Pak. J. Bot., 51(3): 1137-1141, 2019. DOI: 10.30848/PJB2019-3(39)

THE EFFECTS OF MAGNETIC FIELD AND RHIZOBIAL INOCULATION ON NITROGEN FIXATION PROCESS AND GROWTH PARAMETERS IN ALFALFA (*MEDICAGO SATIVA* L.)

NEDA KAZEMI KHALEDI[1], SARA SAADATMAND[1*], RAMAZAN ALI KHAVARI-NEJAD[1] AND TAHER NEJADSATTARI[1]

[1]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[*]*Correspondence author's email: sara.Saadatmand7@gmail.com*

Abstract

The present study aimed to investigate the effects of magnetic field and rhizobial inoculant on the Alfalfa's growth parameters (leaves, nodules number, shoot and root lengths) and nitrogen fixation process. Inoculated and non-inoculated seed with *Sinorhizobium meliloti* were subjected to 0.75mT and 1.5mT intensities of magnetic field. Plants were analyzed after 60 days growing. Findings showed 1.5mT magnetic intensity reduced growth parameters, nitrate reductase (NR), nitrite reductase (NIR) and ammonium content, while nitrate and nitrite content increased in comparison with the control group. Application of rhizobial inoculant with 1.5mT intensity increased growth parameters, NR, NIR activity, nitrite, and ammonium content, while it reduced nitrate accumulation in comparison with the single application which was received just 1.5mT intensity. A separate application of 0.75mT intensity increased growth parameters, but had no effect on NR, NIR activity, ammonium, nitrate and nitrite contents, while using rhizobial inoculant individually and combined with 0.75mT intensity increased growth parameters, NR, NIR activity, and ammonium content. Also, it decreased nitrate and nitrite contents as compared to the control. As a result, it could be concluded that rhizobial inoculant application, alone or in combination with a suitable magnetic intensity (0.75mT), could be used as a biofertilizer for vegetable production in sustainable and ecological agricultural systems.

Key words: Magnetic field, Inoculant, Alfalfa, Nitrate, Nitrite, Ammonium.

Introduction

Alfalfa (*Medicago sativa* L.) is one of the most important forage crops with high protein and highly digestible fiber contents (Li *et al.*, 2010). Because of its ability to fix atmospheric nitrogen, Alfalfa is considered to protect cropping system productivity. The specific bacteria on its nodulated roots are able to convert the atmospheric N2 to NH3-a form that can be used by plants. This process is largely limited to legumes, such as alfalfa, which has ability to form a symbiotic relationship with the soil bacteria. Collectively, it is termed as rhizobia.

Rhizobia may be introduced to legumes by seed or soil inoculation (Deaker *et al.,* 2004), which is called rhizobial inoculation. Rhizobial inoculation has been widely used in controlled conditions as a substitute for chemical fertilizers to increase plants growth and productivity (Fall *et al.,* 2016). It is a significant technology for rhizobia manipulation to improve crop productivity and soil fertility (Lamptey *et al.,* 2014). Optimal performance of rhizobium inoculation is dependent on many environmental factors such as magnetic field. Environmental factors play an important role on plant growth and development (Rachmawati *et al.,* 2014; Bielach *et al.,* 2017).

The present magnetic field has been increased in the environment because of the development of wireless technology, including cell phones, Wi-Fi, and other kinds of devices. The interaction between magnetic field and rhizobial inoculation may induce significant changes in alfalfa performance. Therefore, the present study tried to clear the influence of magnetic field and rhizobial inoculation on alfalfa (*Medicago sativa* L.) and determine the effect of interactions between them on nitrate, nitrite, ammonium content, NR and NIR activity in alfalfa. It also aimed to determine how alfalfa seedlings respond to these factors. Findings could be used in plant physiology and agriculture researches to improve crops quality.

