Responses of alfalfa influenced by magnetic field and rhizobial inoculant

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Abstract

Plants are generally subjected to a combination of different conditions such as magnetic field and soil bacteria in their life. The present investigation tried to compare the effects and interactions of magnetic field and rhizobial inoculant in alfalfa. A pot experiment was performed under a natural condition by a factorial design to investigate the influences of magnetic field with 0.75 and 1.5 mT intensities on treated and untreated alfalfa seed with *Sinorhizobium meliloti*. Results showed that 1.5 mT magnetic intensity reduces growth parameters, protein content, catalase, ascorbate peroxidase, and peroxidase activity as a stress factor while superoxide dismutase activity, malondialdehyde, and reducing sugars content increased. Interaction of rhizobial inoculant and 1.5 mT intensity can reduce the damage caused by magnetic field generated in the plant. Application of 0.75 mT intensity and rhizobial inoculant (individual and together) led to a significant increase in growth parameters, protein content, catalase, ascorbate peroxidase, peroxidase, and superoxide dismutase enzyme activity while malondialdehyde and reducing sugars content reduced. Therefore, as an eco-friendly technique in agriculture, the application of 0.75 mT and rhizobial inoculant might improve the plant quality. This method could be used as a biofertilizer for vegetable production which reduces the environmental pollution caused by the application of biochemical fertilizers.

Keywords: magnetic field; rhizobial inoculant; alfalfa; growth parameters; physiological indicators

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Introduction

Medicago sativa L. which is commonly known as 'Alfalfa', belongs to the fabaceae family. Alfalfa has been used in traditional herbal medicine for a very long time (over 1500 years), having a high concentration of proteins, minerals such as calcium, and vitamins like E, K (liposoluble vitamins) B, and C (Radu et al., 2010). It is considered as one of the most important crops because it can release nitrogen to soil and improve soil structure for future crops. Unlike other crops, it does not need nitrogen fertilizer. Nodules on Alfalfa roots have a kind of bacteria, performing nitrogen fixation through taking nitrogen gas from the air and converting it into the nitrogen which could be used by plants. These bacteria promote plant growth either directly (through nitrogen

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fixation) or indirectly (by suppression of plant pathogenic organisms and induction of resistance in host plants against plant pathogens and abiotic stresses), and they are referred to as plant growth promoting rhizobacteria (PGPR) (Gopalakrishnan et al., 2015). PGPR can be added to the plant by rhizobial inoculation technique which is a significant technology for manipulation of rhizobia to improve crop productivity and soil fertility (Lamptey et al., 2014).

Generally, rhizobium inoculation in legumes is recognized for stimulating growth and is known to be a substitute to the costly inorganic nitrogen fertilizers (Tairo and Ndakidemi, 2013). Rhizobial inoculant application can be considered as an important strategy to maximize agricultural productivity with minimum soil loss by decreasing the use of chemical fertilizers and pesticides (Alves et al., 2004; Adesemoye et al., 2009; Hungria et al., 2010a, 2013b; Souza et al., 2015).

Success and efficiency of PGPR as rhizobial inoculant for agricultural crops are influenced by various factors (Souza, 2015) such as plant species, soil type and environmental conditions. One of the environmental conditions, which might influence the success and efficiency of PGPR is magnetic field. Magnetic field (MF) is an unavoidable environmental factor for all living organisms because they are exposed to Earth's MF (geomagnetic, GMF) and artificial magnetic fields which are widespread in the modern environment.

Study of the magnetic fields has been the subject of many research studies where the effects of magnetic fields were investigated on physiological and biochemical changes in plants. It is hypothesized that plants respond quickly to varying MF by altering their gene expression and phenotype (Maffei, 2014). They affect the activities of some enzymes (Aleman et al., 2014) and biochemical processes in the seeds (Asadi Samani et al., 2013). MF pre-sowing includes stimulation or inhibition depending on intensity (from mili T to T ranges), duration (second to hours or continuous), frequency (acute or chronic), species and experimental conditions.

