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Effects of high frequency electromagnetic wave and salicylic acid on the growth and some physiological and biochemical parameters in *Lycopersicon esculentum*

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Abstract. Salicylic acid in a phenolic phytohormone with roles in plant growth and development.It also acts an endogenous signaling during biotic and abiotic stresses. Among various stresses the impacts of high frequency Electromagnetic waves (hfew) on plant growth and development have not been studied extensively.in the present study, the effect of 0.0, 0.05 and 0.1 mM salicylic acid or pretreatment of seed for 48 hrs with electromagnetic waves of high frequency (940 MHz) on plant growth and some biochemical parameters were investigated.hydrogen peroxide increased at 0.05 and 0.1 mM salicylic acid compared to control. Hydrogen peroxide also increased when seeds were pre-treated with HFEW. In contrast, carotenoids and anthocyanin content decreased in the presence of salicylic acid. Chlorophyll and carotenoids declined while antocyanin increased in pre-treated seeds with HFEW.Malondialdehyde content, as a sign of lipid peroxidation, increased in the presence of both salicylic acid and HFEW. It is concluded that high salicylic acid and HFEW cause oxidative stress in plant which can result in reduced plant growth and development.

Keywords: Lycopersicon esculentum, High frequency electromagnetic wave, Biochemical parameters,Salicylic acid

1. INTRODUCTION

Salicylic acid,from latin salix, is a monohydroxybenzoic acid (Popova et al.,1997). It is a phytohormone with various activities such as seed germination, fruit ripening, glycolysis, flowering and also is involved in systemic acquired resistance (chen et al. 2007). SA is synthesized through two distinct and compartmentalized pathways that employ different precursors: the phenylpropanoid route in the cytoplasm initiates from phenylalanine, and the isochorismate pathway takes place in the chloroplast. Most of the SA synthesized in plants is glucosylated and/or methylated. Glucose conjugation at the hydroxyl group of SA results in formation of the SA glucoside [SA 2-*O*-β-D-glucoside] as a major conjugate, whereas glucose conjugation at the SA carboxyl group produces the SA glucose ester in minor amounts. These conjugation reactions are catalysed by cytosolic SA glucosyltransferases that are induced by SA application or pathogen attack in tobacco and *Arabidopsis* plants (song 2006).

Different kinds of the electromagnet waves are existed around us and the other creatures of the earth perpetually. All live creatures are affected by them. Mobile is one of the generators of electromagnet waves with frequency band 900 Megahertz and Base BTS Transceiver Station masts with frequency 1800 to 2200 Megahertz which are the reinforce masts for mobile are located in cities and sensitive place including primary schools, kindergartens and people environments greatly. These electromagnet waves affect alive creatures destructively such as plants because of the daily usage (Ayrapetyan, 2006).

It is displayed in some surveys that plants in electromagnet fields affect the germination speed of seeds, length and diameter of growth seeds (Kresimir et al., 2009). Different researches show electromagnet waves are effective on different process of plants live including germination and generation growth, structure and action of herbal cells (Huge, 2009).The results demonstrate that electromagnet radiations especially with lower frequency impress the electrical characteristics of membrane and ever existent water in seed and it causes the acceleration of biological process and material exchange in seed cells. This intensification originates the entrance of nourishing materials to the whole volume of seed and because of this action seeds have more speed and percentage for germination (Alorainy, 2003;

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Ayrapetyan, 2006). On the studies have been done about microwave, this device has produce the electromagnet waves about 2.45 gigahertz which it is able to absorb the water of materials and if they are impressed under these waves the irrecoverable effects will be made (Davies, 1996).

In a laboratory study, the lettuce seeds have shown the remarkable increase in the electromagnet field 10 mT. The researchers have deduced that the electromagnet field changes the watery connection in seed and this effect may explain the change germination speed on treatment seeds under electromagnet field partly (Aladjadjiyan, 2002). Garcia and his colleagues have observed the speed increase of water and germination on lettuce seed under electromagnet field from 1 to 10mT and presented that it may be possible that changes on the inner parts of cell, calcium ion congestion and other ions including potassium over cell membrane cause the change in the osmotic pressure and the power of cell tissues for absorbing water (Arza and Garcia, 2001).

