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ORIGINAL ARTICLE

Photosynthetic Characteristics, Membrane lipid levels and Protein content in the *Phaseolus vulgaris* L. (cv. Sadri) exposed to Magnetic Field and Silver Nanoparticles

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ABSTRACT
In order to study the effect of silver nanoparticles and magnetic field on Phaseolus vulgaris, experiments were conducted. Experiment was carried out with four treatments in 10 days. Treatments were including (T1) control, (T2) magnetic field with B=1.8 mT for 1h per day in 10 days, (T3) silver nanoparticles (50 ppm), (T4) magnetic field and silver nanoparticles. Results showed that Plants treated with combined of magnetic field and silver nanoparticles increased chlorophyll a, b and carotenoid content, whereas, this factors decreased in group of treated with alone magnetic field. Also, protein content increased in group of treated with silver nanoparticles. Magnetic field increased rate of Malondialdehyde. Physical damage and gene mutation as a result of magnetic field are generally restricted to the plant regeneration. Decreased of protein content may be due to produced free radicals by magnetic field stress. Increasing the concentration of free radicals enhances stress response and some chemical and biological reactions, creates oxidative stress.
Key words*: Malondialdehyde , Pigment content, Proline content, Protein content, Magnetic field, Silver nano particles, Phaseolus vulgaris.*

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INTRODUCTION

The recent decade was a rage of advanced chemical and physical application such as silver nanoparticles and magnetic field in agriculture as well in other and different areas modern biology systems. Physical and chemical factors have had many uses in ancient and modern society. Plant species vary in their sensitivity and response to environmental and biosystems stresses because they have various reactions for stress perception, signaling and response [1]. Physical methods are not only cost effective, they also significantly changes the yield with positive and negative impacting the environment. They influence the physiological, biochemical and metabolisms process and interactions in the seeds. Nanotechnology has a significant effect in agriculture and main areas of the food industry. Engineered nano materials have received a particular attention for their positive impact in improving many sectors of economy and trade, including consumer products, loom, pharmaceutics, consmetics, transportation, energy and agriculture etc., and are being increasingly produced for a wide range of applications within industry [2,3]. Silver nanoparticles influences a variety of plant functions such as growth [4, 5] and chlorophyll content [6]. In contrast, magnetic field influences on growth [7,8], chlorophyll content [9, 10], MDA content and protein content [11]. It was shown that the natural geomagnetic field has an important role on biological systems. Such models have been introduced for at least 20 years,such as (1) torque on ferromagnetic particles, (2) modulation of chemical reaction rates (radical-pair mechanism), and (3) modulation of transport rates and binding by the ICR (ion-cyclotron resonance) mechanism but they have received limited attention best from plant physiologists. Because the energy content of weak magnetic fields is too low to break chemical bonds, other physical and engineering mechanisms capable of triggering a biological response are required [12]. The objective of this study was to determine the effect of magnetic field and silver nanoparticles on photosynthesis pigments, proline, MDA and protein content. In these experiments, *Phaseolus vulgaris* were exposed to combined of silver nanoparticles and magnetic field for highly efficient biochemical factors regeneration. The regenerated plants were examined to determine the

effects of the magnetic field and silver nanoparticles combined, on regeneration chlorophyll, MDA, proline and protein content.

MATERIAL AND METHODS

Seed pretreatment with MF

Seeds of *Phaseolus vulgaris* (cv.sadry) were obtained from Esfahan in Research Center in 2012, Iran. Seeds of *Phaseolus vulgaris* have been used for investigating the influence of magnetic field on the development of plants. The induction of magnetic field has been *B*= 1.8 mT, measured with a digital tesla-meter (PHYWE, Germany). Magnetic-field-induction value has been chosen according to the opinion that weaker magnetic field has stronger effect on plant productivity. The healthy uniform dry seeds 8.6% of moisture content were selected and seeds kept at the geometric Centre of coil assemblies. Control seeds were kept under similar condition local geomagnetic field only but in the absence of magnetic field. Exposure was 10 days for 1 h per day and control seeds were kept under the similar condition in the absence of the MF. The experiments have been performed in 2012 under laboratory conditions. The natural light cycle was 16 h– light/8h darkness with daily temperature 25°Cand night temperature 22°C.

Seed pretreatment with silver nanoparticles

silver nanoparticles were prepared by means of the biological reduction of metal salt precursor (silver nitrate, $AgNO_3$) in water with aqueous extract of manna of hedysarum plant in the presence of extract of soap-root plant as a stabilizer (13Forough and Farhadi, 2010). Briefly, 10 ml of freshly prepared extract of soap-root plant as a stabilizer agent was added to 100 ml of 3 mM aqueous silver nitrate solution and incubated in a rotary shaker for 2 h in dark conditions at 25 ◦C, and then 15 ml of the aqueous extract of manna of hedysarum plant as a reducing agent was added into the mixture at 86 ◦C. The mixture obtained, was purified by repeated centrifugation at 12,000 rpm for 20 min to obtain the fresh biologically Ag nanoparticles solution.