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 separately exposed to 0.75mT and 1.5mT magnetic fields for four days, 30 minutes per day. Four days after treatments, seeds treated with magnetic field were mixed with the rhizobial inoculant and a decent amount of untreated seeds with magnetic field was mixed with the rhizobial inoculant. Commercial rhizobial inoculant used in this study was 'Biomedica' and contained *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,
T5: treated with rhizobial inoculant.

Then, each treatment was sown separately in pots under randomized and natural condition during the spring season. 60 days after sowing, samples were collected from each treatment to determine growth parameters, nitrate reductase (NR), nitrite reductase (NIR), nitrate, nitrite and ammonium content in alfalfa leaves (*Medicago sativa* L.) (Fig. 1).

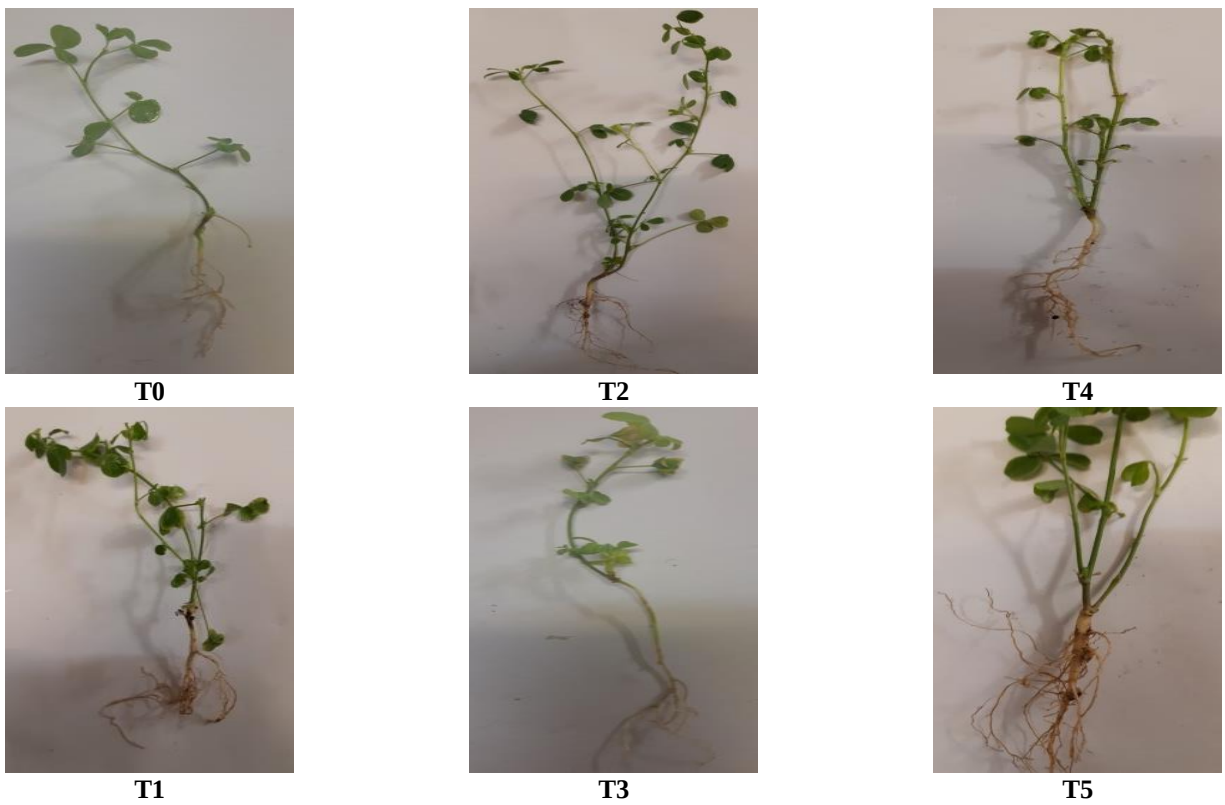

Fig. 1. All treatments (T0-T5), 60 days after sowing

Note that in this study, there was no nitrogen application in mineral form. In fact, the nutrient was provided through biological nitrogen fixation.

