The effects of magnetic fields on growth and enzyme activities of *Helianthus annuus* L. seedlings

Maryam Peyvandi*, Neda Kazemi Khaledi, Sedighe Arbabian

Department of Biology, Faculty of Biology, Islamic Azad University, Tehran-North Branch, Tehran, Iran

Abstract

In recent years, many research studies have been conducted on the effects of magnetic field on plants. But certain mechanisms regarding magnetic influences have not been defined yet. In this study, effects of magnetic fields (MF) on the growth parameters and enzyme activities of *Helianthus annuus* L. were investigated. Sterilized seeds in sterile conditions were exposed to 0, 0.5, 1 and 1.5 mT of MF. One day after the treatments percentage of seed germination was increased at 0.5 mT. Different MF densities had no significant effects on root length and fresh weight of leaves. The highest level of shoot length was gained at 1 mT. In this treatment the activity of auxin oxidase was significantly decreased compared with that of the control samples. The activity of the catalase and peroxidase were decreased by MF. The minimum level of protein content and catalase and peroxidase activity were observed in the samples exposed to 1 mT of MF while there were no significant differences in the ascorbate peroxidase activity of different treatments. MF caused a reduction in chlorophyll *a*, *b* in all treatments compared to the control. The Results confirmed that the intensity of the MF had different effects on the growth parameters and enzyme activity.

Keywords: Helianthus annuus L.; magnetic fields; growth parameters; antioxidant enzymes; auxin oxidase; chlorophyll

M. Peyvandi, N. Kazemi Khaledi and **S. Arbabian.** 2013. 'The effects of magnetic fields on growth and enzyme activities of *Helianthus annuus* L. seedlings'. *Iranian Journal of Plant Physiology* 3(3), 717-724.

Introduction

All organisms during their life are faced with two types of magnetic field, one of them is natural magnetic field that is the result of earth magnetic field and the amount is between 0.03-0.07 mT, another is artificial magnetic field which is the result of application of electrical power at homes and industrial workshops (Williams et al., 2002).

Magnetic field which is produced in a wide range has positive and negative effects on animal and plant life. Today an important question is if magnetic field has any distinctive effect on biological systems. There are reports about the short-term MF exposure. MF influences a variety of plant functions such as growth (Racuciu et al., 2006), development (Yano et al., 2004; Rakosy-Tican et al., 2005), protein biosynthesis (Alikamanoglu et al., 2011) and enzyme activity (Alikamanoglu et al., 2011). But the interaction of such fields with the living cells is still unclear (Atak, 2003). MF causes an oxidative stress, that is, increases the activity,

*Corresponding author
E-mail address: m_peyvandi@iau-tnb.ac.ir
Received: November, 2012
Accepted: April, 2013

concentration, and lifetime of free radicals which are highly reactive byproducts of normal metabolism and immune defense (Scaiano, 1994). Accumulation of reactive oxygen species which are generated during stress can harm many cellular components such as lipids, proteins, carbohydrates and nucleic acids (Halliwell, 1982). Therefore, MF could change the antioxidant enzyme activity (Sahebjamei, 2007; Alikamanoglu, 2011).

The purpose of this study was to investigate if MF has any considerable effect the growth parameters and enzyme activities of *Helianthus annuus* L..

Materials and Methods

Sunflower seeds (*Helianthus annus* L.) cv. *Progress* were provided from Oilseeds Cultivation Company in Qom. Seeds were sterilized with sodium hypochlorite 0.5% (20 min) and washed with distilled water (3 times). The seeds were placed on sterilized Petri dishes with two pieces of filter paper and 5 ml sterilized water was added to each Petri dish (15 seeds per Petri dish). The dishes were then exposed to 0, 0.5, 1.0, 5.0 mT of MF for one hour per day during four days. At the end of the first day of applying MF, the percentage of germination was measured. Four days after treatments, seedlings were transferred to the pots with peat-perlite. Fourteen-day-old plants were used for measurement of growth parameters, enzymes activity, and chlorophylls contents.

Magnetic field generating device

To create a uniform and constant MF, a P.V.C cylinder with the diameter of 160 mm was used. On this cylinder, 350 rings of copper wire with a diameter of 9.0 mm was rolled which created about 4 ohms resistance. Bases with a height of 2 cm were considered for the coil in order to maintain air ventilation and stabilize the temperature inside the chamber. Fortunately, during the experiment, significant changes were not observed in the space inside the coil which shows properly designed coil in wire diameter, size and mode of ventilation. The coil was then connected to an electric power source equipment (MEGA TEG Company).

