# MAGNETIZED PHOSPHORUS SOLUTION AND MYCORRHIZATION WITH Diversispora versiformis AFFECT P USE EFFICIENCY, GROWTH AND PHOTOSYNTHETIC PARAMETERS IN SWEET BASIL (OCIMUM BASILICUM)

Edris SHABANI<sup>1</sup>\*, Sahebali BOLANDNAZAR<sup>2</sup>, Seyed Jalal TABATABAEI<sup>3</sup> <sup>1</sup>Shahid Chamran University of Ahvaz, Ahvaz, Iran <sup>2</sup>Faculty of Agriculture, University of Tabriz, P.B. 51664, Tabriz, Iran <sup>3</sup>Faculty of Agriculture, Shahed University, Tehran Qome High Way, Tehran, Iran

Received: September 2018; Accepted: October 2019

## ABSTRACT

In order to consider phosphorus (P) limitations in agriculture, research has been carried out on the methods that can improve plant growth and increase the efficiency of P use. A pot experiment was conducted to find the effects of magnetized  $Ca(H_2PO_4)_2 \cdot H_2O$  solutions as P source at concentrations 0, 5, 10, 20 and 40 mg·dm<sup>-3</sup> and inoculation with arbuscular mycorrhizal fungi *Diversispora versiformis* on P use efficiency, growth and photosynthetic pigments in sweet basil. P solutions were treated with magnetic field of 110 mT at 3 dm<sup>3</sup>·min<sup>-1</sup> volumetric flow rate. The results indicated that the growth of basil plant, the number of leaf, leaf area, harvest index and chlorophyll a and b contents significantly increased in the result of fertilization with magnetized P solutions and mycorrhizal inoculation as compared to the control. The application of magnetized P solution at 10 mg P·dm<sup>-3</sup> and inoculation of mycorrhizal fungi increased P use efficiency by 18.9% and 23.5%, respectively. Findings of the experiment clearly showed that the use of magnetization of P fertilizer and mycorrhization potentially represent natural ways of promoting growth, P status and chlorophyll content in sweet basil.

Keywords: Arbuscular mycorrhizal fungi, chlorophyll, magnetic field, Ocimum basilicum, phosphorus

## INTRODUCTION

Phosphorus (P) has a dominant role in plant reproduction, affecting cellular metabolism and several biochemical and structural functions (Jokubauskaitė et al. 2015). The main source of P is rock phosphate, and agriculture is by far the main user of mined P globally, accounting for 80– 90% of the total world demand (Childers et al. 2011). A conservative analysis using industry data suggests that the peak in global P production could occur by 2033 (Cordell et al. 2009). Recent scientific debates warn of future supplies of phosphate rock becoming scarcer and more expensive to mine within the next 30–50 years. The amount of P concentration in soil solution ranges from 0.001 to 1 mg·dm<sup>-3</sup> and the mobility of plant-available P in soil (3 mm per year) is very low (Shabani et al. 2018). On the other hand, even with optimum management, the efficiency of plant uptake of P is very low, usually less than 20% (MacDonald et al. 2011). All of this means that P can be a major limiting factor for plant growth. As a result, a holistic understanding of P-use efficiency is necessary for optimizing P management, aiming at reducing consumption of chemical P fertilizer, maximizing exploitation of the biological potential of root/rhizosphere processes for efficient mobilization, and acquisition of soil P by plants (Shen et al. 2011). Phosphorus use efficiency decides on the ability of the plant to grow and yield well at suboptimal P-availability situations. P-use efficiency can be evaluated by the amount of dry matter produced for a given P concentration in the plant dry tissue (Akhtar et al. 2009).

In the last few years, there has been a growing interest in the use of physical methods in plant growing stimulation (Soltani et al. 2006; Aladjadjiyan 2007; Hajnorouzi et al. 2011; Ghanati et al. 2015). Vegetable production with using physical methods, especially magnetic fields, is being considered as a means of increasing crop quantity and quality (Shabani et al. 2017). A few articles suggested that magnetic field increases the uptake of P (Hilal et al. 2002; Maheshwari & Grewal 2009). Our finding demonstrated that exposure of P solution to 110 mT of magnetic field can increase water-soluble P in the soil and P concentration in sweet basil shoot by 30% and 13%, respectively, in comparison to the control (Shabani et al. 2018). Previous studies showed that magnetic field induces development of photosynthesis pigments in maize (Ghanati et al. 2015), water holding capacity of soil (Al-Khazan et al. 2011), mobility and uptake of nutrient elements in the root zone (Hilal et al. 2002), soil electrical conductivity (Maheshwari & Grewal 2009) and bean plant growth (Podleśny et al. 2004). Water is the most abundant compound in plant cells and has a crucial role in plant metabolism. It has been found that magnetic field can have an influence on microscopic structures and macroscopic properties of water. It enhanced the clustering structure of hydrogenbonded chains and polarization features of water to be more stable (Pang & Deng 2008), as well as changed water properties to become more active, soft, energized and able to flow (Zhang et al. 2014).

In recent years, great effort has been devoted to the study on the effects of arbuscular mycorrhizal colonization on macro- and micronutrient uptake and translocation, in particular, P (Zarei et al. 2006), water uptake, plant growth and yield (Miransari 2010; Colla et al. 2008). In general, the literature review reveals that very few publications can be found about the main effect of magnetized P solution and arbuscular mycorrhizal fungi on sweet basil growth. The aim of this study was to investigate whether passing of P solution through magnetic field and inoculation of sweet basil roots by arbuscular mycorrhizal fungi can improve P use efficiency, growth and photosynthetic pigments of sweet basil.