Create 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. To maintain constant temperature inside the cylinder, a base with a height of 50 mm was applied to allow air ventilation. During the experiment, the temperature inside the coil was measured. Results showed that the temperature was constant. The coil was connected to a DC power supply.

Growth parameters: sixty-day-old plants were used for measurement of growth parameters (leaves and nodules number, shoot and root lengths).

Nitrate reductase (NR) determination: The assay of NR activity was done by Hageman & Hucklesby method (1971) with slight modifications. Leaf discs (2 mm slices) were incubated in a medium containing 0.05M of potassium phosphate buffer (pH 7.8) and 0.4M KNO_3 solution. Samples were incubated in water bath and boiled to stop the enzyme activity, and also to complete the leaching of the nitrite in the medium (Ahmad *et al.,* 2010). Nitrite concentration was estimated by Evans & Nason method (1953). The absorbance was measured at 540 nm and compared with that of $NaNO_2$ standard.

Nitrite reductase (NIR) determination: Nitrite reductase activity assays were carried out according to Losada and Paneque (1971). The extraction was added to the medium containing 50 mM of phosphate buffer (pH 7.5) with 2.3mM methyl viologen and 100mM $NaHCO_3$ with 86.15 mM sodium dithionate. After incubation and boiling, the absorbance was measured at 540 nm and compared with that of $NaNO_2$ standard.

Ammonium determination: Ammonium was determined by Baethgen & Alley method (1989). One mL of sample was added to 5.5 mL of buffer solution (0.1M Na_2HPO_4, 5% Na-K tartrate, 5.4% NaOH). After mixing, 4mL of salicylate /nitroprusside solution (5%-0.03%) and 2mL of hypochlorite solution was added and mixed well. Then, after 45 minutes, the absorbance was read at 650nm. Different concentrations of ammonium sulfate $(NH_4)_2SO_4$ were used for standard curve.

Nitrate determination: The nitrate contents were determined using rapid colorimetric method given by Cataldo *et al*., (1975). 100mg of dry powder was suspended in 10ml of deionized water. After incubating and centrifuging, the supernatant was taken for analysis in a medium with salicylic acid 5% (w/v) in concentrated H_2SO_4 (pH above 12). The absorbance was measured at 410 nm by spectrophotometer, and different concentrations of KNO_3 were used for standard curve.

Nitrite determination: Griess reagents were prepared for nitrite analysis as described in Grasshoff *et al*., (1983). Sulphanilamide reagent and N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) reagent were mixed in equal proportions and used as Griess-reagent.

One mL of sample was added to 50µL of Griess-reagent and mixed. After 20 minutes, the absorbance was measured at 540 nm. Different concentrations of $NaNO_2$ were used for standard curve (García-Robledo *et al.,* 2014).

Result and Discussion

The highest growth parameters were observed in T2 treatment (Figs. 2-5), which may be due to changes induced by magnetic field at Ca^{+2} level (Florez *et al.*, 2007).

Magnetic field may increase metabolism and improve the growth by inducing energy between atoms.

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, which may be due to increased reserves of nutrients such as nitrogen and phosphorus in the plant (Zaidi, 2003).

T2 and T5 treatments by increasing nitrate and nitrite reductase activity (Figs. 6-7), reducing the accumulation of nitrate and nitrite (Figs. 8-9), lead an increase ammonium content (Fig. 10, T5) of the plant for many metabolic processes.

Therefore, rhizobial inoculation with the effect on nitrogen fixation through the formation of root's nodules (Fig. 5) led to positive effects on growth parameters in alfalfa.

As shown in figures 6-10, the most statistically significant and the highest level of NR, NIR activity and ammonium as well as the lowest level of nitrate and nitrite contents were obtained for T2 treatment. It could be concluded that the interaction between 0.75mT intensity and rhizobial inoculant was the maximum increase in activity of NR, NIR activity and ammonium content.

Therefore, rhizobial inoculant is a positive factor promotes plant growth directly (Gopalakrishnan *et al.*, 2015) through developing the process of nitrogen fixation.