Growth parameters

On the first day of treatment the percentage of germination was measured. Fourteen-day-old plants were then used for measurement of growth parameters (leaf fresh weight, shoot and root length)

Assay and protein extraction

Frozen leaves (0.5 g fresh weight) were homogenized in 5 ml Tris- Glycine buffer (pH 8.3). The homogenate was then centrifuged at 12000 g for 10 min. All operations were performed at 4 °C. Protein contents were determined by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard.

Catalase activity

The activity of catalase was measured in a reaction mixture consisting of a Tris-Glycine buffer (50 mM, pH 7.5), H_2O_2 (10 mM) and enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm by a spectrophotometer (Genway Genova) (Pereira et al., 2002).

Peroxidase activity

The peroxidase activity was measured in a reaction mixture consisting of acetate buffer (0.2 mM, pH 4.8), hydrogen peroxide (0.1 mM), benzidine (0.04 M) and enzyme extract. Enzyme activity was measured by a spectrophotometer (Genway Genova) at 530 nm (Koroi, 1989).

Ascorbate peroxidase activity

The ascorbate peroxidase activity was measured according to the method of Nakano and Asada (1981). The reaction mixture consisted of enzymatic extract, L-1 sodium phosphate buffer (50 mM, pH 7), ascorbate (0.5 mM), hydrogen peroxide (0.1 mM), EDTA (0.1 mM) and enzyme extract. The reaction started after the hydrogen peroxide addition, and the absorbance

was measured by a spectrophotometer (Genway Genova) at 290 nm. The molar extinction coefficient 2.8 $mmol^{-1}$ cm^{-1} was used to calculate ascorbate peroxidase activity.

Auxin oxidase activity

Auxin oxidase enzyme activity was measured by Gordon and Weber (1951) method. This mixture consisted of 5.5 ml phosphate buffer (0.02 M, pH 6.1), 2 ml of indole acetic acid (10^{-3} M), 1 ml of manganese chloride (10^{-3} M), 1 ml of 2,4-Dichlorophenol (10^{-3} M) with 0.5 ml of enzyme extract. Enzyme activity was measured by a spectrophotometer (Genway Genova) at 530 nm.

Measurement of chlorophyll *a* and *b*

Young leaves were homogenized with acetone 80% in a mortar. The absorbance was measured at 663 nm and 645 nm by a spectrophotometer (Genway Genova). Chlorophyll content was calculated according to the method of Arnon (1949).

Statistical analysis

Experiments followed a randomized complete block design. Three explants per pot and three replications per treatment were tested. Analysis of variance was performed by the General Linear Model procedure (SPSS ver. 16) and differences among treatments were evaluated by Duncan Test ($p \leq 0.05$).

Results

Germination percentage

One day after treatments, different intensities (0.5, 1, 5 mT) of MF increased germination percentage, in comparison with control (0 mT). The highest and the lowest percentage of germination were observed in 0.5 mT treatment (88.88%) and the control (73.33%), respectively (Fig. I).

