds and control in growth parameters including seed germination, root length, shoot length was campared . Leaf samples of 30 day seedling were chosen for measurement of fresh weight, dry weight, leaf area, photosynthetic pigments and antioxidant activity assay.

2.3 Determination of total flavonoid

Aliuminum chloride colorimetric method was used for flavonoids determined [11]. Each extract of the plant material (0.5ml of 1:10 g/ml) in methanol was separately mixed with 1.5ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water. The extract remained at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with UV-VIS spectrophotometer . The calibration plot was generated by using quercetin solutions. Total flavonoid values are expressed mg g^{-1}dw.

Table 2 Effect of low frequency electromagnetic radiation on rate of enzymatic and non-enzymatic antioxidant in *S .bachtiarica*

	Phenol (mg g^{-1}dw)	Flavonoid (mg g^{-1}dw)	Proline (µM g^{-1} fw)
Control	1.37±0.02	1.43±0.04	0.04±0.002
Treatment	2.48±0.09 *	2.05±0.15 *	0.07±0.004*

Results are means ±SE of 3 replicates. Significant level for Student test is shown of P<0.05.

2.4 Determination of total phenol

Total phenol content was determined by Folin Ciocalteu reagent [27]. A dilute solution of extract (0.5 ml of 1:10 g ml^{-1}) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5ml ,1:10 diluted with distilled water) and aqueous Na2CO3 (4ml,1M). The mixture was allowed to stand for 15 min and the phenols were determined by colorimetry at 765 nm.The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg ml^{-1} solutions of gallic acid in methanol:water (50:50, v/v).Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1}dw).

2.5 Determination of proline content

Free proline content in the leaves was determined following the method of Bates et al. [6]. Leaf samples (0.5g)were homogenized in 5mL of sulfosalycylic acid (3%) using mortar and pestle. 2 ml of the extract were taken in a test tube and to it 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added. The reaction mixture was boiled in a water bath at 100 ^{o}C for 30min. After cooling the reaction mixture, 6ml toluene were added and then transferred to a separating funnel. After thorough mixing,

the chromophore containing toluene was separated and absorption was read at 520 nm. Toluene was used as blank. Concentration of proline was estimated by referring to a standard curve of proline. Calculate the absorbance of the diluted sample and it was converted to µM g^{-1} fw.

2.6 Statistical analyses

analyzes of variance (ANOVA) followed by Duncan's multiple range test were performed using the SPSS 18.0 for Windows statistical software package. Differences were considered significant at the P<0.05 level.

3 RESULTS

3.1 Growth characteristics

Morphological observations in our study showed that, according to (table. 1) in the irradiation samples in comparison to control, the percentage of seed germination increased that was significantly. The average of root length increased .This different in root length was not significantly. EMF exposure, caused significant increase in mean of shoot length (fig. 2). A significant decrease in rate of Leaf area, fresh weight and dry weight was observed in comparison to control (fig. 3).

Table 1. Effect of low frequency electromagnetic radiation on growth parameters of *S.bachtiarica* seedlings.

	root length cm	Shoot length cm	Fresh weight g $plant^{-1}$	Dry weight g $plant^{-1}$	Leaf area cm^2 $plant^{-1}$
Control	3.65±0.24	5.82±.10	2.16±0.09	0.53 ±0.05	2.81±0.13
Treatment	4.29±0.14	4.20±0.16*	1.48±0.14*	0.35 ±0.03*	2.32 ±0.05*

Results are means ±SE of 3 replicates. Significant level for Student test is shown of P<0.05.

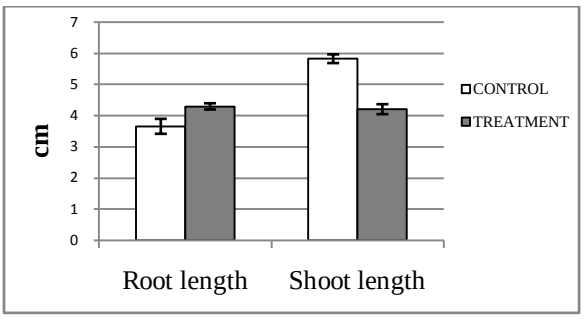

Fig. 2 Effect of low frequency electromagnetic radiation on growth parameters in S.bachtiarica. Results are means ±SE of 3 replicates. Significant level for Student test is shown of P<0.05.

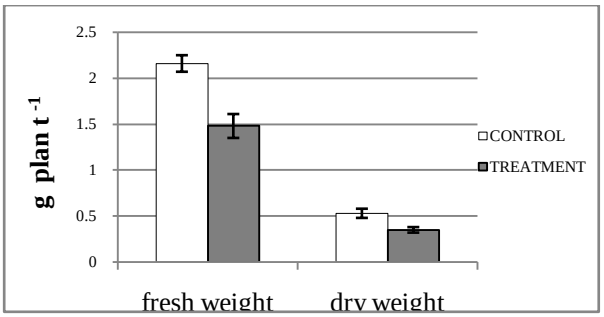

Fig. 3 Effect of low frequency electromagnetic radiation on Fresh and dry biomass weight in S.bachtiarica. Results are means ±SE of 3 replicates. Significant level for Student test is shown of P<0.05.

3.2 Non-enzymatic antioxidant activity assays

In S.bachtiarica plants EMF exposure caused significant increase in activity non-enzymatic antioxidant such as phenol, flavonoids(fig. 4).The increasing in the level of phenol and flavonoids are considered as an important responses of EMFr. Our study showed that the content of proline significantly increased in irradiation plants. In fact electromagnetic radiation exposure induced an increase in the content of this compound comparison to the control plants (fig. 5).