In this research, authors investigated the influences of magnetic field and rhizobial inoculation on alfalfa (*Medicago sativa* L.) and tried to determine the effects of interactions between magnetic field and symbiotic bacteria on growth parameters, protein content, antioxidant enzymes activity, malondialdehyde content, and reducing sugars in alfalfa. The study also was set to evaluate how alfalfa seedlings adapt and respond to these factors. The results can be used in plant physiology and agriculture research to know better about defense mechanisms of plants against abiotic stresses.

Materials and Methods

Plant material

Alfalfa seeds (*Medicago sativa* L.) were provided from Pakan Bazr company in Iran. Seeds were placed on petri dishes and then were separately exposed to 0.75 mT and 1.5 mT magnetic fields for four days, 30 minutes per day. The two intensities of MF were chosen according to the previous studies (Peyvandi et al., 2013). Four days after treatments, seeds treated with magnetic field along with the untreated seeds with magnetic field were mixed with the rhizobial inoculant. Commercial rhizobial inoculant 'Biomedica' was used in the study, which contains *Sinorhizobium meliloti* (Culture Forming Unit 108 ml^{-1}).

The experiment was conducted in three replications as a completely randomized design. The factors were used as follows:

T0**:** no magnetic field and no rhizobial inoculant (control or normal condition),

T1: treated with 0.75 mT,

T2: treated with 0.75 mT and rhizobial inoculant,

T3: treated with 1.5 mT,

T4: treated with 1.5 mT and rhizobial inoculant, and

T5: treated with rhizobial inoculant.

Then, each treatment was sown separately in pots under randomized and natural conditions during spring season.

Sixty (60) days after sowing, samples were collected from each treatment to determine

growth parameters, protein content, antioxidant enzymes activity, reducing sugars, and malondialdehyde content in alfalfa (*Medicago sativa* L.) leaves.

Magnetic field device

To create a uniform magnetic field, a DC coil was used with 350 copper ring wires rolled around a PVC cylinder with 160 mm diameter. In order to maintain constant temperature inside the cylinder, a base with a height of 20 mm was considered to allow air ventilation. During the experiment, the temperature inside the coil was measured. Results showed that the temperature did not change. The coil was connected to a DC power supply made by MEGA TEG company.

Assays

Sixty-day-old plants were used for measurement of growth parameters (leaves fresh and dry weights, roots fresh and dry weights). Also a number of assays were carried out in the study as detailed below.

Protein content was determined by Bradford method (1976). In sum, a sample of frozen leaves (0.5 g) was ground in a mortar with 5 ml Tris- Glycine buffer (pH 8.3). The homogenate was centrifuged at 12000 g for 10 min at 4° C. Bovine serum albumin (BSA) was used as a standard.

Catalase activity was measured according to Pereira's method (2002). The assay contained Tris-Glycine buffer (50 mM, pH 7.5), H_2O_2 (10 mM), and enzyme extract. Catalase activity was determined by decreasing H_20_2 absorbance at 240 nm. Enzyme activity was expressed as changes in unit absorbance per minute gram fresh weight.

Peroxidase activity was measured according to Koroi's method (1989) in a reaction mixture consisting acetate buffer (0.2mM, pH 4.8), hydrogen peroxide (0.1mM), benzidine (0.04 M), and enzyme extract. Then, the absorbance was read at 530nm. Enzyme activity was expressed as changes in unit absorbance per minute gram fresh weight.

Ascorbate peroxidase activity was assayed according to Nakano and Asada (1981). The reaction mixture contained enzymatic extract, L^{-1} sodium phosphate buffer (50 mM, pH 7), hydrogen peroxide (0.1 mM), ascorbate (0.5 mM), EDTA (0.1 mM), and enzyme extract. The absorbance was measured in a spectrophotometer at 290 nm. Enzyme activity was expressed as changes in unit absorbance per minute gram fresh weight.