The changes in the Amylase enzymes and Nitrate Reductase activities have been reported on the germinating seed under electromagnet treatment (Yinan et al., 2005). The study of respiratory characteristics in the seed with 10mT represents that the outgoing intensity and amount of dioxide carbon in the treatment condition increase about 70 to 100%. There are some evidences that the electromagnet field has caused the content decreasing of photosynthesis pigments (Chlorophyll a,b) in the bean leaves (Belyavskaya, 2004). It has been represented in such survey that plants under the radiation of electromagnet waves, have more length and diameter (Goldworthy, 2006). According to this view, the present research results are parallel to these ideas and the waves of mobile increase the germination speed and the diameter of treatment sample vessels than control group. The cytochemical studies have represented that root cells under weak magnet field than control group cells have shown calcium saturation manner in all their organs and cytoplasm. The magnet field can increase the release of free radicals and producing tensions in plant, while calcium ions aid in some plant growth process and react to tension. Therefore, this subject is an explanation for calcium increase in plant in magnet filed condition (Dhawi et al., 2009).The positive effect of magnet field has shown in proteins biosynthesis, cell multiplication, biochemical activities, respiration measure, enzymes activities, Nucleic Acid and development and growth period (Cakmak et al., 2009). Cells have been watched with the increase in the content of DNA in root meristems and bud embryo in the bulb after being under the magnet field (Ayrapetyan, 2006). It is reported that positive effects of magnet field treatment may be in connection with paramagnet of atoms in herbal cells and pigments such as chloroplast (Aladjian, 2010).It is shown in the studies which have been done in the seaweed cells in California that seaweeds cells are damaged and demolished under electromagnet waves. It has been seen in this research that the amount of abnormal cell has increased under electromagnet waves.The effect of magnetic fields are studied on enzymes activities of Antioxidant including Peroxidase, Polyphenol Oxidase and Catalase in herbal cells and it has been showed that the magnet filed can affect the Antioxidant system and increase of activities of cell free radicals in herbal cells like animals and human (Belyavskaya and Kondrachuk, 2004).

So, our purpose in these laboratory researches is to survey the effects of electromagnetic waves with high frequency 900-1000 MHz on biochemical and physiology factors of *Lycopersicun esculentum.*

2. MATERIALS AND METHODS

In this research seeds were bought from Hungary by Shiraz Zare Seed Company. For doing researches and study the mobile waves on the biochemical and physiological responses of the *Lycopersicun esculentum* plant, the mobile simulator device should be provided which was controllable according to radiation and frequency arrangement.

Making the mobile simulator device by Electrical and Computer Department of Shiraz University with such characteristics: the designed high frequency system in this project has characteristics as follows:

the production of sinus wave of narrow band with the facility to regulate the frequency about 800 to 1000 MHz.. The possibility of regulating exhausted potency more than 3 watt and the resistance of device is considered 5 ohm too.

3. THE PREPARATION OF THE SEEDS

For surveying the pre-treatment effect of Salicylic Acid on some of growth parameters, Peroxidase enzyme activities and the contents of MDA, the experiment was done as an accidental complete plan with 4 treatments in 4 repetitions. The considered sample seeds were washed with distilled water for 5 minutes after disinfection soaked on Salicylic Acid with 0, 0/05 and 0/1 concentration and natural water separately for 3 hours. Next, soaked seeds in Salicylic Acid solution were transferred to sterilization petri dishes with Whatman filter paper number 1 and after 3 days the plants were transferred to plastic pots with perlite. 5o ml of half-power Hoagland nutrient was given to each pot daily. After 21 growth days, the plants were harvested for analysis. The soaked seeds were put in natural water for electromagnet treatment and then seeds were transformed to little pots by 250 cc perlite. Daily, they were irrigated by 5o cc of water with half-power Hoagland nutrient. After a week, *Lycopersicon esculentem* seedlings were located in the mobile simulated device with high frequency 900 MHz for 0, 3, 5 hours in a day for a week. A control was located in the simulated device with 4 repetitions about 5 hours daily for a week in off mode. During the experiment the degree was 28 ^{c} and the humidity 31%. The inside temperature of the device was measured before and after treatment and the difference from 0/1 to 0/2 centigrade was observed. After 21 days, the plants were harvested for analysis and the aerial parts were separated from root and they were freezed by liquid Nitrogen and they were kept in freezer -20^{c} until experiment time.