Rhizobia inoculation enhanced nitrogen fixation (Koskey *et al.*, 2017), which improved shoot nitrogen nutrition (Thuita *et al.*, 2011; Ulzen *et al.*, 2016).

Several studies reported a positive effect of inoculation leading to a significant improvement in grain yield. (Ouslim *et al.*, 2015).

The results showed that a separate application of 0.75mT intensity (T1) increased shoot, root lengths and leaves number compared to control (Figs. 2-4) but had no significant effect on NR, NIR activity, nitrate, nitrite and ammonium content (Figs. 6-10), which might be due to the absence of root's nodule.

As shown in Figures 2-10, the lowest level of shoot and root lengths, number of leaves, NR, NIR activity, ammonium content and the highest level of nitrate content were obtained by 1.5mT intensity (T3) which affected as a stress factor, leading to an increase in the level of ROS (Wang *et al.*, 2008; Saha *et al.*, 2017).

The application of rhizobial inoculant with 1.5mT intensity (T3), increased growth parameters (Figs. 2-5), NR, NIR activity, ammonium, nitrite content and reduced nitrate accumulation compared to separate application of 1.5mT intensity (Figs. 6-10). Rhizobial inoculant, as a positive factor interacting with unfavorable environmental conditions, decreased the damage caused by 1.5mT intensity. It can be concluded that rhizobial inoculant plays a protective role against ROS under stress condition (Durán *et al.*, 2016) and improves plant quality (Medina & Azcón, 2010) although the coordination between the antioxidants is complex, as reported by Mittler *et al.*, (2004).

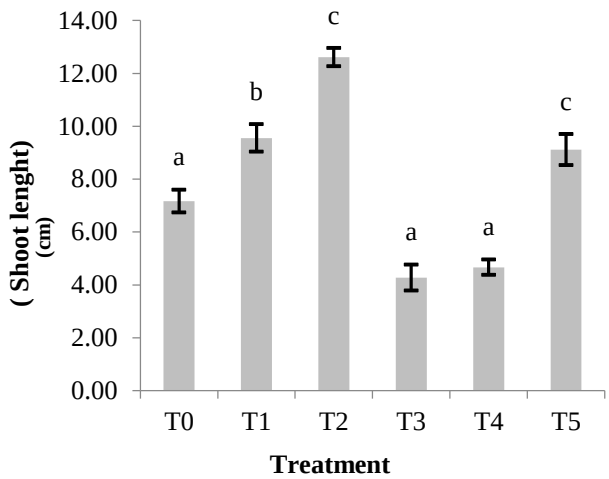

Fig. 2. Mean of shoot length (cm) in response to magnetic field and rhizobial inoculant (Individual and together) (mean ± SE) (grouped by Duncan test ($p \leq 0.05$). Different letters indicate significant differences between the means.

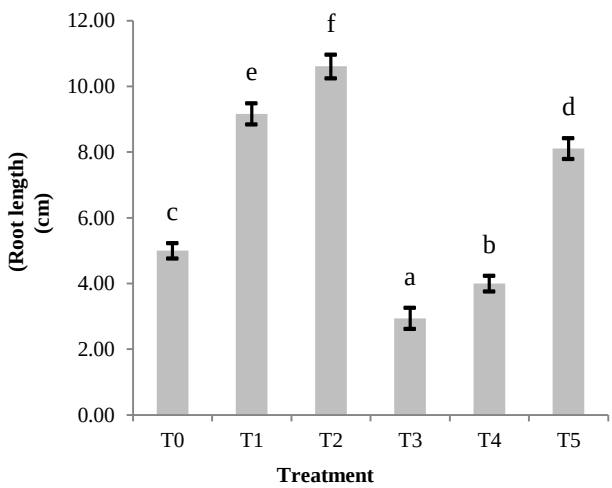

Fig. 3. Mean of root length (cm) in response to magnetic field and rhizobial inoculant (Individual and together) (mean ± SE) (grouped by Duncan test ($p \leq 0.05$).

