Advances in Bioresearch
Adv. Biores., Vol 7 (3) May 2016: 100-108

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV Value 8.21 [2014]

Advances in Bioresearch

ORIGINAL ARTICLE

Effects of Electromagnetic Fields on Seed Germination and Weight, Phenol and Flavonoid Content and Antioxidant System of *Portulaca oleracea*

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ABSTRACT
Electromagnetic field is an inescapable environmental factor for living beings which many investigations have been conducted to evaluate its effect. Portulaca belongs to Portulacaceae family which widespread as a weed in the world. In this study the effects of Electromagnetic fields on seed germination, dry and wet weight, shoot and root length, phenol and flavonoid content and antioxidant activity of Portulaca oleracea L. were studied. The seeds were exposed to electromagnetic field by a magnitude of 1200, 1800, 2400, 3600 G at 20 min in the electromagnetic field generator apparatus. Untreated seeds were used as control under similar condition. The effect of electromagnetic fields on seed germination was evaluated. Germination was quatified as the percentage of germinated seed grains per 100 evaluated, thus, dry and wet weight, shoot and root length were evaluated. Plants were treated with different electromagnetic field (1200, 1800, 2400, 3600 G at 20 min) and the contents of phenolic compounds, total flavonoid, antioxidant activity were investigated. The results were analyzed using ANOVA statistical to analyses of the treatments. The results of experiments suggested that treated seeds had 100% germination and length of root and shoot increased in all treated samples with electromagnetic fields, dry weight of seeds were reduced significantly compared to the control seeds. Treatment plants an significant increase in the total phenol content and antioxidant activity in 2400 and 3600G in comparison with control samples (p< 0.05).These results showed that the electromagnetic fields probably degenerate proteins in the early stages of seedlings ontogeny and the treated Purslanes can increase antioxidant activity by decreasing free radicals against electromagnetic fields tension. Therefore, electromagnetic fields have effects on the structure and developmental characteristic and phytochemical factors of plants.
Keywords; *Electromagnetic Field, Germination, Phenol, Portulaca oleracea*

Received 18/01/2016 Accepted 04/04/2016

How to cite this article:
Shabnam M, Leila A, Maryam K. Effects of Electromagnetic Fields on Seed Germination and Weight, Phenol and Flavonoid Content and Antioxidant System of *Portulaca oleracea*.Adv. Biores.Vol 7 [3] May 2016: 100-108**.** DOI: 10.15515/abr.0976-4585.7.3.100108

INTRODUCTION

Common purslane (*Portulaca oleracea* L.), which is a member of the Portulacaceae, is widespread as a weed and has been stratified the eight most common plants in the world [1]. It has a long history of use for animal feed, human food and medical purpose. It is self-compatible and produces many numbers of seeds that have a long viability. Recent researches indicate that purslane offers better nutrition than the major cultivated vegetables. In particular, it has a great percentage of a-linolenic acid, a-tocopherols, ascorbic acid, β-carotene and glutathione). a-linolenic acid, an omega-3 fatty acid, is an essential fatty acid because it cannot be synthesised by humans but has to be ingested. It plays an important role in human growth, development and disease prevention [2]. Purslane has been demonstrated as a ''power food'' of the future because of its high nutritive and antioxidant properties. It is angoodt source of antioxidants such as vitamins A, C and E and β-carotene [1].

Portulaca oleracea L. is of high nutrition with contents of proteins, carbohydrates, Ca, K, Zn, and Na [3]. Flavonoids and polysaccharides are the most many effective components in *P. oleracea*. It is reported that seven kinds of flavonoids were found in *P. oleracea* L., including quercetin, kaempferol, myricetin,

apigenin, luteolin, genistein, and genistin [4, 5]. In addition to flavonoids, coumarins [6] and a monoterpene glycoside [7], alkaloids have also been offered to be important chemical constituents of this plant. In particular, it contains N-trans-feruloyltyr-amine [8], dopamine, dopa and a high concentration of noradrenaline [9]. P. oleracea contains betalains rather than anthocyanins [10]. *P. oleracea* L. has been used traditionally for the treatment of dysentery with bloody stools and externally for boils and sores, eczema, erysipelas, and insect and snake bites [4, 5, 11, 12].