# MATERIAL AND METHODS

# Plant material, growth conditions and inoculation with mycorrhizal fungi

A pot experiment was performed during the summer season 2016 in the greenhouse. Seedlings of sweet basil (Ocimum basilicum L.) 'Mobarakeh', were cultivated in pots, in a sandy-loam soil with values in percentages of: field capacity saturation, organic carbon, calcium carbonate equivalent and total N were 10, 23.5, 0.1, 0.0 and 0.08%, respectively. The concentrations of available P, K, Ca, Mg, Fe, Mn, Zn and Cu in soil were 4.4, 82.6, 49.0, 99.0, 1.8, 1.1, 0.9, and 1.3 mg  $kg^{-1}$ , respectively. The soil extract (1 : 1) pH and electrical conductivity (EC) were 7.25 and  $0.6 \text{ dS} \cdot \text{m}^{-1}$ , respectively. Plants were grown under controlled conditions at 27 °C for 14 h at day time and 18 °C for 10 h in the night, with 4000 ft-c light intensity. Diversispora versiformis [(P. Karst.) Oehl, G.A. Silva & Sieverd.] inoculum, which was used in this experiment, was prepared using a pot culture of sorghum [Sorghum bicolor (L.) Moench.] as host plant. The autoclaved sandy loam soil was inoculated with pure inoculum of D. versiformis. The plants were grown in a greenhouse for four months. After this time, the aerial parts of plants were removed and mixture of pot content, including the spores, hyphae, and finely segmented colonized roots, was maintained at 4 °C until used for inoculation of basil. Plastic pots (20 cm inner diameter, 30 cm depth) were filled with an autoclaved soil (2.5 kg). For each pot, 40 grams of arbuscular mycorrhizal fungi inoculants (with 120 active spores,  $10^4 \,\mathrm{CFU} \cdot \mathrm{g}^{-1}$ ) were distributed over the soil at a depth of 10-15 cm and covered with soil. Eight seeds were planted at 2 cm depth per pot. At the two-leaf stage, the seedlings were thinned to four plants per pot and the soil surfaces were covered with perlite and cork to reduce evaporation.

# Fertilization of plants with magnetized phosphorus solution

Treatments were arranged in a completely randomized design, defined by a factorial combination of five concentrations of P-containing compound in the form of  $Ca(H_2PO_4)_2$ ,  $H_2O(0, 5, 10, 20, and$  $40 \text{ mg} \cdot \text{dm}^{-3}$ ), which means without, low, moderate, high, and very high P level, along with two physical treatments - magnetization or not and two mycorrhizal treatments - with and without arbuscular mycorrhizal fungi inoculation. Each treatment consisted of three pots with four plants for a total of 12 plants. All the pots received 250 mL of plant nutrient solution (g·dm<sup>-3</sup>): potassium sulfate (K<sub>2</sub>SO<sub>4</sub>) 2.22, calcium nitrate [Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O] 12.64, ammonium sulfate [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>] 2.35, magnesium sulfate (MgSO<sub>4</sub>·7H<sub>2</sub>O) 10.13, manganese  $(MnSO_4 \cdot 4H_2O) = 0.4,$ sulfate iron sulfate  $(FeSO_4 \cdot 7H_2O) 0.74$ , copper sulfate  $(CuSO_4 \cdot 7H_2O)$ 0.19, zinc sulfate (ZnSO<sub>4</sub>·7H<sub>2</sub>O) 0.43, boric acid (H<sub>3</sub>BO<sub>3</sub>) 0.05 and sodium molybdate (Na<sub>2</sub>MoO<sub>4</sub>) 0.0001 three times during the first two weeks. The quality of irrigation water was typical of the region. The concentrations of ions expressed as mg dm<sup>-3</sup> were as follows: P (0.05), K (4.3), Ca (42), Mg (11), Fe (0.1), Zn (0.6), and Na (3.5). The values of pH and electrical conductivity (EC) were 7.7 and 0.49  $dS \cdot m^{-1}$ , respectively. The pots were weighed daily by digital weigh balance and the evapotranspiration loss was restored ensuring a stable water content throughout the experiment. In addition, two pots without plants were prepared to monitor water loss by evaporation in the different treatments. Five different concentrations of Ca(H<sub>3</sub>PO<sub>4</sub>)<sub>2</sub> were made in 50-dm<sup>3</sup> tanks at the following concentrations: 0, 5, 10, 20, and 40 mg dm<sup>-3</sup>. P-containing solutions were passed through magnetic field of 110 mT (at 3 dm<sup>3</sup>·min<sup>-1</sup> volumetric flow rate) that was produced by a locally designed apparatus. The main part of the magnetic field device was accelerator sector, which was composed of a paramagnetic tube, around which several strong permanent magnets were arranged alternatively. When water flows along the pipe axis through the device, magnetic field exerts a force perpendicular to the direction of motion of the ions present in the water. Magnetic

force and water velocity deduce the ions to move in a helical motion along the axis (Shabani et al. 2018). Magnetized P solutions were used between weeks five and seven, at 2 days interval, for a total of seven times during the growing cycle.

#### **Growth measurement**

After 8 weeks, the basil plants were harvested and the number of leaves was counted. To determine the leaf area (LA), Li-Cor-Model: Li 1300, USA was used. Basil plants were oven dried at 70 °C for 48 hours to determine the dry weight of plants under different treatments. Harvest index (HI) was calculated from leaf dry weight ratio to total dry weight.