Seed germination and seedling development

MF pretreated and control seeds were surface sterilized with 1% NaOCl (w/v) for 5 min, washed thoroughly 3 times with distilled water and then propagated in pots containing soil and sand mixture (1:2). The pots were maintained under natural photoperiod with 35% (w/w) soil moisture content. Seed germination observed at 7th day, and germination seedlings were uprooted and measured the length, fresh and dry weight of 10 days for both control and treated seedlings.

Pigment contents (chlorophyll a, chlorophyll b and carotenoid)

The photosynthetic pigments e.g., Chl a, b and Car were extracted in 5 ml of chilled 80% acetone by grinding the leaves of salt treated seedlings in a chilled mortar and pestle. The homogenate was centrifuged at 3000 g for 10 min at 4 ◦C. The absorbance of the resulting supernatant was taken at 480, 645 and 663 nm. Different pigments were estimated using the following formula by Arnon as given below:

Chl a (mg/l)= 12.7 (A663) − 2.69 (A645)
Chl b (mg/l)= 22.9 (A645) −4.68 (A663)
Car (mg/l) = 1000A480 – 1.8Chl a-85.02Chl b/198
The pigment concentration was calculated in g/g FW of sample and expressed as percent change [14].

Total protein assay

Folin Lowry method was used for detection of proteins. This method is based on protein hydrolysis and release of the amino acids using Folinciocalteo and then the resulting color is assayed by spectrophotometer. For each population, 0.07 g of shoot was weighed. It was then ground with mortar and pestle and 5 ml Tris-HCl buffer (50 ml Tris 0.2N, 26.08 ml Hcl, 17.2 g sucrose, 1 g ascorbic acid) was added to it. 100 ml solution was obtained by adding sterile water. The solution was then blended and put in the centrifuge 5000 g for 30 minutes. For each case, 1 ml of upper phase was taken up and 4 ml of After 10 minutes, 1.5 ml Folin solutions was added to each case and they were put in dark for 30 minutes. Then by means of a spectrophotometer (Biowave, S2100 Diode Array, UK) absorbance of each case was recorded at 660 nm. Standard curve was depicted and total protein was calculated.

Free proline

Proline was determined following Bates et al.[15]. Fresh plant material (1–0.5 g) was homogenized in 10 ml of 3% sulfosalicylic acid and the homogenate filtered. The filtrate (2 ml) was treated with 2ml acid ninhydrin and 2ml of glacial acetic acid, then with 4ml of toluene. Absorbance of the colored solutions was read at 520 nm.

Determination of malondealdehyde

Malondealdehyde (MDA) levels were measured by the method of Heat and Packer (151968) 0.5 g of fresh leaf was homogenized in 2.5 mL of 0.1% (w/v) trichlroacetic acid (TCA), and centrifuged at 1000 g for 5

min. For every 1 ml of supernatant 4 mL of 20% TCA containing 0.5% TBA was add. The mixture were incubated for 30 min at 95°C, chilled on ice, and centrifuged at 4000 *g* for 10 min. the absorbance of the supernatant was measured at 532 nm using a Philips PU 8620 spectrophotometer. Unspecific absorption at 600 nm was subtracted from the 532 nm values. The concentration of MDA was calculated by using a molar extinction coefficient of 156 mM-1 cm. MDA were estimated using the following formula by Heat and Packer [16] as given below:

MDA equivalents ($nmol.cm^{-1}$) = 1000[(Abs 523- Abs 600nm)/155]

Statistical analysis

The data obtained from the experiments were analyzed and calculated. As the experimental design is completely randomized design and data for each experiment were analyzed by one-way ANOVA with factorial arrangement to determine the effects of magnetic treatment. Means were compared using Duncan's multiple-range test at a 5% level of significance by SPSS software version 16.

RESULT AND DISCUSSION

The results of the magnetic field biological effect depends the field frequency, the accumulation of chemical substances may increase, decrease, or remain unchanged. Also, Silver nanoparticles may be changes the chemical compounds in different concentrations. Chlorophyll is an extremely important biomolecule, critical in photosynthesis, which allows plants to absorb energy light. Chlorophyll absorbs light most strongly in the blue protion of the electromagnetic spectrum, followed by the red portion. Chlorophyll is vital for photosynthesis, which allows plants to absorb energy from light. Chloroplast includes many different part that respond to heavy metal and other stresses such as magnetic field and silver nanoparticles, therefore any decreases in photosynthesis rate used as the index of direct toxic effects of magnetic field. Magnetic field and silver nanoparticles were sufficient to change the content of phytochemical compounds in *Phaseolus vulgaris.*

Table 1. Comparison of Chlorophyll a , b and Carotenoid content (µg/gFw) in different treatments with magnetic field and silver nanoparticles in *Phaseolus vulgaris.*

