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Magnetized nutrient solution and arbuscular mycorrhizal affect essential oil and physiological aspects of sweet basil (*Ocimum basilicum* L.) grown in various P concentrations

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ABSTRACT

Nowadays, finding new ways to motivate the production of pharmaceutical compounds along with promoting growth in vegetables has been received more attention. An experiment was organized to find the effects of magnetic solution, arbuscular mycorrhizal (AM) fungi and phosphorus (P) concentration (0, 5, 10, 20, 40 mg L^{-1}) on essential oil production, nitrate reduction and rhizosphere conditions in sweet basil. The results showed that the growth of basil plant, nitrate content of shoots, rhizosphere conditions were significantly affected by both magnetic solutions and AM compared to the control. The value of basil's nutrition increased by nitrate reduction in magnetic field and AM fungi treatments. The application of magnetic solutions and inoculation of AM fungi increased P content in basil shoots and as a result, essential oil concentration at 10 and 20 mg P L^{-1} increased 70.7% and 102.4%, respectively. The concomitant use of magnetic P solution and AM fungi at 20 mg L^{-1} increased constituent of essential oils such as neral and caryophyllene 102.3% and 19.66%, respectively. Overall, AM fungi and magnetic solution demonstrate a natural way for improving the growth and production of essential oils in plants.

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Arbuscular mycorrhizal fungi; basil; growth; magnetic field;; nutrient solution

Introduction

One of the main essential oils producing species belonging to the genus *Ocimum* is Sweet basil (*Ocimum basilicum* L.) (Sifola and Barbieri 2006). It is commonly used as medicinal herb in preservatives, food flavorings, culinary applications, and pesticides, cosmetic, pharmaceutical, and industrial products (Scagel and Lee 2012). Plant secondary metabolites have been shown to inhibit tumorigenesis, angiogenesis, and metastasis, and many are known to have antifungal, antibacterial, and anti-inflammatory capabilities (Nguyen and Niemeyer 2008). The main secondary metabolites are divided into phenolic compounds (from the shikimic acid pathway), terpenoids (from the mevalonate and methylerythritol phosphate [or Non-mevalonate] pathway), acetate pathways (such as the production of prostaglandins and antibiotics, etc.) and alkaloids (light nitrogen compounds) that produced from some amino acids pathway (Dewick 2002). Terpenoids, especially monoterpenes and sesquiterpenes and phenylpropanoids such as methyl chavicol, form a significant part of basil essential oils (Simon et al. 1999).

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Glandular trichomes in the leaf are specialized structures for the synthesis and storage of essential oils (Sifola and Barbieri 2006). On the other hand, Werker et al. (1993) showed that during the development of a sweet basil leaf, there is a net accumulation of essential oil and methyl chavicol, both per leaf, and on a leaf weight basis. Therefore, the factors affecting leaf growth such as agronomic parameters and growing conditions have significant potential to essential oils production. Nguyen and Niemeyer (2008) reported that P, nitrogen (N), calcium (Ca), and potassium (K) fertilization levels affect the production of secondary metabolites in some plants. The study of nutrient effects on essential oil content in leafy vegetables is very limited. Despite the importance of P on essential oil yield and quality, few studies have been conducted on P fertilization of basil (Toussaint, Smith, and Smith 2007; Scagel and Lee 2012).

In the last decade, there has been growing interest in developing natural and simple methodologies to increase secondary metabolites concentration in vegetables and fruits to further enhance their overall nutritional value (Schreiner and Huyskens-Keil 2006; Li et al. 2007; Nguyen and Niemeyer 2008). The magnetic field is one of these ways which increases the quality and quantity of plants (Aladjadjiyan 2007). Plants of parsley (*Petroselinum crispum*), sweet basil, and celery (*Apium graveolens*) irradiated by GSM-frequency (Global System for Mobile communications) microwaves had greater essential oil, and polyphenolics contents than in the control plants (Lung et al. 2016). The cellular and molecular characteristics of plant tissues could be positively affected as a result of irrigation using water molecules prior modified through magnetic field. It has been demonstrated that magnetized water helps in dissolving minerals by a higher rate than non-magnetic water, moreover dissolving oxygen and enhancing the speed of chemical reactions (Shabani et al. 2018).

Inoculation with beneficial fungi such as arbuscular mycorrhizal (AM) fungi is another natural way to improve rhizosphere conditions, basil plant growth, and phyto-bioactive compounds production. Previous studies demonstrated that AM increases uptake of poorly available nutrients such as P and N (Smith, Smith, and Jakobsen 2003) and change the pH and EC of soil (Baum, El-Tohamy, and Grudac 2015). Yao et al. (2003) showed that AM fungi induce changes in the production and accumulation of secondary metabolites in the host plant roots. Despite the many studies that have been done on the effects of different AM fungi strains on plant growth, there is no complete information on the effect of *Diversispora versiformis* colonization on the cumulation of essential oil in shoots of medicinal vegetables.

Therefore, the aim of this study was to investigate the improvement of mineral distribution, rhizosphere conditions, nitrate reduction, essential oil production and thus improve plant growth under the influence of the passage of P solution through the magnetic field and mycorrhizal fungi.