For measuring the amount of Chlorophyll, Carotenoids, the method of (Lichtenthaler, 1987) was used. 0.02 gram of fresh leaves were grinded in a porcelain mortar containing 15 mM Acetone 80% and after filtering by filter paper, its absorption was read with spectrophotometer UV Visible, SPEKOOL 1500 model made in German at the wave lengths 646/8, 663/20 and 470 nanometer. For resetting the device, the Acetone 80% was used. The concentration of pigments was calculated by the followed formula:

$$Chla = 12.25\ A_{663.2} - 2.79\ A_{646.8}$$

$$Chlb = 21.21\ A_{646.8} - 5.1\ A_{663.2}$$

$$Car = (1000\ A_{470} - 1.8\ chla - 85.02\ chlb) / 198$$

In these formulas, Chla, Chlb, and Car are Chlorophyll a, Chlorophyll b and Carotenoids respectively (including Carotenes and Xanthophyll's). The concentration is determined based on mg.ml^{-1} of sap. Results of photosynthesis pigments measurement are represented and calculated based on mg per gram of moist weight.

For measuring the amount of leaf Anthocyanin, the method of (Wagner, 1979) was used. 0/1 gram of leaf tissue was grinded in a porcelain mortar containing 10 ml acidic Methanol (pure Methanol and pure Choloridric Acid in the content proportion of 1:99) completely grinded and the sap was poured in a Falcon and they were put in the darkness and at 25.^{c} for 24 hours. Then they were centrifuged with 4000rpm, the absorption of over solution was calculated at the wave length 550 nanometer. The extinction coefficient 33000 (ε)M^{-1}cm^{-1} was considered for concentration measurement.

$$A = \varepsilon bc$$

A= Absorption, B= Width of cuvet, C= Concentration of the considered solution

The measurement of Mallondialdehyde concentration (MDA), was done by (Heath and Packer, 1969) method. According to this method, 0/02 gram of fresh leaf tissue was weighed and grinded in a porcelain mortar containing 5 ml Tri Coloro acetic Acid (TCA) 0/1 %. The produced sap was centrifuged in a centrifuge for 5 minutes at 10000gr. 4 ml of TCA 40% with 5% Thiobarbituric Acid (TBA) was added

to 1 ml of over solution. The produced solution was heated for 30 minutes in the temperature of 95.^{c} of hot water bath. Then, they were cold in ice immediately and again it was centrifuged for 10 minutes in 10000gr. The absorption speed of this solution was read by spectro photometer at the wave length 532 nanometer. The considered material for absorption at this wave length is Red Complex (MDA-TBA). For calculating the concentration of MDA, the extinction coefficient equal $M^{-1}cm^{-1}$ $1/56\times10^{5}$ and the measurement results were calculated and presented according to moist weight.

The measurement of Hydrogen Peroxide was done by (Velikova et al, 2000) method. The aerial organs of plant were grinded at the ice bath with Tri Coloristic Acid 1%. The sap was centrifuged at the refrigerated centrifuge with Centrifuge 5804 R-Germany model, from Eppendorf Company at the temperature of 4^{c} in 10000gr for 15 minutes. Then 0/5ml of solution was added to Potassium Phosphate buffered 10 mM (PH=7) and one ml Potassium Iodine one molar. The absorption was read at the wave length 390 nanometer. The amount of Hydrogen Peroxide in each sample was calculated by extinction coefficient $M^{-1}cm^{-1}$ 0/28 and based on micromole on gram of the moist weigh was reported.

4. STATISTICAL ANALYSIS

The experiment was done at the model of accidental compete plan. The data analysis was done by statistical software SPSS 16 and ANOVA test. The comparison of the averages was used for the Duncan test at the probability 5% and for drawing figures Excel software was used.