## Measurement of photosynthesis parameters

At fully expanded leaves, a SPAD value using SPAD-502, Minolta Camera Co., Osaka, Japan was used and photochemical efficiency (Fv/Fm) was recorded by chlorophyll fluorometer (Hansatech Instruments, Handy PEA Chlorophyll Fluorimeter, UK). The SPAD and Fv/Fm indexes were measured twice during the experiment on 10 and 2 leaves per plant, respectively. Determination of chlorophyll a and b content in methanolic extract was performed according to Carter and Knapp (2001) method using 6 leaf discs from fully expanded leaves from each plant (0.04 g). Extract absorption was read at 665.2, 652.4 and 470 nm wavelengths and calculated using the Lichtenthaler and Buschmann equation (2001).

## Measurement of total plant water use

The volumes of total water used by basil plants were recorded by weighing the pots and subtracting the soil water content from field capacity (FC). This calculation has been undertaken for a range of crops such as maize, leek, and winter wheat (Gregory 2006).

# Determination of phosphorus utilization efficiency

P use efficiency (PUE) was determined according to Siddiqi and Glass (1981) as below:

PUE  $(g^2 \cdot mg^{-1}) =$  Shoot dry mass (g per plant)/P concentration in dry mass  $(mg \cdot g^{-1})$ .

#### Statistical analysis

The experiment was arranged in a completely randomized factorial design using three factors (G – magnetization, M – mycorrhization and P concentrations) with four plants per each of the three pot (n = 12 plants) under greenhouse conditions. All data were statistically analyzed by analysis of variance (ANOVA) using the SAS 9.1 software (SAS, USA). All of the data are expressed as mean values  $\pm$  standard deviation (SD). Data analysis was performed using GLM procedure and average comparisons was performed with Duncan's multiple-range test at p = 0.05 on each of the significant variables measured.

## RESULTS

# Effects of phosphorus concentrations in the fertilization solution

In the present study, increasing P levels in the form of  $Ca(H_3PO_4)_2$  from 0 to 40 mg·dm<sup>-3</sup> improved the plant growth and photosynthesis parameters as well as total water use (TWU) (Table 1 & 2). Irrespective of magnetization and mycorrhization, PUE value doubled with P concentration increase in the solution (Table 1).

## **Effects of magnetization**

The magnetization of P-containing solutions increased significantly leaf number (LN), leaf area (LA), harvest index (HI),  $F_v/F_m$ , SPAD, chlorophyll a (Chl a), chlorophyll b (Chl b) and TWU in basil. The only exception was P use efficiency (PUE) (Table 1).

## Effects of mycorrhization

The inoculation with arbuscular mycorrhizal fungi exhibited positive effects on basil plant's growth in terms of LN and HI (Table 1). This factor also increased photosynthesis parameters – SPAD, Chl a, Chl b as well as TWU by 4.9%, 6.5%, 10.7%, and 3.8%, respectively compared to the non-inoculated treatments (Table 1).

Table 1. Mean ( $\pm$ SD) effects of magnetized P solution (G), arbuscular mycorrhizal fungi (M) and different concentrations of P (P) on leaf number (LN), leaf area (LA), harvest index (HI),  $F_v/F_m$ , SPAD, chlorophyll a (Chl a), chlorophyll b (Chl b), TWU and phosphorus use efficiency (PUE) in basil plants

Treat-	LN	LA (cm <sup>2</sup> )	HI	$F_{\rm v}/F_{\rm m}$	SPAD	Chl a	Chl b	TWU	PUE
ment	LN					$(mg \cdot g^{-1})$	$(mg \cdot g^{-1})$	(dm <sup>3</sup> )	$(g^2 \cdot mg^{-1})$
G									
$G_0$	44.20±13.32 <sup>a</sup>	244.57±130.51b	55.28±14.49 <sup>b</sup>	0.79±0.02 <sup>b</sup>	41.53±3.76 <sup>b</sup>	0.75±0.10 <sup>b</sup>	0.55±0.11 <sup>b</sup>	3.15±0.74 <sup>b</sup>	0.83±0.29
$G_1$	43.20±9.75 <sup>b</sup>	288.60±94.59 ª	62.45±9.62 ª	0.80±0.02 ª	46.73±4.18 ª	0.84±0.10 <sup>a</sup>	0.63±0.11 ª	3.47±0.60 <sup>a</sup>	0.84±0.17
Sig.	*	**	**	*	**	**	**	**	Ns
М									
$M_0$	40.80±11.58 <sup>b</sup>	262.31±141.49	57.96±15.04 <sup>b</sup>	$0.80\pm0.02$	43.07±5.34 <sup>b</sup>	0.77±0.11 <sup>b</sup>	0.56±0.12 <sup>b</sup>	$3.25{\pm}0.80^{b}$	0.87±0.29
$\mathbf{M}_1$	46.80±10.97ª	270.86±83.17	59.77±10.05 °	0.80±0.02	45.19±3.82 ª	0.82±0.10 <sup>a</sup>	0.62±0.10 ª	3.37±0.56ª	0.81±0.15
Sig.	**	Ns	*	Ns	**	**	**	*	Ns
Р									
$\mathbf{P}_0$	27.50±6.54 °	$136.08 \pm 70.34^{d}$	44.07±12.61 d	$0.78 \pm 0.02$ °	39.36±4.00 °	0.66±0.04 °	0.46±0.05 °	$2.47{\pm}0.35^{d}$	0.55±0.21°
$\mathbf{P}_1$	$36.50\pm2.73^{d}$	187.99±46.26 °	54.81±8.04 °	$0.79{\pm}0.01$ bc	42.74±2.79 <sup>d</sup>	$0.72{\pm}0.06$ <sup>d</sup>	$0.49{\pm}0.06$ <sup>d</sup>	2.80±0.36°	$0.84{\pm}0.10^{b}$
$\mathbf{P}_2$	45.25±3.53 °	286.14±60.39 <sup>b</sup>	59.32±6.00 °	0.80±0.02 <sup>ab</sup>	44.24±2.67 °	0.80±0.06 °	0.60±0.06 °	3.33±0.23 <sup>b</sup>	0.91±0.17 <sup>ab</sup>
$P_3$	52.50±4.44 <sup>b</sup>	324.75±65.74 <sup>b</sup>	65.47±8.17 <sup>b</sup>	0.81±0.009 <sup>a</sup>	45.77±3.68 <sup>b</sup>	$0.87{\pm}0.07$ <sup>b</sup>	$0.67{\pm}0.07$ <sup>b</sup>	3.92±0.44 <sup>a</sup>	0.93±0.22 <sup>ab</sup>
$\mathbf{P}_4$	57.25±3.35ª	397.96±88.86 <sup>a</sup>	70.66±9.13 <sup>a</sup>	0.80±0.02 <sup>ab</sup>	48.54±4.88 <sup>a</sup>	0.92±0.06 <sup>a</sup>	0.72±0.06 <sup>a</sup>	4.05±0.12 <sup>a</sup>	0.96±0.20ª
Sig.	**	**	**	**	**	**	**	**	**