Materials and methods

Plant material, growth conditions, and AM inoculation

As we described in our last published article (Shabani et al. 2018) the experiment was conducted in a research greenhouse situated at the Faculty of Agriculture, University of Tabriz, Tabriz, Iran (latitude 38°08'N, longitude 46°30'E). Plants were grown under 800 μmol $m^{-2}s^{-1}$ light intensity. The greenhouse was maintained at 27 °C for 14 h per day and 18 °C for 10 h per night and relative humidity was 65%±5. Seeds of sweet basil (*Ocimum basilicum* L.) cv. "Mobarakeh," were cultivated in pots with sandy-loam soil. The studied soil had a sandy loam texture and FC, saturation percentage, organic carbon, calcium carbonate equivalent and total N were 10, 23.5, 0.1, 0.0, and 0.08%, respectively. The concentration of available- P, K, Ca, Mg, Fe, Mn, Zn, and Cu in soil were 4.4, 82.6, 49, 99, 1.8, 1.1, 0.9, and 1.3 mg kg^{-1}, respectively. The soil solution (1:1) pH and electrical conductivity (EC) were 7.25 and 0.6 dS m^{-1}, respectively. Treatments were arranged in a completely randomized design with four replicates. The treatments were defined by a factorial combination of five concentrations of P (0, 5, 10, 20, and 40 mg L^{-1}). Based on daily crop water requirement in the pretest, five levels of P solution (without, low, moderate, high, and very high) were prepared, along with two magnetic P solutions (with Magnetic solution, and without magnetic solution) and two mycorrhizal treatments (with AM, AM_1 and without AM, AM_0). *Diversispora versiformis* was used in this experiment preparing the inoculants using a pot culture of sorghum (*Sorghum bicolor* L.) as a host plant. The autoclaved sandy loam soil was inoculated with pure inoculums of *Diversispora versiformis.* The plants were grown in a greenhouse for four months. After this time, the aerial parts of sorghum plants were removed and the mixture of pot content, including the spores, hyphae, and finely segmented mycorrhizal roots, was maintained at 4 °C until use as inoculants. Plastic pots (20 cm inner diameter, 30 cm depth) were filled with an autoclaved mixture of soil (2.5 kg). In total, 40 grams of arbuscular mycorrhizal fungi inoculants (with 120 active spores, 10^4 CFU 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 in the plastic pots. At the two-leaf stage, the seedlings were thinned to four plants per pot and the pot surfaces were covered with perlite and cork (to reduce evaporation from the soil surface).

Exposure of plants to magnetic phosphorus solution

Each experimental unit consisted of three pots for a total of 12 plants. All the pots received 250 mL of plant nutrient solution [g L^{-1}: potassium sulfate (K_2SO_4): 2.22, calcium nitrate ($Ca(NO_3)_2.4H_2O$): 12.64, ammonium sulfate ($(NH_4)_2SO_4$): 2.35, magnesium sulfate ($MgSO_4.7H_2O$): 10.13, manganese sulfate ($MnSO_4.4H_2O$): 0.4, iron sulfate ($FeSO_4.7H_2O$): 0.74, copper sulfate ($CuSO_4.7H_2O$): 0.19, zinc sulfate ($ZnSO_4.7H_2O$): 0.43, boric acid (H_3BO_3): 0.05 and sodium molybdate (Na_2MoO_4): 0.0001] three times during the first two weeks by 50 mL syringe. The quality of the irrigation water was typical of the region. The concentrations of ions expressed as mg L^{-1} 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 EC were 7.7 and 0.49 dS m^{-1}, respectively. The pots were weighed daily by digital weighing balance and the evapotranspiration loss was restored subtracting the soil water content from FC, ensuring a stable water content throughout the experiment. Also, two pots without plants were prepared to monitor water loss by evaporation in the different treatments. Five different concentrations of P were made in 50-L tanks. P was added as Ca $(H_2PO_4)_2.H_2O$ at the following concentrations of 0, 5, 10, 20, and 40 mg L^{-1}. P solution was passed from the magnetic field of 110 mT (with a speed of flow of 3 L min^{-1}) that was produced by a locally designed apparatus (FAPAN Co. Iran) and eventually was added to each pot. The magnetization amount of the solution was 110 mT, which was measured by a teslameter device (PHYWE, Germany). Magnetic P solution was used between weeks five and seven, at 2 days interval for a total of seven times during the growing cycle. About the operating model of this system as documented in our previous article (Shabani et al. 2018) the main part of the apparatus 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. This motion causes the ion to act as a microscopy stirrer in water.

Growth measurement

At the end of the experiment, the basil plants were harvested and fresh weight of leaves (FWL) was measured by digital weighing balance.

Shoot mineral analysis

The N concentration of shoot tissues was determined after mineralization with sulfuric acid by the"Regular Kjeldahl method" (Bremner 1965). P concentration was measured by a vanadate-molybdate method using a spectrophotometer (Unico, UV-2100, UK). Other mineral compositions (K, Ca, Mg and NO_3) were detected on dried, finely ground (mesh 0.5 mm) samples of basil shoots. Anions and cations were extracted in Milli-Q water (Merck Millipore, Darmstadt, Germany) in a thermostatic bath at 80 C° for 10 minutes (ShakeTemp SW22, Julabo, Seelbach, Germany). After centrifuging at 6000 rpm for 10 minutes, the supernatant has been filtered (0.2 µm) and analyzed by ion chromatography with suppressed conductivity detection using a Dionex ICS-3000 system (Sunnyvale, CA, USA).

