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THE RESPONSE of BEAN (PHASEOLUS VULGARIS L.) PLANTS to ELECTROMAGNETIC FIELD EXPOSURE

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ABSTRACT

The electromagnetic field is a natural component of the environment. Plants are known to recognize different wavelengths of light and respond to them. This paper aims at describing the development of P. vulgaris seeds exposed in EMF (electromagnetic field) using direct visualization and histological sections. Two groups of bean seeds (wet and dry) were subjected to an electromagnetic field of 4mT. Maximum seed germination in the early days occurred when exposed to EMF in wet conditions which increased the germination percentage compared to dry conditions. Considerable differences were found in anatomical features of organs under study in twice EMF treatments compared to control so that it negatively influenced the morphology especially root and leaf. The coherent influence on biomass accumulation was noticed in the case of EMF exposure for all exposure times and all treatments. We found that low-frequency EMFs had an inhibitory effect on early germination but a stimulatory effect on the growth characteristics in vegetative and generative stages. The effects of EMFs on the growth apices of beans varied depending on the level of induction. Treatment of bean seeds with EMF at the time before planting in greenhouse conditions and also the production of fruit. This fact provided a basis for the assumption that it is reasonable to use alternating EMFs for the stimulation of plant development.

Keywords: EMF, Phaseolus vulgaris L., germination, Biomass, anatomy

INTRODUCTION

Electromagnetic is a physical field produced by any wiring or equipment carrying an electric current that may affect living organisms such as plants, animals, and finally humans. Levels of electromagnetic fields (EMF) from artificially man-made sources have increased progressively in the past decades, and human societies are not aware that magnetic fields and plant development are related to each other (1, 2). This relationship can be used in agricultural areas by treating the seeds, water, soil, and nutrients in the soil. The majority of studies have considered that plants are continuously

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exposed to EMF as they cannot avoid them. It has been demonstrated that electromagnetic fields have both positive and negative impacts on the stimulation and inhibition of the development and germination of plants depending on the species, but significant effects of EMF were found mostly on the inhibition of the growth of exposed plants (3). Nearly 75% of the undertaken studies have reported physiological effects at both high and low dosages. Therefore, results are still not decisive and there is some indeterminacy about the low dosages and frequency. On the other hand, observed effects did not always refer to the existence of a dose-effect relationship and there seem to be no specific physical parameters and experimental conditions that seem to determine an effect (3, 4).

Comprehensive research on the effect of static magnetic fields on seeds and seedlings shows that consistent results are lacking (5, 6, 7). As well as the continued lack of good quality data on relevant species means that there are insufficient data to identify whether a single exposure standard is appropriate to protect all environmental species from EMF. Therefore, extensive studies are essential to indicate the mechanism of magnetic field action in plants and identify its benefit applications. The present study was carried out to evolve the effect of electromagnetic fields on some physiological parameters of beans (Phaseolus vulgaris L). Phaseolus vulgaris is one of the major sources of protein in the world which is receiving increasing attention as a functional food (8). To the best of our knowledge, this is the first study to evaluate the long-term effect of electromagnetic fields on the growth, development, and reproduction of beans in a greenhouse condition. Here we focused on the effects of electromagnetic fields on the developmental stages of the bean and evaluated whole bean growth.

MATERIALS AND METHODS

Phaseolus vulgaris L. (var D81083) seeds were prepared from the Khomain research center, Arak, Iran. The seeds were surface-sterilized using sodium hypochlorite at 5% for 10 min, washed twice with distilled water, and dried at room temperature. The seeds were divided into two groups; one group was immersed in distilled water for 12 h (moisture seeds) and the second group remained dry (dry seed). Then each of the two was divided into three subgroups; (Two main subgroups of seeds were treated (once and double treatments), and non-exposed seeds were used as control. The seeds of one treatment were exposed to a magnetic field of 4mT intensity for 45 min, and in the double treatment group, the seeds were exposed to a 4mT magnetic field twice with a two-hours interval, each time for 45 mi. Three replicates were used in the experiment with 30 seeds in each treatment. The magnetic field was performed by a locally designed generator whit its electrical power provided by a 220 V and 0.01 A, AC power supply. This system consists of a PVC cylinder of 20 × 20 cm (diameter and length) and 300 turn coil of copper wire. The samples were placed in the middle of a horizontally fixed coil and exposed. The temperature was measured with a thermometer to be 22+1°C. Then treated and untreated seeds were grown into Petri dishes in a growth room with the temperature controlled at 28±1°C, 16 h light and 8h dark cycle. Three

replicates of 4 seeds were used for each treatment.

The seeds were considered to be germinated when their radicles exceeded a length of 1 mm. The rate of germination obtained after 96 h was considered final and was not changed in the following counts, following which the germination percentage (GP) was calculated from the following equation. The germination rate is determined by calculating the GP at different time intervals after planting and then plotting these data.

