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Magnetic field and silver nanoparticles induced changes on phenolic compound and oxidative status of marigold seedlings

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Abstract
Effect of magnetic fields (MFs) and silver nanoparticles (SNPs) on a biological organism vary depending on their system and applied materials, time and intensity. In recent years, many studies have been conducted on the sensitivity of living organisms to MFs and NPs. For this purpose, an experiment was carried out in 2016 to study the effects of MF and SNPs on marigold (*Calendula officinalis* L.) using randomized complete block design with four treatments and three replications. The treatments were as follows: control, MF with B=3 mT for an hour per day, SNPs (50 ppm) and combination of MF (B=3 mT) and SNPs (50 ppm). Results showed that phenolic content, DPPH radical scavenging, phenylalanine ammonia lyase, polyphenoloxidase, catalase, ascorbate peroxidase, guaiacol peroxidase and glutathione reductase activity were significantly increased by the application of MFs and SNPs as compared to the control group. It seems that the MF+SNPs treatment had the best effect on the antioxidant status of *C. officinalis* L. and may be suitable alternative for chemical compounds used in agriculture.

Keywords: *Calendula officinalis*; Magnetic field; Phytochemical compounds; Silver nanoparticles.

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Introduction

The Earth's magnetic field (MF) has existed since the planet's early days, long before life on earth began. During the evolution process, all living organisms experienced the action of the Earth's MF, which is a natural component of their environment. MFs have had many uses in ancient and modern society (Asadi Samani *et al*. 2013; Maffei 2014). In recent years the number of bioelectromagnetism research reports focusing on the investigation of magneto sensitivity of living organisms have increased (Florez 2011). Nanotechnology is a research field that is associated with physical, chemical and engineering sciences with novel techniques and produces materials at the nano-scale level and is an emerging field in biotechnology. In recent years, noble metal nanoparticles (NPs) have been the subjects of focused researches due to their unique electronic, optical, mechanical, magnetic and chemical properties (Vanaja *et al*. 2013). Nano-biotechnology is presently one of the most dynamic disciplines of research in contemporary material science whereby plants and different plant products are finding an imperative use in the synthesis of NPs (Banerjee1 *et al*. 2014). Stimulatory effects of weak intensity MFs have been reported on seedling growth and antioxidant activities in two *Raphanus sativus* L. varieties (Akacha *et al*. 2013). Seedling growth response to MF pre-sowing include stimulation or inhibition depending on the treatment used. Asadi Samani *et al*. (2013) in an

experiment on cumin observed that MF treatment (25, 50 and 75 mT) recorded the highest seed germination and enzyme activity. The use of MF creates a condition for plant growth just as environmental factors like salinity, drought, UV light, heat and chilling (Celik *et al*. 2009). A MF applied to dormant barley seed was found to stimulate the rate of seedling growth and increase grain yield (Najafi *et al*. 2013).

The use of plant materials in the modern medicine is increasing with the extensive research on process in phytochemistry (Sujatha *et al*. 2011). *Calendula officinalis* L. (marigold) belongs to the Asteraceae (Compositae) family. It is an annual herb, native to Egypt and the Mediterranean countries and is known for its antispasmodic, aperient, cholagogic, diaphoretic and vulnerary properties. Phenolic compounds (flavonoids, phenolic acids) are abundant in *C. officinalis* L. (Butnariu and Coradini 2012; Rigane *et al*. 2013).

The aim of this study was to investigate the influence of MFs and silver nanoparticles (SNPs) on the content of phenolic compounds (total phenol and flavonoid), DPPH radical scavenging and antioxidant enzymes activity of marigold (*C. officinalis* L.) and evaluate the medicinal and defence properties *of C. officinalis* L. in response to SNPs and MFs.

Material and Methods

Preparation of silver nanoparticles and magnetic field

SNPs were prepared by means of biological and green syntheses and the use of reduction in metal salt precursor (silver nitrate, $AgNO_3$) in water with aqueous extract of manna of hedysarum plant and extract of soap-root plant as stabilizer (Forough and Farhadi 2010). In general, 10 mL extract of soap-root as a stabilizer agent was added to 100 mL of 3 mM aqueous silver nitrate solution and incubated at room temperature and dark conditions in a rotary shaker for 2 h. For reduction of Ag^+ ions, 15 mL of the aqueous extract of manna of hedysarum as a reducing agent was added to the mixture at 86 °C. This solution was purified by repeated centrifugation at 12000 g for 20 min and the final solution of SNPs was obtained. The details of the nanoparticles used in this study are explained in Table 1.

In this study, the induction of magnetic field was B= 3 mT, measured with a digital tesla-meter (PHYWE, Germany). This magnetic field induction value was chosen according to the opinion that weaker magnetic field has stronger effect on plant productivity (Akacha Touati 2012).

Table 1. Details of silver nanoparticles used in the experiment.

True density	Purity	Average particle size	Specific surface area	Color	Morphology
10.5 g/cm^3	99.99%	20 nm	~18-20 m^2/g	Yellow	Spherical

Plant material, growth conditions and seedling development

Seeds of *C. officinalis* L. were obtained from Esfahan Research Centre in 2015, Iran. Seeds were preliminarily surface sterilized with 5% NaOCl (vol/vol) for 5 min and thoroughly washed with distilled water. The sterilized seeds were then germinated in an incubator at 25 ˚C for 4 days. After germination, similar seedlings were visually selected and transferred to pots containing the soil and sand mixture (1:2).