Soil pH and EC

At the end of the experiment, the roots were extracted from the soil. Soil samples were passed through a 2-mm sieve and saturated with distilled water. For full immersion of pores and porosity, containers containing soil samples were placed in dark conditions and laboratory temperatures for 24 hours. After 24 hours, the saturated extracts were prepared through the vacuum pump and filtered with filter paper. pH and EC of soil solution measured by pH meter (Model Ezodo, PL-500) and EC meter (Model HANNA), respectively.

AM fungi root colonization

At the end of the experiment, for the determination of root colonization by AM, the root samples were extracted from the soil. Root samples were cleared with 10% KOH, stained with 0.05% trypan blue in lactoglycerol as described by Phillips and Hayman (1970), and microscopically examined for AM fungi colonization by determining the percentage of root segments containing arbuscules + vesicles using a gridline intercept method (Giovannetti and Mosse 1980).

Extraction and assay of essential oil

Fifty grams of air-dried powdered plant materials (aerial parts) were subjected to hydro-distillation for 3 hours using an all–glass Clevenger type apparatus. The oil fraction was collected and subjected to GC-MS device. GC-MS analysis was performed on a GC-MS system (Agilent, 19091S-443, USA) equipped with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The concentration of essential oil was taken by hydro-distillation using Clevenger's device. It was calculated based on the ml of essential oil per 100 g dry weight (% v/w) of the basil plants. Essential oil yield (ml per pot) was calculated based on the multiplication of essential oil percentage and dry weight of aerial parts.

Statistical analysis

All observations and biochemical analyses were conducted with four independent repetitions with the exception of GC-MS analysis. All data were statistically analyzed by analysis of variance (ANOVA) using the SAS 9.1 software (SAS Inc., Cary NC). Duncan's multiple range test was performed at $p = 0.05$ on each of the significant variables measured.

Table 1. Mean effects of magnetic solution (G), arbuscular mycorrhizal fungi (AM) and different concentrations of P (P) on Leaf fresh weight (LFW) Nitrogen (N), P, Potassium (K), Calcium (Ca) and Magnesium (Mg) contents, PH and EC of soil solution, colonization rate (CR) and essential oil yield (E Yield) in basil.

Treatment	LFW (g)	N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	NO_3 (mg g^{-1})	Soil pH	Soil EC (dS m^{-1})	CR (%)	E Yield (ml pot^{-1})
G											
G_0	10.08 ^{b}	28.81 ^{b}	2.26 ^{b}	30.16 ^{b}	7.57	3.76	1.80 ^{a}	7.73 ^{a}	3.62 ^{b}	31.52 ^{a}	0.22 ^{b}
G_1	11.87 ^{a}	29.93^{a}	2.59 ^{a}	32.54 ^{a}	7.27	3.50	1.60 ^{b}	7.64 ^{b}	3.87 ^{a}	26.99 ^{b}	0.30 ^{a}
Sig.	**	**	***	**	Ns	Ns	*	**	**	**	**
AM											
AM_0	10.64 ^{b}	28.89 ^{b}	2.30 ^{b}	31.03	7.30	3.47 ^{b}	1.72	7.72 ^{a}	3.69 ^{b}	0.00 ^{b}	0.21 ^{b}
AM_1	11.32 ^{a}	29.85 ^{a}	2.56 ^{a}	31.68	7.53	3.80 ^{a}	1.68	7.65 ^{b}	3.81 ^{a}	58.51 ^{a}	0.29 ^{a}
ig.	**	*	***	Ns	Ns	*	Ns	**	**	**	**
P											
P_0	4.62 ^{e}	27.63 ^{c}	1.28 ^{e}	24.99 ^{d}	7.06 ^{cd}	3.25 ^{c}	2.13 ^{a}	7.52 ^{e}	3.55 ^{e}	27.49 ^{c}	0.15 ^{d}
P_1	7.92 ^{d}	28.70 ^{bc}	1.85 ^{d}	29.95 ^{c}	7.40 ^{bc}	3.58 ^{bc}	2.00 ^{ab}	7.61 ^{d}	3.65 ^{d}	42.91 ^{a}	0.22 ^{c}
P_2	9.53 ^{c}	30.08 ^{b}	2.35 ^{c}	32.28 ^{b}	7.76 ^{ab}	3.94 ^{ab}	1.79 ^{b}	7.70 ^{c}	3.77 ^{c}	31.31 ^{b}	0.27 ^{b}
P_3	14.92 ^{b}	31.44 ^{a}	3.04 ^{b}	33.44 ^{b}	8.12 ^{a}	4.07 ^{a}	1.45 ^{c}	7.75 ^{b}	3.84 ^{b}	25.83 ^{c}	0.32 ^{a}
P_4	17.89 ^{a}	29.01 ^{bc}	3.62 ^{a}	36.11 ^{a}	6.76 ^{d}	3.33 ^{c}	1.13 ^{d}	7.84 ^{a}	3.93 ^{a}	18.74 ^{d}	0.32 ^{a}
Sig.	**	**	***	**	**	**	**	**	**	**	**

*, ** and *** significant differences between means at 0.05, 0.01, and 0.001 level of probability, respectively; Ns, non-significant. Within each column in G, AM, or P treatments, means followed by the same letters are not significantly different at 5%.

Results

Effects of magnetic solution

The results demonstrate high magnetic solution effectiveness in increasing N, P, and K concentrations, and leaf fresh weight (LFW) of basil (Table 1). The use of magnetic solutions increased N, P (Shabani et al. 2018), K, and LFW 3.88%, 14.60%, 7.89% and 17.75%, respectively, as compared to the control (G_0) (Table 1). Magnetically solution treatment caused an 11.11% reduction in shoot nitrate concentration (Table 1). The use of magnetic field significantly decreased the pH of soil solution and the percentage of root colonization and increased EC of soil solution (Table 1). Magnetic field treatment increased essential oil yield 36.36% in comparison with control (G_0) (Table 1).

