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# Effects of 940 MHz electromagnetic fields on *Malondealdehyde* content in Zeamays

**Habibeh Zare**

Ph.D Biology, Department of Biology, Payame Noor University (PNU) , Iran

**ABSTRACT**

Electromagnetic fields are examples of abiotic stresses. Nowadays, the world sinks in lesser-known species of messages and signals that encompasses the environment. So living creatures are in exposure of electromagnetic fields. Living cells are charged that are created by ions and free radicals. Electromagnetic fields with interaction between the ions particularly fero magnetic materials such as iron affect on living cells. These environmental factors can significantly affect living cells in a short time and low intensity.

In this research, the effects of electromagnetic waves with high frequency of 940 MHZ on biochemical, physiological factors of seedling corn (Zea mays L) have been examined. corn seeding were put for 10 days in medium perlite and Hoagland of ½ strength. After enough growth, group of plants were treated with high-frequency electromagnetic fields with high frequency (940 MHz) for 3, 5, 7 days respectively each day 3, 5 hours. Biochemical and physiological analyzes on the samples after these steps were under control and treatment.

The content of photosynthetic pigment chlorophyll a, b in electromagnetic field treatment was not significantly increased. But and level of the anthocyanin pigments in electromagnetic field treatment was reduced significantly.superoxide dismutase in leaves have been observed in high-frequency electromagnetic fields (940 MHz) compared with the control were significantly increased.

**Keywords:** Zea mays L., Eletromagnetic wave (940MHz), biochemical factor and Growth factor

***Correspondence to Author:**
Habibeh Zare
Ph.D Biology, Department of Biology, Payame Noor University (PNU) , Iran

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**Introduction**:

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).

It has been shown in the same researches on the cells of red corpuscle that electromagnet waves of mobile cause the destruction and deformation of blood red corpuscle (Draper et al., 2005).

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.

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:**

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 17 days of age were hydroponically cultivated and grown in a culture room under standard conditions (30/25°C day/night and 31% relative humidity).

**Total chlorophyll, Chlorophyll a , Chlorophyll b ,Carotenoids and Anthocyanin assay**

Chlorophyll can easily be quantified with a spectrophotometer based on the Beer-Lambert Law and the extinction coefficient for chlorophyll. In a classic paper, Arnon (1949) reported the following equations for quantification of the total chlorophyll, chlorophyll **a** and chlorophyll **b** content in an 80% acetone extract:

Total chlorophyll = 20.2 ($A_{645}$) + 8.02 ($A_{663}$)
Chlorophyll **a** = 12.7 ($A_{663}$) - 2.69 ($A_{645}$)
Chlorophyll **b** = 22.9 ($A_{645}$) - 4.68 ($A_{663}$)

where $A_{663}$ is the solution absorbance at 663 nm and $A_{645}$ is the absorption at 645.

Unfortunately, the Arnon equations are not particularly accurate (Porra, 2002). Other equations have been derived that minimize the problems with the Arnon equations. For example, Lichtenthaler & Welburn (1983) report the following equations to determine chlorophyll **a**, chlorophyll **b** and carotenoid content in 80% acetone extracts:

Chlorophyll **a** (µg/ml) = 12.21 ($A_{663}$) - 2.81 ($A_{646}$)
Chlorophyll **b** (µg/ml) = 20.13 ($A_{646}$) - 5.03 ($A_{663}$)
Carotenoids (µg/ml) = (1000$A_{470}$ - 3.27[chl a] - 104[chl b])/227

And, more recently Porra (2002) reports the following equations in buffered aqueous 80% acetone:

Chlorophyll **a** (µg/ml) = 12.25 ($A_{663.6}$) - 2.55 ($A_{646.6}$)
Chlorophyll **b** (µg/ml) = 20.31 ($A_{646.6}$) - 4.91 ($A_{663.6}$)
Total chl (µg/ml) = 17.76 ($A_{646.6}$) + 7.34 ($A_{663.6}$)

If the absorbance is greater than 0.8 then the solutions should be diluted with fresh 80% acetone and remeasured.

