INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 11, No. 3, p. 31-45, 2017
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Motivate the production of pharmaceutical compounds in *Ocimum basilicum* by magnetic phosphorus solution and Arbuscular mycorrhizal fungi

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Article published on September 17, 2017

Key words: Antioxidant activity, Antioxidant enzymes, Basil, Essential oil, Phenolic compounds.

Abstract

Vegetable production by using of physical methods as a way to increase the quantity and quality of crops are considered. A greenhouse experiment was conducted to assess the effects of magnetic field (G), arbuscular mycorrhizal fungi (M) and phosphorus (P) concentration in the nutrient solution (0, 5, 10, 20 and 40 mg L^{-1}) on the fresh weight, antioxidant enzymes activity, production of phenolic compounds and essential oil components of basil plants. The experiment was designed as a factorial combination and treatments were arranged in a completely randomized design with four replicates. Treatment of basil plants with G, M and P led to increase of the plant growth, carotenoid contents (CAR), antioxidant activity, antioxidant enzyme contents such as catalase (CAT) and proxidase (POD) as well as decrease in phenol and flavonoid compounds content. Methyl chavicol, methyl eugenol and sesquiterpenes like α–bisabolene, α-humulene and caryophyllene content was increased by magnetic P solution and mycorrhizal fungi colonization. Therefore, magnetic P solution and M potentially represent natural ways of promoting growth and motivate the production of pharmaceutical compounds in this important medicinal herb.

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Introduction

Basil (*Ocimum basilicum*) is an aromatic plant, cosmopolitan herb and belonging to the Lamiaceae. Members of this family especially basil and its essential oil have been traditionally used for their medicinal properties (Toussaint *et al.,* 2007) and therapy of many pains, such as diarrhea, coughs, headaches (Javanmardi *et al.,* 2002) rhinitis, dysentery, nausea and mental fatigue colds (Hassanpouraghdam *et al.,* 2011). Nowadays, it is recognized that pharmaceutical properties of basil are due to anthocyanins as well as secondary metabolites such as phenolic compounds (including phenylpropanoids, flavonoids) and essential oil (Toussaint *et al.,* 2007). Essential oils are volatile compounds that their concentrations of individual components and chemical composition are influenced by several unified parameters such as agronomic parameters (macro and micronutrients availability, fertilization and irrigation regime), growing conditions (greenhouse production, different soilless culture systems and wild habitats), geographical and climatological conditions (water availability, soil characteristics, temperature and light quality and quantity), biochemical and genetical criteria (natural hybridization and subspecies) (Hassanpouraghdam *et al.,* 2010).

Many spices belonging to the Lamiaceae family, such as oregano, thyme and sage show strong antioxidant activity (Javanmardi *et al.,* 2003). Oxidation of lipids or other molecules can delay or inhibit by antioxidants compounds. They inhibit the initiation or propagation of oxidative chain reactions (Velioglu *et al.,* 1998). The antioxidative effect is mostly because of phenolic components such as phenolic acids, flavonoids and phenolic diterpenes. It mostly due to their redox properties, which can plays a vital role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Javanmardi *et al.,* 2003).

Phosphorus (P) is a crucial macronutrient that plays a great role in plant growth. P is a component of nucleic acids and adenosine triphosphate (ATP). So, it has a dominant role in plant reproduction, cellular metabolism and output of many biochemically and structural functional portions (Jokubauskaite *et al.,* 2015). Previous finding showed that P nutrition improved biomass and concentration of phytochemical compounds in sweet basil (Toussaint *et al.,* 2007).

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; Ghanati *et al.,* 2007; Hajnorouzi *et al.,* 2011; Ghanti *et al.,* 2015). Vegetable production by using of physical methods especially magnetic fields as a way to increase the quantity and quality of crops are consider (Aladjadjiyan, 2007). Activity of enzymes vitally depends on enhancing of water content in the solvents. In interactions between the enzyme-bound and organic solvent, the importance of water layer is rather than enzyme itself (Zaks and Klibanov, 1988). So, it seems that with changing the properties of water molecules by G, the cellular and molecular characteristics of plant tissues as a result of irrigation water will be affected. Several studies demonstrated that G changes surface tension enthalpies and viscosity of water as well as it increase polarization features of water and clustering structure of hydrogen-bonded chains (Chang, 2006; Toledo *et al.,* 2008; Dong, 2008; Zhang *et al.,* 2014). Previous studies have documented antonym results about the actual effect of G on the activities of antioxidant enzymes and production of phenolic components. Some results shown that exposure of plant cells to G affect adversely the activity of certain antioxidant enzymes involved in scavenging of reactive oxygen species (ROS). For example, Sahebjamei *et al.* (2007) stated that using of G decreased activity of the CAT and ascorbate peroxidase (APX) in maize. Whereas, Belyavskaya (2004) reported that in barley seedlings after 24 h exposure with 10 μT G the CAT activity enhanced slightly, while after 48 and 72 h it increased nearly by 30% and 100%, respectively, compared to control.