GP=100(N'/N)

N: number of germinated seeds, N': total number of seeds

Biomass and water content measurement

Four days after following the germination, seedlings were taken and divided into radicles, cotyledon, and hypocotyl to measure factors such as fresh weight, dry weight, water content (WC), and root and stem length. Different parts of seedlings were immediately weighed (fresh mass, FM) and all samples were dried at 60˚C for 48 h to determine the dry mass (DM). Values of FM and DM were used to calculate WC, using the equation: WC= (FM-DM)/DM. Another group of plants was used for morphological and anatomical studies.

Functional treat measurement

Treated seeds were planted in 20-50 pots filled with natural soil in the greenhouse. After harvest, some yield characteristics such as plant height leaves number, and the number of branches was measured. After completing the developmental stages, fruit and seed numbers were measured. These measurements were carried out in ten replicates (ten plants) for each treatment.

Histology

Different parts (leaf, root, petiole, stem) of plant in the vegetative stage fixed in 70% FAA. Fixed material was dehydrated through an ethanol series and 15 µm sections were cut by the handle. Handling of small, delicate specimens was facilitated by the use of pipettes and moving specimens to small tubes closed at one end with fritted glass that allowed drainage of solutions and stained with 0.5% methylene blue and safranin. Specimens were viewed with a digital camera on a Zeiss Axiophot light microscope at the same magnification.

Statistical Analysis

Experiments followed a randomized complete block design. Three explants per petri dish and three replications per treatment were tested. Analysis of variance was performed by the General Linear Model procedure and Duncan's test (SPSS ver. 20).

RESULTS

Effect of electromagnetic field on plant growth

Germination percentage is an estimate of the viability of a population of seeds. The evaluation of the germination rate showed the treatments used in this study have a significant impact on this parameter so that significant difference was observed on the first day. Germination percentage in treated groups rather than in control is lower (Figure 1). While on the fourth day, the germination percentage of all treatments is 97 or 100, that is electromagnetic

treatments postpone the germination starting time and reduce the germination rate but it does not affect the germination percentage. The minimum and maximum germination rates related to dry seeds (Once and twice-treated seeds) and untreated wet seeds respectively (Figure 1).

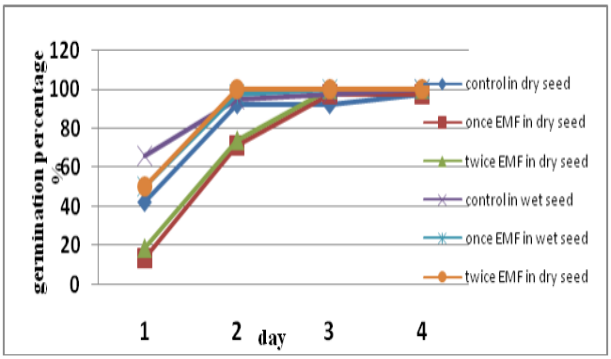


Figure 1. effect of different treatments on seed germination of bean (Phaseolus vulgaris L.). Values represent mean ±SE of 10 replicates. Symbols having the same letter are not significantly different at the 0.05 level.

Variance analysis and Average Comparison of the measured parameters are observed in followed tables. The mean germination time of Phaseolus vulgaris was significantly reduced when seeds were exposed to the electromagnetic field. Results obtained for all of the treatments showed that electromagnetic field has an influence on early seed germination which reveals that electromagnetic treatment causes an inactivation on early germination of exposed seeds. Variance analysis of fresh weight showed all studied factors except the electromagnetic field and interaction between electromagnetic field- tissues more significantly affected the fresh weight (Table 1). In other words, wet and tissue type are all factors that affect fresh weight. Radicle tissue's fresh weight is the lowest in dry conditions. Applying wet increased the radicle fresh weight, whereas the magnetic field has the opposite effect. The highest fresh weight is related to the cotyledon tissue and results showed no significant difference between wet and dry conditions as well as the magnetic field. In the case of hypocotyl, it is noteworthy that fresh weight differences in dry conditions were not affected by the electromagnetic field but in wet conditions, the electromagnetic field had a significant effect on fresh weight.

Table 1. Analysis of variance for fresh weight, dry weight, and water content character. Ns: represented no significant effect, *: significant at 0.05 level of significance and **: significant at 0.001 level of significance

Source of changes	frequency	wet	magnetic field	Tissue type	Wet× magnetic field	Wet× tissue type	Magnetic field × tissue type	Wet× magnetic field × tissue type
Fresh weight	0.79^{ns}	54.7^{**}	2.98^{ns}	206.5^{**}	6.56^{**}	11.65^{**}	2.34^{ns}	3.28^{*}
Dry weight	0.51^{ns}	26.77^{*}	0.23^{ns}	145.89^{*}	0.32^{ns}	9.79*	0.70^{ns}	0.47^{ns}
Content	1.33^{ns}	56.5^{*}	4.2^{*}	123.5^{*}	7.1^{*}	8.4^{*}	3.4^{*}	5.1^{*}

Variance analysis of dry weight showed that wet, tissue type and wet-tissue interaction parameters affected it significantly while the magnetic field have no effect. Due to the significance of wet and tissue-type parameters, an average comparison was done based on these factors. Variance analysis of water content showed all factors have a significant effect on this parameter (Table 1). The average comparison of fresh weight, dry weight, and water content is presented in tables 2.