Superoxide dismutase activity was determined as described by Gianopolitis and Ries (1977). The reaction mixture (3 ml) consisted of 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionin, 75 mM nitro blue tetrazolium (NBT), 2 µm riboflavin, and 0.2 ml of enzyme extraction. The absorbance was measured in the spectrophotometer at 560 nm. One unit of superoxide dismutase was defined as the amount of enzyme that inhibited NBT photo reduction by 50% at 25° C.

Leaf tissues (1 g) were extracted in 4 ml ethanol (80%) at 90 °C for five min and then centrifuged. The supernatant was used to determine reducing sugars as described by Nelson's method (1944). One mL of copper reagent was added to 1 mL of plant extract. After mixing, the tube was placed in a boiling water bath for 20 min and was quickly cooled for five min. One mL of arsenomolybdate reagent was added and shaken. Tubes were left for 15 min for the blue color to appear. After that, the absorbance was read at 500 nm. Sugars reduction was estimated as µg glucose g^{-1} fresh weight.

Lipid peroxidation level was determined in terms of malondialdehyde content by Heath and Packer (1968) with slight modifications. Fresh samples (0.2 g) were homogenized with 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) before they were centrifuged. Then, 1 mL of supernatant was mixed with 4 mL of 20% trichloroacetic acid containing 5% (w/v) thiobarbituric acid (TBA). The mixture was incubated at 100 °C for 30 min and were quickly cooled and again centrifuged. Finally, the absorbance of the supernatant was determined at 532, 600 nm.

To calculate the concentration of malondialdehyde, an extinction coefficient equal to 155 $mM^{-1}cm^{-1}$ was used and the results of measurement were calculated in terms of Nano moles per gram fresh weight.

