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STUDY OF ANTIOXIDANT ACTIVITY AND BIOCHEMICAL CHARACTERISTICS OF *PHASEOLUS LANATUS* IN THE PRESENCE OF SILVER NANOPARTICLES AND MAGNETIC FIELD

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Abstract

This study is about to investigate the effect of silver nanoparticles and magnetic field on *Phaseolus lanatus*. So, experiments including four treatments were carried out 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 nano particles (50 ppm), (T4) magnetic field and silver nanoparticles. Obtained showed that silver nanoparticles (T3) lead to the highest changes of biochemical composition while rate of this factors in group of treated by magnetic field (T2) was the lowest. It seems that silver nanoparticles (50 ppm) had the best effect on phenol and flavonoid content, antioxidant activity such as CAT and GPX. content DPPH increased in Plants treated with both of magnetic field and silver nanoparticles.

Keywords : Antioxidant activity, biochemical factors, Magnetic field, Silver nanoparticles, *Phaseolus lanatus*

Introduction

Phaseolus lanatus is a perennial herbaceous plant of the legumes family and indigenous to parts of Brazil and Preu.The present study investigate the effect of magnetic field and silver nanoparticles treatments on the antioxidant activity and some biological characteristics of *Phaseolus lanatus*. With regard to vertical farm of the XXIst century, for productive growth of cultivated plants, using techniques and tods which are environmentally safe, noticeably has been increased. Studied carried showed that technique in which silver particles and some magnetic fields are used, leading to preparation of high-quality and specially prepared seed is an important. These techniques have influence on yield-forming and performance factors cultivation of many species of agricultural plants. The chemical and physical treatments evaluated were able to eliminate or satisfactorily change the physiological and chemical factors which consist mainly in treating the seed with various chemicals and physical methods. Both chemically and physically pre-sowing seed treatment like magnetic fields electrical, nanoparticles, microwave and irradiation positive or negative effect modifications of plant metabolisms and seed preferment that could be favorable or inappropriate to plant growth and yield. Magnetic field and nanoparticles stimulation seem to be especially promising. However, that how plants respond to magnetic field is under study. function of composition in living systems also could be affected by Magnetic field (Atak *et al*., 2003; Carbonell *et al*., 2000; Stage *et al*., 2002). However, since magnetic field applications causes harmful effect on biological systems, it is difficult to choose individuals in required characteristics. Magnetic field is considered as a physical factor due to it's biological effects (Matsuda *et al*., 1993). Magnetic field became a part of the environmental stress and source of energy that functions and metabolisms in cell could be influenced by it (Aladjadjiyan, 2007). In addition, magnetic field effects on macromolecules (Paul *et al*., 2006). Florez *et al*. (2007) reported that magnetic field treatment effects on biological systems. Nanotechnology in agriculture will significantly affect the plants. In addition, some nanoparticles such as silver nanoparticles seems to be useful for plant biochemical factors, however, phytotoxicity cannot be neglected.

Material and Methods

Phaseolus lanatus growth condition and treatments

Seeds of *Phaseolus lanatus* were obtained from Esfahan in Research Center, Iran. measured. The healthy uniform day seeds contains 8.6% moisture were selected. For sterilization, treatments seeds of control, silver Nanoparticle, MF, MF and silver Nanoparticle were surface sterilized with 1% NaOCl (w/v) for 5 min, with distilled water washed thoroughly 3 times and then propagated in pots containing soil and sand mixture (1:2). Then, under natural photoperiod with 35% (w/w) soil moisture content the pots were maintained. Then, we measured the biochemical factors from *Phaseolus lanatus* leaves after 10 days.

Seed pretreatment with silver nanoparticles

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 silver nanoparticles were prepared (Forough and Farhadi, 2010). Briefly, in 100 ml of 3 mM aqueous silver nitrate solution, 10 ml offreshly prepared extract of soap-root plant as a stabilizer agent was added and incubated in a rotary shaker in dark conditions at 25 ºC for 2 h, and then as a reducing agent15 ml of the aqueous extract of manna of hedysarum plant was added into the mixture at 86 ºC. By repeated centrifugation at 12,000 g for 20 min to obtain the fresh biologically Ag nanoparticles solution, the mixture obtained was purified.