Table 2. Average Comparison of fresh and dry weight and water content. Values are mean ± SE (n = 3). Symbols having the same letter are not significantly different at the 0.05 level

	Dry seed			Wet seed		
	No magnetic field	One magnetic field	Twice magnetic field	No magnetic field	One magnetic field	Twice magnetic field
radicle	0.12^{h}	0.11^{h}	0.13^{gh}	0.84^{cd}	0.18^{fg}	0.23^{ef}
Cotyledon	0.87^{ab}	1^{ab}	0.92^{ab}	0.87^{ab}	1.045^{a}	1.00^{ab}
Hypocotyl	0.23^{ef}	0.3^{de}	0.25^{ef}	0.81^{ab}	0.33^{de}	0.68^{bc}

	Dry seed	Wet seed
radicle	0.022^{c}	0.034^{b}
Cotyledon	0.34^{a}	0.3^{a}
Hypocotyl	0.02^{c}	0.046^{b}

	Dry seed			Wet seed		
	No magnetic field	One magnetic field	Twice magnetic field	No magnetic field	One magnetic field	Twice magnetic field
radicle	0.093^{c}	0.087^{c}	0.112^{c}	0.36^{c}	0.138^{de}	0.192^{de}
Cotyledon	0.573^{b}	0.592^{b}	0.588^{b}	0.615^{ab}	0.713^{ab}	0.703^{ab}
Hypocotyl	0.21^{de}	0.278^{cd}	0.225^{de}	0.758^{a}	0.39^{cd}	0.627^{ab}

Variance analysis of root and stem length proved that electromagnetic field, wet conditions, and their interaction have a significant effect on these traits (Table 3).

Table 3. Analysis of variance root length and areal portion length.

Source of variance				
Character	Frequency	Wet	Magnetic field	Wet × magnetic field
Root length	1.27^{ns}	15.6**	12.6**	24.01**
areal portion length	1.19^{ns}	89.87*	22.17*	10.34*

So, the minimum and maximum root length is related to no electromagnetic field dry and twice electromagnetic field wet conditions respectively. Similarly, the minimum and maximum stem lengths were observed in no magnetic field dry and twice magnetic field wet conditions respectively. A positive effect of the electromagnetic treatments on root and stem length was significant (Tables 4).

Table 4. Average Comparison of root length and areal portion. Values are mean ± SE (n = 3). Symbols having the same letter are not significantly different at the 0.05 level.

	No magnetic field	One magnetic field	Twice magnetic field
Dry seed	6.15^{d}	7.18^{c}	9.25^{b}
Wet seed	10.73^{a}	7.51^{c}	9.55^{b}
	No magnetic field	One magnetic field	Twice magnetic field
Dry seed	3.06^{d}	4.36^{c}	5.03^{c}
Wet seed	6.55^{b}	5.18^{c}	8.31^{a}

In the vegetative and maturation stage some traits such as plant height, leaf number, shoot number, fruit number, and seed number in the pod were measured (Table 5). Growth and morphology in treatments showed significant differences under electromagnetic different treatments. All of the measured parameters mentioned above in dry conditions gradually increased with increasing exposing time, and no significant difference was observed between treatments with wet conditions. Therefore, exposing dry beans to electromagnetic increased crop yields. In wet conditions, once electromagnetic seeds had the most yields. Therefore, both the electromagnetic time and exposing condition (wet and time) had a significant effect on the growth and yield of bean plants.

Table 5. Average Comparison of measured functional traits. Values are mean ± SE (n = 3). Symbols having the same letter are not significantly different at the 0.05 level. (A: No magnetic field, B: One magnetic field, C: Twice magnetic field, D: No magnetic field, E: One magnetic field, F: Twice magnetic field)

	Leaf number	Plant height	Shoot number	Seed number in fruit
A	13.2 ± 2.8 a	23.7 ± 2.9 a	3.6 ± 0.9 a	7 ± 1.6 a
B	19.2 ± 5.9 b	28.8 ± 2.9 b	5.8 ± 1.9 b	7.8 ± 2.7 a
C	23.6 ± 6.5 b	29.2 ± 3.1 b	7.2 ± 2.2 b	8.6 ± 1.5 a
D	11.6 ± 1.3 a	22.2 ± 1.7 a	3.2 ± 0.4 a	6.4 ± 0.5 a
E	26.6 ± 8.6 b	38.8 ± 4.8 c	8.2 ± 2.9 b	8 ± 2.4 a
F	24.2 ± 5.8b	40.2 ± 3.2 c	7.4 ± 1.9 b	7.8 ± 0.8 a