\*, \*\* significant differences between means at 0.05 and 0.01 level of probability, respectively; Ns – non-significant. Within each column in G, M or P treatments, means followed by the same letters are not significantly different at 5%.

Treatment	LN	LA (cm <sup>2</sup> )	HI	SPAD	Chl a (mg·g <sup>-1</sup> )	Chl b (mg·g <sup>-1</sup> )	TWU (dm <sup>3</sup> )
G×M							
$G_0M_0$	40.00±13.93 <sup>d</sup>	227.62±165.42 <sup>b</sup>	51.76±18.12°	39.47±3.49°	$0.70{\pm}0.08^{d}$	$0.49{\pm}0.09^{d}$	2.99±0.87°
$G_0M_1$	48.40±11.54 <sup>a</sup>	261.54±83.64 <sup>ab</sup>	58.80±8.77 <sup>b</sup>	43.59±2.89 <sup>b</sup>	0.80±0.10°	0.60±0.10°	3.31±0.56 <sup>b</sup>
$G_1M_0$	41.60±8.92°	297.01±105.81ª	64.17±7.43 <sup>a</sup>	46.67±4.37 <sup>a</sup>	$0.84{\pm}0.10^{b}$	$0.62{\pm}0.12^{b}$	3.51±0.64 <sup>a</sup>
$G_1M_1$	45.20±10.42 <sup>b</sup>	280.20±83.79ª	60.74±11.34 <sup>ab</sup>	46.79±4.08ª	0.84±0.10 <sup>a</sup>	0.64±0.10 <sup>a</sup>	$3.44{\pm}0.53^{ab}$
Significance	**	*	**	**	**	**	**
G×P							
$G_0P_0$	25.00±8.71 <sup>h</sup>	99.97±48.41 <sup>i</sup>	37.41±13.65 <sup>e</sup>	$36.45 \pm 3.74^{\rm f}$	$0.64{\pm}0.04^{j}$	$0.44{\pm}0.07^{j}$	2.27±0.38
$G_0P_1$	$36.50{\pm}3.46^{\rm f}$	$158.71{\pm}50.80^{h}$	52.53±6.80 <sup>cd</sup>	41.33±2.19e	$0.67{\pm}0.04^{i}$	$0.46{\pm}0.04^{i}$	2.54±0.36
$G_0P_2$	$46.50 \pm 3.54^{d}$	$252.71 \pm 56.41^{ef}$	$56.01 \pm 6.49$ <sup>cd</sup>	$42.63 \pm 2.74^{de}$	0.75±0.05 <sup>g</sup>	$0.55{\pm}0.05^{\rm f}$	3.21±0.17
$G_0P_3$	54.50±4.95 <sup>b</sup>	294.22±43.26 <sup>de</sup>	$60.42{\pm}10.98^{ab}$	43.16±2.35 <sup>de</sup>	$0.82{\pm}0.06^{e}$	$0.62{\pm}0.07^{e}$	3.80±0.55
$G_0P_4$	58.50±3.81ª	417.28±125.00 <sup>a</sup>	$68.03{\pm}12.54^{ab}$	44.09±2.35 <sup>cd</sup>	0.88±0.06 °	$0.68{\pm}0.06^{\circ}$	3.94±0.56
$G_1P_0$	30.00±1.19g	172.19±72.64 <sup>gh</sup>	$50.74 \pm 7.29^{d}$	42.28±0.89 <sup>cd</sup>	$0.69{\pm}0.003^{h}$	$0.49{\pm}0.002^{\text{ h}}$	2.66±0.19
$G_1P_1$	$36.50{\pm}2.00^{\rm f}$	217.29±6.61 <sup>fg</sup>	57.09±8.96 <sup>cd</sup>	44.14±2.71 <sup>cd</sup>	$0.88{\pm}0.003~{\rm f}$	$0.53{\pm}0.05^{g}$	3.06±0.93
$G_1P_2$	44.00±3.25 <sup>e</sup>	319.58±45.58 <sup>cd</sup>	62.64±3.17 <sup>ab</sup>	45.86±1.36°	$0.85{\pm}0.004^{d}$	$0.65{\pm}0.005^{d}$	3.44±0.24
$G_1P_3$	50.50±2.92°	355.28±72.53 <sup>bc</sup>	68.52±1.17 <sup>ab</sup>	$48.39 \pm 2.80^{b}$	$0.93{\pm}0.002^{b}$	$0.73{\pm}0.001^{b}$	4.04±0.29
$G_1P_4$	56.00±2.44 <sup>b</sup>	378.66±21.06 <sup>ab</sup>	73.28±2.35ª	53.00±0.54ª	0.96±0.003ª	0.76±0.01ª	4.16±0.43
Significance	**	*	*	**	**	**	Ns
M×P							
$M_0P_0$	$23.50{\pm}7.15^{h}$	115.51±93.73 <sup>e</sup>	39.79±15.98 <sup>e</sup>	37.70±5.03	$0.64 \pm 0.04^{j}$	$0.44{\pm}0.07^{h}$	2.29±0.45
$M_0P_1$	$34.50{\pm}1.60^{f}$	1671.17±56.89 <sup>de</sup>	$58.04 \pm 9.44^{bc}$	41.93±3.32	$0.70{\pm}0.08^{h}$	$0.45{\pm}0.03^{h}$	2.70±0.49
$M_0P_2$	$42.50 \pm 2.82^{d}$	266.35±79.40°	55.445.59 <sup>cd</sup>	43.44±3.27	$0.77{\pm}0.08^{f}$	0.57±0.08e	3.32±0.34
$M_0P_3$	49.00±1.30°	322.26±71.94 <sup>bc</sup>	$65.73{\pm}11.14^{ab}$	44.80±4.28	$0.84{\pm}0.09^{d}$	$0.64{\pm}0.09^{d}$	3.90±0.65
$M_0P_4$	54.50±1.30 <sup>b</sup>	440.27±112.88 <sup>a</sup>	70.81±10.91ª	47.48±5.80	$0.89 \pm 0.07^{\circ}$	0.69±0.07°	4.04±0.16
$M_1P_0$	31.50±2.00 <sup>g</sup>	156.66±29.11 <sup>de</sup>	48.35±6.60 <sup>d</sup>	41.03±1.61	$0.68{\pm}.0.01^{i}$	$0.49{\pm}0.003^{g}$	2.65±0.12
$M_1P_1$	38.50±2.07e	$208.83{\pm}18.90^{d}$	51.58±5.06 <sup>cd</sup>	43.54±2.04	$0.75 {\pm} 0.03^{g}$	$0.54{\pm}0.03^{f}$	2.90±0.15
$M_1P_2$	48.00±1.19°	305.94±24.79 <sup>bc</sup>	$63.20{\pm}3.40^{ab}$	45.04±1.77	0.83±0.02 <sup>e</sup>	$0.63{\pm}0.02^{d}$	3.33±0.67
$M_1P_3$	56.00±3.54 <sup>b</sup>	327.24±63.81 <sup>b</sup>	65.20±4.36 <sup>ab</sup>	46.74±2.92	$0.91{\pm}0.02^{b}$	$0.71 {\pm} 0.02^{b}$	3.94±0.64
$M_1P_4$	60.00±2.26 <sup>a</sup>	355.67±9.49 <sup>b</sup>	70.50±7.70 <sup>a</sup>	49.61±3.85	0.95±0.01ª	0.76±0.01ª	4.06±0.63
Significance	**	**	*	Ns	**	**	Ns