Seed pretreatment with MF

Seeds of *Phaseolus lanatus* 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. Seeds of control and T3 groups were kept under condition of laboratory. Group of T2 (exposure with magnetic field) and T4(exposure of magnetic field and treatment with silver nanoparticles) exposed to MF 1 h per day for 10 days, so that the seeds were kept in the geometric Centre of coil assemblies. The natural light cycle was 16 h–light/8 h

darkness with daily temperature 25°C and night temperature 22°C. in the magnetic set, the maximum field intensity occurs in the middle of coil and confirms 1.8 m Tesla of the measured magnetic flux density.

Activity of enzymes

Enzyme extraction and enzyme assays

Phaseolus lanatus leaf fresh tissues (500 ml) were used to prepare enzyme extract. Plant materials were ground in 3 ml of 50mM tris-HCl (pH 7.5) buffer containing 3 mM $MgCl_2$, 1 mM EDTA, using pre cooled mortar and pestle. Then the mixture centrifuged at 5000 g at 4 ˚C for 20 min. The supernatant was used for determination of enzyme activity.

Catalase (CAT) (EC 1.11.1.6)

CAT activity was determined using the method of Aebi. The reaction mixture contained 2.5 ml of 50mM phosphate buffer (pH 7.0) with 0.2 ml hydrogen peroxide (1%) and 0.1 ml enzyme extract. CAT activity was measured by monitoring a decrease in absorbance of H_2O_2 using an extinction coefficient 0.0436/ (mM cm) at 240 nm within 1 min(Maehly and Chance, 1959).

$$\text{Unit (mM/min)} = \frac{\frac{d_{OD}}{\min(\text{slope})} \times \text{Vol of assay}(0.0003)}{\text{Extinction coefficient}(0.0436)}$$

Guaiacol peroxidase (GPX) (EC 1.11.1.7)

GPX activity was measured by the method of Upadhyaya. 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. Activity was determined using an extinction coefficient 26.6 (mM/cm) at 420 nm within 1 min (Upadhaya *et al*., 1985).

$$\text{Unit (mM/min)} = \frac{\frac{d_{OD}}{\min(\text{slope})} \times \text{Vol of assay (0.0001)}}{\text{Extinction coefficient (26.6)}}$$

Total phenol

Total phenol was determined spectrophotometrically using Folin–Ciocalteu's reagent as described by Bonilla et al. (2003). Briefly, 4 g fresh *Vigna radiata* (the seed discarded) were ground in liquid nitrogen. A sample was then extracted in 2% HCl in methanol for 24 h in the dark and at room temperature. After centrifugation at 12,000 g for 20 min at 4 ºC, the supernatant was diluted with the same extract solvent at a suitable concentration for assaying total phenol. Two hundred microliters of diluted extraction was introduced into a 5.0 ml test tube. One milliliters of Folin–Ciocalteu reagent and 0.8 ml sodium carbonate (7.5%) were then added and the contents mixed and allowed to stand for 30 min. Absorption at 765 nm was measured in a Shimadzu UV–Vis spectrophotometer (Shimadzu UV-1601).Total phenol content was expressed as gallic acid equivalents (GAE) in milligrams per gram of sample using a standard curve generated with 50, 100, 150, 200, 250, 300, 350, 400, and 500 mg/l of gallic acid (Bonilla *et al*., 2003).

DPPH radical-scavenging activity

Various concentrations of *Phaseolus lanatus* extracts (0.3 mL) were mixed with 2.7 mL of a methanolic solution containing DPPH radicals (6 × 10–5 mol/L). The mixture was shaken vigorously and incubated, allowed to stand in the dark for 60 min until stable absorption values were obtained. The reduction of DPPH radicals was determined by measuring the absorption at 517 nm. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation RSA% = [(ADPPH – AS)/ADPPH] × 100, where AS is the absorbance of the solution when the sample extract has been added at a particular level and ADPPH is the absorbance of the DPPH solution (Blank *et al*., 1994). The extract concentration providing 50% inhibition was calculated from the graph of the scavenging effect percentage against the corresponding extract concentration. BHA was used as the reference compound.

Determination of flavonoid content

The flavonoid contents of the extracts were determined by the colorimetric method measurement with some modifications (Jerman *et al*., 1989). The *Phaseolus lanatus* extract (0/1 mL) was mixed with 1.25 mL of distilled water and 75 µL of a 5% $NaNO_2$ solution. After 5 min, 150 µL of a 10% $AlCl_3\,H_2O$ solution was added. After 6 min, 500 µL of 1 M NaOH and 275 µL of distilled water were added to the mixture. The solution was mixed well and the intensity of the pink color was measured at 510 nm. The results were expressed as milligrams of catechin equivalents per gram of extract (mg CEs/g extract).