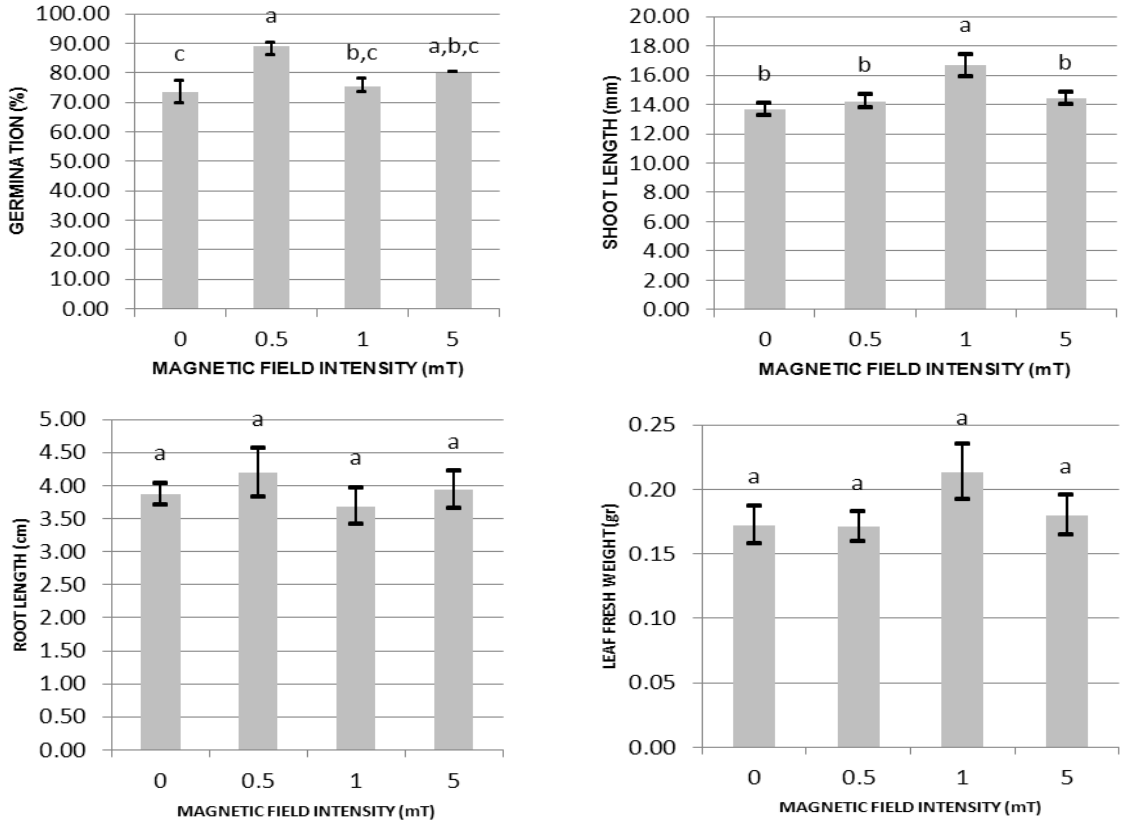


Fig. I. Mean of seed germination percentage, shoot length (cm), root length (cm) and leaf fresh weight (g) in response to MF intensity (mean ± SE) grouped by Duncan test ($p \leq 0/05$); Means with the same letter have no significant different.

Shoot length

There were no significant differences between control, 0.5 and 5 mT. Maximum and minimum shoot lengths were observed in 1 mT treatment (16.67 cm) and the control (13.68 cm), respectively (Fig. I).

Root length

Results showed that different intensities of MF had negligible effect on root length. Maximum and minimum root lengths were observed in 0.5 mT treatment (4.19 cm) and in 1 mT treatment (3.69 cm, respectively (Fig. I).

Leaf fresh weight

Maximum and minimum leaf fresh weights were observed in 1 mT treated seedlings (0.21 g/seedling) and 0.5 mT treated seedlings (0.17 g/seedling), respectively although the difference was not significant (Fig. I).

Leaf protein content

Results showed that protein content decreased in the treated plants in comparison with the control group. Maximum and minimum leaf protein contents were observed in the control (6.55 $mg.g^{-1}$. FW) and 1 mT treatment (3.75 $mg.g^{-1}$.FW), respectively (Fig. II).

Catalase activity

Catalase activity decreased in treatment plants in comparison with the control. Maximum and minimum leaf catalase activities were