Harmful environmental factors generate of reactive oxygen species (ROS), such as singlet oxygen (O_2), superoxide anion (O_2•), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH). Levels of ROS are regulated by their rates of generation, the rate of reaction with target substances, such as proteins, lipids, and/or nucleic acids, their potential rate of degradation, and their rate of scavenging/neutralizing by enzymatic and/or non-enzymatic antioxidants [13]. Stresses produce intracellular signals, such as calcium transients, that lead to modifications of growth or morphogenesis of the all plants [14]. Abiotic stress results in the formation of ROS in plants which creates a case called oxidative stress that can harm cellular components [15]. Oxidative stress arises when there is a main imbalance between the production of reactive oxygen species (ROS) and antioxidative defence. Exposure to electromagnetic field can product ROS and lead to cell death as a result of increase in free oxygen radicals and DNA damage [15].

Datillo *et al*. (2005) demonstrated that an alternating magnetic field enhanced the anomalies pollen tube in *Actinidia deliciosa* plant [16]. A study has showed that electromagnetic fields increased lipid peroxidation and hydrogen peroxide content in *Lemna minor* L. [17]. Sandu *et al*. [18] showed a decrease in chlorophyll content in leaves of *Robiniapseudo acacia* [18]. Majd and Shabrangi [19] showed that external magnetic fields effect on the germination, ontogeny growth and anatomical structure of *Lens culinaris* L. Their results suggested that stele and xylem vessels develop and grow more than control and parenchyma cells are larger than control. The greatest difference was observed in leaf section. Air chambers and parenchyma cells were larger than control. Their results suggested that some intensities of magnetic field improve significantly seed germination and growth of plants. Majd *et al*. [20] demonstrated treatment under *Achillea wilhelmsii* pollens with electromagnetic fields 2mT-10,20min have few changes, but treatment under pollens with electromagnetic fields 4mT-20min become abnormal, shrinkage and fragile. the percentage of pollen germination and viability decreased that was significantly($p< 0.05$).

Thus, The present work was investigated the effects of electromagnetic field radiation on seed germination and weight, biochemistry factors of *Portulaca oleracea* at 20min after electromagnetic field radiation (1200, 1800, 2400, 3600 Gauss) exposure.

MATERIALS AND METHODS

Seeds Preparation and Treatments

Purslane seeds (*Portulaca oleracea* L.) were supplied from Pakanbazr company (Isfahan, Iran). The seeds were saturated with 13% Sodium hypochlorite solution for 10 min and were soaked in distilled water for 2h.

Exposure to electromagnetic field was performed using a locally designed electromagnetic field generator (Magnet Bruker, B-E 10, Germany). 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. To ward production of field with intensity of 1200, 1800, 2400, 3600 G for 20min was transmitted flow between the coils. The seeds and seedlings 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. The samples placed in the middle of a horizontally fixed coil and were exposed [21].

SEED GERMINATION

Three replicates were used in the experiment with 12 seeds in each treatment. In case of seed treatments, the seeds were spread on the filter papers in Petri dishes and then placed in the middle of a horizontally fixed coil and were exposed to electromagnetic field by a magnitude of 1200, 1800, 2400, 3600 G at 20 min in the electromagnetic field 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. The effect of electromagnetic fields on seed germination was evaluated. All treatments were replicated three times and placed in a completely randomized design at 25˚C.Seed germination was determined daily (24, 48, 72h) and hypocotyl and radicle lengths were determined, thus wet and dry weight of seeds were determined 7 days after treatment [21].

BIOASSAY AND EXTRACTION

Seeds were germinated in pots containing perlite and cocopeat (1:1) and watered with half-strength Hogland nutrient solution for 20 day. The plants were grown at 25°C temperature, with a 16/8 h day/night photoperiod. Seedling at the two-leaf stage treated at different electromagnetic fields, include: 1200, 1800, 2400, 3600 G at 20 min.

Plants aerial parts were collected and air-dried under shade and ground in to fine powder using electric blender. Then, 0.2 gr of each powder were extracted with 20ml methanol 80% for 48 hours. The mixtures were filtered with whatman filter paper and extracts were stored in the dark kept at 4˚C for further studies [20].

TOTAL PHENOL DETERMINATION

Total phenols were determined by FolinCiocalteu [22, 23]. Different values of each plant extract (50, 100, 200, 300, 400 µl) or gallic acid (standard phenolic compound) was mixed with FolinCiocalteu reagent (1 ml, 1:10 diluted with distilled water) and 7% Na_2CO_3 (1 ml). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg L^{-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} of dry mass), which is a common reference compound.