5. .RESULT

5.1. The effect of the Salicylic Acid and electromagnet 940MHZ on the Carotenoids concentration

As it is shown at the figure 1, the Salicylic Acid treatment 0/1 and 0/05 millimolar caused the decreasing of Carotenoid than control group plant and the least content was related to treatment of Salicylic Acid 0/1 mM. But any meaningful difference in the amount of Carotenoid was not shown in the electromagnet treatment.

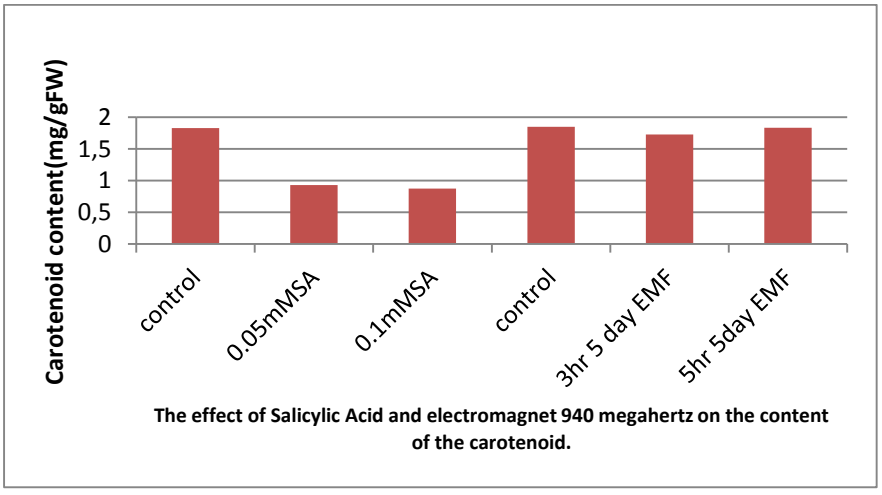

Figure 1. Effects of Salicylic Acid and electromagnetic wave 940 megahertz on the content of the carotenoids. The amounts were the average of 4 repetitions comparison of the averages was done based on Duncan test at $P<0.05$.

5.2. The effect of the Salicylic Acid and electromagnet 940MHZ on the Anthocyanin concentration

The increase of Anthocyanin concentration caused the meaningful decrease of Anthocyanin at the plant. As it was shown at the figure 2, the concentration of Salicylic Acid 0.1 mM allocated the lowest concentration to itself. The treatment of the electromagnet 940 MHz caused the significantly decrease of the Anthocyanin at the *Lycopersicon esculentum* plant.