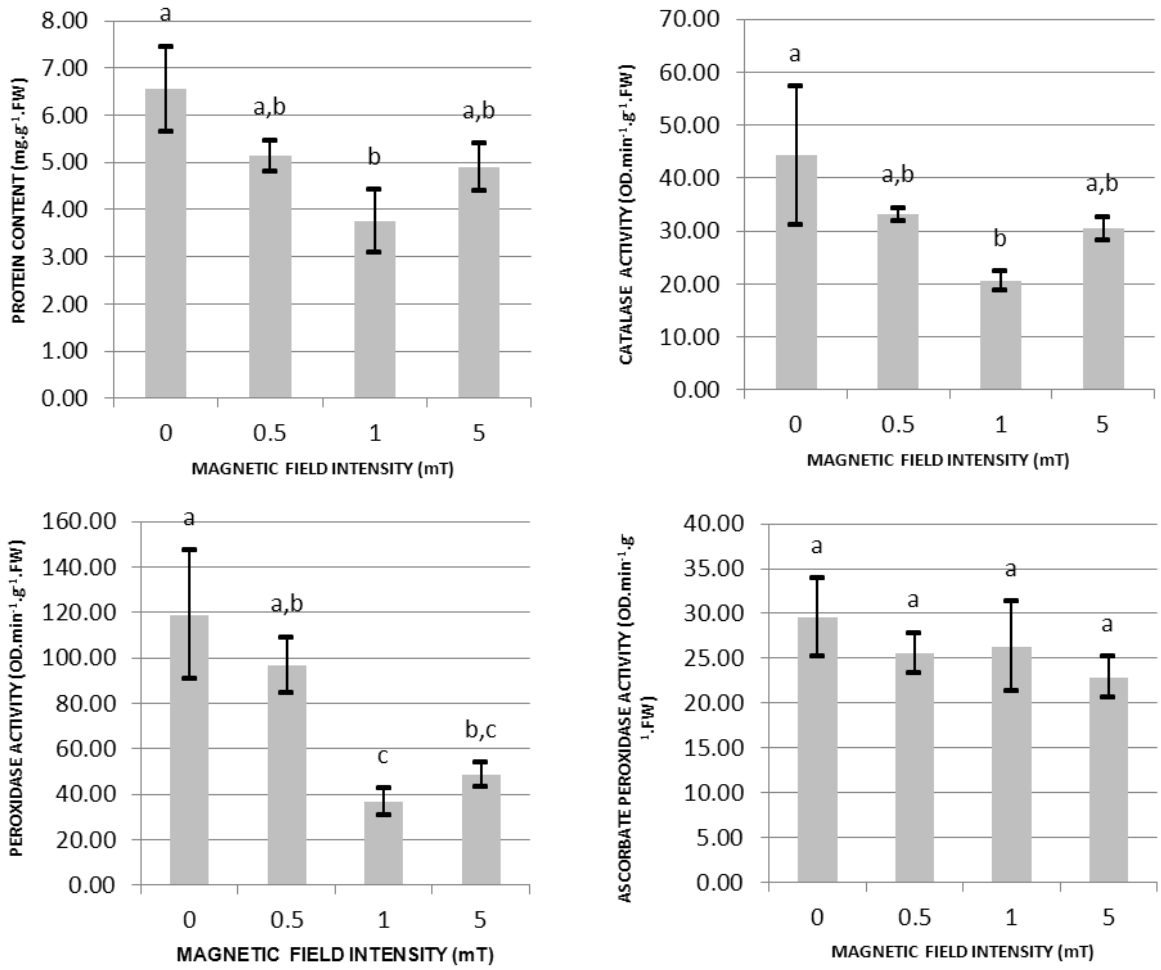


Fig. II. Mean of protein content ($mg.g^{-1}$.FW), catalase, peroxidase and ascorbate peroxidase activity (OD $min^{-1}.g^{-1}$.FW) in response to MF intensity (mean ± SE) grouped by Duncan test ($p \leq 0/05$); Means with the same letter have no significant difference.

observed in control plants (44.25 OD $min^{-1}.g^{-1}$ FW) and 1 mT treatment (20.55 OD $min^{-1}.g^{-1}$ FW), respectively (Fig. II).

Peroxidase activity

Peroxidase activity decreased in the treated plants in comparison with the control group. Maximum and minimum leaf peroxidase activities were observed in the control (119.00 OD $min^{-1}.g^{-1}$FW) and 1 mT treatment (36.66 OD $min^{-1}.g^{-1}$ FW), respectively (Fig. II).

Activity of ascorbate peroxidase

Ascorbate peroxidase activity slightly decreased in the treated plants in comparison with the control group. Maximum and minimum leaf ascorbate peroxidase activities were observed respectively in the control (29.53 OD $min^{-1}.g^{-1}$ FW) and 5 mT treatment (22.86 OD $min^{-1}.g^{-1}$ FW) (Fig. II).

Effect of magnetic field on auxin oxidase activity in leaf

Reduced auxin oxidase activity was observed in all treatments in comparison with control. Maximum leaf auxin oxidase activity was observed in the control (0.37 OD $min^{-1}.g^{-1}$.FW) while minimum activity was measured in 1 mT treatment (0.16 OD $min^{-1}.g^{-1}$.FW) (Fig. III). At this intensity maximum level of shoot growth and minimum of root growth were obtained in the study.