In order to investigate the effect of SNPs and MF on marigold, an experiment was carried out using randomized complete block design with four treatments and three replications. Treatments were as follows: 1) Control, 2) MF with B= 3 mT for an hour per day, 3) SNPs (50 ppm) and 4) MF (B= 3 mT) + SNPs (50 ppm). Plants were grown in the greenhouse for 30 days with diurnal regime of 16 h light at 25 ˚C and 8 h dark at 22 ˚C, light intensity of µmol $m^{-2}s^{-1}$ and relative humidity of 30-40%. After over a month, samples were uprooted and the length and fresh and dry weight of shoots were measured for the control and treated groups.

Determination of total phenolic content (TPC)

For extracting, 0.5 g of leaf fresh weight with 10 mL of 80% methanol was pulverized in a mortar. The homogenate was centrifuged at 8000 g for 15 min at 4 ˚C. The total phenolic content was assayed colorimetrically by means of the Folin–Ciocalteu method, as modified by Marinova *et al*. (2005). One mL extract was added into a flask containing 9 mL of distilled water. Then 1 mL of Folin-Ciocalteu's phenol reagent was added and the mixture was mixed thoroughly. After 5 min, 10 mL of 7% sodium carbonate was added. The mixture was diluted to 25 mL with the addition of 4 mL of distilled water and allowed to stand at room temperature for 90 min. The absorbance of the solution was determined at 750 nm using a spectrophotometer (WPA, S2100, UK). The TPC was expressed as mg gallic acid equivalents (GAE)/100 g FW.

Determination of total flavonoid content (TFC)

TFC was determined as the method described by Chang *et al*. (2002). One mL of a sample was mixed with 3 mL of 95% ethanol (v/v), 0.2 mL of 10% aluminium chloride, 0.2 mL of 1 mol L^{-1} potassium acetate and 5.6 mL water. After incubation at room temperature for 45 minutes, the absorbance of the reaction mixture was measured at 415 nm. The TFC was expressed as mg catechin equivalents (CE)/100 g FW.

DPPH radical scavenging activity

The free radical scavenging capacity of extracts was determined using DPPH (Burits and Bucar 2000). Two mL of freshly prepared methanol solution of DPPH (0.004%) was added to 20 µL of extracts and allowed to stand at room temperature for 30 min. The absorbance of sample solution was measured at 517 nm, compared with that of control solution. The reduction of DPPH radicals was determined by measuring the absorption at 517 nm. The radical scavenging activity was calculated as a percentage of DPPH discoloration using the following equation:

DPPH radical scavenging% = $[(A_0 – A_1)/A_0] \times 100$

where A_0 is the absorbance of the DPPH solution and A_1 is the absorbance of the sample.

Phenylalanine ammonia lyase (PAL) activity

PAL activity was assayed as described by Ke and Saltveit (1986). Two g of leaf samples with 8 mL of 50 mM borate buffer (pH= 8.5) containing 5 mM 2-mercapto ethanol and 2% polyvinyl pyrolydin were homogenized in a chilled mortar. The resulting mixture was filtered through four layers of clean cloth and was centrifuged at 8000 g for 20 minutes at 4 °C. To 5 mL of the supernatant, 0.55 mL L-phenylalanine (100 mM) was added and incubated for 60 min at 40 °C. Then, absorbance of the reaction mixture was measured at 290 nm.

Polyphenoloxidase (PPO) activity

Detection of PPO activity was performed according to the method of Siriphanich and Kader (1985). In assaying for PPO activity, 1 mL of reaction mixture containing 100 µL enzyme extract and 50 mM phosphate buffer (pH= 7.0) was preparde. Each sample was aerated for 2 min in a small test tube followed by the addition of pyrocatechol, as the substrate, at a final concentration of 20 mM. PPO activity was determined as the change in one unit of absorbance at 420 nm per minute per gram fresh weight of sample.

Catalase (CAT) activity

The activity of catalase was determined according to Aebi (1984). The mixture reaction included 5.2 mL of 50 mM phosphate buffer (pH= 7.0), with 0.2 mL hydrogen peroxide (1%) and 0.3 mL of enzyme extract. The activity of catalase was estimated by the decrease of absorbance at 240 nm for 1 min as a consequence of H_2O_2 consumption (Havir and McHale 1987).

Ascorbate peroxidase (APX) activity

Activity of APX was measured according to Nakano and Asada (1981) with minor modification. The reaction mixture contained 2.5 mL of 50 mM phosphate buffer (pH= 7.0) with 0.1 mL H_2O_2 (1%) and 0.1 mL enzyme extract. The oxidation of ascorbate was followed by the decrease in the absorbance at 240 nm.

Guaiacol peroxidase (GPX) activity

GPX activity was measured by the method of Upadhyaya *et al*. (1985). The reaction mixture including 2.5 mL of 50 mM phosphate buffer (pH= 7.0) contained 1mL guaiacol (1%), 1 mL H_2O_2 (1%) and 0.1 mL enzyme extract**.** Enzymatic activity was determined by measuring the increase in absorbance at 420 nm for guaiacol with a spectrophotometer.