	Chlorophyll a content (µg/gFw)	Chlorophyll b content (µg/gFw)	Carotenoid content (µg/gFw)
Control	1.22±0.03	4.94±0.06	1.37±0.05
magnetic field	6.09±0.07	3.81±0.10	0.49±0.01
silver nanoparticles	9.04±0.04	3.43±0.09	0.40±0.00
Magnetic field and silver nanoparticles	1.62±0.05	4.12±0.07	1.10±0.02

Each value is expressed as mean±standard error, n=3, P<0.05

As shown as Table 1, the chlorophyll a content significantly decreased in magnetic field treatment, similar results were obtained by other researchers. In generally, magnetic field and silver nanoparticles have increased the retardation of seedling growth, the degradation of chlorophyll. Furthermore, the results of combined exposures have indicated that silver nanoparticles exposures suppressed the magnetic field effects. This case had been reported in some plants such as dates [17,10] and soybean [10]. The absorbed energy can effect on intercellular organelles such as chloroplast magnetic moments and disturb photosynthetic pigments. Dhawi and Al-Khayri [18]showed that long exposure of magnetic field had a negative effect on pigment content. Thus might be the result of their oxidation by magnetic field induced free radicals and ROS accumulation. Decrease in chlorophyll content may be due to reduce in the source of essential metal that involved in chlorophyll synthesis such as Fe^{+2} and Zn^{+2}[19, 20].

Table2. Comparison of Protein content (mg/gFwt), MDA content (µmol/gFw) and Proline content (µg/gDw) in different treatments with magnetic field and silver nanoparticles in *Phaseolus vulgaris.*

	Protein content(mg/gFwt)	MDA content(µmol/gFw)	Proline content(µg/gDw)
Control	9.96±0.41	0.41±0.01	10.11±0.21
magnetic field	1.06±0.31	0.59±0.02	5.89±0.42
silver nanoparticles	1.43±0.24	0.57±0.01	9.91±0.30
Magnetic field and silver nanoparticles	1.15±0.57	0.51±0.00	7.14±0.17

Each value is expressed as mean±standard error, n=3, P<0.05

Silver nanoparticles improved protein rate of *Phaseolus vulgaris* seed, while effect of magnetic field decreased the protein rate of seeds. Silver nanoparticles had the most effective in increasing protein rate of seed irrespective of magnetic field had negative effective on protein rate. Crnobarace et al. [11] reported an increase in yield of soybean from 5-25% with a higher quantity of oil and protein and at sunflower from 13.2-17%. Magnetically induced mutagenesis has been reported in some systems. Physical damage and gene mutation as a result of magnetic field are generally restricted to the plant regeneration. Decreased of protein content may be due to produced free radicals by magnetic field stress [21]. Magnetic field effects on protein biosynthesis and alternation in cell membrane lipids On tissue and cellular levels [22-24]. Malondialdehyde (MDA) concentration is a widely used stress indicator of plant membrance. The results are presented in detail Table 2 the results suggest that lipid peroxidation may be corrected against interfering compounds. In some studies, glutathione S-transferase(GTS) detoxify peroxidised lipids and reduce lipid peroxidation in plants under magnetic field stress[25]. Dhawi and Al-Khayri [18] showed that long exposure of magnetic field had a negative effect on proline content. Cerdonio et al. [26] reported that magnetic field changed the growth of seeds in a parallel and antiparallel orientation, obtaining a difference response in plant growth with a significant inhibition for antiparallel seeds. The data obtained previously has indicated that magnetic field has some negative effects on the biological system including carcinogenesis at the high magnetic field energy level or at close around the energy source [27]. The biological effects of magnetic field depend on the exposure time, waveform and frequency of the source electrical voltage, energy level, distance of target from energy source and structure of organism [28]. In general, magnetic field alters the chemical reaction rates and electron spins of molecules, especially ionic forms, modulation of transport rates and binding by the ion-cyclotron resonance(ICR) [29]. Jajte [30] and seaiano et al. [31] have observed that magnetic field exposure may be due to both the increase in the concentration and oscillating of free radicals, respectively. Magnetic field increase the concentration of oxygen free radicals, affect radical pair recombination in living systems [30]. Increasing the concentration of free radicals enhances stress response and some chemical and biological reactions, creates oxidative stress. The reason of the increase in oxygen free radicals into more stable and less reactive forms under low magnetic field energy [31]. The mechanism could explain the reducing effect of silver nanoparticles on the oxidative effect of magnetic field. The data obtained from this study may be helpful to explain the mechanism of biological effects of magnetic field and silver nanoparticles alone or combined of magnetic field with silver nanoparticles. In sum, it can be concluded that the applied magnetic field intensities decreased growth parameters and development at the first stages of growth in *Phaseolus vulgaris*.

CONCLUSION

In our study it has been found that silver nanoparticles changes the effect of magnetic field in a positive way as a result combined application of magnetic field and silver nanoparticles. This stresses had different effects on Photosynthetic characteristics, MDA, proline and protein content. In general, for increasing of chemical compounds in *Phaseolus vulgaris.* Need to vastly examined, because various treatments differently effects on different seeds.

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