In recent years, great effort has been devoted to the study about the effects of arbuscular mycorrhizal colonization on the accumulation of active phytochemicals in aerial parts of some medicinal plants. Several publications indicated that M can induce changes in the accumulation of secondary metabolites, including phenolic compounds, in host plant (Devi and Reddy, 2002; Yao *et al.,* 2003; Rojas-Andrade *et al.,* 2003; Toussaint *et al.,* 2007; Mollavali *et al.,* 2016). Generally, very few publications can be found about the main effect of magnetic P solution and M on essential oil and enzyme activity. As first report, we have investigated whether passing of P solution from G and colonization of basil by M can improve the growth, activity of certain free radical scavenger enzymes, production of phenolic compounds and essential oil composition.

Material and Method

Plant growth conditions and greenhouse experiment

A pot experiment was performed during June to August 2016 on a sandy-loam soil. Physical and chemical characteristics of the soil were shown in Table 1. *Diversispora versiformis* (obtained from the Soil Biology Laboratory, University of Tabriz, Iran) was used in this experiment, its inoculants were prepared by pot culture of sorghum (*Sorghum bicolor* L.) as the host plant. Plastic pots (20 cm inner diameter, 30 cm depth) filled with autoclaved soil (2.5 kg) and forty grams of M inoculants were distributed over the soil at a depth of 10-15 cm and covered with soil. Basil seeds were planted at 2 cm depth in plastic pots. At the two-leaf stage, the seedlings were thinned to four plants per pot and the pot surfaces were covered with perlite medium grain and cork (to reduce evaporation from the soil surface). All of plants were grown under controlled conditions at 27◦C for 14 h per day and 18◦C for 10 h per night with 800 µmol $m^{-2}s^{-1}$ light intensity. Based on soil analysis, the field capacity (FC) of soil was 10 percent. So, all the pots received 250 mL of plant nutrient solution (g L^{-1}: K_2SO_4: 2.22, $Ca(NO_3)_2.4H_2O$: 12.64, $(NH_4)_2SO_4$: 2.35, $MgSO_4.7H_2O$: 10.13, $MnSO_4.4H_2O$: 0.4, $FeSO_4.7H_2O$: 0.74, $CuSO_4.7H_2O$: 0.19, $ZnSO_4.7H_2O$: 0.43, H_3BO_3: 0.05 and Na_2MoO_4: 0.0001) three times during the first two weeks by 50 mL syringe. Tap water was used to prepare a nutrient solution and watering plants. Chemical characteristics of this water measured that include: (pH: 7.7, EC: 0.49 dS m^{-1}, P: 0.05, K: 4.3, Ca: 42, Mg: 11, Fe: 0.1, Zn: 0.6, Mn: 0.0, Cu: 0.0 and Na: 3.5 mg L^{-1}). The plants were irrigated a few days more than field capacity in order to better plant establishment. After this time, the pots were weighed daily by digital weigh scale and water loss by evapotranspiration was added by syringe until FC to each pot. In addition, two pots with no plants were prepared to monitor water loss by evaporation for each treatment. Five different concentrations of P were made in 50-liter barrels. P was added as $Ca(H_2PO_4)_2.H_2O$ at concentrations of 0, 5, 10, 20 and 40 mg L^{-1}. P solution was passed from MF 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. Magnetic P solution was used between 5th to 7th weeks every two days (7 times). When water flows along the pipe axis through the device, G 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 helical motion along the axis. This motion causes the ion to act as a microscopy stirrer in water.

Measurement of fresh weight (FW)

Plants were harvested 56 days (8 weeks) after cultivation and fresh weight of aerial part of basil was determined by digital weigh scale.

Determination of total carotenoid contents

According to the method of Lichtenthaler and Buschmann (2001) the quantitative determination of total carotenoids in a whole pigment extract of green plant tissue was measured by spectrophotometer (UV-2100, China) at 470 nm. In this method, methanol was used as solvent. Total carotenoids were expressed based on mg/g fresh weight of basil leaf.

Extract preparation

Fresh samples of basil leaves were frozen with liquid N_2 and transferred to laboratory. To frozen leaf material (0.2 g) was added 0.5 N HCl in methanol/Milli-Q water (80% v/v). The mixture was incubated overnight at 4 °C and then centrifuged for 20 min at 4 °C and 20000g. Supernatant was recovered and this hydroalcoholic extract was used for antioxidant capacity, total phenolics and flavonoids assays (Cantin *et al.,* 2009).

Estimation of total antioxidant activity by using DPPH scavenging assay

Antioxidant activity was determined by reacting 50 µL of the extracts with 950 µL of 60 µM 2,2-diphenyl-1-picryl-hydrazyl (DPPH) as described by Koleva *et al.* (2002) with minor modifications. The solution was mixed by vortex and left at room temperature for 30 min in dark. Absorbance was measured at 515 nm with spectrophotometer (Unico, UV-2100, UK). The antiradical activity was calculated by the following ratio:

$$\text{Antioxidant activity} = A_{control} - A_{sample}/A_{control} \times 100$$

($A_{control}$: absorbance of the DPPH solution A_{sample}: absorbance of the DPPH solution after the addition of the sample).

Determination of total phenolic contents

Total phenolic contents of basil samples in methanol extracts were determined by the Folin–Ciocalteu method as described previously with minor modifications (Waterhouse, 2001). Briefly, 30 µL of the extract was mixed with 75 µL Folin–Ciocalteu reagent, 225 µL 20% sodium carbonate and 1.1 mL distilled water, and placed at 40 °C for 30 min. Absorbance of samples was measured at 765 nm. A standard curve was constructed with different concentrations of gallic acid. The results were expressed in milligrams (mg) of gallic acid equivalents (GAE) per 100 g of FW (mg GAE 100 g^{-1} FW).