Effects of AM fungi

The inoculation with AM fungi represented positive effects on basil crop production in terms of LFW (Table 1). Except for Ca and NO_3, the highest concentrations of N, P (Shabani et al. 2018), and Mg were recorded on AM_1 compared to AM_0 plants (Table 1). As well as magnetic solution treatment, the inoculation of basil plants with AM reduced pH and increased the EC of soil solution (Table 1). As expected, the highest percentage of root colonization was recorded in mycorrhizal treatments, and no AM fungi colonization was recorded in roots of control plants (Table 1). Inoculation of basil plants with AM increased essential oil yield 38.09% in comparison with control (AM_0) (Table 1).

Effects of P concentrations in the nutrition solution

In this study, LFW increased with increasing P concentration from 0 to 40 mg L^{-1} (Table 1). In P treatments, N, P (Shabani et al. 2018), K, Ca and Mg increased quadratically with increasing the P content in the nutrient solution and the highest concentration of these elements was observed in 20 mg P L^{-1} (Except for P and K at 40 mg P L^{-1}) (Table 1). The results also indicated that increasing the P concentration from 0 to 40 mg L^{-1} in the nutrient solution decreased the NO_3 concentrations ($r = -0.59$) and the highest concentration of nitrate was observed at

Table 2. Leaf Fresh Weight (LFW), Nitrogen (N), Phosphorus (P), Potassium (K), and Magnesium (Mg) contents, PH and EC of soil solution and colonization rate (CR) as affected by magnetic solution (G), arbuscular mycorrhizal fungi (AM), and P concentration interactions in basil plants.

Treatment	LFW (g)	N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	Mg (mg g^{-1})	NO_3 (mg g^{-1})	soil pH	soil EC (dS m^{-1})	CR (%)
GAM									
G_0AM_0	9.16^{c}	28.29	2.02^{b}	28.98^{b}	3.37^{b}	1.68^{ab}	7.72^{a}	3.49^{c}	0.00^{c}
G_0AM_1	11.01^{b}	29.34	2.50^{a}	31.35^{a}	4.15^{a}	1.91^{a}	7.74^{a}	3.76^{b}	63.04^{a}
G_1AM_0	12.12^{a}	29.49	2.58^{a}	33.08^{a}	3.56^{b}	1.75^{a}	7.72^{a}	3.89^{a}	0.00^{c}
G_1AM_1	11.63^{ab}	30.36	2.61^{a}	32.01^{a}	3.45^{b}	1.46^{b}	7.56^{b}	3.86^{a}	53.99^{b}
Sig.	**	Ns	***	*	**	**	**	**	**
GP									
G_0P_0	3.30	27.44^{d}	1.24^{g}	22.03^{d}	3.35	2.18	7.59^{e}	3.40^{h}	28.32^{de}
G_0P_1	7.09	28.38^{cd}	1.64^{f}	27.95^{c}	3.64	2.08	7.66^{d}	3.49^{g}	45.82^{a}
G_0P_2	8.81	28.80^{cd}	2.37^{d}	30.95^{bc}	4.10	1.94	7.75^{c}	3.67^{f}	32.62^{c}
G_0P_3	14.07	30.13^{bc}	2.75^{c}	36.28^{a}	4.20	1.55	7.81^{b}	3.75^{e}	30.00^{cd}
G_0P_4	17.15	29.33^{cd}	3.31^{b}	33.61^{ab}	3.53	1.22	7.86^{a}	3.81^{d}	20.82^{f}
G_1P_0	5.95	27.82^{d}	1.33^{g}	27.95^{c}	3.14	2.08	7.46^{f}	3.70^{ef}	26.66^{e}
G_1P_1	8.75	29.01^{cd}	2.06^{e}	31.95^{b}	3.52	1.92	7.56^{e}	3.81^{d}	40.00^{b}
G_1P_2	10.25	31.36^{ab}	2.33^{d}	33.61^{ab}	3.78	1.65	7.65^{d}	3.88^{c}	30.00^{cd}
G_1P_3	15.78	32.76^{a}	3.33^{b}	35.94^{a}	3.95	1.35	7.69^{d}	3.94^{b}	21.66^{f}
G_1P_4	18.64	28.70^{cd}	3.93^{a}	33.28^{ab}	3.12	1.03	7.82^{ab}	4.06^{a}	16.66^{g}
Sig.	Ns	*	***	*	Ns	Ns	*	**	**
AMP									
AM_0P_0	3.55^{g}	27.47	1.16^{g}	24.03	3.13	2.12	7.55	3.37^{f}	0.00^{f}
AM_0P_1	7.54^{e}	28.52	1.84^{e}	29.95	3.50	2.05	7.64	3.52^{e}	0.00^{f}
AM_0P_2	9.23^{cd}	29.12	2.34^{d}	32.61	3.91	1.86	7.76	3.76^{cd}	0.00^{f}
AM_0P_3	14.90^{b}	30.80	2.72^{c}	35.94	3.85	1.41	7.79	3.85^{b}	0.00^{f}
AM_0P_4	17.98^{a}	28.56	3.43^{b}	32.61	2.94	1.14	7.86	3.94^{a}	0.00^{f}
AM_1P_0	5.70^{f}	27.79	1.40^{f}	25.95	3.36	2.15	7.50	3.72^{d}	54.98^{c}
AM_1P_1	8.30^{de}	28.87	1.86^{e}	29.95	3.66	1.94	7.57	3.77^{cd}	85.82^{a}
AM_1P_2	9.83^{c}	31.04	2.36^{d}	31.95	3.97	1.72	7.64	3.79^{bc}	51.66^{d}
AM_1P_3	14.95^{b}	32.09	3.35^{b}	36.28	4.30	1.49	7.71	3.84^{b}	51.66^{d}
AM_1P_4	17.80^{a}	29.47	3.81^{a}	34.28	3.72	1.11	7.83	3.92^{a}	37.48^{e}
Sig.	*	Ns	***	Ns	Ns	Ns	Ns	**	**