Glutathione reductase (GR) activity

GR activity was measured by recording the increase in absorbance in the presence of oxidized glutathione (GSSG) and 5,5-dithiobis-2-nitrobenzoic acid (DTNB) as described by Sairam *et al*. (2002). The reaction mixture contained 1 ml of 0.2 M potassium phosphate buffer (pH= 7.5) incuding 0.1 mM EDTA, 0.5 ml of 3 mM DTNB in 0.01 M potassium phosphate buffer (pH= 7.5), 0.1 ml of 2 mM NADPH, 0.1 ml enzyme extract and distilled water to make up a final volume of 2.9 ml.

Reaction initiated by adding 0.1 ml of 2 mM GSSG. The increase in absorbance at 412 nm was recorded at 25 °C over a period of 5 min on a spectrophotometer.

Statistical Analysis

The statistical analysis included the analysis of variance and comparison of the means. Means were compared using Tukey's test at the 5% level of probability by SPSS software, version 21.0.

Results and Discussion

Phenolic compounds and DPPH free radical scavenging

The assessment of total phenol and flavonoid content showed that the seedlings of marigold which treated with the combination of MF and SNPs, had the highest phenol and flavonoid proportion (Figure 1). The level of phenolic compounds significantly increased in response to MF and SNPs.

Plants have the ability to adjust their metabolism according to changing environmental conditions. They accelerate their metabolism and grow faster when optimal conditions develop. However, when a stress condition arises, plants generally decelerate their metabolism and limit their growth (Cakmak *et al*. 2012). In this study, total phenol and flavonoid increased in response to MF (Figure 1 A, B). Dhawi (2014) reported that MF increases phenylalanine ammonialyase activity and phenolic compound concentration. The increase in phenol and flavonoeid concentration or any antioxidant compounds may be the result of accumulation of by-products which lead to redirection of synthesis pathway.

Under stress conditions, plants generally increase the activity of one or more antioxidant molecules, and the elevated activity levels usually correlate with increased stress tolerance (Mittler 2002). Therefore, resistance to stress is related to the plant's antioxidant capacity. Our results showed that a possible relationship may exist between total phenol and flavonoeid content of marigold leaves with the application of MF and SNPs. Several investigators indicated the stimulatory role of MFs on phenolic compound of different plant species. Our results are in good harmony with those obtained by Amira *et al*. (2010). They have shown that, irrigated lentil plants with magnetized water showed an increase in carotenoid and total phenol. Moreover, Atak *et al*. (1997) and Goodman *et al*. (1995) described the role of MF in changing the characteristics of cell membrane, affecting the cell metabolism. Therefore, the increase in total phenol under this study may be attributed to the role of MF in changing the cell membrane properties.

Phenolic compounds significantly contribute to the antioxidant activity in plants. The highest increase of antioxidant activity was significantly found in the group treated with SNPs plus MF in comparison to other treatments. Kaimoyo *et al*. (2008) and Dannehl *et al*. (2011) have found that sub-lethal levels of electric current can be used to induce plant defence reactions and activity as an abiotic elicitor to enhance the secondary metabolite production in fenugreek, chickpea roots and tomatoes. Phenolic compounds play an important role in growth and reproduction, providing protection against pathogens and predators (Balasundram *et al*. 2005). Secondary metabolites

present in plant systems may be responsible for the reduction of silver and synthesis of nanoparticles (Savithramma *et al*. 2011).

The results of measuring the capacity of DPPH free radical scavenging in marigold's shoot showed that the samples treated with the combination of MF and SNPs were significantly higher than the control group (Figure 1C). DPPH radical scavenging activity is one of the most widely used methods for screening the antioxidant activity of plant extract (Naskar and Mazumder 2015). The antioxidant activity of *C. officinalis* extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals (Stoilova *et al*. 2007). Krystofova *et al*. (2013) have shown that the use of metal NPs such as Fe NPs and Ag NPs increased the antioxidant activity and DPPH free radical scavenging. Also, Nabizadeh *et al*. (2014) demonstrated the effect of the magnetic field of 2 mT in *Cucurbita maxima* L. that led to an increase in DPPH radical scavenging.

Phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) activity

PAL enzyme activity in treatments with MF and SNPs was significantly increased as compared to the control plants. Also, there was close relationship between phenol content and PAL activity (Figures 1A and 2A). Figure 2B shows that the exposure to MF and SNPs had a significant effect on PPO activity in the seedlings. PPO activity was significantly increased in MF and SNPs treatments as compared with the control treatment. The largest increase was observed in the plants treated with SNPs.

PAL is the key enzyme linking primary metabolism of aromatic amino acids with secondary metabolic products in plants. PAL plays a key regulatory role in controlling biosynthesis of all phenylpropanoid products. As the entry point into the pathway, PAL catalyses the non-oxidative deamination of phenylalanine to trans-cinnamic acid and ammonia. Phenylpropanoid pathway produces variable phenolic compounds and many of them are involved in plant defence reactions and scavenging of reactive oxygen species (Danaee *et al*. 2013). PAL is also an important enzyme in plant development and defence against pathogens. In all plants, it is encoded by a multi-gene family, ranging in copy number from four in *Arabidopsis* to a dozen or more copies in some higher plants (Chang *et al*. 2008). In addition, there is close relationship between phenol content and PAL activity (Schovankova and Opatova 2011) (Figures 1A, 2A). Jones *et al*. (1986) have shown that levels of PAL can be increased in cells of *Phaseolus vulgaris* L. (French bean) in response to complex pulsed electromagnetic field.