Table 2. Interaction of magnetized P solution (G), arbuscular mycorrhizal fungi (M), and phosphorus concentration (P) on leaf number (LN), leaf area (LA), harvest index (HI), SPAD, chlorophyll a (Chl a), chlorophyll b (Chl b) and total water use (TWU) as affected by in basil plants

\*, \*\* significant differences between means at 0.05 and 0.01 level of probability, respectively; Ns – non-significant. Within each column in GM, GP or MP treatments, means followed by the same letters are not significantly different at 5%.

# Combined effects of magnetization, mycorrhization and P concentration (Table 2)

When magnetization of P-containing solutions was applied together with mycorrhization, all parameters were in higher amounts, but LN was not influenced. The magnetization analyzed together with P increasing concentrations usually gave higher values in comparison with the not magnetized ones. The only exception was TWU. In this value, an increase was observed but differences were not significant.

Similar results were obtained in the treatments with mycorrhization when analyzed together with increasing concentration of P. Dominant effect of P caused significant increase of growth and photosynthesis parameters with the exception of SPAD and TWU.

Important result is that at all concentrations of P, a significant increase in P use efficiency was caused by both magnetization and mycorrhization (Fig. 1 & 2). The biggest differences were recorded at 10 mg  $\cdot$  dm<sup>-3</sup>.



Fig. 1. Phosphorus use efficiency (PUE) as affected by magnetized P-fertilizer solution (G) and phosphorus concentration in the nutrient solution. Different letters indicate significant differences according to Duncan's multiple-range test ( $P \le 0.05$ , n = 8).



Fig. 2. Phosphorus use efficiency (PUE) as affected by mycorrhization (M) and phosphorus concentration in the nutrient solution. Different letters indicate significant differences according to Duncan's multiple-range test ( $P \le 0.05$ , n = 8).

## DISCUSSION

The results of our experiment showed that all three factors studied – the magnetization of P-containing solutions, arbuscular mycorrhizal fungi inoculation and various concentrations of P affected plant growth, the photosynthetic status, water absorption and P use efficiency in sweet basil pot culture.

Phosphorus is an essential macronutrient for plants. It is a component of nucleic acids, lipoproteins and enzymes connected with energy supply. This means that its availability is immanently connected with plants' life. Nowadays, it is a matter of concern in agriculture because of the need for its economical use (Jokubauskaite et al. 2015).

In our experiment, increasing supply of P played the main role in the increase of growth and photosynthetic parameters of basil young plants. With higher P availability, its share in the dry matter of basil young plants has decreased, which means that for the same dry mass, less P was necessary. Grant et al. (2001) pointed out that P is indispensable during early growth. Because of the amount of P in soil being finite and reserves are depleted continuously, several attempts have been undertaken to increase the efficiency of its use by plants. The most hope for more effective use of P from soil is associated with microorganism, for example mycorrhizal fungi, that can make P easily available (Grant et al. 2001; Zarei et al. 2006; Miransari 2010).