*, ** and *** significant differences between means at 0.05, 0.01, and 0.001 level of probability, respectively; Ns, non-significant. Within each column in G, AM, or P treatments, means followed by the same letters are not significantly different at 5%.

$G_0AM_0P_0$ $G_0AM_1P_1$ $G_1AM_1P_1$

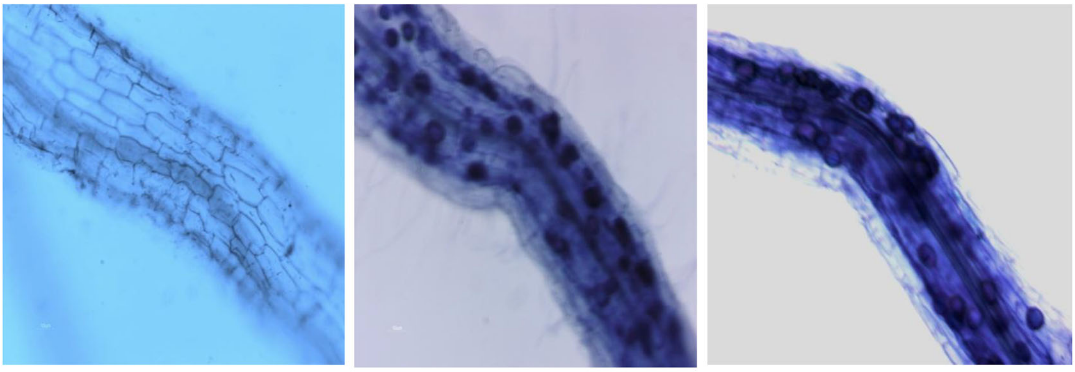
low levels of P. Increasing P concentration from 0 to 5 mg L^{-1} significantly increased percentage of root colonization and decreased again with increasing of P concentration. Increasing P concentration up to 20 mg P L^{-1} increased the essential oil yield by 113.33% in comparison with control plants. The highest essential oil yield was observed in treatment with 20 mg P L^{-1} (Table 1).

Interaction effects of magnetic solution and AM fungi

Interaction of magnetic solution and mycorrhizal fungi significantly impress growth parameters such as LFW. The highest contents of P, K, and Mg were recorded on G_1AM_1, G_1AM_0, and G_0AM_1 and the lowest content of NO_3 were observed in G_1AM_1 compared to control plants (G_0AM_0), respectively (Table 2). The application of magnetic solution and inoculation of basil plants with AM reduced pH and increased the EC of soil solution (Table 2). Treatment of G_1AM_1 decreased the colonization rate of basil roots in comparison with G_0AM_1 (Table 2). The interaction of magnetic field and AM fungi on Ca content and essential oil yield was not significant. Therefore, they are not shown in Table 2.

Interaction effects of magnetic solution and the P concentrations in the nutrient solution

The results of this experiment showed that the interaction of the magnetic field and P increasedthe N, P (Shabani et al. 2018), and K content of shoots, pH, and EC of soil solution and



$G_0AM_0P_0$ $G_0AM_1P_1$ $G_1AM_1P_1$

Figure 1. Colonization rate of basil roots in different treatments ($G_0AM_0P_0$= 0.00%, $G_0AM_1P_1$= 91.65% and $G_1AM_1P_1$= 80.00%, G_0= without magnetic field, G_1= with magnetic field, AM_0= without mycorrhizal fungi, AM_1= with mycorrhizal fungi, P_0 and P_1= 0 and 5 mg L^{-1} of phosphorus, respectively).

decreased the colonization percentage of basil roots (Table 2). The highest amounts of N were 31.36 and 32.76 mg g^{-1} in treatments of 10 and 20 mg P L^{-1}, respectively when the P solutions were passed from 110 mT magnetic field (Table 2). The interaction of magnetic field and P on Ca content and essential oil yield was not significant. Therefore, they are not shown in Table 2.

Interaction effects of AM fungi and the P concentration in the nutrient solution

Inoculation of basil plants with AM fungi and nutrition with different concentrations of P increased growth, P content of shoot, and EC of soil solution (Table 2). In AM fungi treatments, the increase of P concentration from 0 to 5 mg L^{-1} resulted in an increase of about 56% colonization of basil roots and decreased again with increasing of P concentration (Table 2). The interaction of AM fungi and P on Ca content and essential oil yield was not significant. Therefore, they are not shown in Table 2.

The triple effects of treatments showed that the percentage of basil root colonization was significantly affected not only in the presence of mycorrhizal fungi but also in the presence of magnetic field (Figure 1).