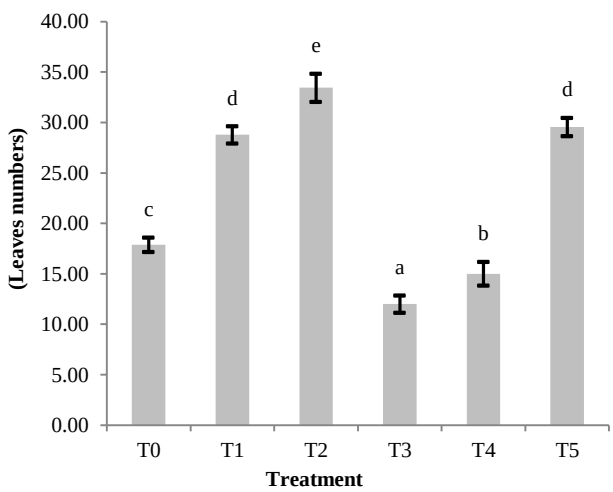

Fig. 4. Mean of leaves number in response to magnetic field and rhizobial inoculant (Individual and together) (mean ± SE) (grouped by Duncan test ($p \leq 0.05$).

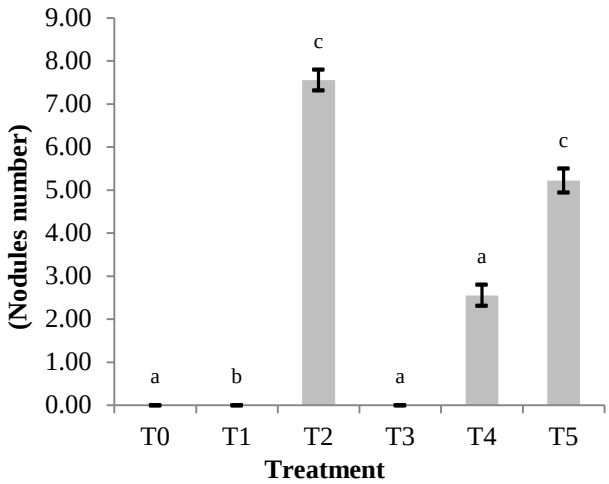

Fig. 5. Mean of nodules number in response to magnetic field and rhizobial inoculant (Individual and together) (mean ± SE) (grouped by Duncan test (p≤0.05).

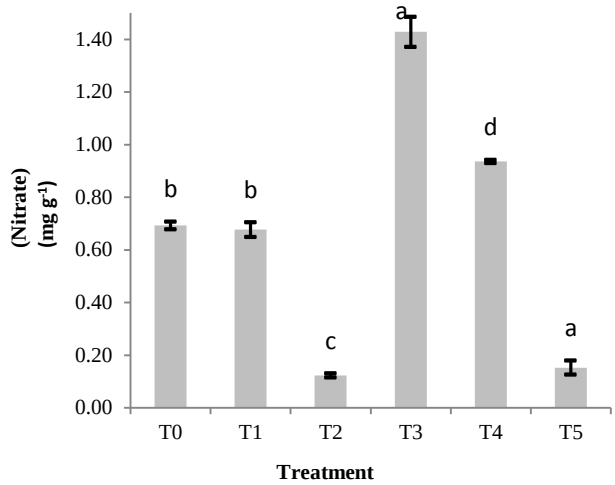

Fig. 8. Mean of nitrate (mg g^{-1}) in response to magnetic field and rhizobial inoculant (Individual and together) (mean ± SE) (grouped by Duncan test (p≤0.05).