The most direct effects of bean plants under EMF treatments were an increase in the number of green leaves; an increase in plant height and an increase in yields except twice EMF treatment that decreased these parameters. The results showed that the length of plants under EMF treatment increased so it is a stimulation for the increase in length. Table 5 showed that treatment of EMF increase leaf number in all treatments compared to the control. Also, this increase of number leaves is considerable in wet conditions, whereas the twice EMF treatment led to a decrease in the number of leaves compared to once EMF. The data in Table 5 showed there was a significant difference in these parameters between plants under wet and dry conditions. Under dry conditions, all of the measured functional parameters were higher than in wet conditions.

Microscopy study and changes in plant anatomy

The general arrangement of tissues in the leaf blade, petiole, stem, and root is completely similar in all samples of the treatments that are exposed to electromagnetic and had no effect on the general arrangement. The cross-section of the leaf reveals a typical bifacial structure and possesses an upper and lower epidermal layer in all treatments (Figure 1). The epidermal layer of the midrib was thin with small oval-shaped cells and a thin cuticle. The ground tissue consists of four or five layers of polyhedral, thin-walled, compact parenchyma cells. The vascular bundle was uncordially large and in shape. It was collateral with the adaxial xylem and abaxial phloem (Figure 1). The xylem elements are in clusters with wide, thick-walled, angular metaxylem elements and one or two protoxylem elements. The cells of metaxylem elements are 10-20 µm wide. The upper and lower epidermal layers are unstratified and composed of oval-shaped cells in the transverse section. The epidermis is covered with a thin waxy cuticle which is comparatively thinner when compared with the lower epidermis. The shape and size of upper and lower epidermal cells vary in the treatments so the size of upper epidermal cells is larger than the lower epidermis. Both the epidermal layers are unstratified and constituted of parenchymatous cells that appear rectangular in the cross-section of the leaf. The mesophyll is clearly differentiated into two regions: palisade and spongy. The palisade parenchyma that developed on the adaxial side is stratified and contains rectangular elongated cells. Palisade region is one- layer or in some treatments, it is two-layered. The palisade parenchyma cells in twice electromagnetic treatments in both wet and dry conditions are irregular and disorganized with larger size and color of less compared to control and once electromagnetic treatments (Table 6). In twice electromagnetic field treatments, the palisade cells are less elongated and somehow rounded or oval-shaped and loose (Figure 1 B, D), whereas, in the once electromagnetic field, they are more elongated and compact (Figure 1 A, C). The spongy parenchyma is organized with loosely arranged polygonal cells in treatments with twice electromagnetic fields compared to one electromagnetic field and control. The mesophyll cells became smaller, lesser, and out of shape because of the long duration of EMF treatment, but the cellular structure was very clear and organized under mild EMF. There is a single collateral and opened vascular bundle in

the midrib and also there are two small vascular bundles in the leaf blade in all treatments. The vascular bundle appears to be conical. There is a difference in shape, size, and cell organization in the vascular bundles of treatments (Table 6). The diameter of xylem vessel elements such as metaxylem and protoxylem decreased in all treatments compared to the control. Cell wall thickening of collenchyma cells was considerably lower in the leaf of plants treated with EMF in wet conditions compared to control and dry plants. These changes were also observed in the cell wall thickening of parenchyma tissues (Figure 2).

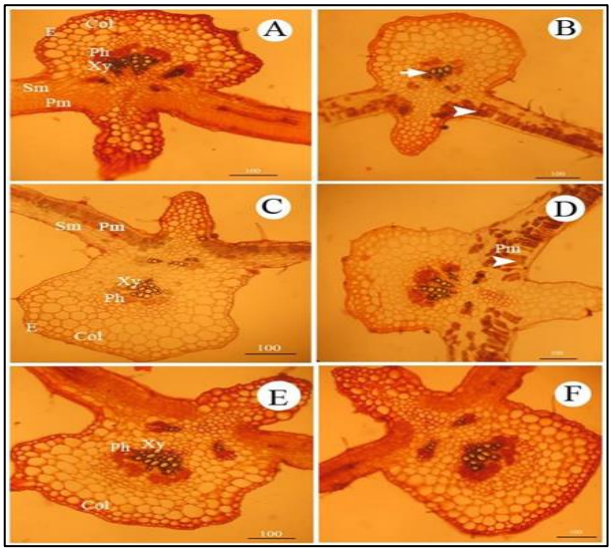


Figure 2. Cross sections through the leaf's midrib of bean exposed in different treatments. A: once electromagnetic field dry seed, B: twice electromagnetic field dry seed, C: once electromagnetic field wet seed, D: twice electromagnetic field wet seed, E: no electromagnetic field dry seed, F: no electromagnetic field wet seed. Abbreviations: E: epidermis, Col: collenchyma tissue, Xy: xylem tissue, Ph: phloem tissue, Pm: palisade mesophyll, Sm: spongy mesophyll. Scale bar: 100 µm