Plants have their own enzymatic resources such as the metalloenzymes PPO to prevent oxidative damages (Celik *et al*. 2009). The significant increase of PPO activity in the shoots of *C. officinalis* by application of MF and SNPs in this study, could represent an adaptive response against MF and SNPs. The increase in PPO activity indicated that *C. officinalis* had the capacity to adapt to MF and SNPs treatment by increasing its phenolic compounds and removing ROSs. Other researchers have also reported the enhancement of PPO activity with exposure of plants to the MF in *Valeriana officinalis* L. (Farzpourmachiani *et al*.

2015) and *Glycine max* L. (Radhakrishnan and Ranjitha Kumari 2013). Polyphenols have the capacity to reduce lipid peroxidation by neutralizing and detoxifying the radicals produced in the process of exposure to oxidative stress (Xin *et al*. 2009).

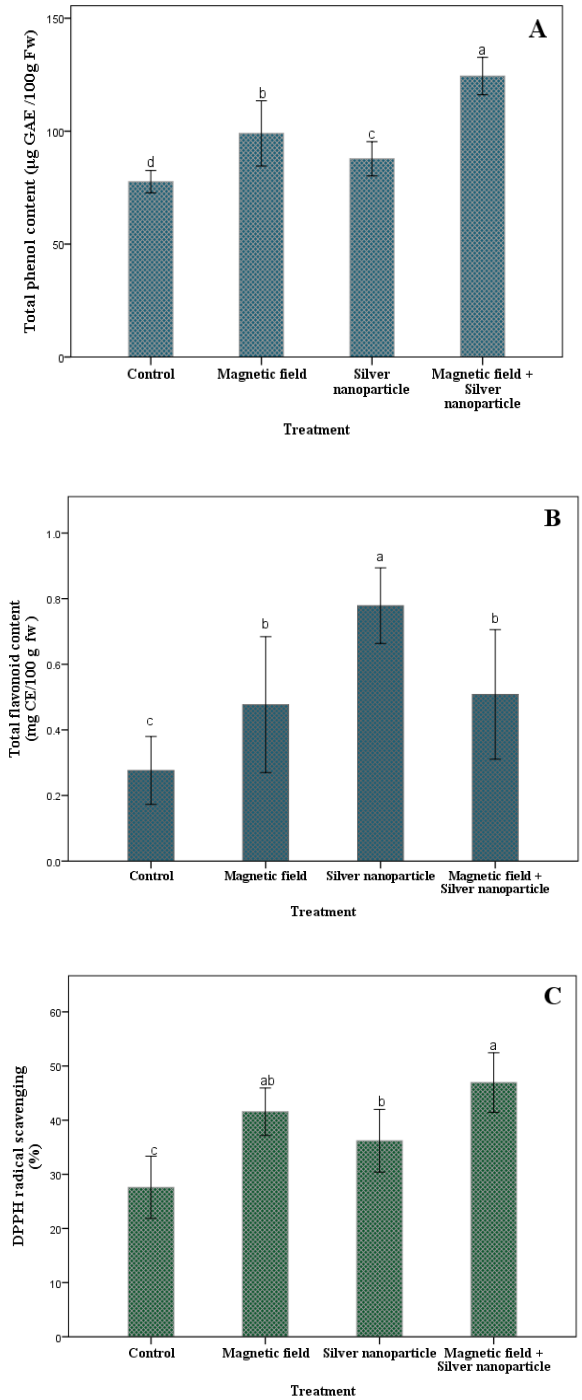

Figure 1. Influence of MF, SNPs and MF+SNPs on total phenolic content (A), total flavonoeid content (B) and DPPH radical scavenging (C) of *Calendula officinalis.* Bars represent means ± standard error. Means followed by the same letter are significantly different ($p \leq 0.05$) based on Tukey's test.

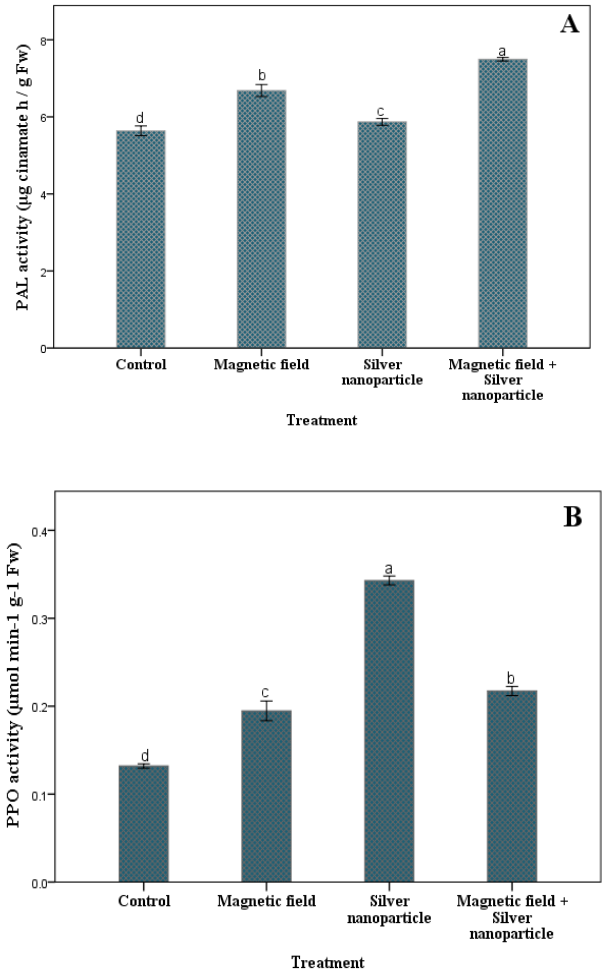

Figure 2. Influence of MF and SNPs on PAL activity (A) and PPO activity (B) of *Calendula officinalis* shoots. Bars represent means ± standard error. Means followed by the same letter are significantly different ($p \leq 0.05$) based on Tukey's test.