Sims and Gamon (2002) used a solution acetone/Tris buffer (80:20 volume; pH = 7.8) to extract tissue and reported equations corrected for the presence of anthocyanins. They report:

anthocyanin (µmol $ml^{-1}$) = 0.08173 $A_{537}$ - 0.00697 $A_{647}$ - 0.002228 $A_{663}$
Chl **a** (µmol $ml^{-1}$) = 0.01373 $A_{663}$ - 0.000897 $A_{537}$ - 0.003046 $A_{647}$
Chl **b** (µmol $ml^{-1}$) = 0.02405 $A_{647}$ - 0.004305 $A_{537}$ - 0.005507 $A_{663}$
Carotenoids (µmol $ml^{-1}$) = ($A_{470}$ - (17.1 x (Chl **a** + Chl **b**) - 9.479 x anthocyanin))/119.26

Since the anthocyanin concentration estimated in the extraction medium (80% acetone) is not reliable, they report equations for determining anthocyanin in methanol/HCL/water (90:1:1, vol:vol:vol):

Anthocyanin absorbance (corrected) = $A_{529}$ - (0.228 $A_{650}$) and then insert the corrected anthocyanin absorbance in the Beer-Lambert expression, A = ecl, assuming a molar absorbance coefficient at 529 nm or 30,000 l $mol^{-1}$ $cm^{-1}$.

**Enzyme assays:**

Specific SOD activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium chloride, as described by Giannopolitis and Ries (1977). The assay mixture consisted of 50 µL of the enzyme extract, 50 mM phosphate buffer (pH= 7.8), 0.1 µM EDTA, 13 mM methionine, 75 µM nitroblue tetrazolium and 2 µM riboflavin in a total volume of 1.5 mL. Riboflavin was added last and tubes were shaken and placed under fluorescent lighting from two 20 W tubes. The reaction was allowed to proceed for 15 min, after which the lights were switched off and the tubes covered with a black cloth. Absorbance of the reaction mixture was read at 560 nm, and one unit of SOD activity (U) was defined as the amount of enzyme required to cause 50 % inhibition of the nitroblue tetrazolium photoreduction rate. The results were expressed as $U.mg^{-1}$ protein.

**Estimation of lipid peroxidation:**

The level of lipid peroxidation was measured by estimating the MDA content according to the method proposed by Heath and Packer (1986). 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 ºC 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.