Our funding indicated that the inoculation of basil with arbuscular mycorrhizal fungi enhanced the chlorophyll concentration, which is in agreement with the results of other studies (Sannazzaro et al. 2006; Colla et al. 2008; Sheng et al. 2008). The results of Sheng et al. (2008), unlike as in our experiment, showed that the mycorrhizal fungi increased the Fv/Fm index compared to the control treatment. Light energy absorbed by chlorophyll molecules can undergo one of the three fates: it can be used to drive photosynthesis (photochemistry), excess energy can be dissipated as heat, or it can be re-emitted as light-chlorophyll fluorescence (non-photochemistry) (Sheng et al. 2008). It seems that the mycorrhizal fungi in our experiment, although not effective for the non-phytochemical properties of the plant but they were effective in photochemical efficiency. Arbuscular mycorrhizal fungi symbiosis improved the photosynthetic capacity of maize leaves, mainly through elevating the capacity of gas exchange and the efficiency of photochemistry and non-photochemistry of PSII and regulating the energy division between photochemical and non-photochemical events (Sheng et al. 2008).

Magnetization of P fertilizer increased several parameters of growth and photosynthesis in basil. The several physiological mechanisms (Aliverdi et al. 2015) and growth regulators balance of plant tissues (Turker et al. 2007) could be positively affected by magnetic field, which results in growth stimulation. Racuciu et al. (2008) reported that exposure of maize to magnetic field of 50 mT revealed an increase in chlorophyll content in comparison with control. They stated that magnetic field had stimulatory influence on fresh weight; assimilatory pigments level as well the chlorophyll ratio, average nucleic acid level, increase in the average plants height. According to Maheshwari & Grewal (2009), magnetic field decreases the pH of cell wall, affects the metabolism of meristem cells, increases the absorption and assimilation of nutrients and improves photosynthetic activity (Belyavskaya 2004). In our study, P-fertilizer magnetization and mycorrhization caused better water uptake by basil plants. Lin and Yotvat (1990) reported that magnetization of irrigation water increased water productivity in crop production or water use efficiency (Aliverdi et al. 2015). This may be related to an increase of root growth (Maheshwari & Grewal 2009) and stomatal conductance (Sadeghipour & Aghaei 2013), increasing absorption and assimilation of nutrients, and as a result, improved the process of water communication and water absorption by plants. In this case, improving the growth and physiological conditions of the basil plants can be attributed to the improvement of root development (Shabani et al. 2018), and the production and formation of photosynthetic pigments resulted from magnetization.

The simultaneous use of magnetization and mycorrhization increased the content of chlorophyll, SPAD index and the total water use of basil plants. These positives probably were resulted from the stimulating effect of the magnetic field on the production of photosynthetic pigments, the meristem cell metabolism, increased absorption and assimilation of nutrients and the positive effect of mycorrhizal fungi on increasing water absorption, leaf area development and photochemical efficacy.

Mycorrhizal fungi can increase the concentration of chlorophyll in plants by 40% as a result of increasing P concentration in nutrient solution (Augé 2001). The results of increasing chlorophyll concentration in pepper leaves have also been reported due to mycorrhizal coexistence (Demir 2004). It can increase the rate of photosynthesis and carbon fixation by increasing chlorophyll concentration (Feng et al. 2002). Moreover, it is well established that mycorrhizal fungi can remarkably enhance plant tolerance to different abiotic stresses due to the production of compounds like glomalin and the formation of extensive hyphal networks (Miransari 2010). Plant roots become a strong sink for carbohydrates when colonized by arbuscular mycorrhizal fungi, as they can consume up to 20% of the host photosynthates. Thus, mycorrhizal fungi modulate these source-sink relations by enhancing the exchange of carbohydrates and mineral nutrients and can stimulate the rate of photosynthesis sufficiently to compensate for fungal carbon requirements (Porcel et al. 2015). Therefore, non-stomatal factors such as higher Chl a content and rubisco activity could enhance net photosynthetic activity of mycorrhizal plants (Chen et al. 2014). Increasing rate of photosynthesis occurs by increasing leaf area and increasing the amount of CO<sub>2</sub> fixation (Porcel et al. 2015). Also, Sheng et al. (2008) showed that in the presence of mycorrhizal fungi, maize plants had higher stomatal conductance, higher transpiration rate, higher net photosynthetic rate and lower intercellular CO2 concentration compared with nonmycorrhizal ones.

The results of the present study showed that the main effects of the magnetized P solutions, mycorrhizal fungi and their interaction on the PUE were not significant. It was caused by the fact that these effects were covered by the interaction of above factors with P concentration ( $P \le 0.01$ ). Our results clearly showed that in the presence of the magnetized solution and mycorrhization, increasing P-fertilizer concentration up to 10 mg·dm<sup>-3</sup> increased the PUE, and by increasing the levels of P to 20 and 40 mg  $dm^{-3}$ , the PUE remains at the same level. In fact, the use of magnetic field and mycorrhizal fungi at low concentrations of P (10 mg dm<sup>-</sup> <sup>3</sup>) significantly increased the dry matter percentage (Shabani et al. 2018), and consequently, increased its PUE. Although higher P levels (>10 mg  $\cdot$  dm<sup>-3</sup>) in the nutrient solution increased P concentration in tissues, accumulation and plant uptake efficiency (Shabani et al. 2018), the dry matter accumulation, partitioning and PUE were not affected (Fig. 1 & 2). Plants develop physiological mechanisms to increase the PUE, such as changes in P uptake kinetics and in the plant capacity to maintain normal metabolic levels when P concentrations in tissues are low, ensuring a satisfactory dry matter or grain production (Machado & Furlani 2004). These results indicated that at low concentrations of P, in addition to the intrinsic properties of the plant, the role of these stimulants in increasing dry matter percentage and PUE is undeniable.