Determination of total flavonoid contents

Colorimetric aluminum chloride method was used for flavonoid determination (Chang *et al.,* 2002). Briefly, 100 µL solution of each plant extracts in methanol were separately mixed with 100 µL of distilled water, 600 µL of methanol 95%, 40 µL of 10% aluminum chloride, 40 µL of 1 M potassium acetate and 1120 µL of distilled water again, the solution was mixed by vortex and left at room temperature for 40 min; the absorbance of the reaction mixture was measured at 415 nm with spectrophotometer (Unico, UV-2100, UK). Total flavonoid contents were calculated as quercetin from a calibration curve. The results were expressed in mg of quercetin equivalents (QE) per 100 g of FW (mg QE 100 g^{-1} FW).

Extraction and assay of the enzymes of antioxidant system

To measure the specific activities of POD, CAT and PPO, frozen samples (200 mg fresh weight of leaf) were powdered in liquid nitrogen and homogenized in 1.3 ml of 0.05 M potassium phosphate buffer of pH 7.2 containing 1 mM ethylene diamine tetra acetic acid (EDTA) by a homogenizer into microtubes. The homogenate was centrifuged at 14000g for 16 min. All operations were performed at 4°C and the supernatant used as the source of enzyme extraction. Enzyme activities were expressed based on change in absorbance against protein content. Protein contents were determined by the method of Bradford (1976), using bovine serum albumin (BSA) as a standard.

Activity of POD was measured in a reaction mixture consisted of 100 µL of enzyme extraction, 1780 µL of 0.05 M potassium phosphate buffer, 20 µL of 20 mM guaiacol, 100 µL of 10 mM H_2O_2. The reaction was initiated by adding 100 µL of H_2O_2 with concentration of 10 mM. The reaction rate was monitored by the decrease in absorbance at 470 nm in 1 minute (Chance and Maehly, 1955).

Activity of CAT was measured in a reaction mixture consisted of 100 µL of 30 mM H_2O_2, 1700 µL of 0.05 M potassium phosphate buffer and 200 µL of enzyme extraction. The decomposition of H_2O_2 was followed by the decrease in absorbance at 240 nm in 1 minute (Aebi, 1984).

Activity of PPO was measured in a reaction mixture consisted of 100 µL of enzyme extraction, 700 µL of 0.05 M potassium phosphate buffer and 700 µL of 10 mM pyrocatechol. The reaction rate was monitored by the increase in the absorbance at 410 nm in 1 minute (Mayer *et al.,* 1966).

Extraction and assay of essential oil

Fifty grams of air dried powdered plant materials (aerial parts) were subjected to hydro-distillation for 3 h using an all–glass Clevenger type apparatus. GC-MS analysis was carried out on a GC-MS system (Agilent, 19091S-443, USA) equipped with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The oven temperature was programmed as follows: 70°C (held 3 min) raised to 120°C at a rate of 10°C/min (held 2 min), then heated to 150°C at the 10°C/min rate (held 2 min) and finally increased to 240°C at 7°C/min and held 5 min in this temperature. Transfer line temperature was 160 °C. The components of the essential oil were identified by comparison of their mass spectra with those of similar compounds from the NIST library databases.

Statistical analysis

The experiment was arranged in a completely randomized factorial design using three factors (G, M and different concentrations of P) with four replications under greenhouse conditions. A statistical analysis was made by using analysis of variance in the SAS 9.1 software and means were compared using Duncan's multiple range tests at 5% level of significance.

Results

Magnetic solution

The results indicated that the effects of magnetic solution on all the measured characteristics were significant ($P<0.01$). Based on the results shown in Table 2, G_1 had beneficial effect on vegetative and biochemical parameters of basil in comparison with G_0. Using of magnetic solution increased FW 14.18 % as compared to the control. Although, using of magnetic solution significantly ($P<0.01$) decreased phenol and flavonoid contents whereas it intensely increased ($P<0.01$) CAR and enzymes of antioxidant system like POD, CAT, PPO and as a result increased total antioxidant activity. In comparison with the G_0, passing of P from G increased 14.81, 47.91, 50, 15.38 and 11.67%, CAR, POD, CAT and PPO, respectively.

Table 1. Physical and chemical characteristics of the soil at the experiment.

Sand (%)	Clay (%)	Silt (%)	pH (1:1)	EC (dS m^{-1}) (1:1)	FC	Saturation Percentage	Organic Carbon	Calcium carbonate equivalent (%)	Total N
76.78	16.55	6.67	7.25	0.6	10	23.5	0.1	0.0	0.08
P	K	Ca	Mg	Fe	Mn	Zn	Cu		
				Available(mg kg^{-1})					
4.4	82.6	49	99	1.8	1.1	0.9	1.3		

In fact, magnetic solution was more efficient on POD and CAT than PPO (Table 2).

The GC and GC/MS analysis exhibited that the amount of compounds such as neral, geranial, α-bergamotene, α-humulene and caryophyllene oxide decreased although the amount of compounds such as methyl chavicol, methyl eugenol and caryophyllene increased in basil plants exposed to magnetic solution (Table 5).