**Result:**

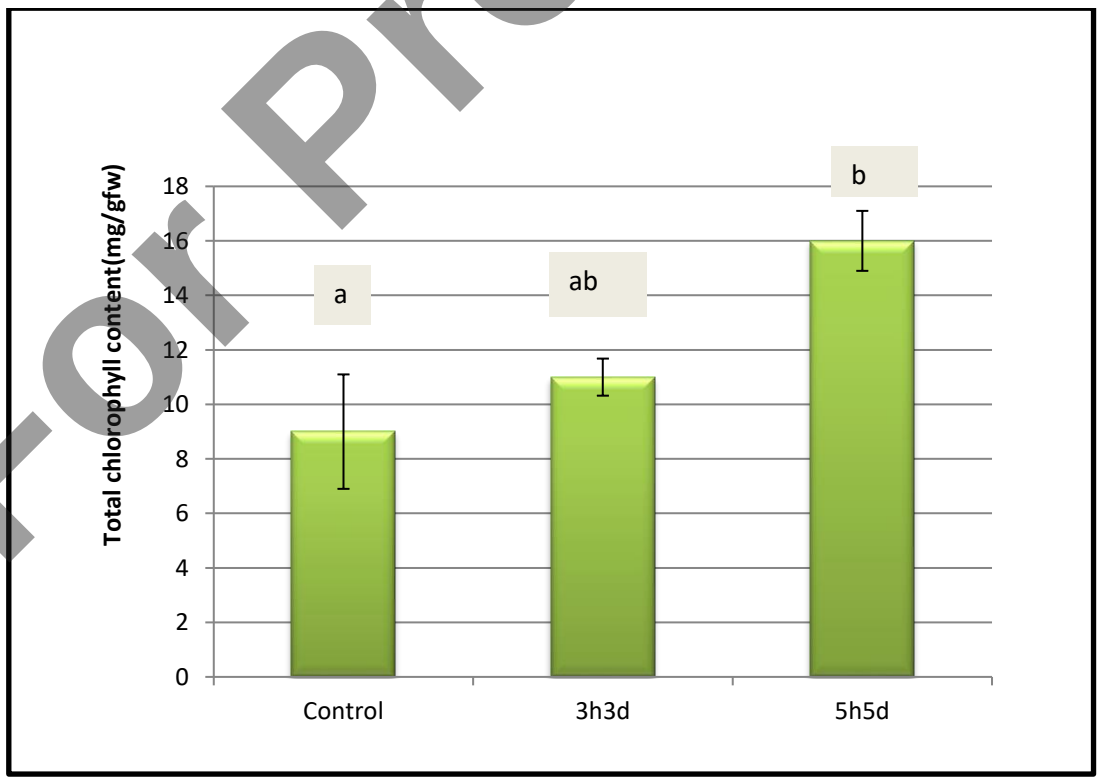


Figure 1. Effects of high frequency electromagnetic wave (940 MHz) on total chlorophyll in *Zea mays* L. Result are means±SE for 4 replicates. Significant level for tuky test is shown at p≤0.05

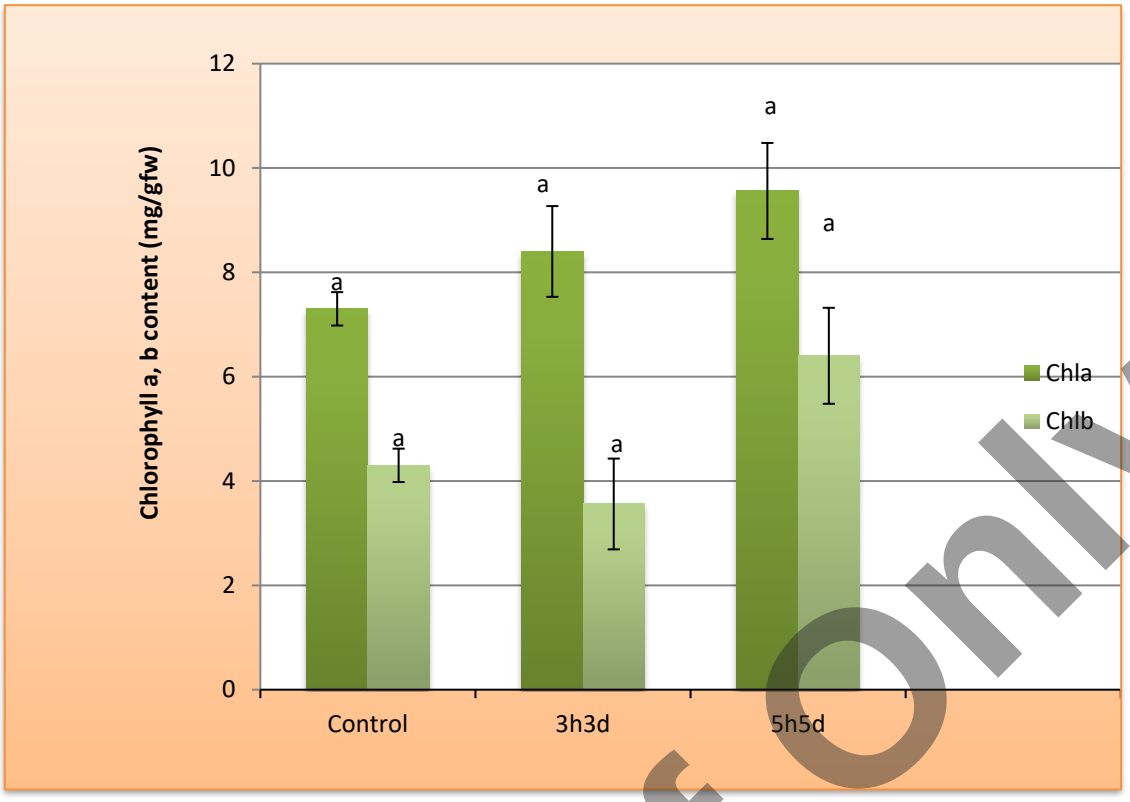


Figure 2. Effects of high frequency electromagnetic wave (940 MHz) on chlorophyll a,b in *Zea mays* L. Result are means±SE for 4 replicates. Significant level for tuky test is shown at p≤0.05