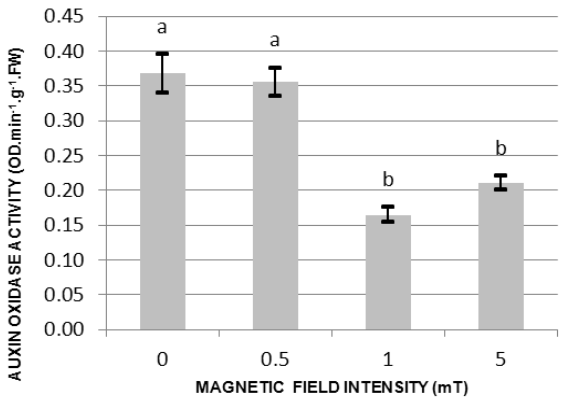
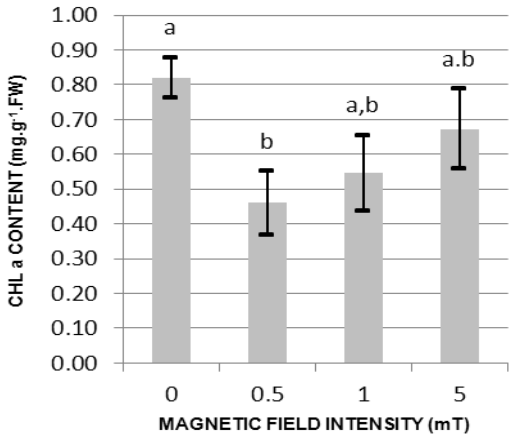


Fig. III. Mean of leaf auxin oxidase activity in response to MF intensity (mean ± SE) grouped by Duncan test ($p \leq 0/05$); Means with the same letter have no significant difference.

Effect of magnetic field on chlorophyll *a* and chlorophyll *b*

MF with different intensities decreased chlorophyll *a* and *b* in all treatments as compared with the control. Maximum levels of chlorophyll *a* and *b* contents were achieved in control plants. Maximum and minimum chlorophyll *a* contents were observed in the control plants (0.82 $mg.g^{-1}$.FW) and 0.5 mT treatment (0.46 $mg.g^{-1}$.FW. Furthermore, maximum and minimum chlorophyll *b* levels were observed in the control (0.55 $mg.g^{-1}$.FW) and 0.5 mT treatment (0.31 $mg.g^{-1}$.FW), respectively (Fig. IV).



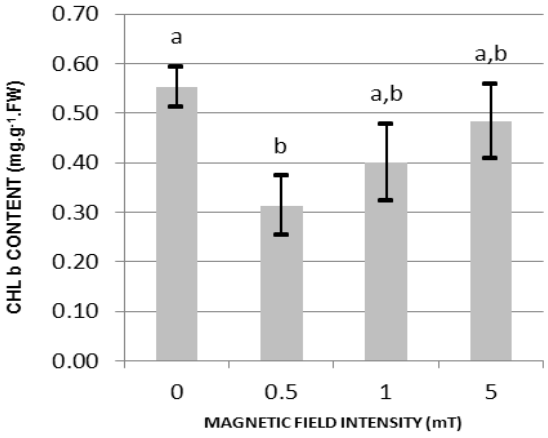


Fig. IV. Mean of chlorophyll a and chlorophyll b content in sunflower in response to MF intensity (mean ± SE) grouped by Duncan test ($p \leq 0/05$); Means with the same letter have no significant difference.

Discussion

The results of the present study indicated that 0.5 mT, MF intensity has different effects on growth parameters in sunflower plant. Germination percentage in 0.5 mT MF was higher than the control. Increase in germination of the treated seeds with MF has been observed in some plants such as maize (Aladjadjiyan, 2002), rice (Carbonel et al., 2000), wheat (Martinez, 2002) and tobacco (Aladjadjiyan, 2003) which are consistent with the findings of this study.

Maximum level of shoot length was gained in 1 mT treatment. This parameter in all treatments was higher than the control. Increase in shoot length had been observed in some plants such as peas, lentils (Martinez et al., 2009) and wheat (Harichand et al., 2002) which are consistent with the obtained results in this study.

Maximum average root length was observed in 0.5 mT treatment, although the difference was not significant. Maximum average leaf fresh weight was observed in 1 mT treatment. This index in all treatments was higher than the control. Maximum average root number was observed in 5 mT treatment, although the difference was not significant.