Arbuscular mycorrhizal fungi

Inoculation of basil plants with M significantly increased FW ($P<0.05$), CAR, CAT, PPO and DPPH ($P<0.01$), but it show no significant effect on POD activity and significantly decreased phenol and flavonoid contents ($P<0.01$) (Table 2). The results indicated that M effectively influence on the amount of CAR and activity of CAT in comparison with other enzymes and antioxidant compounds (Table 2).

Table 2. Effect of magnetic solution (G), arbuscular mycorrhizal fungi (M) and phosphorus (P) on FW, CAR, POD, CAT, PPO, DPPH, Phenol and Flavonoid in basil.

Treatment	FW (g/pot)	CAR (mg/g)	POD (U/mg pro)	CAT (U/mg pro)	PPO (U/mg pro)	DPPH (%)	Phenol (mgGA/100g FW)	Flavonoid (mg QE/100g Fw)
G								
G_0	16.39[b]	0.253 [b]	0.025 [b]	0.028 [b]	1.21 [b]	52.79[b]	321.06 [a]	3.67 [a]
G_1	19.10[a]	0.297 [a]	0.048 [a]	0.056 [a]	1.43 [a]	59.77[a]	266.92 [b]	3.48 [b]
Significance	**	**	**	**	**	**	**	**
M								
M_0	16.87[b]	0.268 [b]	0.0375	0.041 [b]	1.31 [b]	55.06[b]	301.72 [a]	3.69 [a]
M_1	18.62[a]	0.281 [a]	0.0370	0.043 [a]	1.33 [a]	57.50[a]	286.26 [b]	3.46 [b]
Significance	*	**	ns	**	**	**	**	**
P								
P_0	8.42 [e]	0.208 [e]	0.013 [e]	0.012 [e]	1.07 [e]	43.06[e]	404.68 [a]	4.54 [a]
P_1	14.15[d]	0.234 [d]	0.028 [d]	0.032 [d]	1.21 [d]	51.07[d]	339.69 [b]	4.15 [b]
P_2	18.73[c]	0.272 [c]	0.041 [c]	0.047 [c]	1.37 [c]	57.10[c]	307.36 [c]	3.87 [c]
P_3	22.14[b]	0.310 [b]	0.050 [b]	0.058 [b]	1.48 [a]	62.56[b]	239.07 [d]	2.97 [d]
P_4	25.27[a]	0.351 [a]	0.053 [a]	0.061 [a]	1.46 [b]	67.61[a]	179.16 [e]	2.35 [e]
Significance	**	**	**	**	**	**	**	**

* and ** 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; values followed by the same letter are not significantly different ($p \leq 0.05$) using Duncan's multiple range test.

The GC and GC/MS analysis exhibited that the amount of compounds such as methyl chavicol, lemonal, neral and methyl eugenol decreased by mycorrhizal inoculation whereas the amount of compounds such as ß-cubebene, caryophyllene, α-bergamotene, ß-farnesene, α-humulene, germacrene-D and α-bisabolene increased (Table 4).

Different concentrations of P

Increasing P levels in soil improved FW, CAR, POD, CAT, PPO and DPPH significantly ($P<0.01$), whereas phenol and flavonoid content was decreased significantly ($P<0.01$).

The results showed that all P levels were better than the control (P_0) and level of 40 mg L^{-1} was higher than other levels (except for phenol and flavonoid). Using of P fertilizer increased antioxidant power by 36.31% compared to the control (P_0). Based on the results shown in Table 2 different concentrations of P had more impact on amount of CAR (40.74%), POD activity (75.47%) and CAT (80.32%) than PPO activity (26.71).

The GC and GC/MS analysis exhibited that lemonal, geranial and caryophyllene oxide was decreased while the amount of compounds such as methyl chavicol, neral, methyl eugenol ß-cubebene, caryophyllene, α-humulene, germacrene-D and α-bisabolene was increased by application of P to basil plants (Table 4).

Application of magnetic solution and arbuscular mycorrhizal fungi

The concomitant use of magnetic solution and M significantly increased all measured traits (except phenol and flavonoid) compared with non-magnetic and inoculated treatment (G_0M_0) (Tables 3). The highest amount of growth parameter, CAR (Fig. 1), activity of antioxidant enzymes and antioxidant capacity observed in G_1M_1 and G_1M_0 (Tables 3 and Fig. 1).

Using a combination of magnetic P solution and M showed that the amount of compounds such as Neral, Geranial, α-Bergamotene and Caryophyllene Oxide was decreased whereas the amount of compounds such as Methyl Chavicol, Methyl Eugenol, α–Bisabolene, α-Humulene and Caryophyllene was increased distinctly.

Application of magnetic solution and different concentrations of P

All measured characteristics (except phenol and flavonoid) in present study were increased significantly by application of G in different levels of P (Tables 3) and (Fig. 2).

The highest amount was observed in FW, antioxidant compounds and enzymes when the P solutions were passed from 110 mT magnetic field. The highest change was observed in treatments G_0P_3, G_0P_4, G_1P_3 and G_1P_4 for FW, G_1P_3 and G_1P_4 for CAR (Fig. 2), POD, CAT and PPO (Table 3). However, both of magnetic and non-magnetic treatment in different concentration of P decreased phenol and flavonoid contents.

Application of arbuscular mycorrhizal fungi and different concentrations of P

Interaction effect of M and P on FW and POD was not significant (Tables 3). Whereas CAR, CAT, PPO and DPPH were significantly increased (Fig. 3 and Table 3). The highest activity of antioxidant enzymes was observed in G_1P_3 treatment (Table 3). Like other treatments, application of M and different concentrations of P decreased phenol and flavonoid contents (Table 3).