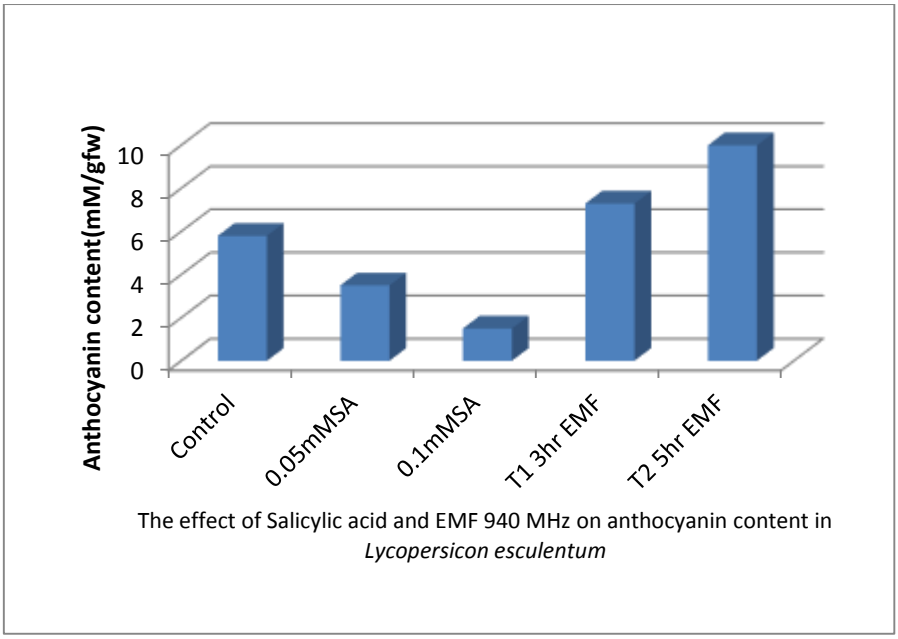

Figure 2. Effect of Electromagnetic Field and Salicylic acid on the anthocyanin content. The amounts were the average of 4 repetitions and the comparison of the averages was done based on Duncan test at P<0.05.

5.3. The effect of the Salicylic Acid and electromagnet 940 MHz on the Mallondialdehyde concentration

As it is shown at the figure 3, the increase of Salicylic Acid concentration caused the meaningful decrease of Mallondialdehyde at the plant. But the treatment of the electromagnet waves caused the meaningful increase of the lipid Peroxidation amount of the membrane.

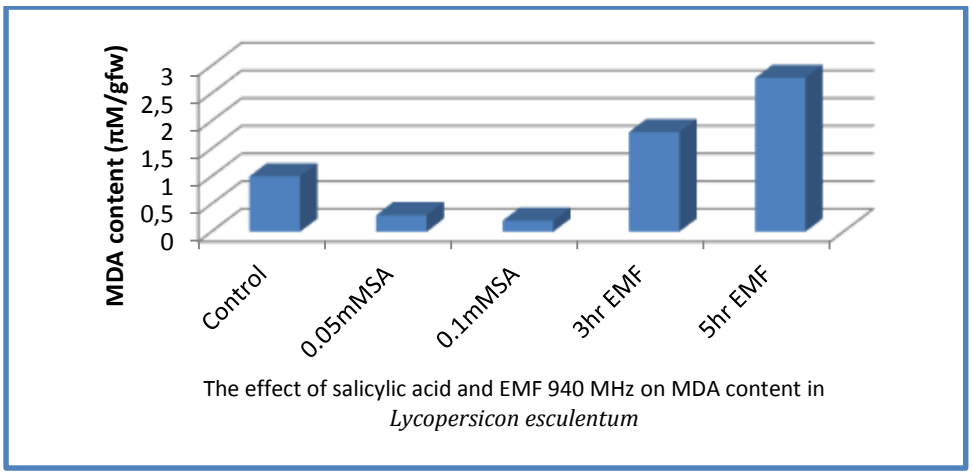

Figure 3. Effect of Electromagnetic wave and Salicylic acid on the content of Mallondialdehyde. The amounts were the average of 4 repetitions and the comparison of the averages was done based on Duncan test at P<0.05.

5.4. The effect of the Salicylic Acid and electromagnet wave on the Hydrogen Peroxide concentration

Salicylic treatments 0/05 and 0/1 mM caused the increase of Hydrogen Peroxide content than control group and the highest amount of the content was related to the treatment Salicylic Acid 0.05 mM (fig 4). Also, the treatment of electromagnet waves 940 MHz caused the increase in the amount of the Hydrogen Peroxide than control group and the highest content was related to the treatment of 5 hours daily for a week.