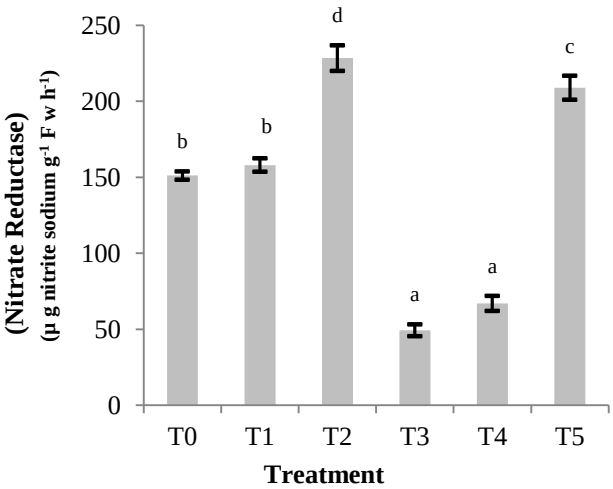

Fig. 6. Mean of nitrate reductase (NR) (µg nitrite sodium g^{-1}Fw h^{-1}) in response to magnetic field and rhizobial inoculant (Individual and together) (mean ± SE) (grouped by Duncan test (p≤0.05).

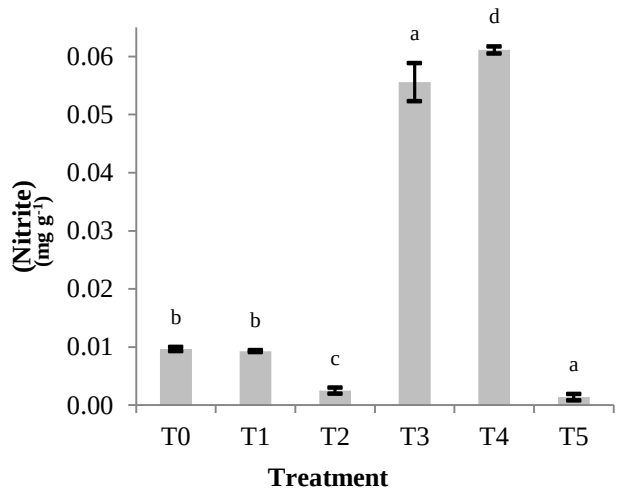

Fig. 9. Mean of nitrite (mg g^{-1}) in response to magnetic field and rhizobial inoculant (Individual and together) (mean ± SE) (grouped by Duncan test (p≤0.05).

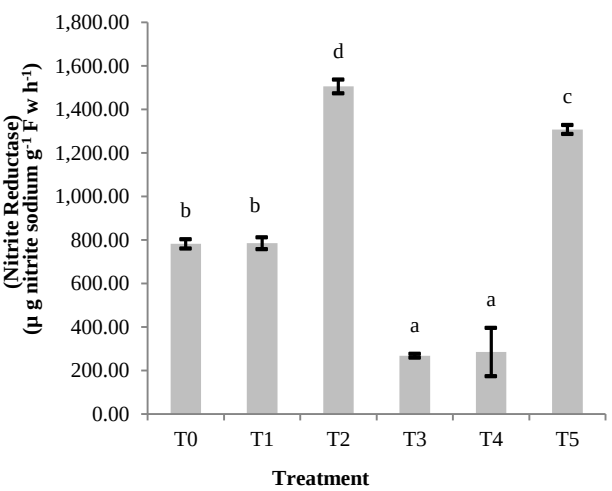

Fig.7. Mean of nitrite reductase (NIR) (µg nitrite sodium g^{-1}Fw h^{-1}) in response to magnetic field and rhizobial inoculant (Individual and together) (mean ± SE) (grouped by Duncan test (p≤0.05).