TOTAL FLAVONOID DETERMINATION

Aluminum chloride colorimetric method was used for flavonoids determination [24, 25]. Each plant extracts (50, 100, 200, 300, 400 µl) in methanol were separately mixed with 1.5 ml of methanol, 1 ml of 2% aluminum chloride, 6 ml of 5% potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 40 min; the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing rutin solutions at concentrations 12.5 to 100 g ml^{-1} in methanol.

FREE RADICAL SCAVENGING ACTIVITY DETERMINATION

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts. Different values of each extract (50, 100, 200, 300, 400 µl) were added, at an equal volume, to methanolic solution of DPPH (0.004g per 100 ml). After 120 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals [26].

STATISTICAL ANALYSIS

The experimental design was a split plot in a randomized complete block design with three replications. The presented data included means of three separate experiments ± SD. In order to analyze the data, SPSS software and ANOVA test were used. Thus, the statistical significance between phytochemical activities values of the extracts was evaluated with a LSD test. P values less than 0.05 were considered to be statistically significant.

RESULTS

The treatment plants with electromagnetic fields (1200, 1800, 2400, 3600 G) not showed a significant different on germination percentage of *Portulaca oleracea* seeds. Germination percentage for 4 treatment and control sample at different three times (24, 48, 72 h) was 100% (Figure 1).

In addition, the treatment plants with electromagnetic fields increased in shoot and root length in contract to control plants ($P<0.05$). In treatment plants with electromagnetic field 2400 G showed the highest amount of shoot length among the another samples. Thus, In treatment plants with electromagnetic field 1200G showed the highest amount of root length among the another samples (Figure 2).

Also, the treatment plants with electromagnetic fields decreased dry weight and increased wet weight in contract to control plants ($P<0.05$) (Figure 2).

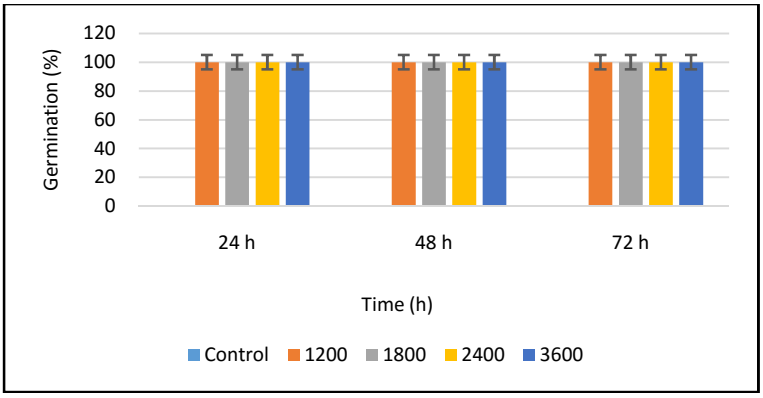

Figure 1. Electromagnetic fields effects on germination percentage *Portulaca oleracea* at three times

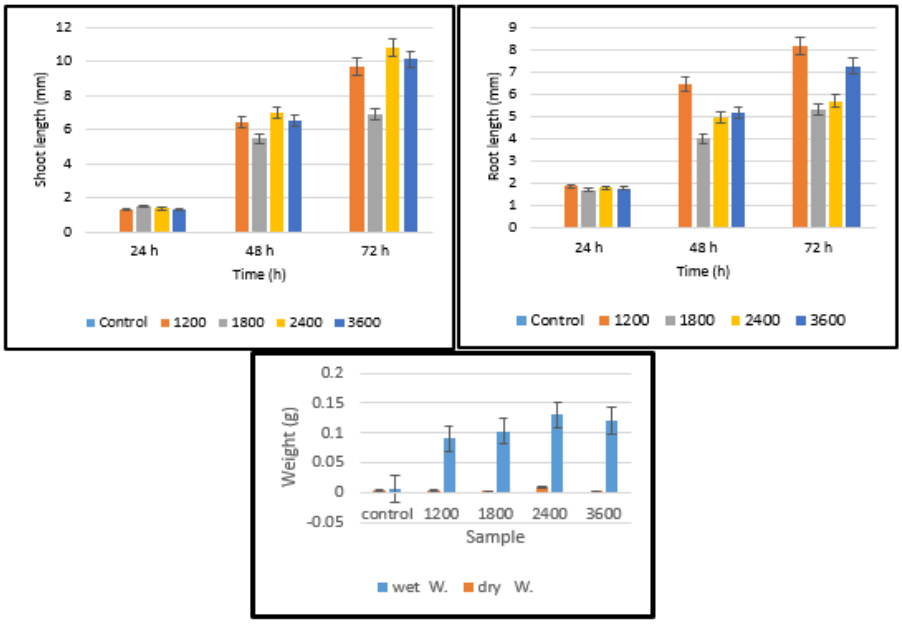

Figure 2. Electromagnetic fields effects on shoot and root length and dry, wet weight of *Portulaca oleracea* at three times