Catalase (CAT), ascorbate peroxidase (APX), guiacol peroxidase (GPX) and glutathione reductase (GR) enzymes' activity

The activity of scavenging enzymes (CAT, APX, GPX, GR) was evaluated on the marigold's root and shoot extracts. Results are shown in Tables 2 and 3. CAT and APX activity showed a significant increase in the shoots and roots of MF and SNPs treatments as compared to the control, and the highest value was observed for the MF+SNPs treatment. GPX activity in the shoots and roots of plants treated with SNPs showed the largest increase when compared with other plants. As shown in Tables 2 and 3, the exposure to MF and SNPs increased the GR activity in shoots and roots and the maximum activity in shoots was observed in the group treated with SNPs. However, there was no significant difference among plants treated with MF and SNPs in terms of GR activity in the roots.

Present study shows that *C. officinalis* responds to MF and SNPs with changes in the levels of antioxidant enzymes. The plants have

oxidative defence system that protects them against ROS such as superoxide, hydroxyl and hydrogen peroxide. Oxidative defence system includes antioxidants and scavenging enzymes such as peroxidases, superoxide dismutase (SOD), CAT and GR with detoxification ability of toxic oxygen forms in plants under environmental stresses (Ali *et al.* 2003). The enhanced activity of APX and CAT may be attributed to the increased activity of SOD. Scavenging of H_2O_2, produced by SOD, can be attained by either nonenzymatic antioxidants or scavenging enzymes, e.g., CAT and APX (Sahebjamei *et al*. 2007). CAT and APX are involved in detoxification and scavenging of H_2O_2 and they have distinct affinity levels with H_2O_2. CAT has been reported as a primary enzyme that effectively eliminates the bulk of H_2O_2 while APX can scavenge low levels of H_2O_2 that is not removed by CAT as it has higher affinity for H_2O_2 as compared to CAT (Cakmak *et al*. 2012). Probably, MFs by affecting the spatial structure of enzymes or changing the amount and speed of substrate binding, impose their effects on enzyme activity (Batcioglu *et al*. 2002). Thus, due to the presence of iron in the structure of APX and CAT, one possible mechanism to alter the activity of these enzymes in response to MF, is having impact on iron and therefore changing the enzyme's spatial structure (Ghanati *et al*. 2007).

The conversion of glutathione disulfide (GSSG) to glutathione (GSH) which is catalysed by GR is related to the change in GSH/GSSG mole ratios that plays an important role in the cellular redox status and in signal transduction of several transcription and metabolic processes (Hatata and Adel Abdel-Aal 2008). As mentioned before, in

Table 2. Specific and total activity of catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR) in the root extracts of *Calendula officinalis*.

Treatment	CAT activity (μ mol min^{-1} g^{-1}fw)	APX activity (μ mol min^{-1} g^{-1}fw)	GPX activity (μ mol min^{-1} g^{-1}fw)	GR activity (μ mol min^{-1} g^{-1}fw)
Control	0.055 ± 0.006^{d}	0.088 ± 0.002^{d}	0.020 ± 0.003^{c}	0.065 ± 0.006^{b}
Magnetic field	0.133 ± 0.005^{c}	0.144 ± 0.005^{c}	0.082 ± 0.008^{b}	0.134 ± 0.005^{a}
Silver nanoparticles	0.184 ± 0.003^{b}	0.250 ± 0.008^{b}	0.247 ± 0.009^{a}	0.129 ± 0.006^{a}
Magnetic field + silver nanoparticles	0.214 ± 0.006^{a}	0.283 ± 0.004^{a}	0.114 ± 0.005^{b}	0.133 ± 0.004^{a}

Data represent means ± standard error. Means followed by the same letter are significantly different ($p \leq 0.05$) based on Tukey's test.

Table 3. Specific and total activity of catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR) activity in extract shoot of *Calendula officinalis*.