Table 3. The results of interaction between magnetic solution (G), arbuscular mycorrhizal fungi (M) and phosphorus (P) on FW, POD, CAT, PPO, DPPH, Phenol and Flavonoid in basil.

Treatment	FW (g/pot)	POD (U/mg pro)	CAT (U/mg pro)	PPO (U/mg pro)	DPPH (%)	Phenol (eqGA/100g FW)	Flavonid (mg QE/100g Fw)
GM							
G_0M_0	14.60 ^{b}	0.024 ^{d}	0.025 ^{d}	1.16 ^{d}	49.75 ^{c}	345.71 ^{a}	3.95 ^{a}
G_0M_1	18.18 ^{a}	0.026 ^{c}	0.031 ^{c}	1.27 ^{c}	55.82 ^{b}	296.40 ^{b}	3.54 ^{b}
G_1M_0	19.14 ^{a}	0.050 ^{a}	0.058 ^{a}	1.39 ^{b}	60.37 ^{a}	257.72 ^{d}	3.42 ^{c}
G_1M_1	19.06 ^{a}	0.047 ^{a}	0.054 ^{b}	1.47 ^{a}	59.17 ^{a}	276.12 ^{c}	3.39 ^{c}
Significance	*	**	**	**	**	**	**
GP							
G_0P_0	7.34 ^{g}	0.011 ^{i}	0.010 ^{j}	1.03 ^{i}	37.36 ^{f}	452.49 ^{a}	4.77 ^{a}
G_0P_1	13.06 ^{e}	0.018 ^{g}	0.017 ^{h}	1.13 ^{h}	48.06 ^{e}	382.36 ^{b}	4.18 ^{c}
G_0P_2	15.71 ^{d}	0.028 ^{f}	0.029 ^{g}	1.24 ^{g}	55.42 ^{d}	329.17 ^{d}	3.95 ^{d}
G_0P_3	21.93 ^{bc}	0.037 ^{d}	0.043 ^{e}	1.35 ^{d}	58.99 ^{c}	259.02 ^{g}	3.03 ^{f}
G_0P_4	23.93 ^{ab}	0.034 ^{d}	0.040 ^{f}	1.32 ^{e}	64.11 ^{b}	182.26 ^{i}	2.44 ^{g}
G_1P_0	10.08 ^{f}	0.016 ^{h}	0.013 ^{i}	1.12 ^{h}	48.75 ^{e}	356.86 ^{c}	4.32 ^{b}
G_1P_1	15.22 ^{ed}	0.038 ^{d}	0.048 ^{d}	1.30 ^{f}	54.08 ^{d}	297.02 ^{e}	4.12 ^{c}
G_1P_2	21.39 ^{c}	0.055 ^{c}	0.065 ^{c}	1.51 ^{c}	58.78 ^{c}	285.55 ^{f}	3.79 ^{e}
G_1P_3	22.27 ^{bc}	0.069 ^{a}	0.079 ^{a}	1.62 ^{a}	66.13 ^{b}	219.12 ^{h}	2.92 ^{f}
G_1P_4	26.54 ^{a}	0.066 ^{b}	0.076 ^{b}	1.59 ^{b}	71.12 ^{a}	176.06 ^{j}	2.26 ^{h}
Significance	*	**	**	**	**	**	**
MP							
M_0P_0	7.44 ^{e}	0.014 ^{e}	0.012 ^{g}	1.05 ^{h}	40.06 ^{f}	410.64 ^{a}	4.59 ^{a}
M_0P_1	13.10 ^{d}	0.028 ^{d}	0.031 ^{f}	1.20 ^{f}	50.73 ^{d}	351.92 ^{c}	4.44 ^{b}
M_0P_2	17.53 ^{c}	0.042 ^{c}	0.047 ^{d}	1.38 ^{d}	56.43 ^{c}	320.22 ^{e}	4.06 ^{c}
M_0P_3	21.22 ^{b}	0.053 ^{a}	0.060 ^{b}	1.48 ^{ab}	61.67 ^{b}	244.54 ^{g}	2.98 ^{f}
M_0P_4	25.07 ^{a}	0.050 ^{b}	0.057 ^{c}	1.45 ^{c}	66.42 ^{a}	181.27 ^{i}	2.38 ^{g}
M_1P_0	9.97 ^{e}	0.013 ^{e}	0.012 ^{g}	1.10 ^{g}	46.05 ^{e}	398.71 ^{b}	4.50 ^{ab}
M_1P_1	15.18 ^{d}	0.028 ^{d}	0.034 ^{e}	1.23 ^{e}	51.41 ^{d}	327.47 ^{d}	3.86 ^{d}
M_1P_2	19.57 ^{c}	0.041 ^{c}	0.047 ^{d}	1.37 ^{d}	57.77 ^{c}	294.50 ^{f}	3.68 ^{e}
M_1P_3	22.98 ^{b}	0.053 ^{a}	0.063 ^{a}	1.49 ^{a}	63.45 ^{b}	233.60 ^{h}	2.97 ^{f}
M_1P_4	25.40 ^{a}	0.049 ^{b}	0.059 ^{b}	1.46 ^{bc}	68.81 ^{a}	177.05 ^{j}	2.32 ^{g}
Significance	ns	ns	*	**	*	**	**

* and ** 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% using Duncan's multiple range test.