Magnetic solution, AM fungi and the phosphorus concentration interrelationships on essential oil

Triple effects of magnetic P solution and AM fungi were significant in the concentration of essential oil. So, it is shown in Figure 2 only in the triple effects. Measurement of basil essential oil concentration showed that by passing various concentrations of P from the magnetic field of 110 mT and inoculation with AM fungi, the highest concentration of essential oil in basil shoot was observed at concentrations of 10 and 20 mg P L^{-1} ($G_1AM_1P_2$ and $G_1AM_1P_3$) (Figure 2). The GC/MS output indicated that the highest value of caryophyllene, methyl chavicol, and neral were observed in $G_0AM_1P_4$, $G_1AM_0P_3$, $G_1AM_1P_3$, and, respectively (Figure 3). Also, the GC/MS output showed that the value of compounds such as caryophyllene oxide decreased by mycorrhizal inoculation in non-magnetic and magnetic treatments (G_0AM_1P and G_1AM_1P) (Figure 3). All compounds of basil essential oil under these treatments were reported in previous findings (Shabani et al. 2017).

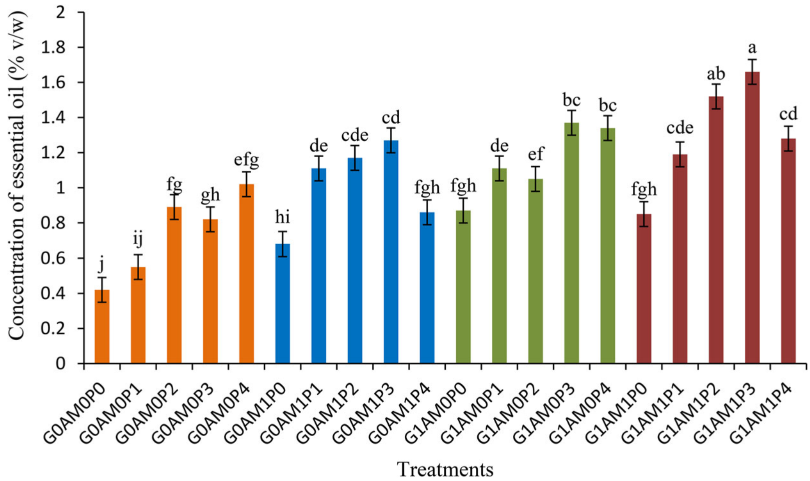


Figure 2. The concentration of essential oil (%, ml per 100 g dry wt.) in basil plants as affected by magnetic solution (G), arbuscular mycorrhizal fungi (AM), and different concentrations of P (P). Histograms with a differing letter are significantly different by Duncan's multiple range test (* $p < 0.05$).

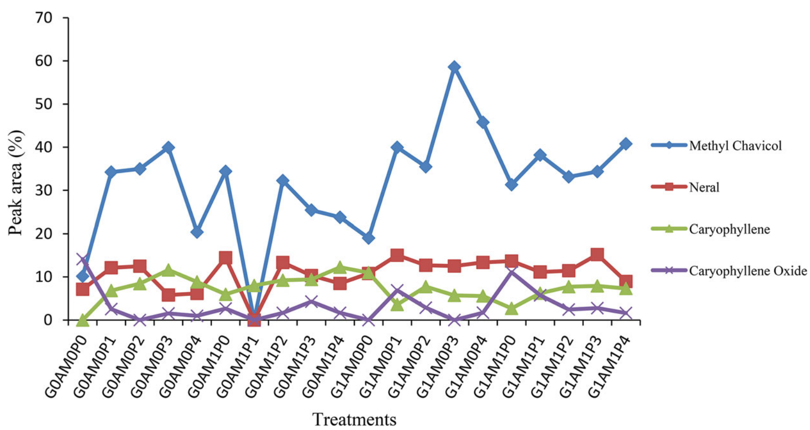


Figure 3. The most important compositions of the essential oils of basil plants as affected by magnetic solution (G), arbuscular mycorrhizal fungi (AM), and different concentrations of P (P). The treatments which tangents to the X-axis were *not detected.*

Discussion

The present study showed that the magnetic field can positively influence on basil performances, in particular on LFW content. Previous studies have pointed to various reasons for improving the plant growth by magnetic fields, including stimulatory influence on fresh weight, average nucleic acids level and assimilatory pigments level as well the chlorophyll ratio (Racuciu, Creanga, and Horga 2008); phyto-hormone production (Maheshwari and Grewal 2009); water use efficiency, stomatal conductance and cell division (Aliverdi, Parsa, and Hammami 2015). The use of magnetic field created a better position to absorb N, P and K. Some previous studies hypothesized that magnetic field decreases the pH of the cell wall (Racuciu, Creanga, and Horga 2008), impresses the structure of cell membranes, enhances their permeability and ion transport through ion channels (Balouchi and Modarres Sanavy 2009) and increases the absorption and assimilation of nutrients (Racuciu, Creanga, and Horga 2008), and as a result increases the availability of mineral elements and plant growth. The above statements imply that the magnetic solution probably alters something in the water, makes the water more functional within the plant system, and therefore probably influences the plant growth at cell level (Maheshwari and Grewal 2009).

Therefore, it can be expected that the magnetic field with the effect on plant growth can play a key role in increasing the essential oil of the basil plants.