Table 6. Analysis of variance and Average Comparison of some characters in the leaf of treatments. Values are mean ± SE (n = 3). Symbols having the same letter are not significantly different at the 0.005 level. *: significant at 0.005 level of significance and **: significant at 0.001 level of significance. (A: Source of change, B: Control in dry Seed, C: Control in wet Seed, D: once EMF in dry seed, E: twice EMF in dry seed, F: once EMF in wet seed, G: twice EMF in wet seed)

Traits	Upper parenchyma layer thickness	lower parenchyma layer thickness	Phloem tissue thickness	lower parenchyma layer thickness	Phloem tissue thickness	xylem tissue thickness	palisade parenchyma thickness	Spongy parenchyma thickness
A	42038**	655**	34999**	2060**	710**	8467**	1783**	859**
B	78.14 ± 5.6 d	38.41 ± 4.2 b	434.28 ± 9.8 a	167.49 ± 6.3 ab	73.70 ± 4.1 a	113.9 ± 7.2 b	61.40 ± 2.7 b	70.01 ± 6.1 b
C	69.24 ± 4.9 d	41.63 ± 4.8 b	398.85 ± 10.1 a	159.25 ± 7.7 b	74.47 ± 5.9 a	108.1 ± 8.1 b	75.34 ± 3.9 a	76.42 ± 6.7 b
D	189.6 ± 10.6 c	38.75 ± 3.6 b	262.28 ± 9.06 b	171.96 ± 8.1 a	56.40 ± 3.4 b	81.42 ± 5.2 d	58.54 ± 3.7 b	60.16 ± 5.3 bc
E	306.12 ± 12.5 a	29.31 ± 2.1 b	259.34 ± 8.4 b	128.59 ± 5.7 c	50.15 ± 5.2 bc	93.54 ± 6.9 c	94.12 ± 6.3 a	73.45 ± 4.9 b
F	291.87 ± 11.2 a	59.78 ± 3.8 a	249.5 ± 11.8 bc	151.50 ± 7.5 b	67.43 ± 5.4 a	187.40 ± 9.3 a	71.35 ± 4.7 a	67.87 ± 6.8 a
G	224.72 ± 10.6 b	36.00 ± 3.4 b	227.54 ± 10.2 c	179.77 ± 8.7 a	44.96 ± 3.7 c	122.52 ± 7.9 b	61.28 ± 2.4 b	54.45 ± 4.3 a

Stem diameter was influenced by electromagnetic fields and the condition of exposing the seed so the increasing duration of exposure decreased the diameter of the stem (Figure 3, Table 7 rows 1-6). Similar alterations in the anatomy including xylem and phloem disorganization and reduction of vascular bundles were observed in the stem of treatments. All these changes were observed in the petiole. In the stem and petiole of EMF-treated plants, few anatomical differences were observed compared to the control plant (Figure 4, Table 7 rows 7-11).

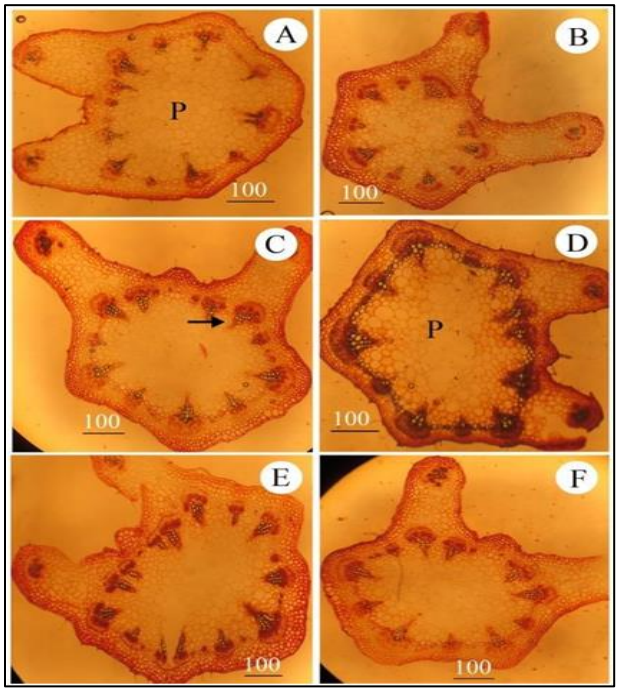


Figure 3. Cross sections through the stem of the bean exposed in different treatments. A: once electromagnetic field dry seed, B: twice electromagnetic field dry seed, C: once electromagnetic field wet seed, D: twice electromagnetic field wet seed, E: no electromagnetic field dry seed, F: no electromagnetic field wet seed. Abbreviations: P: pith parenchyma. Scale bar: 100 µm