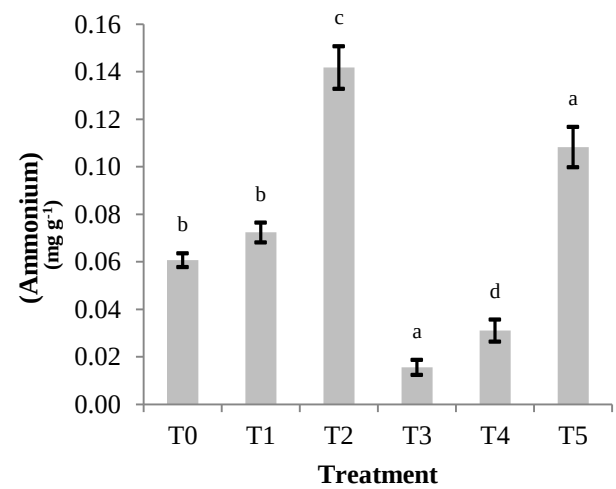

Fig. 10. Mean of ammonium (mg g^{-1}) in response to magnetic field and rhizobial inoculant (Individual and together) (mean ± SE) (grouped by Duncan test (p≤0.05).

Conclusions

It is concluded that the application of magnetic field and rhizobial inoculant on alfalfa seed have a significant effect on growth parameters, nitrate reductase (NR), nitrite reductase (NIR), ammonium, nitrate and nitrite contents in the plant.

1.5mT magnetic field has strong effects on the plant as a stress factor, but rhizobial inoculant application could reduce the damage caused by 1.5mT magnetic field. The present experimental results suggest that 0.75mT magnetic field and rhizobial inoculant treatment (individual or together) improve the plant quality (through nitrogen fixation), growth parameters and could be considered as a promising technique for partial agricultural improvements.

The present findings might be helpful in increasing the general knowledge about the mechanisms of alfalfa response to magnetic field and rhizobial inoculant treatments, but these phenomena are still unclear and require more research.

Reference

Ahmad, S., I.S. Fazili, R. Haque, S.N. Khan and M. Zainul Abdin. 2010. Standardization and estimation of nitrate reductase activity in the leaves of *Ammi Majus* L. (Bishops weed) in relation to sulphur deficiency and seed yield. *Aust. J. Crop Sci*., 4(7): 515-5.

Baethgen, W.E. and M.M. Alley. 1989. A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant kjeldahl digests. *Comm. Soil. Sci. Plant. Anal*., 20: 961-969.

Bielach, A., M. Hrtyan and V. B. Tognetti. 2017. Plants under stress: involvement of auxin and cytokinin. *Int. J. Mol. Sci*., 18: 1427.

Cataldo, D.A., M. Haroon, L.E. Schrader and V.L. Youngs. 1975. Rapid Colorimetric Determination of Nitrate in Plant Tissue by Nitration of Salicylic Acid. *Comm. Soil. Sci. Plant. Anal*., 6: 71-80.

Deaker, R., R.J. Roughley and I.R. Kennedy. 2004. Legume Seed Inoculation Technology – a review. *Soil Biol Biochem*., 36: 1275-1288.

Durán, P., J.J. Acuña, E. Armada, O.M. López-Castillo, P. Cornejo, M.L. Mora and R. Azcón. 2016. Inoculation with selenobacteria and arbuscular mycorrhizal fungi to enhance selenium content in lettuce plants and improve tolerance against drought stress. *J. Soil Sci. Plant Nutr.*, 16(1): 201-225.

Evans, H.J. and A. Nasan. 1953. Pyridine nucleotide nitrate reductase from extracts of higher plants. *Plant Physiol*., 28: 233-254.

Fall, D., N. Bakhoum, S.N. Sall, A.M. Zoubeirou, S.N. Sylla and D. Diouf. 2016. Rhizobial inoculation increases soil microbial functioning and gum arabic production of 13-year-old *Senegalia senegal* (L.) britton, trees in the north part of senegal. *Plant Sci*., 7: 1355.

Florez, M., M.V. Carbonell and E. Martinez. 2007. Exposure of maize seeds to stationary magneticfield: effects of germination and early growth. *Environ. Exp. Bot*., 59: 68-75.

García-Robledo, E., A. Corzo1 and S. Papaspyrou. 2014. A Fast and direct spectrophotometric method for the sequential determination of nitrate and nitrite at low concentrations in small volumes. *Mar. Chem.*, 162: 30-36.