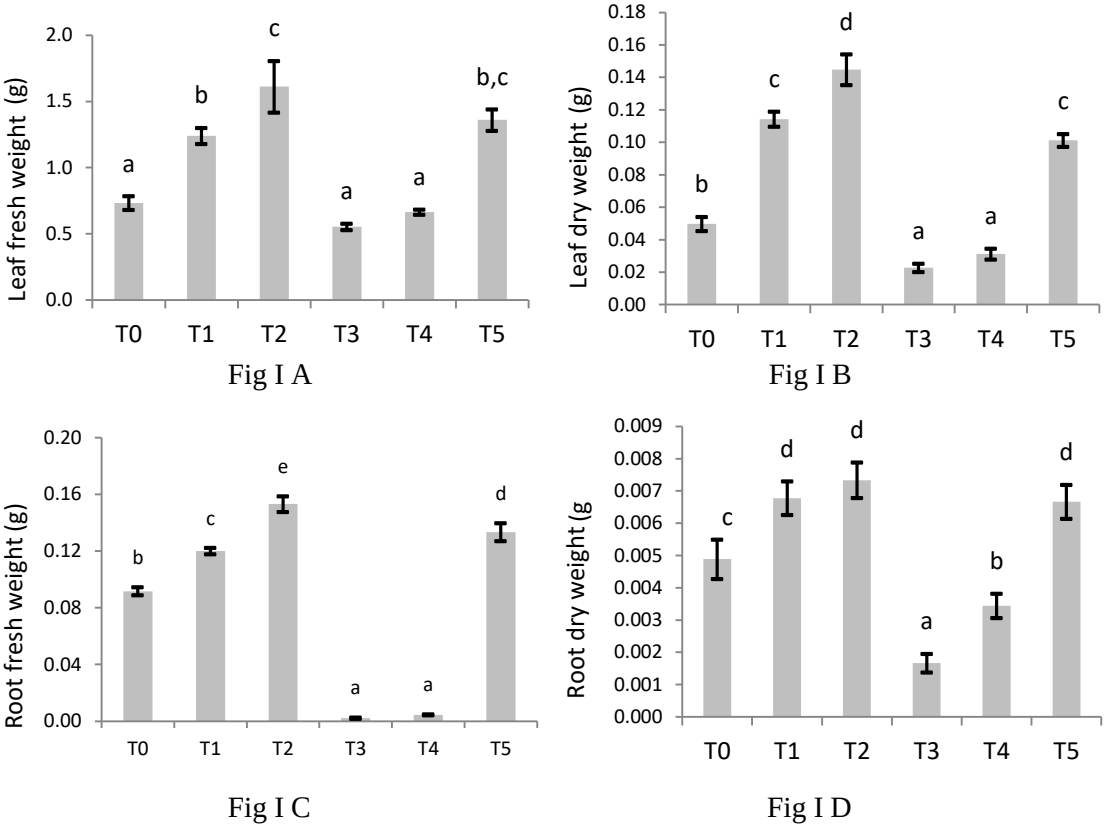

Fig I. Means of leaf fresh weight (A) and dry weight (B) (g), root fresh weight (C) and dry weight (D) in response to magnetic field and rhizobial inoculant (mean ± SE) grouped by Duncan test ($p \leq 0.05$); different letters indicate T0: Control, T1: 0.75 mT, T2: 0.75 mT + Inoculant, T3: 1.5 mT, T4: 1.5 mT + Inoculant, and T5:Inoculant means.

Results

According to the results of this study, application of rhizobial inoculant, individually (T5) and in combined with 0.75mT magnetic field (T2) increased all growth parameters compared to control (Figs. I. A-D); also, 0.75 mT intensity of magnetic field (T1) led to a remarkable increase in protein content and antioxidant enzymes activity (Figs. II and III. A-D) which decreased reducing sugars and malondialdehyde content (Figs. IV A, B) in comparison with the control.

Application of rhizobial inoculant combined with 0.75 mT magnetic field (T2) had the same effect as the magnetic field with 0.75 mT intensity (T1). Also, 1.5 mT magnetic field (T3) reduced growth parameters (Figs. I. A-D), protein content (Fig. II) as well as catalase (Fig. III. B), ascorbate peroxidase (Fig. III. D), and peroxidase activity (Fig. III. C) in *medicago sativa* L. while superoxide dismutase activity (Fig. III. A) increased

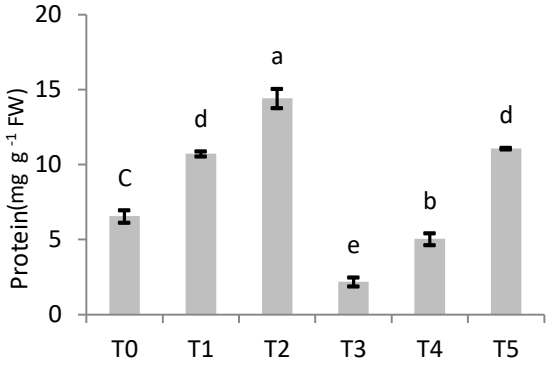

Fig II. Mean protein content (mg g^{-1} FW) in response to magnetic field and rhizobial inoculant (mean ± SE) grouped by Duncan test ($p \leq 0.05$); different letters indicate T0: Control, T1: 0.75 mT, T2: 0.75 mT + Inoculant, T3: 1.5 mT, T4: 1.5 mT + Inoculant, and T5: Inoculant means.