Table 7. Analysis of variance and Average Comparison of some characters in the stem of treatments. Values are mean ± SE (n = 3). Symbols having the same letter are not significantly different at the 0.05 level. *: significant at 0.05 level of significance and **: significant at 0.001 level of significance. (A: Source of change, B: Control in dry Seed, C: Control in wet Seed, D: once EMF in dry seed, E: twice EMF in dry seed, F: once EMF in wet seed, G: twice EMF in wet seed)

Traits	A	B	C	D	E	F	G
Stem thickness	417789**	2221.2 ± 237.4 a	2346 ± 218 a	1895.9 ± 87. bc	1455.1 ± 77.0 d	2032.2 ± 124.4 ab	1761.5 ± 187.7 c
Collenchyma layer thickness	3344**	78.27 ± 5.2 b	81.63 ± 9.1 b	68.72 ± 4.6 b	131.28 ± 10.1 a	83.20 ± 5.8 b	69.89 ± 3.49 b
parenchyma layer thickness	1491**	151.30 ± 9.5 a	147.85 ± 10.13 a	151.12 ± 9.6 a	130.99 ± 12.4 b	129.43 ± 11.4 b	110.07 ± 14.2 c
Phloem tissue thickness	452**	95.3 ± 9.4 a	89.4 ± 8.6 a	70.70 ± 13.4 b	81.8 ± 6.4 ab	81.6 ± 8.7 ab	73.8 ± 10.7 b
xylem tissue thickness	678**	124.0 ± 10.9 bc	131.5 ± 11.2 bc	112.6 ± 18.1 d	164.8 ± 59.3 a	142.7 ± 12.6 b	119.2 ± 13.8 c
Pith parenchyma width	227147**	1724.1 ± 76.2 a	1752.4 ± 81.1 a	1353.5 ± 68.2 c	1150.9 ± 61.9 d	1533.2 ± 94.3 b	1479.8 ± 79.9 bc
Collenchyma layer thickness	2235**	82.3 ± 7.4 bc	81.4 ± 9.1 a	70.1 ± 5.4 bc	55.2 ± 77.0 d	107.9 ± 7.6 a	98.1 ± 8.4 ab
parenchyma layer thickness	12723**	224.3 ± 19.4 b	237.8 ± 21.2 b	285.4 ± 39.2 a	189.7 ± 22.1 bc	147.7 ± 21.3 c	207.6 ± 34.8 bc
Phloem tissue thickness	428^{ns}	87.5 ± 8.4 a	89.4 ± 8.1 a	64.70 ± 7.4 a	65.8 ± 6.7 a	70.6 ± 6.2 a	73.8 ± 9.4 a
xylem tissue thickness	4858**	114.2 ± 8.5 b	121.5 ± 9.2 b	103.3 ± 8.1 b	114.8 ± 9.3 b	178.4 ± 13.6 a	106.2 ± 7.8 b
Pith parenchyma width	96237**	899.9 ± 66.8 ab	879 ± 81.4 ab	626.7 ± 64.2 c	894.3 ± 71.8 b	919.9 ± 84.2 ab	987.9 ± 97.9 a
Root thickness	425193**	4447.6 ± 327.4 c	4456.5 ± 312.1 c	4522.4 ± 295.2 bc	4895.3 ± 341.2 b	4652.9 ± 296.4 bc	5127.8 ± 246.8 a
parenchyma layer thickness	15763**	478.2 ± 29.5 b	467.5 ± 32.2 b	479.7 ± 39.6 b	735.2 ± 42.3 a	811.2 ± 41.2 a	414.1 ± 24.9 b
Phloem tissue thickness	9614**	125.9 ± 3.7 c	120.5 ± 5.2 c	214.8 ± 11.7 a	169.2 ± 27.5 b	231.6 ± 45.8 a	152.2 ± 22.0 bc
xylem tissue thickness	13613**	206.1 ± 15.6 b	201.2 ± 22.7 b	191.9 ± 35.6 b	266.7 ± 69.5 a	157.1 ± 14.4 b	281.5 ± 18.0 a
Pith parenchyma width	427135**	2837.6 ± 96.3 a	2826.4 ± 85.7 a	2845.1 ± 108.6 a	2862.9 ± 98.9 a	2988.2 ± 112.7 a	2244.4 ± 89.7 b