Analysis of data on total phenol showed that treatment plants with electromagnetic fields of 1200, 1800 G caused reduction in polyphenol content compared to control plants, but this decline was not significant (P>0.05), whereas in treatment plants with electromagnetic fields of 2400, 3600 G were observed significantly increase in polyphenol content compared to control plants (p< 0.05) (Figure 3).

Table 1 show the content of total phenols that were measured by FolinCiocalteu reagent in terms of gallic acid equivalent (standard curve equation: y = 29.85x +0.043, r2= 0.990). The total phenol varied from 27.41 ± 0.01 to 52.03 ± 0.05 mg/g GA in samples. The treatment plants with electromagnetic fields of 3600 with total phenol content of 52.03 ± 0.05 mg/g GA had the highest amount among the plants in this study (Figure 2).

Table 1. Total phenol content in the studied plants

Groups	1Mean Total phenol ±SD
Control	433.5±0.2
Treatment 1200	328.81±0.05
Treatment 1800	127.41±0.01
Treatment 2400	48.52±0.02
Treatment 3600	52.03±0.05

[1]Each value in the table was obtained by calculating the average ofthree experiments ± standard deviation

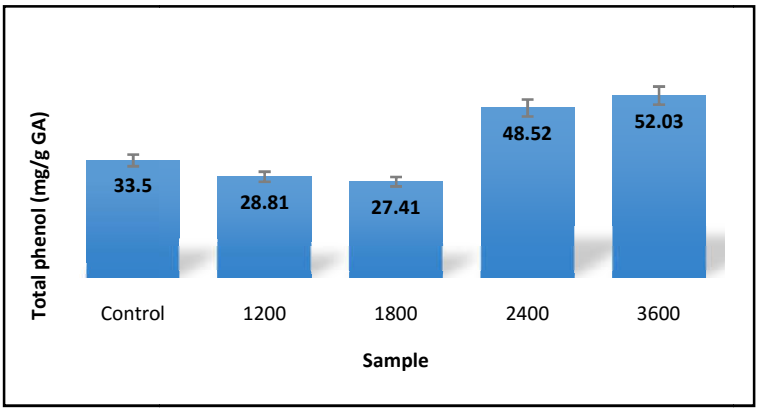

Figure 3. Significant increase of total phenol (mg/g GA) inunder treatment with electromagnetic fields 2400 and 3600 G in compared with control .

Bars are least significant differences where p<0.05hen electromagnetic fields were increased in treatments, the flavonoids content were increased compared to control plants, but this increase was not significant (P>0.05) (Figure 4).
he flavonoid content of the plants in terms of Rutin equivalent (the standard curve equation: y = 0.0603x + 0.0007, r_2 = 0.985) were between 3.573 ± 0.002 and 3.988 ± 0.025 (Table 2, Figure 5).

Table 2. Total flavonoid content in the studied plants

Groups	1Mean Total flavonoid ±SD
Control	3.738±0.003
Treatment 1200	3.573±0.002
Treatment 1800	3.546±0.8
Treatment 2400	3.822±0.013
Treatment 3600	3.988±0.025

[1]Each value in the table was obtained by calculating the average of Three experiments ± standard deviation

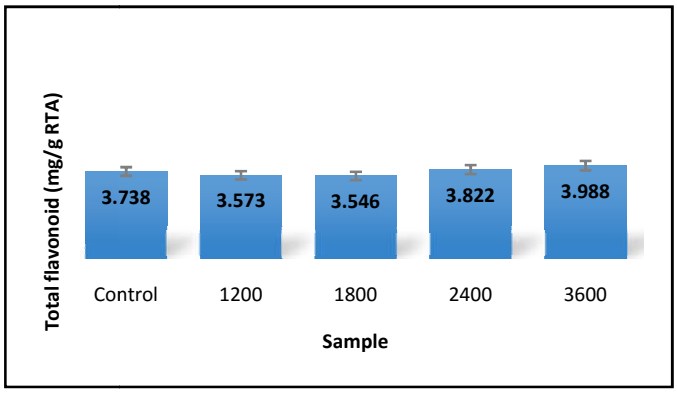

Figure 4. Increase of total flavonoid (mg/g RTA) of under treatment plants with 2400 and 3600 G in compared with control plants