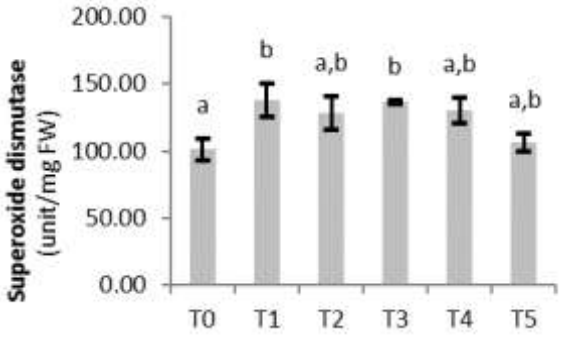

Fig III A

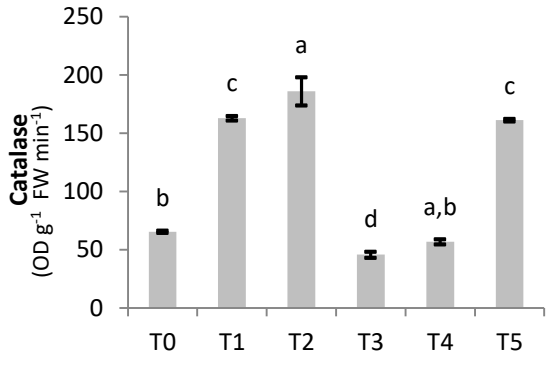

Fig III B

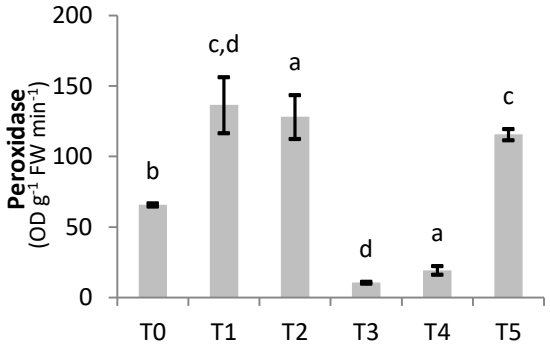

Fig III C

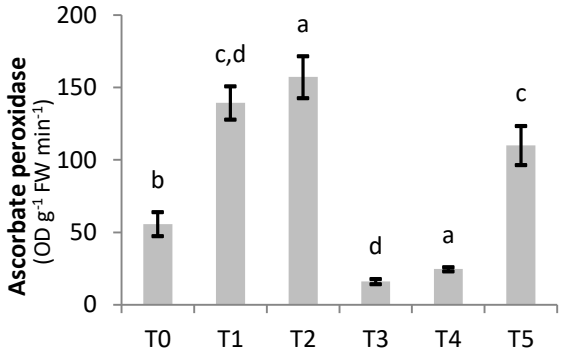

Fig III D

Fig. III. Means of superoxide dismutase (A), catalase (B), peroxidase (C) and ascorbate peroxidase (D) activities in response to magnetic field and rhizobial inoculation (mean ± SE) grouped by Duncan test (p ≤0.05); different letters indicate T0: Control, T1: 0.75 mT, T2: 0.75 mT + Inoculant, T3: 1.5 mT, T4: 1.5 mT + Inoculant, T5: Inoculant means.

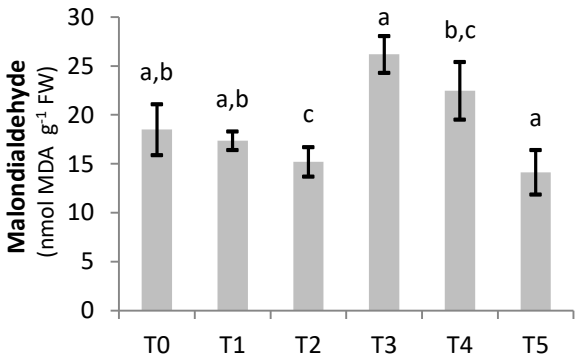

Fig IV A

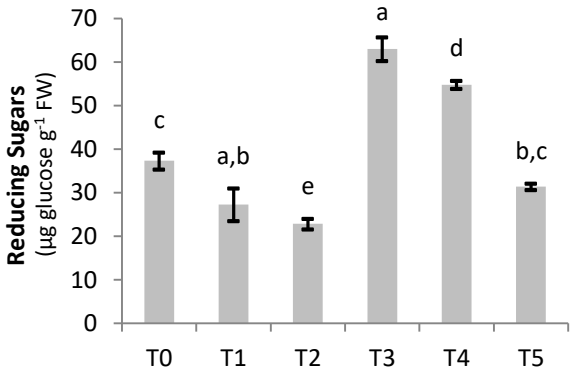

Fig IV B

Fig IV. Means of Malondialdehyde (A) (nmol MDA g $^{-1}$ FW) and reducing sugars (B) (µg glucose g $^{-1}$ FW^{-1}) in response to magnetic field and rhizobial inoculation (mean ± SE) grouped by Duncan test (p ≤0.05); different letters indicate T0: Control, T1: 0.75 mT, T2: 0.75 mT + Inoculant, T3: 1.5 mT, T4: 1.5 mT + Inoculant, T5: Inoculant means.