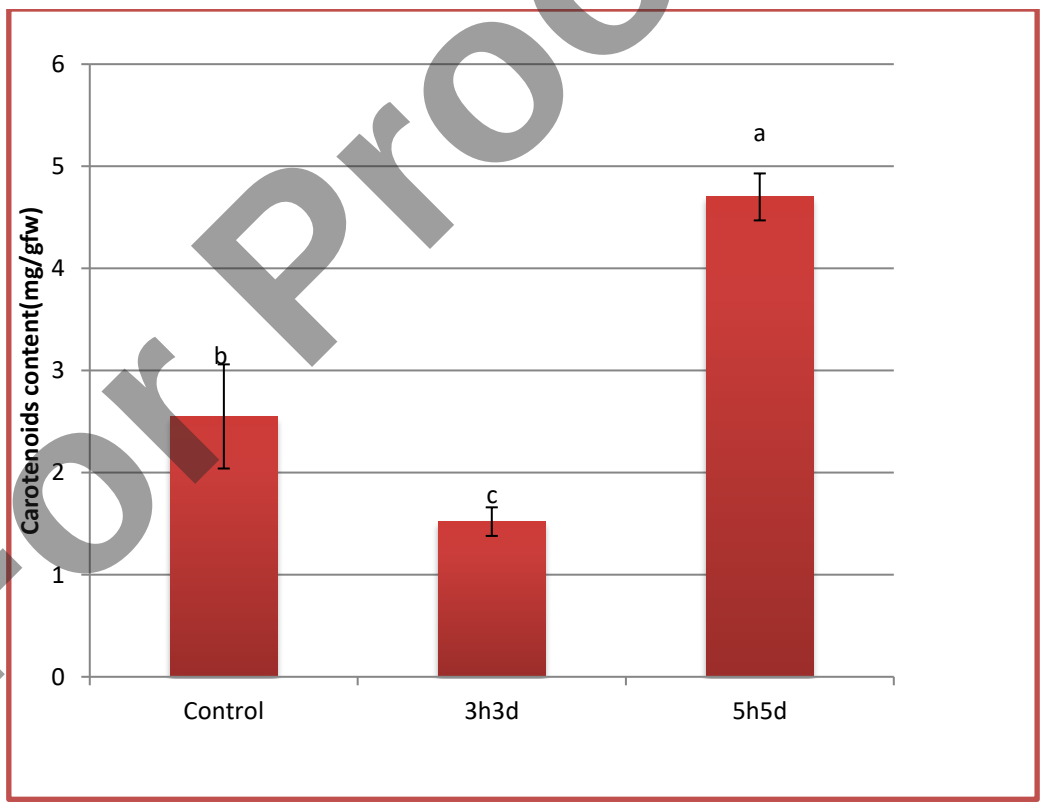


Figure 3. Effects of high frequency electromagnetic wave (940 MHz) on content of carotenoids in *Zea mays* L. Result are means±SE for 4 replicates. Significant level for tuky test is shown at p≤0.05