Figure 5 shows the amount of each plant needed for 50% inhibition (IC_{50}). The highest radical scavenging activity was showed by under treatment plants with 3600 G with IC_{50}=234.13 µg/µl which is higher of control plants (IC_{50}=268.71 µg/µl. The radical scavenging activity decreased in under treatment plants with 1800 G with IC_{50}=291.8 µg/µl (Figure 5).
Results showed that in treatment plants with electromagnetic fields of 2400, 3600 G observe significantly increase in inhibition percentage of free radicals compared to control plants (p< 0.05) (Figure 6). But, when electromagnetic fields decrease in treatments (1200, 1800 G), the inhibition percentage of free

radicles were decreased compared to control plants, but this decrease was not significant (P>0.05) (Figure 6).

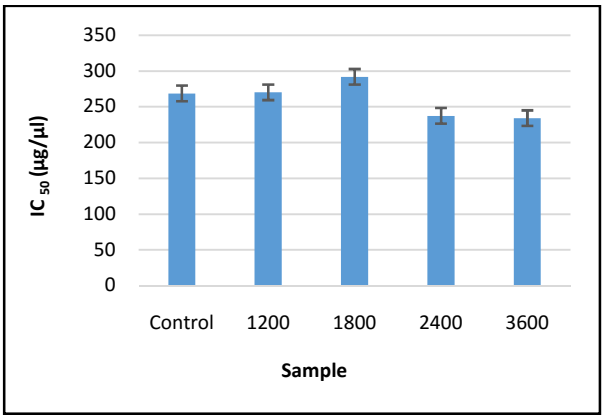

Figure 5. IC_{50} (µg/µl) values of plants for free radical scavenging activity by DPPH radical. Lower IC_{50} value indicates higher antioxidant activity

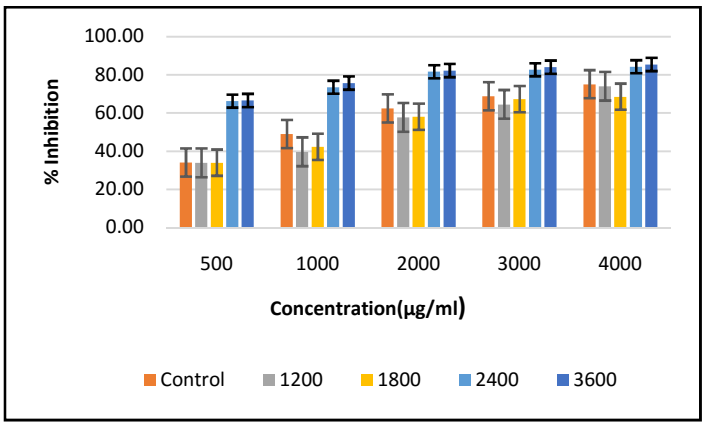

Figure 6. Significant increase of antioxidant activity (concentration and inhibition percentage) of under treatment plants with 2400 and 3600 G in compared with control plants

DISCUSSION

According to our investigation, electromagnetic fields in the applied intensities had effect on Purslane seeds germination. Our results are not compatible with Gusta *et al*. 1978 results that showed exposure of dry seeds of wheat, barley, and wild oats to a magnetic field had no effects on germination [27].

Our study showed that the length of roots and shoots increased in all electromagnetic field at three time (24, 48, 72h), but this increase for roots was more sensible in 1200 G and for shoots was more sensible in 2400 G. It is assume that electromagnetic fields may have caused a change in synthesization of protein. It is deduce that gene expression has changed. The same results reported by Racuciu *et al*. 2006 that said electromagnetic fields can increase roots growth of corn seedlings, that this result is similar to our result [28]. Goodman *et al*., 1995 reported that electromagnetic fields interact with biological molecules via exerting some forces on bound or free charges by changing the size and shape of the energy levels or chemical processes [29]. Then a series of fall events at the molecular, cellular and tissue level take place and induce biological effects [30].