Treatment	CAT activity (μ mol min^{-1} g^{-1} fw)	APX activity (μ mol min^{-1} g^{-1} fw)	GPX activity (μ mol min^{-1} g^{-1} fw)	GR activity (μ mol min^{-1} g^{-1} fw)
Control	0.111 ± 0.003^{d}	0.198 ± 0.007^{d}	0.155 ± 0.006^{d}	0.117 ± 0.001^{c}
Magnetic field	0.295 ± 0.008^{b}	0.444 ± 0.020^{b}	0.266 ± 0.011^{c}	0.240 ± 0.004^{b}
Silver nanoparticles	0.254 ± 0.004^{c}	0.343 ± 0.010^{c}	0.491 ± 0.010^{a}	0.432 ± 0.004^{a}
Magnetic field + Silver nanoparticles	0.376 ± 0.008^{a}	0.598 ± 0.006^{a}	0.309 ± 0.008^{b}	0.228 ± 0.0034^{b}

Data represent means ± standard error. Means followed by the same letter are significantly different ($p \leq 0.05$) based on Tukey's test.

our study, the GR activity in roots and shoots of marigold increased under the MF and SNPs treatments. Maffei (2014) suggested that exposure to MF causes accumulation of reactive oxygen species and alteration of GR and other antioxidant enzymes' activities. They also proposed that apoplastic constituents may work as potentially important redox regulators sensing and signalling MF changes. The polarization effect of high voltage electric field upon dielectric substance can cause the hydrogen bonding in water to bend or break (Chaplin 2005). It is assumed that the electromagnetic field exposure of seedlings can bend or break the hydrogen bonding in ultrastructrural elements of the cell, such as enzymes, resulting in structural alteration of the macromolecules. This structural alteration may increase enzyme activity or cause enzyme denaturing, depending on the strength of the electromagnetic field and time of exposure (Huang *et al*. 2006). Farzpourmachiani *et al*. (2015) on *Valeriana officinalis* L., Celik *et al*. (2008) on *Glycine max* L., Cakmak *et al*. (2012) on *Allium ascalonicum* L., Akacha *et al*. (2013) on *Raphanus sativus* L. and Huang *et al*. (2006) on cucumber also have reported that exposure to MF increased the antioxidant enzyme activity.

SNPs are effective against free radicals including ROSs and organic radicals (Duran *et al*. 2010). Priyadarshini *et al*. (2012) reported that higher concentrations of SNPs enhanced the activity of H_2O_2-metabolizing enzymes. In our research, activity of antioxidant enzymes (CAT, APX, GPX, GR) was increased by the employment of 50 ppm SNPs, implying less ROS formation and less toxicity to the plants. It can be noted that SNPs release Ag^+ ions, which interact with cytoplasmic organelles and nucleic acids to inhibit respiratory enzymes and interfere with cellular functions such as membrane leakage (Lu *et al*. 2010). Similar results were reported by Krishnaraj *et al*. (2012) and Hatami *et al*. (2014) that high levels of CAT and APX activity were recorded from leaf samples of plants subjected to SNPs treatment.

Conclusion

The present study indicated that uses of MF and SNPs were effective on the increase of antioxidant activity of marigold's shoots. The results showed increased phenolic compounds, improving the antioxidant defence system by enhancing the activity of scavenging enzymes and also DPPH radical scavenging under the MF and SNPs treatments. It was found that the accumulation and uptake of SNPs was dependent on the exposure concentration. The use of SNPs with a concentration of 50 ppm and MF with low-intensity (B=3 mT) had positive effect on the marigold's defence mechanism. Thus, weak MF and SNPs can be used as an effective means for augmenting plant's resistance to different stress factors.

References

Aebi H, 1984. Catalase in-vitro. Methods in Enzymology 105: 121-126.

Akacha Touati M, Ghanem Boughanmi N, Ben Salem M and Haouala R, 2013. Effects of moderate static magnetic field presowing treatment on seedling growth and oxidative status in two *Raphanus sativus* L. varieties. African Journal of Biotechnology 12: 275-283.

Ali BMP, Vajpayee RD, Tripathi UN, Rai SN and Singh SP, 2003. Phytoremediation of lead, nickel and copper by Salix acmophylla Boiss.: role of antioxidant enzymes and antioxidant substances. Bulletin of Environmental Contamination and Toxicology 70: 462-469.

Amira MS, Qado SA and Hozayn M, 2010. Magnetic water technology, a novel tool to increase growth, yield and chemical constituents of lentil (*Lens esculenta*) under greenhouse condition. Agricultural and Environmental Sciences 7: 457-462.

Asadi Samani M, Pourakbar L and Azimi N, 2013. Magnetic field effects on seed germination and activities of some enzymes in cumin. Life Science Journal 10: 323-328.

Atak C, Danilov V, Yurttas B, Yalcn S, Mutlu D and Rzakoulieva A, 1997. Effect of magnetic field on soybean (*Glycine max* L. Merrill) seeds. JINR Communication, Dubna, 1-13.

Balasundram N, Sundram K and Samman S, 2005. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence and potential uses. Food Chemistry 99: 191-203.

Banerjeel P, Satapathy M, Mukhopahayay A and Das P, 2014. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresources and Bioprocessing 1(3): 1-10.

Batcioglu K, Ozturk K, Atalay S, Dogan D, Bayri N and Demirtas H, 2002. Investigation of time dependent magnetic field effects on superoxide dismutase and catalase activity. Journal of Biological Physics and Chemistry 2: 108-112.

Burits M and Bucar F, 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy Research 14: 323-328.

Butnariu M and Coradini CZ, 2012. Evaluation of biologically active compounds from *Calendula officinalis* flowers using spectrophotometry. Chemistry Central Journal 6(35): 1-7.

Cakmaka T, Cakmak ZE, Dumlupinar R and Tekinay T, 2012. Analysis of apoplastic and symplastic antioxidant system in shallot leaves: impacts of weak static electric and magnetic field. Journal of Plant Physiology 169: 1066-1073.