Our funding demonstrated that the AM fungi symbiosis could enhance the fresh weight of basil leaves, which is in agreement with the results of other studies (Smith, Smith, and Jakobsen 2003; Copetta, Lingua, and Berta 2006; Toussaint, Smith, and Smith 2007; Scagel and Lee 2012). This may due to increased uptake of nutrients with low mobility, such as P, Fe, Cu, and Zn. Also, physiological processes like water absorption capacity of plants can improve by AM fungi due to enhanced root hydraulic conductivity and favorably adjusting the osmotic balance and composition of carbohydrates (Colla et al. 2008). Almost all previous researches indicated that root colonization by AM can alter plant biomass, nutrient status, and carbon use and therefore may also alter the production and composition of secondary metabolites in plants (Copetta, Lingua, and Berta 2006; Toussaint, Smith, and Smith 2007; Scagel and Lee 2012).

Our findings showed that when the concentration of P increased from 0 to 40 mg L^{-1}, the LFW, N, P, K, Ca, and Mg contents in plant tissues and pH and EC of soil solution increased linearly. Similar results have been reported by Colla et al. (2008) in zucchini plants at two P concentrations (0.3 and 1 mM). Also, NO_3 content and colonization rate of basil decreased with increasing of P in nutrition solution. Apparently, under different P levels, EC and pH changes could be one of the main parameters in increasing of the mineral elements in basil tissues (Akhtar, Oki, and Adachi 2009). The content of essential oil can enhance by P fertilizer as well as caused changes in the balance between carbon use in growth and secondary metabolite production (Nguyen and Niemeyer 2008).

Interaction between magnetic field and AM indicated that improvement of basil leaf growth not only was affected by the percentage of root colonization, but also influenced by changes in soil rhizosphere such as pH and EC, and consequently, the amount of P adsorbed in the shoot (Table 2). Acidification of the rhizosphere by H^+-efflux can probably increase orthophosphate availability and thus lead to the above results (Akhtar, Oki, and Adachi 2009). Compared to the control treatment (G_0AM_0), concomitant use of magnetic field and AM fungi (G_1AM_1), reduce and increase the pH and EC of soil solution, respectively (Table 2). Also, the average LFW of basil plants in G_1AM_0 and G_1AM_1 was 1.3 and 1.2 times that of the control, respectively. Shoot nitrate concentrations showed that the lowest amount of nitrate in basil plants was observed when the magnetic field and AM fungi were simultaneously used. Concomitant use of the magnetic field and AM fungi reduced 13.09% nitrate content of shoots. However, in separate use of the magnetic field and AM fungi (G_1AM_0 and G_0AM_1), the results are contrary to the above findings (Table 2). It seems that the magnetic field and AM fungi probably play an effective role in the regeneration of nitrate and N assimilation, leading to the production of secondary metabolites, amino acids, and other N compounds. It requires further studies on the cellular and molecular level for a detailed study of the reasons.

Although the interaction between magnetic solution and P concentrations on LFW was not significant, but the highest amount of shoot fresh weight (data not shown) was observed in their combined effects ($p \leq 0.05$). Each level of P in magnetic treatment compared to non-magnetic treatment had a lower soil pH and percentage of root colonization and a higher level of soil EC. Also, N, P, and K amount of shoots were strongly influenced by the magnetic field and P concentrations. A similar finding by Maheshwari and Grewal (2009) in snow pea showed that magnetically treated potable and recycled water resulted in a significant increase in soil $EC_{1:5}$ and a significant decrease in soil $pH_{1:5}$ values after the harvest of snow pea plants. They stated that organic acids released in the rhizosphere by celery and snow pea plants irrigated with magnetically treated water may be influencing desorption of P and K from soil adsorbed P on colloidal complex, and thus making these nutrients more available to plants. Also, other scientists reported that positive combined effects of magnetic solution under and P concentrations could be related to direct influence of magnetic field on the function of ion channels (Ghanati et al. 2015) or originate from their affection on the physical and chemical properties of the rhizosphere (e.g., pH and EC) (Hozayn and Abdul-Qados 2010). These researchers deduced that the magnetic solution enhanced the soil water holding capacity and as a result the availability of soil nutrients. On the other hand, it has been reported that the rate of P supply in the soil and the ability of plants to absorb P increased by reduction of soil acidity close to neutral soil pH values (Jokubauskaite et al. 2015). So, it reasonable that pH changes could be one of the main factors associated with an enhancing of P ($r = 0.65$) in basil tissues under different P magnetic solutions (Shabani et al. 2018).

The interaction of AM and P demonstrated that the growth of basil plants has been influenced by P ($r = 0.91$) more than any other mineral. In the interaction of AM and P, despite the decrease in colonization rate, P content and leaf growth showed an increasing trend (Table 2). It seems that a sudden increase in P from 0 to 5 mg L^{-1} acts such a starter and, by improving plant growth and providing a carbon source for the fungus, increases the growth of the fungus, the production of mycelium and as a result increases the colonization rate of basil plants (56% more than control). The previous reports showed that high P can prevent AM colonization of plant roots, decrease the formation of entry joints and vesicles, and reduce the length of external hyphae associated with AM (Colla et al. 2008). LFW increased in the AM treatments even under high P concentrations, demonstrating that AM colonization remained mostly high even under 20 and 40 mg L^{-1} of P (51.66 and 37.48%, respectively). It seems that this ability is related to the amount of external mycelium produced by each AM fungus and to the redundancy of root colonization in terms of active and alive fungal structures (Baum, El-Tohamy, and Grudac 2015). The reason for the increase in plant growth can be that the high daily additions of high soluble P concentrations (20 and 40 mg L^{-1}) did not fully remove AM colonization. As a result, high P availability was temporary and thus allowed AM colonization to endure even at high concentrations of P (Colla et al. 2008). Therefore, it seems that increasing the P content of basil shoots is associated to changing of the pH and enhancing of the organic acids excretion (Maheshwari and Grewal 2009) or improving physical exploration of the soil pore by AM fungi (Kapoor, Giri, and Mukerji 2004) which leads to improving P uptake. According to the insignificance of soil pH in AM and P interaction (Table 2), the effect of AM colonization on root growth and absorption of water and nutrient mineral is inevitable. In this regard, Kapoor, Giri, and Mukerji (2004) suggested that the extent of mycorrhizal colonization is not a guide to the efficiency of the mycorrhizal associations. Therefore, improving basil growth can be associated with positive mycorrhizal effects, such as the formation of extensive hyphal networks, increasing water and nutrients uptake and translocation (especially P) to the plant, improving soil structure and photosynthesis in the host plant (Miransari 2010) and higher leaf water potential and osmotic adjustment (Aroca, Vernieri, and Ruiz-Lozano 2008).