Vashisth & Nagarajan, [32] observed an increase in the total length of *Helianthus annuus* L. by magnetic field [31]. Electromagnetic fields probably affect the plant growth regulators like auxin and cytokinin and can be effective on the plant growth and development. Electromagnetic fields probably increase auxin rate and are effective on genes activity which produce growth proteins in nucleus and so increase the protein production and lead to growth. Also they can increase the ATPase pumps activity in cell wall and peroxides so they can extend the cell wall totality and lead to cells growth. They can be effective on genes regulators like cytokinin and increase mitosis divisions in shoot and root meristems.

A high number of reports about the mechanism of electromagnetic fields interaction have pointed to the effects of the fields on the calcium channels. According to the reports, the magnetic and electric fields

affect the electrical charges of the calcium channels and cause the opening of the channels and increasing the intracellular concentration of calcium [5]. Calcium as a second messenger is involved in regulation at all stages of plant growth and development, including growth and differentiation, photomorphogenesis and embryogenesis, the self-incompatibility responses in pollen-pistil interactions, perception of symbiotic signals, hypersensitive responses induced by pathogens and elicitors, gravitropism and phototropism, assembling and disassembling of cytoskeleton elements, perception of red and blue light, cyclosis and movement of stomatal cells [33]. Accordingly Ca2+ is the most investigated ion for the stress [34].

Germana *et al*. (2003) demonstrated that a electromagnetic eld can increase the transport of calcium across the cell membrane and alter pollen germination [35]. It is well known that Ca^{2+} is fundamental in the regulation of the cell cycle and it has been recently suggested that its fast oscillation is necessary for centrosome duplication [16]. Chiabrera *et al*. (1984) showed that Ca^{2+} changes the cytoplasm stimulate the depolarization of tubulin, which is the basic protein of the microtubules [36].

Dry biomass weight of seeds were reduced significantly compared to the control seeds, and wet biomass weight of seeds were increased significantly compared to the control seeds. Different growth rate in different growth condition suggested that response to electromagnetic treatment would be different. Therefore, the response depends not only on the electromagnetic induction and its gradient, but also on the physiological state of experimental organism as proposed by Smith *et al*. [37]. As a result we need to concentrate on the environmental conditions accompanying the response to electromagnetic.

Electromagnetic fields perhaps increase mineral elements absorption, water absorption and enzymes activity so they lead to increase plants biomass. On the other hand, they probably affect mRNA, gene expression and cell division and lead to increase growth, fresh and dry weight. Florez *et al*. indicated that electromagnetic fields increased enzymes activity and protein contents and lead to raise biomass of plants [38]. Yinan *et al*. showed that biomass of the *Cucumissativus* L. increased by electromagnetic fields [39]. Radhakrishnan and Kumari reported an increase in the fresh and dry weight and mineral accumulation by pulsed magnetic field [40].

Radhakrishnan and Kumari reported that pulsed magnetic field increased protein content and catalase enzyme activity. Electromagnetic fields stress especially in dry condition increased catalase activity. Plants begin to enhance oxidative enzymes activity like catalase against electromagnetic fields stress and free radicales which has protective role against electromagnetic fields and detoxification of H_2O_2 [40]. These results consent with Kursevich and Travkin and Atak *et al* [41, 42]. That found magnetic field treatment higher levels of catalase, peroxidase, and superoxide dismutase and glutathione reductase in electromagnetic fields-treated seedlings cause to delay senescence. Catalase stops H_2O_2 accumulation in cells and supports plants against ROS [43].

Treatment samples in our study experienced an significant increase in the total phenol content and antioxidant activity in 2400 and 3600G in comparison with control samples, whereas, total flavonoid content increase in 2400 and 3600G in comparison with control samples, but this increase was not significant. 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. Phenols accumulate in plant tissues during stress and due to oxidant damage. Phenols concentration also depends on the competition for the allocation of photosynthetically fixed carbon to growth or defense. Phenols could also be an important part of the plants defense system against biotic and abiotic stresses [44].

We observed a high amount of phenol and antioxidant changes in Purslane plant, thus confirming similar observations reported for plants and increase of plants defense against environmental stresses.

CONCLUSION

Results showed that treated seeds had 100% germination. Our study showed that the length of roots and shoots increased in all treated samples with electromagnetic fields at three time (24, 48, 72h), thus, dry weight of seeds were reduced significantly compared to the control seeds, and wet weight of seeds were increased significantly compared to the control seeds. Treatment samples an significant increase in the total phenol content and antioxidant activity in 2400 and 3600G in comparison with control samples. It has been known that different electromagnetic field intensities cause various reactions in plants. These reactions have no linear effect on strength and period of exposed to magnetic field.

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