Celik O, Büyükuslu N, Atak C and Rzakoulieva A, 2009. Effects of magnetic field on activity of superoxide dismutase and catalase in *Glycine max* (L.) Merr. Roots. Polish Journal of Environmental Studies 18: 175-182.

Chang A, Lim HL, Lee SW, Robb EJ and Nazar RN, 2008. Tomato phenylalanine ammonia-lyase gene family, highly redundant but strongly underutilized. Journal of Biology Chemistry 48: 591-601.

Chang CC, Yang MH, Wen HM and Chern JC, 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10: 178-182.

Chaplin C, 2005. Magnetic and electric effects on water. http://www. Lsbu ac.uk/water/magnetic. Retrieved at html, May 28, 2005.

Dannehl D, Huyskens Keil, Eichholz I, Ulrichs C and Schmidt U, 2011. Effects of direct-electric-current on secondary plant compounds and antioxidant activity in harvested tomato fruits (*Solanum lycopersicon* L.). Food Chemistry 126: 157-165.

Dhawi F, 2014. Why magnetic fields are used to enhance a plant's growth and productivity? Annual Research and Review in Biology 4(6): 886-896.

Duran N, Marcato PD, De Conti R, Alves OL, Costa FTM and Brocchib M, 2010. Potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanisms of action. Journal of the Brazilian Chemical Society 21: 949-959.

Farzpourmachiani S, Majd A, Arbabian S, Dorranian D and Hashemi M, 2015. Effect of extremely low frequency electromagnetic fields on antioxidant enzymes in valerian (*Valeriana officinalis* L.). Iranian Journal of Plant Physiology 5: 1465-1472.

Florez M, Martinez E and Carbonella MV, 2011. Effect of magnetic field treatment on germination of medicinal plants *Salvia officinalis* L. and *Calendula officinalis* L. Polish Journal of Environmental Studies 21: 57-63.

Forough M and Farhadi K, 2010. Biological and green synthesis of silver nanoparticle. Turkish Journal of Engineering and Environmental Sciences 34: 281-287.

Ghanati F, Abdolmaleki P, Vaez Zadeh M, Rajabbeigi E and Yazdani M, 2007. Application of magnetic field and iron in order to change medicinal products of *Ocimum basilicum* L. Environmentalist 27: 429-434.

Goodman EM, Greenabaum B and Morron TM, 1995. Effect of electromagnetic field on molecules and cells. International Review of Cytology 158: 279-325.

Hatami M and Ghorbanpour M, 2014. Defence enzyme activities and biochemical variations of *Pelargonium zonale* in response to nanosilver application and dark storage. Turkish Journal of Biology 38: 130-139.

Hatata MM and Adel Abdel-Aal E, 2008. Oxidative stress and antioxidant defence mechanisms in response to cadmium treatments. American-Eurasian Journal of Agricultural and Environmental Sciences 4(6): 655-669.

Havir AE and McHale NA, 1987. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiology 84: 450-455.

Huang R, Sukprakarn S, Phavaphutanon L, Juntakool S and Chaikul C, 2006. Changes in antioxidant enzyme activity, lipid peroxidation and seedling growth of cucumber seed induced by hydropriming and electric field treatments. Kasetsart Journal 40: 825-834.

Jones DH, 1984. Phenylalanine ammonia-lyase: regulation of its induction and its role in plant development. Phytochemistry 231: 1349-1359.

Kaimoyo E, Farag MA, Sumner LW, Wasmann C, Cuello JL and van Etten H, 2008. Sub-lethal levels of electric current elicit the biosynthesis of plant secondary metabolites. Biotechnology Progress 24: 377-384.

Ke D and Saltveit ME, 1986. Effect of calcium and auxin on russet spotting and phenyl alanine ammonia-lyase activity in iceberg lettuce. HortScience 21: 1169-1171.

Krishnaraj C, Jagan EG, Ramachandran R, Abirami SM, Mohan N and Kalaichelvan PT, 2012. Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* L. Wettst. plant growth metabolism. Process Biochemistry 47: 651-658.

Krystofova O, Sochor J, Zitka O, Babula P, Kudrle V, Adam V and Kizek R, 2013. Effect of magnetic nanoparticles on tobacco BY-2 cell suspension culture. International Journal of Environmental Research and Public Health 10: 47-71.

Lu J, Cao P, He S, Liu J, Li H, Cheng G, Ding Y and Joyce DC, 2010. Nano-silver pulse treatments improve water relations of cut rose cv. Movie Star flowers. Postharvest Biology and Technology 57: 196-202.

Maffei ME, 2014. Magnetic field effects on plant growth, development and evolution. Frontiers in Plant Science 4(5): 445.

Marinova D, Ribarova F and Atanassova M, 2005. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy 40: 255-260.

Michalak A, 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies 15(4): 523-530.

Mittler R, 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7(45): 5-10.

Nabizadeh S, Majd A, Arbabiyan S, Mirzai M and Sharifnia F, 2014. Assessment of the effect of electromagnetic fields on biochemical and antioxidant parameter changes of *Cucurbita maxima* Duchesne. Advances in Environmental Biology 8(24): 185-193.

Najafi S, Heidari R and Jamei R, 2013. Influence of the magnetic field stimulation on some biological characteristics of *Phaseolus vulgaris* in two different times. Global Journal of Science Engineering and Technology 11: 51-58.