Discussion

The effect of magnetic field on basil growth

The present study demonstrated the beneficial effects of G on basil performances, in particular on FW content.

The positive effects of G have been demonstrated previously on maize (Turker *et al.,* 2007) snow pea and chickpea (Grewal and Maheshwari, 2011) as well as on soybean (Aliverdi *et al.,* 2015).

Table 4. GC-MS determined composition of the essential oils of basil under treatments of magnetic solution (G), arbuscular mycorrhizal fungi (M) and phosphorus (P).

Compound	Area (%)									
	$G_0M_0P_0$	$G_0M_0P_1$	$G_0M_0P_2$	$G_0M_0P_3$	$G_0M_0P_4$	$G_0M_1P_0$	$G_0M_1P_1$	$G_0M_1P_2$	$G_0M_1P_3$	$G_0M_1P_4$
Methyl chavicol	10.15	34.23	34.97	39.93	20.39	34.40	-	32.26	25.42	23.75
Lemonal	-	15.75	-	7.34	7.85	19.55	15.96	15.93	12.58	11.38
Neral	7.13	12.13	22.49	5.81	6.17	14.46	-	13.35	10.32	8.49
Methyl eugenol	3.70	3	3.51	6.08	27.16	6.98	6.97	2.55	5.38	2.87
Geranial	10.80	-	1.28	-	6.23	-	-	-	-	
α-Copaene	0.67	-	1	-	0.94	-	-	-	1.37	1.44
ß-Cubebene	-	1.14	1.34	1.53	1.32	-	-	1.20	1.71	1.86
Caryophyllene	-	6.83	8.44	11.61	8.85	5.97	8.01	9.23	9.41	12.25
α-Bergamotene	1.58	-	3.45	-	2.50	2.38	-	2.49	3.53	3.96
ß-Farnesene	-	-	-	1.32	0.95	-	-	1.21	1.33	1.78
α-Humulene	0.71	3.77	5.87	5.63	4.51	3.64	7.53	4.42	5.98	6.91
Humulene oxide II	4.22	1.39	-	-	-	1.33	4.02	-	-	0.72
Germacrene-D	-	1.96	5.41	4.08	5.12	1.33	2.65	4.04	3.08	7.26
α-Bisabolene	-	5.63	7.54	9.57	6.16	5.19	12.17	6.81	7.17	8.98
ß-Bisabolene	-	-	-	-	-	-	-	-	1.03	0.73
Caryophyllene Oxide	14.13	2.55	-	1.53	0.98	2.67	-	1.66	4.28	1.67
4,6-bis(4'-Methylpent-3'-en-1'-yl)-6-methylcyclohexane	2.13	4.91	2.42	3.60	2.96	2.19	-	4.85	2.98	4.28
Geranic acid	4.63	1.20	-	-	-	-	-	-		-
AlphaCubebene;	-	-	-	-	-	-	-	-	-	-
Nerol	-	-	-	-	0.8	-	-	-	-	-

This might be related to several physiological mechanisms such as increase of water use efficiency, permeability of membrane, cell division (Aliverdi *et al.,* 2015), stomatal conductance (Sadeghipour and Aghaei, 2013), indole-3-acetic acid (Turker *et al.,* 2007), gibberellic acid contents (Dalia *et al.,* 2009), leading to a higher water absorption capacity (Garcia-Reina and Pascual, 2001).

Also, an improvement in P and nitrogen (data not shown) absorption by magnetic treatment of solution was observed which can be effective on basil plant growth.

The effect of arbuscular mycorrhizal fungi on basil growth

Almost all previous researches indicated that the beneficial role of M symbiosis in increasing of plant growth was mainly associated to a better uptake of nutrients characterized by low mobility, such as P, Fe, Cu, and Zn through an increase in root growth (Bolandnazar *et al.,* 2007; Colla *et al.,* 2015).

Moreover, it is well established that M can remarkably enhance plant tolerance to different abiotic stresses due to production of biochemical like glomalin and the formation of extensive hyphal networks (Miransari, 2010).

The effect of different P concentrations on basil growth

Also in the present experiment significant positive effects were observed with increasing the P concentration in the nutrient solution.

Table 5. GC-MS determined composition of the essential oils of basil under treatments of magnetic solution (G), arbuscular mycorrhizal fungi (M) and phosphorus (P).