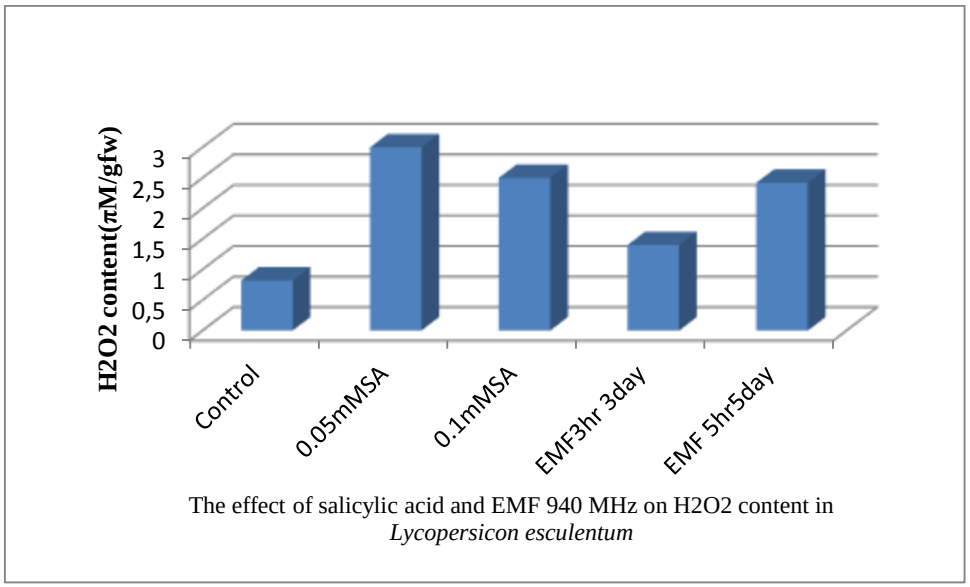

Figure 4. Effect of Electromagnetic wave and salicylic acid on the content of Hydrogen Peroxide. The amounts were the average of 4 repetitions and the comparison of the averages was done based on Duncan test at $P<0.05$.

6. DISCUSSION

6.1. The discussion about the effect of Electromagnet waves on *Lycopersicon esculentum* seedlings

The studies have shown that electromagnet field effect on nuclear spin of paramagnet articles. This mechanism has important effect on the syntax of ions order and paramagnet components that can increases the mid-life of free radicals and damaging potential and with this effect, they have destructive impress on Antioxidant system inside the cell.

Epidemiological and experimental data have attracted attention to the biological effects of EMF.Primary action of EMF in biological systems is the induction of electrical charges and currents [Roy and Repacholi, 2005]. One of the major molecular effects of MFs is their influence on nuclear spins of paramagnetic molecules. This mechanism plays an important role when in the course of a chemical reaction the chemical bound is disrupted and two molecules with unpaired electrons are formed (a pair of radicals). Depending on their spin orientations, radical recombination or diffusion and formation of free radicals (e.g., Oxygenradicals) may take place [Kula et al., 2002; Sobczaket al., 2002]. The radical pairs may be affected for a long and continuous time interval by the MF(Zmyslony et al., 2000). This may extend the lifetime of the free radical and its potential damage could be exaggerated.

Although formed in normal cell metabolism, free radicals are potentially damaging and can initiate chain reactions to form new free radicals. A main protective role against free radicals is attributed to SOD in catalyzing and dismutation of superoxide anions to O2.and H2O2 (Sreenivasulu et al., 2000).

6.2. Malondialdehyde (MDA) as a lipid peroxidation marker

Free radicals generate the lipid peroxidation process in an organism. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress and the antioxidant status in cancerous patients.(Gaweł et al 2004)

In this project, the treatment with Salicylic Acid has caused the decrease of Carotenoids. Carotenoids could get the high energy of the short waves and change the unique Oxygen to triplex one and perform its Anti-Oxidant impress by getting produced Oxygen radicals (*Qinghua and Zhujun, 2008*). Usually, Salicylic Acid has regulated most of the physiologic and growth process of plant with effect on Abscisic Acid Hormones and Ethylene (Zhu, 2001). It has caused the acclimatization of the plant to environmental tensions including with the effect on Abscisic Acid hormones and the aggregation of this hormone in plant. Carotenoids are a pre material for Abscisic Acid synthesis. So the observed decrease in the amount of Carotenoid in this project can be for changing Carotenoids to Abscisic Acid. The existed Anthocyanin in the plant operates as the free radicals recipient and protects plants against Oxidative tensions (*Sairam et al, 199*8). Salicylic Acid causes the decrease of Anthocyanin. The reason has been related to the Etylin synthesis (*Qinghua and Zhujun, 200).* It is probable that Ethylene with effect on biosynthesis way of Anthocyanin and Flavonoids such as Phenyl Alanine Ammonia Liaz, causes the aggregation of the Anthocyanin's in the plant (Hydro and Yang, 1997).

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