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

Naskar S and Mazumder UK, 2015. Antioxidant potential and hepatoprotectivity of hydromethanolic extract of *Litchi chinensis* fruits: *in vivo* and *in vitro* studies. Iranian Journal of Pharmacology and Therapeutics 14: 1-9.

Priyadarshini S, Deepesh B, Zaidi MGH, Pardha Saradhi P, Khanna PK and Arora S, 2012. Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea* L. Applied Biochemistry and Biotechnology 167: 2225-2233.

Radhakrishnan R and Ranjitha Kumari BD, 2013. Influence of pulsed magnetic field on soybean (*Glycine max* L.) seed germination, seedling and soil microbial population. Indian Journal of Biochemistry and Biophysics 50: 312-317.

Rigane G, Younes B, Ghazghazi H and Ben Salem R, 2013. Investigation into the biological activities and chemical composition of *Calendula officinalis* L. growing in Tunisia. International Food Research Journal 20: 3001-3007.

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

Sairam RK, Rao KV and Srivastava GC, 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Science 163: 1037-1046.

Savithramma N, Rao ML, Rukmini K and Devi PS, 2011. Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. International Journal of Chemtech Research 3: 1394-1402.

Schovankova J and Opatova H, 2011. Defensive reactions of apple cultivars Angold and HL 1834 after fungal infection. HortScience 38: 87-95.

Siriphanich J and Kader AA, 1985. Effects of CO_2 on cinnamic acid 4-hydroxylase in relation to phenolic metabolism in lettuce tissue. Journal of the American Society for Horticultural Science 110: 333–335.

Stoilova I, Krastanov A, Stoyanova A, Denev P and Gargova S, 2007. Antioxidant activity of a ginger extract (*Zingiber officinale* L.). Food Chemistry 102: 764-770.

Sujatha K, Chitra K, Polisetti H, Karri S, Nimmalapudi S and Reddy CU, 2011. Standardization of *Calendula officinalis* Linn with reference to quercetin by high performance thin layer chromatography. International Journal of Research in Pharmacy and Chemistry 1(4): 789-792.

Upadhyaya A, Sankhla D, Davis TD, Sankhla N and Smith BN, 1985. Effect of paclobutrazol on the activities of some enzymes of activated oxygen metabolism and lipid peroxidation in senescing soybean leaves. Journal of Plant Physiology 121: 453-461.

Vanaja M, Gnanadhas G, Paulkumar K, Rajeshkumar S, Malarkodi C and Annadurai G, 2013. Phytosynthesis of silver nanoparticles by *Cissus quadrangularis*: influence of physicochemical factors. Journal of Nanostructure in Chemistry 3: 1-7.

Xin M, Lin DH, Yi X, Wu YY and Tu YY, 2009. Effects of phenanthrene on chemical composition and enzyme activity in fresh tea leaves. Food Chemistry 115: 569–573.

ارزیابی تغییرات القاشده میدان مغناطیسی و نانوذرات نقره بر ترکیبات فنلی و خواص پاداکسایشی گیاه همیشه بهار (*Calendula officinalis* L.)

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چکیده

تاثیر میدان‌های مغناطیسی و کاربرد نانوذرات نقره بر یک ارگانیسم زیستی، بسته به نوع مواد و سیستم و برنامه‌ی بکار رفته و زمان و شدت کاربرد آن متفاوت است. در سال‌های اخیر تحقیقات زیادی در زمینه حساسیت موجودات زنده نسبت به میدان‌های مغناطیسی و نانوذرات انجام شده است. به همین منظور، برای ارزیابی تأثیر میدان مغناطیسی و نانوذرات نقره روی فعالیت‌های پاداکسایشی گیاه همیشه بهار (*Calendula officinalis* L.) آزمایشی درسال ۱۳۹۶ در گروه زیست شناسی دانشگاه ارومیه با استفاده از طرح بلوک‌های کامل تصادفی با چهار تیمار و سه تکرار انجام شد. تیمارها به این شرح بودند: شاهد، میدان مغناطیسی (با شدت ۳ mT) به مدت یک ساعت در روز، نانو ذرات نقره (۵۰ ppm) و ترکیب این دو تیمار. نتایج نشان داد که در گیاهان تحت تیمار با میدان مغناطیسی، نانوذره نقره و میدان مغناطیسی + نانوذره نقره، محتوای فنل کل، فلاونویید و ظرفیت جاروب کنندگی رادیکال آزاد DPPH، میزان فعالیت آنزیم‌های فنیل آلانین آمونیالیاز، پلی فنل اکسیداز، کاتالاز، آسکوربات پراکسیداز، گایاکول پراکسیداز و گلوتاتیون ردوکتاز نسبت به گروه شاهد افزایش معنی‌دار در سطح احتمال ۰/۰۵ داشت. تیمار میدان مغناطیسی و نانوذره‌ی نقره واجد بهترین تأثیر روی وضعیت پاداکسایشی گیاه همیشه بهار بود و می‌تواند به عنوان جایگزینی مناسب برای ترکیبات شیمیایی مورد استفاده در کشاورزی قرار گیرد.

واژه‌های کلیدی: ترکیبات فیتوشیمیایی؛ میدان مغناطیسی؛ نانوذرات نقره؛ همیشه بهار