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 catalase Guaiacol peroxidase activities of the samples have been given in Table 1, It has been observed that CAT and GPX activities of the silver nanoparticles samples have been induced while magnetic field exposures have decreased the CAT activities. Reduction of enzyme activity and gene expression may cause oxidative stress and free radicals produced by magnetic field. In some studies, Nano-TiO_2 enhanced photosynthesis and other metabolisms in Spinach (Yang *et al*., 2005). Magnetic field as a stressful environmental factor increase the amount of free radicals and reactive oxygen species. It has generally been agreed that magnetic field-induced cellular damage is brought about through indirect effects by formation of various toxic molecular species, including free radicals and catalase that are generated tissues. Silver nanoparticles changes the effect of injury caused by magnetic field in oxidative way and partially protects the biological system from this kind of damage. A range of responses to environmental stresses has been reported to exist between magnetic field and susceptible plants in the strong frequency and intensity as well as between salt-tolerant susceptible plants and these differences have been explained in terms of radical metabolisms. Magnetic field induced primary damage by ionizing and modifying in enzymatic repair processes (Ahnstrom, 1977). Also, magnetic field and silver nanoparticles changed protein biosynthesis and enzyme activities (Tenforde, 1996) only small damage remains to be realized in a biological change in

the combined application of magnetic field and silver nanoparticles. Arababian *et al*. (2001) observed that esterasis enzyme in pre-treated seed were changed by magnetic field during germination. Phirke *et al*. (1996) reported that magnetic field altered enzyme activities in plant. Magnetic field produced free radicals and ROS (Scaiano *et al*., 1997) which have an essential role in electron transfer and chemical reaction. ROS which have unpaired electrons magnetic materials can be oriented in external magnetic field. Reaction between the external magnetic field and the orientation on movement of unpaired electrons absorb energy. Maneticfied could be protects the living systems for this kind of injuries (Scaiano *et al*., 1995, Suri *et al*., 1996). Also, magnetic field causes oxidative injury by accelerating free radical generation in biological systems. The results of this study showed that, in contrast to other environmental stresses, silver Nanoparticle in optimum concentration can be applied to agriculture to induce the biosynthesis of phenolic and flavonoid content. From this result it can be concluded that the effects of an applied magnetic field also depend on the specific plant species, field frequency, accumulation of the chemical substances. Furthermore, it might be assumed that the magnetic field treatments influenced the phenolic and flavonoid content. The effect of magnetic field are various on growth of plants in some situations, leading to inhibition, and in others to stimulation, while sometimes no effect had been reported.

Table 1 : comparison of phenol content(mg GAEs/g extract), flavonoid content(mg CEg/g extract), DPPH scavenging (%), and Activities of antioxidant enzymes such as CAT and GPX activity in different treatments with magnetic field and silver nanoparticles in *Phaseolus lanatus.*

Treatment	Phenolic content (mg GAEs/g extract)	Flavonoid content (mg CEg/g extract)	DPPH scavenging (%)	CAT activity (µmol min^{-1}/gFw)	GPX activity (µmol min^{-1}/gFw)
Control	8.48±3.65	2.99±1.01	4.49±1.27	5.28±1.12	1.39±0.05
magnetic field	1.52±3.82	2.08±3.99	3.79±0.93	2.24±1.33	1.00±0.02
silver nanoparticles	8.51±3.64	3.15±3.07	6.35±0.35	6.61±1.54	1.59±0.05
Magnetic field and silver nanoparticles	5.71±4.41	2.62±3.0	6.65±0.62	3.88±2.55	1.32±0.05

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

Conclusion

The obtained results in this study, indicated that magnetic field and silver nanoparticles had different effects on biochemical factors and antioxidant activity in *Phaseolus lanatus*. Generally, it can be concluded that the applied magnetic field decreased biochemical factors in *Phaseolus lanatus* plant, while silver nanoparticles treatment increased biochemical factors and antioxidant activity.

Also, this research, despite the fact that the interaction of metal nanoparticles with plants, had a positive effect on the biological factors, however, it is necessary to do more examine such as studying different concentrations, standards and methods of nanoparticles in the plant.

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