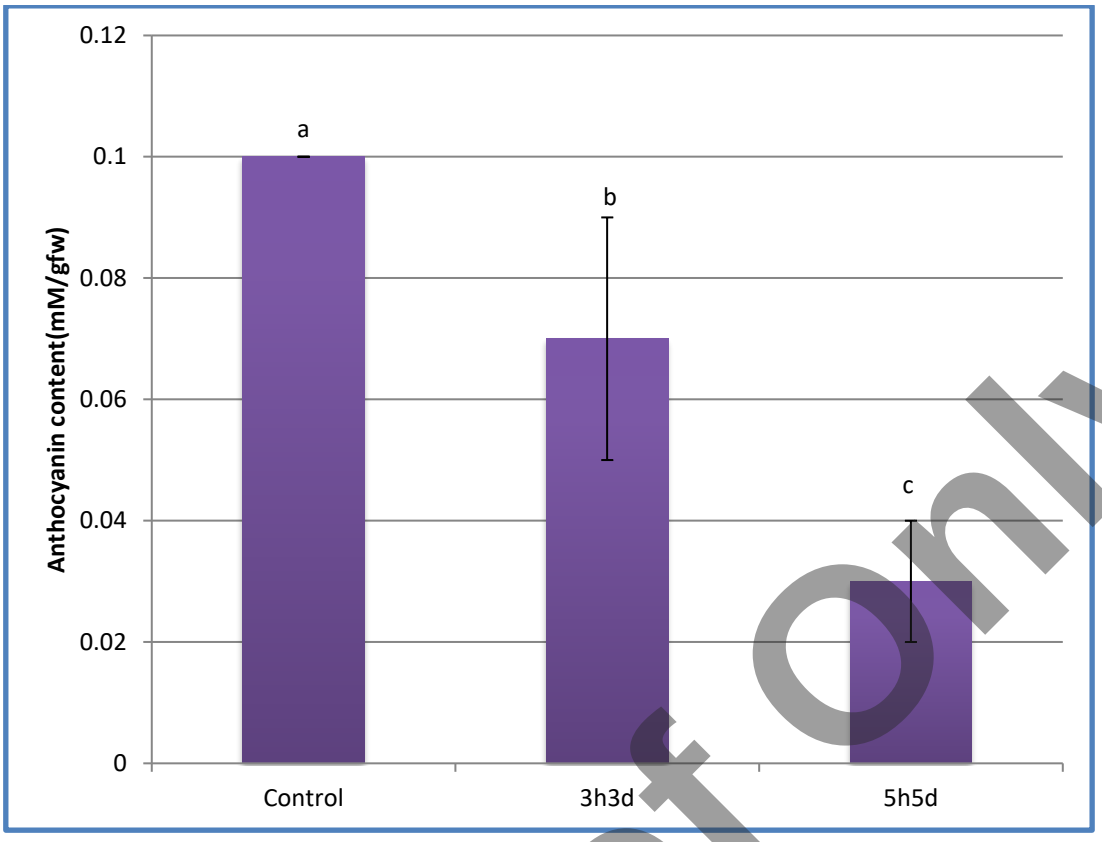


Figure 4. Effects of high frequency electromagnetic wave (940 MHz) on anthocyanin in *Zea mays* L. Result are means±SE for 4 replicates. Significant level for tuky test is shown at p≤0.05