Compound	Area (%)									
	$G_1M_0P_0$	$G_1M_0P_1$	$G_1M_0P_2$	$G_1M_0P_3$	$G_1M_0P_4$	$G_1M_1P_0$	$G_1M_1P_1$	$G_1M_1P_2$	$G_1M_1P_3$	$G_1M_1P_4$
Methyl chavicol	19.01	39.97	35.46	58.59	45.77	31.31	38.19	33.13	34.35	40.78
Lemonal	12.54	-	-	-	13.52	-	-	-	-	-
Neral	10.80	15.03	12.70	12.52	13.36	13.67	11.15	11.44	15.18	8.95
Methyl eugenol	2.30	2.72	3.68	4.18	4.36	3.39	4.78	3.49	3.09	5.43
Geranial	-	18.27	14.71	13.44	-	17.07	13.19	14.04	17.68	10.98
α-Copaene	-	-	1.13	-	-	1.13	1.33	0.95	-	0.79
ß-Cubebene	-	-	1.61	-	1.72	-	-	1.51	-	1.32
Caryophyllene	10.98	3.60	7.74	5.72	5.58	2.71	6.22	7.73	7.91	7.33
α-Bergamotene	4.05	2.69	2.64	-	1.76	3.16	2.95	2.58	2.69	2.53
ß-Farnesene	-	-	1.15	-	-	-	0.81	0.97	-	1.06
α-Humulene	7.13	2.07	4.40	2.88	-	1.77	3.88	4.44	4.52	4.33
Humulene oxide II	-	-	-	-	-	-	-	-	-	-
Germacrene-D	4.22	-	3.64	-	-	-	-	3.45	3.10	4
α-Bisabolene	10.72	2.91	5.80	2.68	4.02	2.39	5.03	6.16	5.67	6.65
ß-Bisabolene	-	-	-	-	-	-	-	-	-	-
Caryophyllene Oxide	-	6.89	2.87	-	1.71	11.09	5.75	2.44	2.77	1.64
4,6-bis(4'-Methylpent-3'-en-1'-yl)-6-methylcyclohexne	5.82	2.29	2.46	-	-	3.37	2.53	4.01	3.03	4.20
Geranic acid	-	-	-	-	-	-	-	-	-	-
AlphaCubebene;	-	1.02	-	-	-	-	-	-	-	-
Nerol	-	-	-	-	-	-	-	-	-	-

It is well known that P has a predominant role in cellular metabolism and as an important part of many structural and biochemically functional components (Akhtar *et al.,* 2009).

The effect of treatments on phenolic components, total antioxidant capacity and antioxidant enzymes

As seen in tables 1 and 2, despite the decrease in phenol and flavonoid, the highest amount of total antioxidant capacity and activity of antioxidant enzymes were observed when magnetic solution, M inoculation and different P concentration were adopted. It seems that enhancing of certain secondary compounds and CAR amounts of treatments increased total antioxidant capacity which also supports photosynthetic membranes from peroxidation and cause growth improvement (Ghanati *et al.,* 2015).

Interaction between GM and GP indicated that positive effects on basil growth.

It could be attributed to their influence on the physical and chemical characteristics of the soil (e.g. pH) or originates from direct influence of G at sub-cellular level (e.g. operation of ion channels) (Ghanati *et al.*, 2015). Different reports confirm this result and suggests that G treatment of water induced even small changes in pH that may influence biological activity in seeds and emerging plants (Grewal and Maheshwari, 2011) or phyto-hormone production leading to developed cell activity and plant growth (Ahmed Ibrahim, 2013). Therefore, it needs to be clarified by further investigations at cell level.

Interaction between treatments showed that using of P magnetic solution and M can be useful tool for increasing of total antioxidant capacity and production of secondary metabolites. Previous results showed that under stress condition, mycorrhizal inoculation can increase tolerance of host plants by enhancing or decreasing the activity of antioxidant enzymes such as SOD, CAT, POD, and APX (Mollavali *et al.,* 2016).

During normal metabolism of the plant cells, ROS are formed by partial reduction of molecular oxygen during electron transfer chain in respiration or photosynthesis (Ghanati *et al.,* 2015). Scavenging and detoxification of ROS is executed by the activity of non-enzymatic antioxidants (e.g. phenolic compounds) and enzymatic antioxidants (e.g. CAT, SOD, APX, POD and PPO) and protects plant cells from oxidative damage (Mollavali *et al.*, 2016).

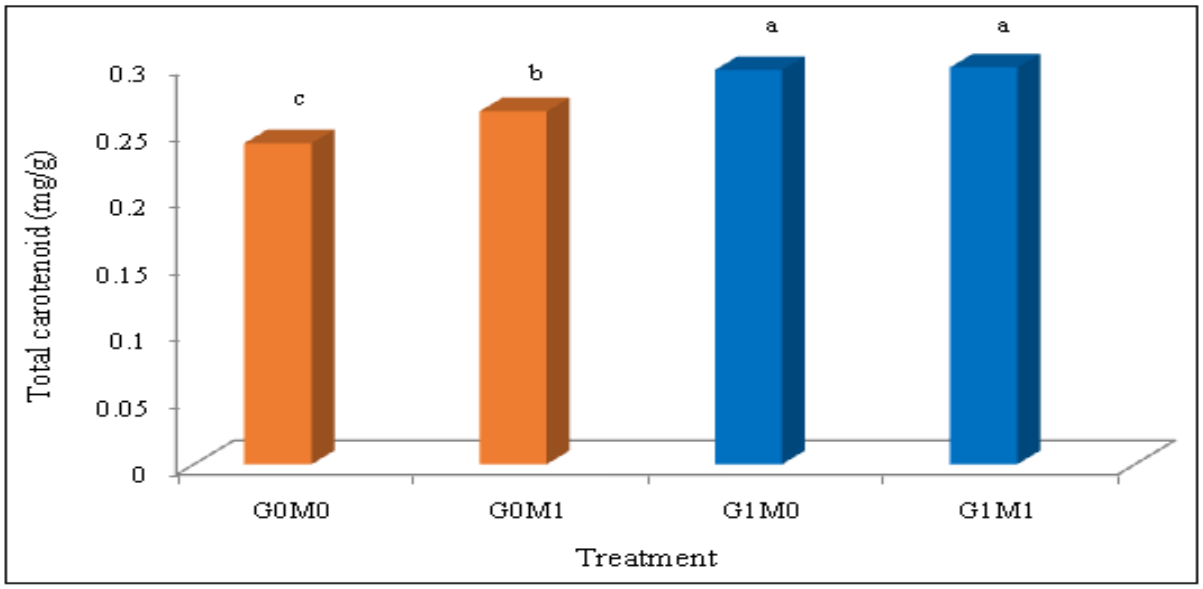

Fig. 1. Total carotenoid contents as affected by magnetic solution (G) and arbuscular mycorrhizal fungi (M). Different letters indicate significant differences according to Duncan's multiple-range test (P = 0.05).