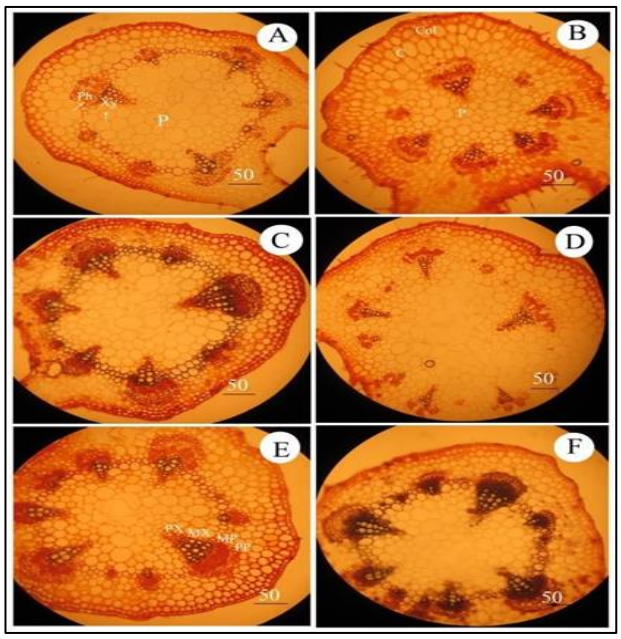


Figure 4. Cross sections through the petiole of bean exposed in different treatments. A: once electromagnetic

field dry seed, B: twice electromagnetic field dry seed, C: once electromagnetic field wet seed, D: twice electromagnetic field wet seed, E: no electromagnetic field dry seed, F: no electromagnetic field wet seed. Abbreviations: Col: collenchyma tissue, C: cortex, Xy: xylem tissue, Ph: phloem tissue, P: pith parenchyma, PX: protoxylem, MX, metaxylem, MP, Meta phloem, PP, proto phlome. Scale bar: 50 µm

Root anatomy was studied in all treatments. In once and twice electromagnetic field treatments, a reduction in the size of tracheary elements and a change in their organization as an aggregation of xylem vessel elements was observed in contrast to the control (Figure 5). This aggregation of elements is stronger in the twice electromagnetic field rather than the once electromagnetic field. The area of the xylem and phloem also the cortex appeared to be expanded in control. The thickness of the cell wall of collenchyma and parenchyma cells was considerably higher in the root of EMF treatments compared to the control (Table 7 rows 12-16).

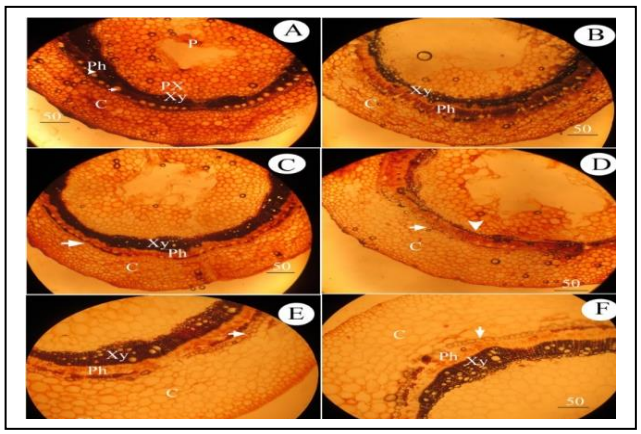


Figure 5. Cross sections through the root of the bean exposed in different treatments. A: once electromagnetic field dry seed, B: twice electromagnetic field dry seed, C: once electromagnetic field wet seed, D: twice electromagnetic field wet seed, E: no electromagnetic field dry seed, F: no electromagnetic field wet seed. Abbreviations: C: cortex, Xy: xylem tissue, Ph: phloem tissue, P: pith parenchyma, PX: protoxylem. Scale bar: 50 µm

DISCUSSION

The effect of weak electromagnetic fields (EMFs) on biological systems draws more and more attention from researchers (10). It has been proven that plants can perceive the different types of EMFs with an extensive range of frequencies (23,3). In general, developmental studies on plant responses have been done at different electromagnetic intensities. It was observed that an electromagnetic field with relatively low intensity could be effective in stimulating or inhibiting plant development (6). The present study was conducted to explore the effects of the electromagnetic field in wet and dry conditions on the early development and vegetative stages of the bean plant. Statistically significant results demonstrated the effect of alternating EMF with different duration of exposure on the rate of seed germination that is depending on the seed state (dry or moistened). EMF treatments are effective at different stages of plant development such as seeds, seedlings, or whole plants, and may influence different organs or developmental processes such as seed germination, stem and root growth, and generative stages so indicating that biological organisms can respond to EMF. Igbal et al (2012) reported the seeds of a pea when exposed to EMF with 60, 120, and 180 mT intensity for 5, 10, and 15 min prior to sowing showed a significant increase in germination. Furthermore, it was found that exposure of 0 min for electromagnetic with 60 and 180 mT intensity significantly enhanced the germination parameters of the pea. These results are not consistent with our results, as our results showed that electromagnetic field delays the germination rate in dry seeds. Different intensities of EMF (4 or 7 mT) were tested on seed germination and seedling growth of bean or wheat seeds in different media. The greatest germination and growth rates in both plants