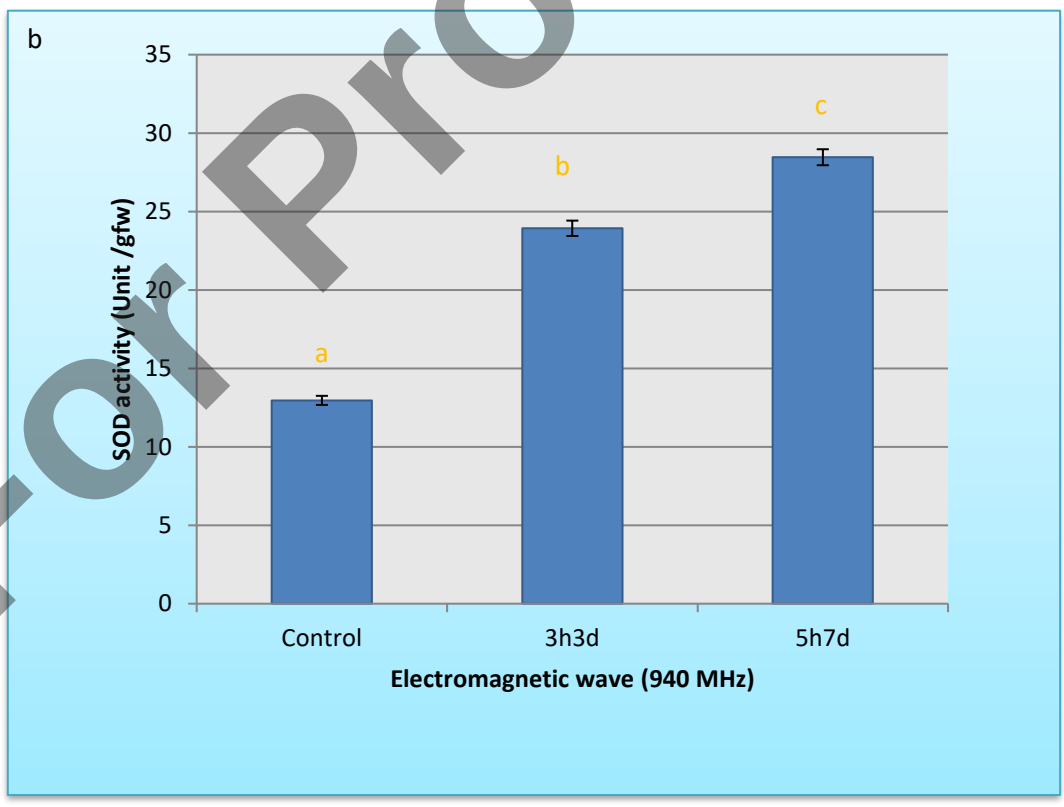


Figure 5. Effects of high frequency electromagnetic wave (940 MHz) on SOD activity in *Zea mays* L. Result are means±SE for 4 replicates. Significant level for tuky test is shown at p≤0.05

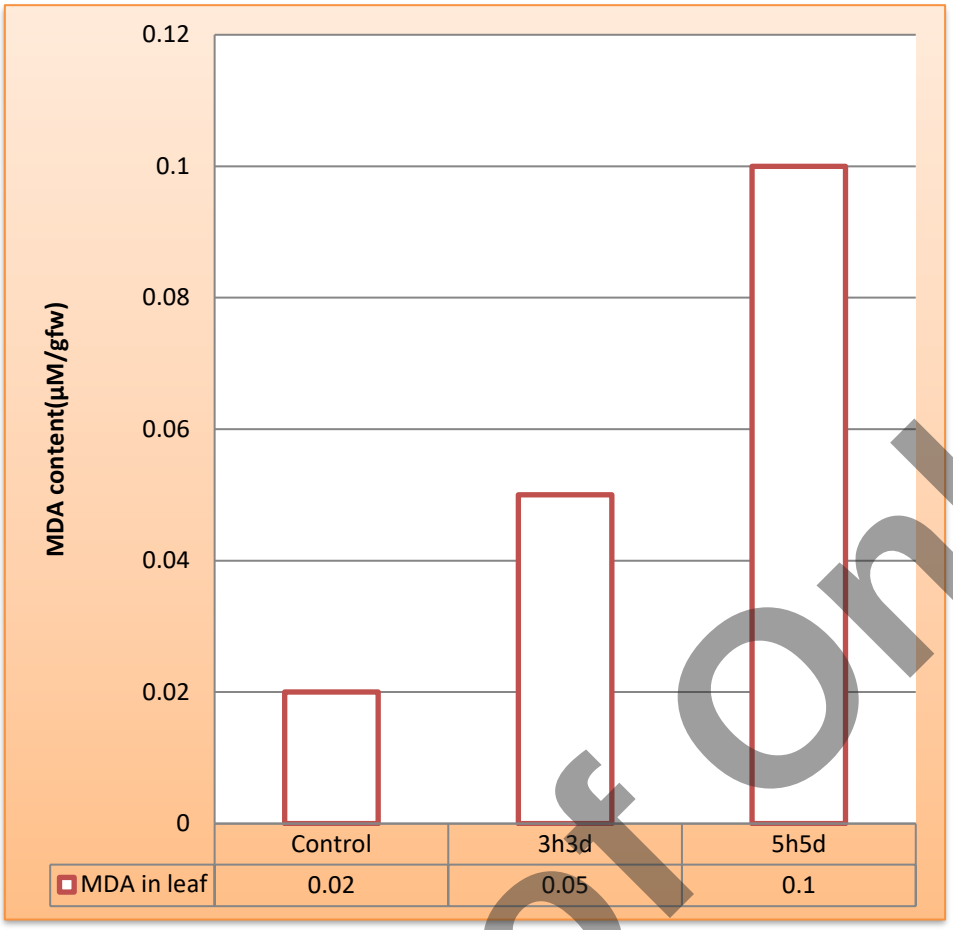


Figure 5. Effects of high frequency electromagnetic wave (940 MHz) on MDA content in *Zea mays* L. Result are means±SE for 4 replicates. Significant level for tuky test is shown at p≤0.05



**Result and discussion:**

**Effects of electromagnetic waves (940 MHz) on the amount of Leaves Chlorophylls and Carotenoids**

The results presented show that the amount of total chlorophyll in leaves increased significantly P<0.05.The total carotenoids content increased.