In sum, it can be concluded that the applied MF intensities increased growth parameters and development at the first stages of growth in sun flower plant. This may result from changes at the level of Ca^{+2} induced by MF (Florez et al., 2007) which controls many processes in plants.

The results showed that different intensities of MF (0.5, 1.5 mT) caused a decrease in leaf protein in comparison with control. Reduction of protein content may cause oxidative stress and free radicals produced by MF (Aladjadjiyan, 2002). Reactive oxygen species and free radicals cause changes in enzyme activity, gene expression and also affect membrane structure and DNA.

MF with different intensities (0.5, 1.5 mT) reduced activity of antioxidant enzymes including catalase, peroxidase and ascorbate peroxidase in all treatments in comparison with control. MF as a stressful environmental factor increases the amount of reactive oxygen species. The biological effect of MF depends on the energy level, exposure time, distance of target from the energy source, and structure of organisms. In general, MF as a stressful environmental factor alters electron spins of molecule so that it can increase the concentration of free radical and reactive oxygen species. Plants have enzymatic defense systems such as catalase, peroxidase and ascorbate peroxidase to reduce the ROS.

The auxin oxidase enzyme activity was reduced in 1.5 mT treatment. There is an inverse relation between auxin oxidase activities and auxin content. The mechanism of the root and shoot elongation by auxin has been difficult to demonstrate, perhaps because auxin induces the production of ethylene, a root growth inhibitor. However, even if ethylene biosynthesis is specifically blocked, low concentrations (10^{-10} to 10^{-9} *M*) of auxin promote the growth of intact roots, whereas higher concentrations (10^{-6} *M*) inhibit growth. Thus, roots may require a minimum concentration of auxin to grow, but root growth is strongly inhibited by auxin concentrations that promote elongation in stems and coleoptiles (Taiz and Zeiger, 2010).

The results showed that different intensities of MF (0.5, 1.5 mT) caused a decrease in chlorophyll *a* and *b* in all treatments compared with control. This was also the case with studies on some other plants such as bean (Kazimove, 1984), dates (Dhawi et al., 2009), maize (Racuciu et al., 2007), and soybean (Atak et al., 2007).

MF releases free radicals (Scaiano et al., 1994) which have an essential role in electron transfer and chemical reaction. Free radicals which have unpaired electrons with magnetic moments can be oriented in external MF. The reaction between the external MF and the moment of unpaired electrons absorbs energy. The absorbed energy can affect the chloroplast magnetic moment and disturb photosynthetic pigment. The consumption of carotenoids for inhibiting the free radicals can also be a factor in reducing the photosynthetic pigment.

Conclusion

The findings of the present study indicated that, MF with different intensities had different effects on growth parameters in

sunflower plants. Generally, it can be concluded that the applied MF intensities increased growth parameters and plant development at the early stages of growth in sunflower plant and that may result from changes at the level of Ca^{+2} induced by MF (Florez et al., 2007) which controls many processes in plants.

References

Aladjadjiyan, A. and **T. Ylieva.** 2003. 'Influence of stationary magnetic field on early stages of the development of tobacco seeds (*Nicotina tabacum*)'. *J Cent Euro Agri* 4(2): 132–138. Reference format

Aladjadjiyan, A. 2002. 'Study of the influence of magnetic field on some biological characteristics of *Zea mays'*. J Cent Euro Agri 3(2): 89-94.

Alikamanoglu, S. and **A. Sen.** 2011. 'Stimulation of growth and some biochemical parameters by magnetic field in wheat (*Tritium aestivum* L.) tissue cultures'. *African Journal of Biotechnology*, 10(53): 10957-10963.

Arnon, D. I. 1949. 'Copper enzymes in isolated chloroplast, polyphenol oxidase in *Beta vulgaris'*. Plant Physiol. 24: 1-75.

Atak, C. and **O. Emiroglu.** 2003. 'Alikamanoglu S et al. Stimulation of regeneration by magnetic field in soybean (*Glycine max* L. Merrill) tissue cultures'. *J Cell and Mol Biol.* 2: 113-119.

Atak, C., O. Celik and **A. Olgun.** 2007. 'Effect of magnetic field on peroxidase activities of soybean tissue culture'. *Biotechnol*. 21(2): 166-171.