Relative reduction in shoot dry matter due to P-stress can be used as an index in assessing the relative tolerance of cultivars under P stress environments (Akhtar et al. 2009). Increased development of plant parts associated with P-inception and efficient re-distribution of absorbed P within the plants may increase its PUE under P-starved environments. This may be an indication of the ability of the tolerant cultivars to better remobilize P from aerial parts to roots under P-stress environments (Akhtar et al. 2009). As a result, it can be stated that the use of magnetized P fertilizer and mycorrhization increased the possibility of basil plants to survive in conditions of low supply of P, probably due to better inception and remobilization of P in the plants, although recognizing their exact mechanism requires further cellular and molecular studies.

## CONCLUSIONS

The magnetization of  $Ca(H_2PO_4)_2$  solutions used for young basilica plants' fertilization as a phosphorus source as well as inoculation of plants with arbuscular mycorrhizal fungi *Diversispora versiformis* enhanced P use efficiency. This finding in the macro scale of world agriculture can improve P management and reduce the demand for this element.

#### REFERENCES

- Aladjadjiyan A. 2007. The use of physical methods for plant growing stimulation in Bulgaria. Journal of Central European Agriculture 8(3): 369–380.
- Aliverdi A., Parsa, M., Hammami H. 2015. Increased soyabean-rhizobium symbiosis by magnetically treated water. Biological Agriculture and Horticulture 31(3): 167–176. DOI: 10.1080/01448765.2014.996253.
- Al-Khazan M., Abdullatif, B.M., Al-Assaf N. 2011. Effects of magnetically treated water on water status, chlorophyll pigments and some elements content of Jojoba (*Simmondsia chinensis* L.) at different growth stages. African Journal of Environmental Science and Technology 5: 722–731. DOI: 10.5897/ajest11.117.
- Akhtar M.S., Oki, Y., Adachi T. 2009. Mobilization and acquisition of sparingly soluble P-sources by *Bras*sica cultivars under P-starved environment. I. Differential growth response, P-efficiency characteristics and P-remobilization. Journal of Integrative Plant Biology 51(11): 1008–1023. DOI: 10.1111/j.1744-7909.2009.00874.x.
- Augé R.M. 2001. Water relations, drought and vesiculararbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3–42. DOI: 10.1007/s005720100097.
- Belyavskaya N.A. 2004. Biological effects due to weak magnetic field on plants. Advances in Space Research 34: 1566–1574. DOI: 10.1016/j.asr.2004.01.021.
- Carter G.A., Knapp A.K. 2001. Leaf optical properties in highest plants: Linking spectral characteristics to stress and chlorophyll concentration. American Journal of Botany 88(4): 677–684. DOI: 10.2307/2657068.
- Chen Y.-Y., Hu C.-Y., Xiao J.-X. 2014. Effects of arbuscular mycorrhizal inoculation on the growth, zinc distribution and photosynthesis of two citrus cultivars grown in low-zinc soil. Trees 28: 1427–1436. DOI: 10.1007/s00468-014-1046-6.

- Childers D.L., Corman J., Edwards M., Elser J.J. 2011. Sustainability challenges of phosphorus and food: Solutions from closing the human phosphorus cycle. Bioscience 61: 117–124. DOI: 10.1525/bio.2011.61.2.6.
- Colla G., Rouphael Y., Cardarelli M., Tullio M., Rivera C.M., Rea E. 2008. Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. Biology and Fertility of Soils 44: 501–509. DOI: 10.1007/s00374-007-0232-8.
- Cordell D., Drangert J.-O., White S. 2009. The story of phosphorus: Global food security and food for thought. Global Environmental Change 19: 292– 305. DOI: 10.1016/j.gloenvcha.2008.10.009.
- Demir S. 2004. Influence of arbuscular mycorrhiza on some physiological growth parameters of pepper. Turkish Journal of Biology 28: 85–90.
- Feng G., Zhang F.S., Li X.L., Tian C.Y., Tang C., Rengel Z. 2002. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza 12: 185–190. DOI: 10.1007/s00572-002-0170-0.
- Grant C.A., Flaten D.N., Tomasiewicz D.J., Sheppard S.C. 2001. The importance of early season phosphorus nutrition. Canadian Journal of Plant Science 81(2): 211–224. DOI: 10.4141/p00-093.
- Gregory P.J. 2006. Plant roots. Growth, activity and interaction with soils. Blackwell Publishing, 318 p. DOI: 10.1002/9780470995563.
- Ghanati F., Mohamadalikhani S., Soleimani M., Afzalzadeh R., Hajnorouzi A. 2015. Change of growth pattern, metabolism, and quality and quantity of maize plants after irrigation with magnetically treated water. Electromagnetic Biology and Medicine 34(3): 211–215. DOI: 10.3109/15368378.2015.1076453.
- Hajnorouzi A., Vaezzadeh M., Ghanati F., Jamnezhad H., Nahidian B. 2011. Growth promotion and a decrease of oxidative stress in maize seedlings by a combination of geomagnetic and weak electromagnetic fields. Journal of Plant Physiology 168: 1123–1128. DOI: 10.1016/j.jplph.2010.12.003.
- Hilal M.H., Shata S.M., Abdel-Dayem A.A., Hilal M.M. 2002. Application of magnetic technologies in desert agriculture. III. Effect of magnetized water on yield and uptake of certain elements by citrus in relation to nutrients mobilization in soil. Egyptian Journal of Soil Science 42(1): 43–56.
- Jokubauskaitė I., Karčauskienė D., Antanaitis Š., Mažvila J., Šlepetienė A., Končius D., Piaulokaitė-Motuzienė L. 2015. The distribution of phosphorus forms and

fractions in retisol under different soil liming management. Zemdirbyste–Agriculture 102(3): 251– 256. DOI: 10.13080/z-a.2015.102.032.