The essential oil yield of basil was increased significantly by the use of magnetic field, AM symbiosis and P fertilizer independently, while the highest concentration of essential oil was observed in simultaneous use of the magnetic field and AM fungi in concentrations of 10 and 20 mg P L^{-1} ($G_1M_1P_2$ and $G_1M_1P_3$) (Figure 2). On the other hand, the positive role of P in the production of essential oils is inevitable. Methyl chavicol is synthesized through the shikimic acid pathway, which produces the aromatic amino acid precursor phenylalanine, whereas terpenoids are synthesized through the condensation of isopentenyl diphosphate (IPP) and its allelic isomer dimethylallyl diphosphate (DMAPP) (Kapoor, Giri, and Mukerji 2004). Terpenoids require acetyl-CoA, ATP, and NADPH for synthesis. Therefore, the biosynthesis of essential oil is dependent on inorganic P content in the plant (Kapoor, Giri, and Mukerji 2004). Our results demonstrated that magnetic solution and colonization of basil plants resulted in enhanced P concentration in plants over G_0 and AM_0 control plants which in turn ameliorated essential oil content. Chemical analyses exhibited that in basil, methyl chavicol was the most abundant oil, followed by neral, caryophyllene, caryophyllene oxide.

The previous findings showed that the inoculation of basil plants with AM fungi decrease the yield of caryophyllene (Copetta, Lingua, and Berta 2006) and exposure with magnetic field enhanced contents of caryophyllene oxide and resulted the decrement of basil plants growth (Ghanati et al. 2007), While our results showed that the simultaneous use of mycorrhizal fungus and magnetic P solution decreased the amount of caryophyllene oxide and increased caryophyllene, finally enhanced basil growth and main compounds of essential oil in treated plants. The separate use of AM fungi (G_0AM_1P) reduced the methyl chavicol, neral and increased caryophyllene levels. On the other hand, the separate use of the magnetic field (G_1AM_0P) increased the methyl chavicol, neral, and reduced the caryophyllene. Findings by Copetta, Lingua, and Berta (2006) suggested that inoculation of basil plants with *Gigaspora rosea* increased levels of hormones such as gibberellin, auxin and cytokinin. These high levels of hormone related to a greater number of peltate glands. They concluded that the increase in peltate glands by AM fungi was responsible for increasing the production of essential oil in colonized basil. Thus, it seems that AM fungi indirectly increased the number of peltate glands and essential oil by affecting hormonal levels.

Also, simultaneous use of the magnetic solution and AM fungi (G_1AM_1P) reduced the phenylpropanoids such as methyl chavicol and increased monoterpenes and sesquiterpenes such as neral and caryophyllene and also decreased the stress enzymes such as caryophyllene oxide. On the other hand, the treatment of the magnetic field and AM fungi at different levels of P reduced the amount of phenol and flavonoids in the basil plants (data not shown). Alteration of plant response from production of phenolic compounds to essential oil, could be one of the reasons for the relative increase of terpenoids in the simultaneous use of AM fungi and magnetic P solution. This finding is in accordance with the observation of Ghanati et al. (2007) in basil plants treated with magnetic field.

Conclusion

The results of this experiment showed that the magnetic solution and AM play an important role in the growth and production of essential oils of basil plants by improving the conditions of the rhizosphere and increasing the amount of P absorption. The value of basil's nutrition increased by nitrate reduction in magnetic field and AM fungi treatments. The Use of magnetic field and AM colonization increased basal essential oil yield by 36.36% and 38.09% respectively. Also, the application of P fertilizers (20 mg P L^{-1}) increased the yield of essential oil by 113.33% compared to the control treatment. The interaction effects of treatments had no significant effect on essential oil yield. While, the highest concentration of essential oil was observed in the simultaneous use of the magnetic field, AM fungi in concentrations of 10 and 20 mg P L^{-1}. Overall, the results of this experiment showed that magnetic field was effective on the water characteristics and increased solubility of the ions, while the positive effects of the AM fungi were due to their effect on root growth and absorption of water and nutrient mineral. From the practical point of view, the increase in leaf fresh biomass and oil percentage induced by magnetic P solution and AM fungi may represent an innovative way for improving the growth and production of essential oils in plants for growers and owners of pharmaceutical and cosmetic manufacturers.

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Availability of data and material

According to the rules of the university, the submission of thesis data for paper publishers and other universities is prohibited. So the data is confidential.

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