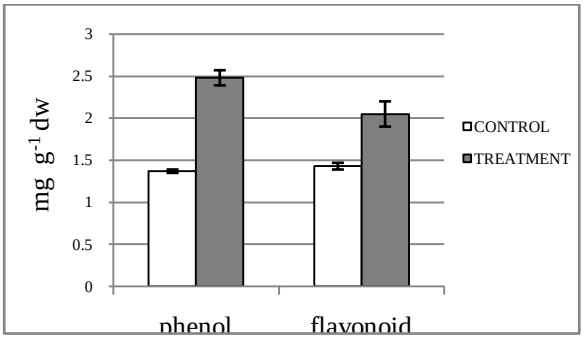

Fig. 4 Effect of low frequency electromagnetic radiation on flavonoid and phenol in S.bachtiarica. Results are means ±SE of 3 replicates. Significant level for Student test is shown of P<0.05.

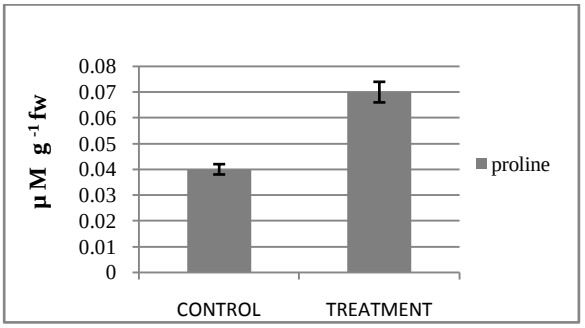

Fig. 5 Effect of low frequency electromagnetic radiation on proline in S.bachtiarica. Results are means ±SE of 3 replicates. Significant level for Student test is shown of P<0.05.

4 CONCLUSION

ROS are usually kept in balance by the antioxidative mechanisms that exist in all living beings. Because ROS have an important signalling role in plants, their concentration must be carefully controlled through adequate pathways [28]. Thus, oxidative stress can be defined as the physiological changes and induces a metabolic response in the plant. [9], [24]. In our research, the treatment samples in comparison to control, showed that the percentage of seed germination increased. The possible reason for intensification of germination, may be the increasing of metabolism in irradiation seeds and increase of rate of substances consumption and more water absorption under effect of EMFr [29], [40]. In the treatment samples , Reduction of shoot length, caused to destruction of the growth regulator indol-3-acetic acid (IAA). Practically inhibition of elongation in EMF irradiation plants might also be due to the action of peroxidases working as IAA-oxidase, causing a decrease in cell wall extensibility [18], [32]. EMF exposure decreased leaf area and this decrease was significantly in radiation exposed plants [30], [44], [42]. Reduce of leaf area under EMF radiation is a photomorphogenic response the can limit the damage to leaf tissue caused by radiation [19]. The decrease in leaf area in response in both the rate and extent of cell division and elongation. EMF radiation decreased the proportion of mitotically active cells and increased the time taken for cell division [17]. Rate of fresh and dry weight and Leaf area in irradiation samples in comparison to control, significantly decreased. The possible reason for Reduction of fresh and dry biomass weight, might be due to reduction of the area leaf [30]. EMF exposure, caused significant increase in activity of non-enzymatic antioxidant. The increase in ROS scavenging capacity brought about by increased intracellular non-enzymatic antioxidant levels could be a key mechanism in reduce of the cellular damage. Flavonoids are one of the largest classes of plant phenolic, perform very different functions such as defense. Plants are able to prevent the dangerous effects of EMFr by synthesizing flavonoids, a class of radiation absorbing compounds located mainly in the epidermis and acting as an internal filter. In higher plants, flavonoids accumulate in large quantities in the vacuoles of epidermal cells of leaves and stems and absorb EMF radiation [13], [26], may offer a measure of protection by screening out harmful EMF radiation [10]. According to Tevini et al. [41], flavonoid accumulation is regarded as a defense mechanism in higher plants to provide protection against radiation, Hence, it is concluded that the EMF treated seedlings may activate a defense mechanism against EMF damage by increasing flavonoid content. Plants produce a large variety of secondary metabolites that contain a phenol group, a hydroxyl functional group on an aromatic ring called Phenol, a chemically heterogeneous group also. Phenols accumulate in plant tissues during stress and due to oxidant damage. Phenols concentration should also depend on the competition for the allocation of photosynthetically fixed carbon to growth or defense [33]. The phenols could be an important part of the plants defense system against biotic and abiotic stresses[28], [38]. EMF exposure, caused significant increase in rate of proline. The accumulation of proline to high levels in plant cells under stress could greatly increase the ROS scavenging capacity of said cells and reduce the potential for oxidative damage. Proline is a proteinogenic amino acid with an exceptional conformational rigidity, and is essential for primary metabolism [36]. Proline could potentially acting as storage reserve of carbon and nitrogen, a compatible osmolyte, a buffer for cytosolic pH, a scavenger of reactive oxygen species (ROS) and as an aid to balancing cellular redox status [15], [36]. proline could act as a molecular chaperone, helping to stabilize the structure of proteins, and as part of the signal transduction chain alerting plant cells to the presence of a stressor and hence triggering adaptive responses [25]. In fact, proline has the potential to reduce ROS levels it could help reduce oxidative damage to vital cellular macromolecules and hence stabilize proteins [4], DNA [23], RNA[20] and lipid membranes [1]. The increase in ROS scavenging capacity brought about by increased intracellular proline levels could be a key mechanism by which proline helps reduce the cellular damage associated. In addition to proline to a solution stabilizes the native structure of protein monomers and protects oligomeric protein complexes from denaturation and dissociation. The accumulation of proline could also be a mechanism to store energy as the oxidation of a single proline molecule can produce up to 30 ATP equivalent [15], [16].

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