Gopalakrishnan, S., A. Sathya, R. Vijayabharathi, R.K. Varshney, C.L.L. Gowda and L. Krishnamurthy. 2015. Plant growth promoting rhizobia: challenges and opportunities. *Biotech*., 5: 355-377.

Grasshoff, K., M. Ehrhardt and K. Kremling. 1983. Methods of Sea Water Analysis. Verlag Chemie, New York.

Hageman, R.H. and D.P. Hucklesby. 1971. Nitrate reductase from higher plants. In: Methods in Enzymology, Ed. San Pietro. Academic Press, London New York. 23: 491-503.

Koskey, G., S.W. Mburu, E.M. Njeru, J.M. Kimiti, O. Ombori and J.M. Maingi. 2017. Potential of native rhizobia in enhancing nitrogen fixation and yields of climbing beans (*Phaseolus vulgaris* L.) in contrasting environments of Eastern Kenya. *Front Plant Sci.*, 8: 443.

Lamptey, S., B.D.K. Ahiabor, S. Yeboah and C. Asamoah. 2014. Response of soybean (*Glycine max* L.) to rhizobial inoculation and phosphorus application. *J. Exp. Biol. & Agri. Sci.*, 2(1): 72-77.

Li, R., F. Shi, K. Fukuda and Y. Yang. 2010. Effects of salt and alkali stresses on germination, growth, photosynthesis and ion accumulation in alfalfa (*Medicago sativa* L.). *J. Soil Sci. Plant Nutr*., 56: 725-733.

Losada, M. and A. Paneque. 1971. Nitrite reductase. *Methods Enzymol*., 23: 487-491.

Medina, A. and R. Azcón. 2010. Effectiveness of the application of arbuscular mycorrhiza fungi and organic amendments to improve soil quality and plant performance under stress conditions. *J. Soil Sci. Plant Nutr*., 10: 354-372.

Mittler, R., S. Vanderauwera, M. Gollery and F. Van Breusegem. 2004. Reactive oxygen gene network of plants. *Trends Plant Sci*., 9: 490-498.

Ouslim, S., M. Lazali, C. Merabet, S. Brahimi, F. Boukhatem and A. Bekki. 2015. Effects of nitrogen fertilization, inoculation with rhizobium sp. on the production of biomass, nitrogen content and yield of bean in oran, algeria. *Int. J. Agri. Crop. Sci*., 8(5): 732-737.

Rachmawati, D., Sudjino and I.J. Asiyah. 2017. Response and stevioside levels of stevia (Stevia rebaudiana Bertoni) grown at different light intensity and water availability. *Pak. J. Bot.*, 49(5): 1689-1695.

Saha, S.K., S.B. Lee, J. Won, H.Y. Choi, K. Kim, G.M. Yang, A.A. Dayem and S. Cho. 2017. Correlation between oxidative stress, nutrition, and cancer initiation. *Int. J. Mol. Sci*., 18: 1544.

Thuita, M., P. Pypers, L. Herrmann, R.J. Okalebo, C. Othieno, E. Muema and D. Lesueur. 2011. Commercial rhizobial inoculants significantly enhance growth and nitrogen fixation of a promiscuous soybean variety in Kenyan soils. *Biol. Fert. Soils*, 48: 87-96.

Ulzen, J., R.C. Abaidoo, N.E. Mensah, C. Masso and A.H.A. Gadir. 2016. Bradyrhizobium inoculants enhance grain yields of soybean and cowpea in northern Ghana. *Front. Plant Sci.*, 7: 1770.

Wang, H.Y, X.B. Zeng, S.Y. Guo and Z.T. Li. 2008. Effects of magnetic field on the antioxidant defense system of recirculation-cultured Chlorella vulgaris. *Bioelectromagnetics*, 29: 39-46.

Zaidi, A., M.S. Khan and M. Amli. 2003. Interactive effect of rhizotrophic microorganism on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). *Eur. J. Agron.*, 19(1): 15-21.

(Received for publication 19 January 2018)