Base on earned results, using Electromagnetic fields with high frequency to exciting initiation plant growth of (*Zea mays* L.) Seed is possible. Electromagnetic field causes a punctual increase seed. on the other hand it increased germination rate punctually, Exciting of plant growth resulted of *Zea mays* L Sides by electromagnetic field traits can has a negative considerable effect in most advanced levels of plants that needs supplementary examinations.

the values of major anthocyanin content due to electromagnetic waves with a frequency of 940 MHz significantly decrease (P<0.05)

In many reports about mechanism of electromagnetic, is mentioned about function of these fields on impact on membranous channels specially channels of calcium transmission. These fields by induction of electric and magnetic fields to these channels that have electric charge cause opening these channels and increasing calcium in the cellules. Base on function and importance of as the secondary guidance which finally causes activation of many factors of gen explanation. These changes cause changes in synthesis of proteins and other metabolic and biological activities of cellules.

**Antioxidants activity:**

Antioxidants of plant cause neutralization of free radicals like major ones as ascorbic acids, toferol, and glutamine(ghanati et al,2012).

These free radicals of oxygen become hydrogen peroxide by dismutase super oxide enzyme, then become water by scorbat peroxides and

glutamine redoctaze in chloroplast. Also, oxygenized water oust to outer part of catalyze enzyme(ghanatti et al,2012).

In cleaning chloroplast species was cleaned by catalyze enzyme in leaf cells.activated oxygen oxides resulted from stress play significant role in rice saltiness(ghanati,2012).In stress time, usually, activities of enzymes like dismutase super oxide, peroxides ascrobat and redoctaze glutamine were stimulated.

***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 (Mansour et al., 2005). In this study, MDA content increased significantly in plants that were treated for longer durations with EMF.

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 (Mansour et al., 2005). 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.

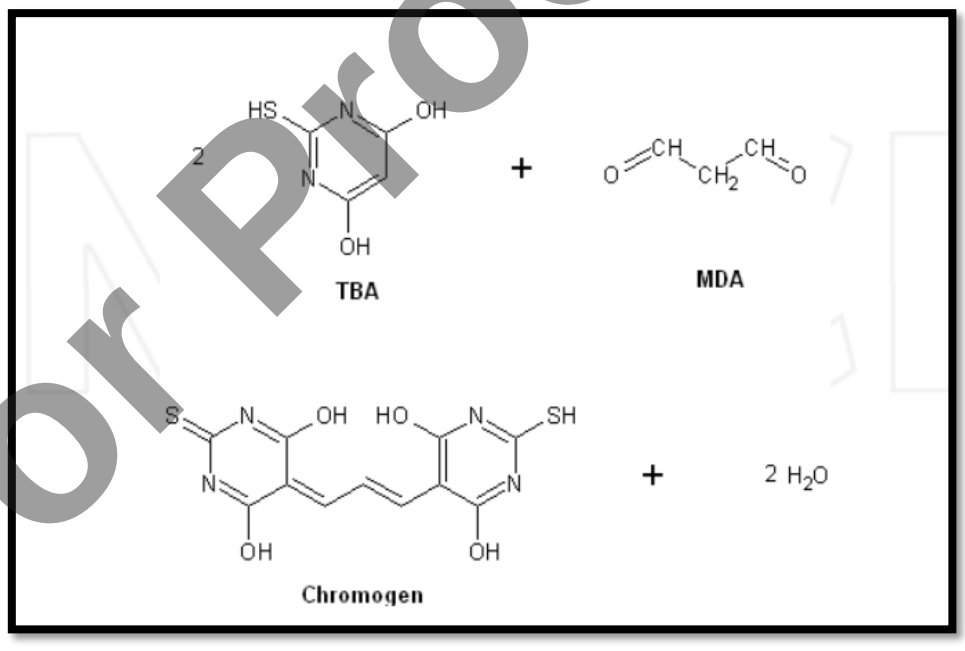


Figure 6.Chromophore produced by a condensation of MDA with TBA



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