Bradford, M. M. 1976. 'A rapid sensitive method for the quantitation of micro program quantities of protein utilizing the principle of protein-dye binding'. *Anal Biochem*. 72: 248-254.

Carbonel, M.V., E. Martinez and **J. M. Amaya, J.M.,** 2000. 'Stimulation of germination in rice (*Oryza sativa*, L.) by a static magnetic'. *Electro- and Magnetobiology*, 19(1):121-128.

Dhawi, F. and **J. M. Al-Khayri.** 2009. 'Magnetic fields induce changes in photosynthetic pigments content in Date Palm (*Phoenix dactylifera* L.) seedlings'. *Open Agri J.* 3: 1-5.

Florez, M., M. V. Carbonell and **E. Martines**. 2007. 'Exposure of maize seeds to stationary magnetic fields: Effects on germination and early growth'. *J Environ Exp Bot*. 59: 68-75.

Gordon, S. A. and **R. P. Weber.** 1952. 'Colorimetric estimation of indole acetic acid'. *Plant Physiol*. 26: 192-195.

Halliwell, B. 1982. 'The toxic effects of oxygen on plant tissues'. In Superoxide Dismutase [L.W. Oberley (ed.)]. Boca Raton, FL: CRC Press, Vol.1: 89-123.

Harichand, K.S., V. Narula and **D. Raj.** 2002. 'Effect of magnetic fields on germination, vigor and seed yield of wheat'. *Seed Res* 30(2): 289-293.

Koroi, S. A. A. 1989. 'Gel elektrophers tische and spectral photometrischoe under uchungen zomein fiuss der temperature auf straktur and aktritat der amylase and peroxidase isoenzyme'. *Physiol* 20: 15-23.

Martinez, E., M. V. Carbonel and **M. Florez.** 2002. 'Magnetic biostimulation of initial growth stages of wheat (*Triticum aestivum*, L.)'. *Electromagn Biol Med.* 21(1): 43-53.

Martinez, E., M. Florez , **R. Maqueda, M. V. Carbonell** and **J. M. Amaya** 2009. 'Pea (*Pisum sativum* L.) and Lentil (*Lens culinaris* Medik*)* growth stimulation due to exposure to 125 and 250 mT stationary fields'. *Polish J. of Environ. Stud*. 18(4): 657-663

Nakano, Y. and **K. Asada.** 1981. 'Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts'. *Plant Cell Physiol* 22: 867-880.

Pereira, G. J. G., S.M.G. Molina and **P.J. Lea.** 2002. 'Activity of antioxidant enzyme in response to cadmium in *Crotalaria juncea'*. *Plant Soil* , 239(1): 123-132.

Racuciu, M., D. E. Creanga and **C. Amoraritei.** 2007. 'Biochemical changes induced by low frequency magnetic field exposure of vegetal organisms'. *Rom J Phys*. 52: 601-606.

Racuciu, M. and **D. E. Creanga.** 2006. 'Plant growth under static magnetic field influence'. Rom J Phys 53(1-2): 353–359.

Rakosy-Tican, L., C. M. Aurori and **V. V. Morariu, .**2005. 'Influence of near null magnetic field on in vitro growth of potato and wild Solanum species'. *Bioelectromagnetics* 26(7): 548-57.

Sahebjamei, H., P. Abdolmaleki and **F. Ghanati .** 2007. 'Effects of magnetic field on the antioxidant enzyme activities of suspension-cultured Tobacco cells'. *Bioelectromagnetics,* 28: 42-47.

Scaiano, J. C., F. L. Cozens, F.L. and **J. McLean.** 1994. 'Model of the rationalization of magnetic field effects in vivo. Application of radical-pair mechanism to biological systems'. *Photochem Photobiol*, 59(1): 585–589.

Taiz, L. and E. Zeiger. 2010. 'Plant Physiology'. (5th Edition). Sinauer Associates Inc.

Williams, B., A. Ventola and **M. Wilson. 2002. '**Magnetic fields and haunting phenomena,a basic primer for paranormal enthusiasts'. Haunting Blog.

Yano, A., Y. Ohashi and **T. Hirasaki.** 2004. 'Effects of a 60 Hz magnetic field on photosynthetic CO_2 uptake and early growth of Radish seedlings'. *Bioelectromagnetics* 25(8): 572-581.