- Lichtenthaler H.K., Buschmann C. 2001. Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. Current Protocols in Food Analytical Chemistry 1(1); F4.3; 8 p. DOI: 10.1002/0471142913.faf0403s01.
- Lin I.J., Yotvat J. 1990. Exposure of irrigation and drinking water to a magnetic field with controlled power and direction. Journal of Magnetism and Magnetic Materials 83: 525–526. DOI: 10.1016/0304-8853(90)90611-s.
- MacDonald G.K., Bennett E.M., Potter P.A., Ramankutty N. 2011. Agronomic phosphorus imbalances across the world's croplands. Proceedings of the National Academy of Sciences 108: 3086– 3091. DOI: 10.1073/pnas.1010808108.
- Machado C.T. de T., Furlani Â.M.C. 2004. Kinetics of phosphorus uptake and root morphology of local and improved varieties of maize. Scientia Agricola 61: 69–76. DOI: 10.1590/s0103-90162004000100012.
- Maheshwari B.L., Grewal H.S. 2009. Magnetic treatment of irrigation water: its effects on vegetable crop yield and water productivity. Agricultural Water Management 96: 1229–36. DOI: 10.1016/j.agwat.2009.03.016.
- Miransari M. 2010. Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. Plant Biology 12: 563–569. DOI: 10.1111/j.1438-8677.2009.00308.x.
- Pang X.-F., Deng B. 2008. The changes of macroscopic features and microscopic structures of water under influence of magnetic field. Physica B 403: 3571– 3577. DOI: 10.1016/j.physb.2008.05.032.
- Podleśny J., Pietruszewski S., Podleśna A. 2004. Efficiency of the magnetic treatment of broad bean seeds cultivated under experimental plot conditions. International Agrophysics 18: 65–71.
- Porcel R., Redondo-Gómez S., Mateos-Naranjo E., Aroca R., Garcia R., Ruiz-Lozano J.M. 2015. Arbuscular mycorrhizal symbiosis ameliorates the optimum quantum yield of photosystem II and reduces non-photochemical quenching in rice plants subjected to salt stress. Journal of Plant Physiology 185: 75–83. DOI: 10.1016/j.jplph.2015.07.006.
- Răcuciu M., Creangă D., Horga I. 2008. Plant growth under static magnetic field influence. Romanian Journal of Physics 53(1–2): 353–359.

- Sadeghipour O., Aghaei P. 2013. Improving the growth of cowpea (*Vigna unguiculata* L. Walp.) by magnetized water. Journal of Biodiversity and Environmental Sciences 3(1): 37–43.
- Sannazzaro A.I., Ruiz O.A., Albertó E.O., Menéndez A.B. 2006. Alleviation of salt stress in *Lotus glaber* by *Glomus intraradices*. Plant and Soil 285: 279– 287. DOI: 10.1007/s11104-006-9015-5.
- Shabani E., Bolandnazar S., Tabatabaei S.J., Najafi N., Alizadeh-Salteh S. 2017. Motivate the production of pharmaceutical compounds in *Ocimum basilicum* by magnetic phosphorus solution and arbuscular mycorrhizal fungi. Journal of Biodiversity and Environmental Sciences 11(3): 31–45.
- Shabani E., Bolandnazar S., Tabatabaei S.J., Najafi N., Alizadeh-Salteh S., Rouphael Y. 2018. Stimulation in the movement and uptake of phosphorus in response to magnetic P solution and arbuscular mycorrhizal fungi in *Ocimum basilicum*. Journal of Plant Nutrition 41(13): 1662–1673. DOI: 10.1080/01904167.2018.1458872.
- Shen J., Yuan L., Zhang J., Li H., Bai Z., Chen X. et al. 2011. Phosphorus dynamics: From soil to plant. Plant Physiology 156: 997–1005. DOI: 10.1104/pp.111.175232.
- Sheng M., Tang M., Chen H., Yang B., Zhang F., Huang Y. 2008. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants

under salt stress. Mycorrhiza 18(6–7): 287–296. DOI: 10.1007/s00572-008-0180-7.

- Siddiqi M.Y., Glass A.D.M. 1981. Utilization index: A modified approach to the estimation and comparison of nutrient utilization efficiency in plants. Journal of Plant Nutrition 4(3): 289–302. DOI: 10.1080/01904168109362919.
- Soltani F., Kashi A., Arghavani M. 2006. Effect of magnetic field on *Asparagus officinalis* L. seed germination and seedling growth. Seed Science and Technology 34: 349–353. DOI: 10.15258/sst.2006.34.210.
- Turker M., Temirci C., Battal P., Erez M.E. 2007. The effects of an artificial and static magnetic field on plant growth, chlorophyll and phytohormone levels in maize and sunflower plants. Phyton, Annales Rei Botanicae 46: 271–284.
- Zarei M., Saleh-Rastin N., Alikhani H.A., Aliasgharzadeh N. 2006. Responses of lentil to co-inoculation with phosphate-solubilizing rhizobial strains and arbuscular mycorrhizal fungi. Journal of Plant Nutrition 29: 1509–1522. DOI: 10.1080/01904160600837667.
- Zhang J., Zhou K., Wang L., Gao M. 2014. Extremely lowfrequency magnetic fields affect pigment production of *Monascus purpureus* in liquid-state fermentation. European Food Research and Technology 238(1): 157–62. DOI: 10.1007/s00217-013-2096-5.