were from the test group exposed to % mT (13). In other experiments, seeds of wheat were soaked in water overnight and then treated with or without a 30 mT static magnetic field (SMF) and a 10 kHz EMF for 4 days, each for 0h. Exposure to MF increased the speed of germination suggesting the promoting effects of EMFs on the growth characteristics of wheat seedlings (14). Bilalis et al (2012) reported that pre-sowing treatment of corn seeds with pulsed EMFs improved germination percentage, leaf area, plant fresh, dry weight, and yields. In this study applying one electromagnetic field in both wet and dry conditions increased characteristics such as plant height, leaf number, number of branches and fruit number increased, while in the twice EMF wet seeds these traits decreased. In previous studies, seeds of maize (Zea mays L.), chickpea (Cicerarietinum L.), and sunflower (Helianthus annus L.) were exposed to different magnetic fields. The maximum enhancement in seedling growth and root features was observed under different combinations of magnetic field and duration time of exposure. The root characteristics of the plants showed an increase in root length, root surface, and volume. In another study, the significant increase in germination, seedlings vigor, and shoot/root growth of one-month-old plants of chickpea seeds exposed to static magnetic fields was also reported (11). The dependence of plant development on the frequency of electromagnetic fields was reported by Murajii et al. (1998). Following exposure experiments with a low-frequency electromagnetic field (10 to 240 Hz) carried out on corn plants, the root growth was stimulated for lower frequencies but inhibited for higher ones. In some studies, opposition results have been reported. For example, the germination percentage of rice (Oryza sativa) seeds exposed to EMF with 125 or 250 mT was significantly reduced compared to controls which indicated this type of magnetic field obviously influences germination and early stages of development of rice plants (16). In different studies, it has been found that the growth and development of plants increased in seeds treated with EMF. For example, in treated corn plants, the total fresh weight, biomass, and height of plants were more than those of the control with the greatest increase being observed in plants exposed to static EMF (17). Treatment of Dioscorea opposite with twice EMF showed that they grow more than the control in the seedling stage, rate of emergence and root number and root length increased (Li, 2000). The effects of pre-sowing magnetic treatments on the growth and yield of tomatoes increased significantly with respect to mean fruit weight, the fruit yield per plant, the fruit Yield per area, and the equatorial diameter of fruits in comparison with the controls. Total dry matter was also significantly more in plants exposed to EMF than in controls (19).

Güvenç and Duman (2010) reported that ecological factors influence some anatomical characteristics such as pubescence, organization of the mesophyll, and distribution of the supporting tissue elements. We noticed that the distribution and density of collenchyma cells and diameter of pith parenchyma of leaf, stem, and root were different in treated and untreated plants. The parenchymatous cells of the stem and leaves of untreated plants are smaller than those of the stressed ones. The density of sclerenchyma tissue in the stem cortex and root of treated plants is higher than in the control; therefore, it appears to be a considerable value (21). In the present study, vessel diameter especially the xylem was smaller in treated plants compared to the control. Ristic and Cass (1991) reported that the vascular tissue size was decreased by stress showing that this difference between treated and untreated plants may function as a tolerance to stress. Also, our results showed that compressing of vascular bundles under EMF treatment was not associated with the development of wider tracheary elements.

CONCLUSION

In this study, we assessed the impact of low-frequency electromagnetic fields on the most sensitive stages of plant development such as seed germination, and seedling growth, and its long-term effect in the vegetative stage. The dry bean seeds were the most sensitive to the effect of EMF. The stimulatory effect of EMF was observed throughout the experiments, until the vegetative and seed formation stage. Exposure to EMF in dry seeds at the stage of early germination led to a 31% and 43% decrease in the rate of germination percentage in once and twice electromagnetic fields compared to the control, respectively. This decrease in germination in wet seeds was lower. Statistically significant results demonstrating the effect of alternating EMF with different duration of exposure on the rate of seed germination depending on seed state (dry or moistened) were obtained for these treatments. In general, the plant's vegetative growth was improved under the electromagnetic field. We also found that Low-frequency electromagnetic fields in dry seeds had an inhibitory effect on early germination but it has a stimulatory effect on the growth characteristics in vegetative and generative stages. Treatment of bean seeds with the electromagnetic field at the time before planting in field conditions influences also the production of fruit.

Many of the experiments on the biological effects of alternating electromagnetic fields appear to give inconsistent results. There are many reasons for this, including differences in the genetic make-up, physiological condition, and the history of the test material. The exposure to electromagnetic fields of low power density has elicited detectable responses in the bean plants cultivated in wet and dry conditions. Direct measurements of the levels of functional traits showed a stimulatory effect on the developmental processes from vegetal green tissues depending on the exposure duration. In about two third of the reviewed studies, ecological effects of RF-EMF were reported at high as well as at low dosages. The very low dosages are compatible with real field situations and could be found under environmental conditions. However, a lack of standardization and a limited number of observations limit the possibility of generalizing results from an organism to an ecosystem level. We propose future standards for reporting EMF-relevant physical parameters in both laboratory and field studies.

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