Results showed that the application of rhizobial inoculant combined with 1.5 mT magnetic field (T4), reduces the damage caused by magnetic field in the plant.

rhizobial inoculant individually (T5), as a Plant Growth Promoting Rhizobacteria (PGPR) increases growth parameters, protein content, catalase, peroxidase, ascorbate peroxidase and superoxide dismutase activity which is in agreement with the result of Stefan et al. (2013) in runner bean. But in this study, reducing sugars and malondialdehyde content decreased as a result of rhizobial inoculant treatment (T5).

Discussion

The highest level of growth parameters were observed in T2 treatment (Figs. I A-D), which may be due to the transfer of energy from magnetic field to the plant. This energy by creating physiological or biochemical changes, leads to improved growth parameters.

Improvement of growth parameters may be due to the rhizobial inoculant function for greater plant access to nutritional elements.

In this study, antioxidant enzymes changes occurred which are also observed in onion plant (Alikamanoğlu et al., 2007) and tobacco cells (Sahebjamei et al., 2007). Antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, and peroxidase are a defense system to control ROS amount induced by environmental factors. ROS metabolism is very often activated after plant exposure to EMF (Vian et al., 2016). As excess ROS is harmful to the plant, to restore the cellular redox balance, both enzymatic and nonenzymatic systems are activated to detoxify the toxic levels of ROS (Caverzan et al., 2016). Therefore, to improve tolerance against environmental factor and detoxify oxidative damage, the plant has increased antioxidant enzyme activity. Higher antioxidant enzyme activity and lower peroxidation (lower malondialdehyde) signify the importance of plant adaptive response to new condition. It seems that rhizobium leads to membrane protection and consequently reduces the electrolyte leakage and lipid peroxidation by making changes in roots (Han and Lee, 2005).

Application of 1.5 mT magnetic field (T3) may be considered as a stress factor which may damage the plant. It could also induce oxidative stress in plants by generation of excessive reactive oxygen species that can increase the level of lipid peroxidation, reducing sugars, and damaging protein content.

The antioxidant defense system is responsible for scavenging excessive ROS which is produced during stress. Superoxide dismutase is the first antioxidant enzyme that acts against ROS to change them from superoxide anions to hydrogen peroxide and oxygen. Increased superoxide dismutase activity may be due to the higher production of superoxide o^{2-} from oxidative stress caused by the magnetic field.

Catalase and peroxidase are notable antioxidant defense enzymes involved in the detoxification of H_2O_2 by converting free radicals to water (H_2O) and oxygen (O_2) (Priyanka et al., 2016). Uncoordinated antioxidant enzymes activity by the 1.5 mT magnetic field reduce resistance to oxidative stress and reduce the life span of plant cells.

It seems that rhizobial inoculant by increasing the antioxidant enzymes activity reduces oxidative stress damage (Saravanakumar, 2010) caused by 1.5 mT magnetic field. These growth Promoting Rhizobacteria (PGPR) by releasing organic acids such as carboxylic acid, pH reduction, and hormone secretion lead to physico-chemical changes in rhizosphere and improve the plant defense system (Yang, 2009).

Conclusions

Findings indicated that the application of magnetic field and rhizobial inoculant on alfalfa seed had a significant effect on growth parameters, protein content, antioxidant enzymes activity, lipid peroxidation, and reducing sugars. While 1.5mT magnetic field had strong effects on the plant as a stress factor, rhizobial inoculant application could reduce the damage caused by 1.5m T magnetic field. The present experimental results suggest that 0.75 mT magnetic field and rhizobial inoculant treatment (individually or in combination) improve the plant quality (through nitrogen fixation) and growth parameters and could be considered as a promising technique for partial agricultural improvements

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