It has been already shown that CAT removes the bulk of H_2O_2 (Dat *et al.,* 2001). All H_2O_2 scavenger enzymes act in a synergistic or cooperative way for the survival of the cell even under normal conditions. Although enzymes such as POD also convert H_2O_2 to water, but CAT is the key enzyme that effectively eliminates H_2O_2 (Sahebjamei *et al.,* 2007). Besides enzymatic antioxidant reactions, active free radicals can be scavenged by small molecules such as carotenoids, ascorbate and thiol compounds (Larson 1988). Thus, the balance between free radical generation and free radical defense determines the survival of the system (Sinha and Saxena, 2006).

The lowest content of total phenol and flavonoids were observed in lowest concentrations of P, without magnetic solution and arbuscular mycorrhizal fungi. Base on the carbon/nutrient balance (CNB) hypothesis (Bryant *et al.*, 1983) under limited nutrient conditions, plants increase their production of carbon-based compounds, particularly secondary metabolites.

Similar results with our findings were report in Dark Opal basil (Nguyen and Niemeyer, 2008). Significantly higher phenolic levels are observed when nutrient availability is limited at the lowest (0 and 5 mg/L) applied P treatments.

The effect of treatments on Essential oils composition

Essential oils are originated from two biosynthetic pathways: mevalonate pathway and shikimic acid pathway (Gang *et al.,* 2001).

Mevalonate pathway is connected with photosynthesis, assimilation and growth and results in production of terpenoides (however terpenoides are produced also by methylerythritol phosphate pathway) while shikimic acid pathway is mediated by the activity of phenyl alanine ammonialyase and leads to production of phenolic compounds. The GC/MS analysis exhibited that the amount of methyl chavicol, neral and caryophyllene were major components of essential oil of all treatments.

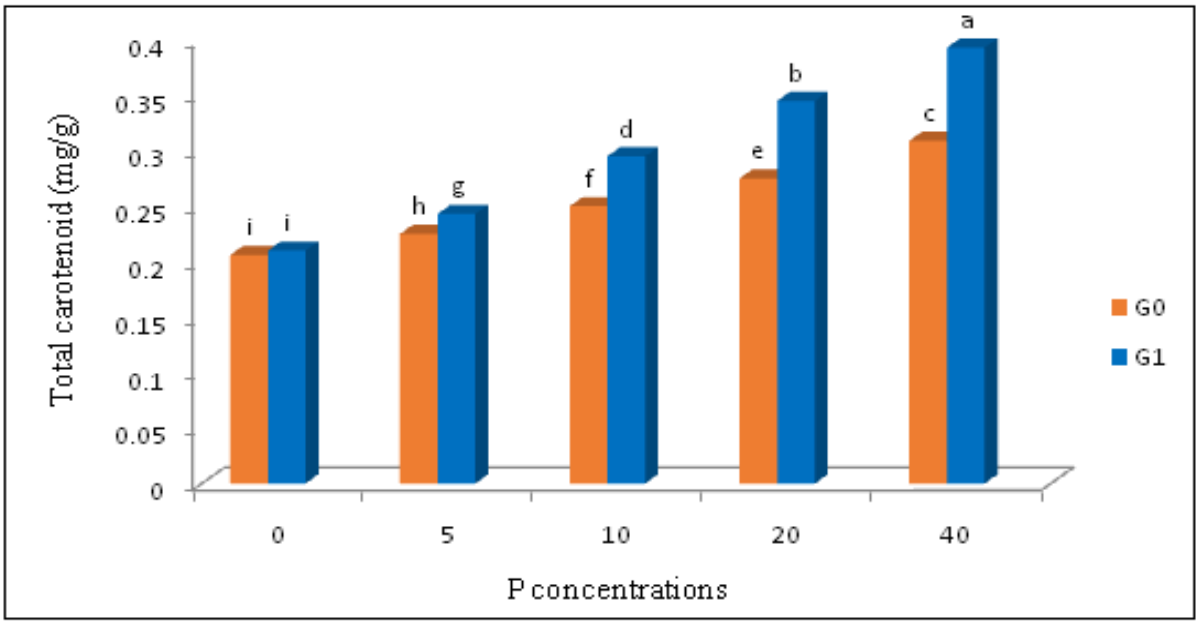

Fig. 2. Total carotenoid contents as affected by magnetic solution (G) and phosphorus concentrations in the nutrient solution. Different letters indicate significant differences according to Duncan's multiple-range test (P = 0.05).

Findings of this research show that basil plants under treatments of magnetic solution and M have shifted their metabolism from biosynthesis of phenolic compounds to production of essential oils, so that major compounds of essential oil in plants treated with magnetic solution and M increased and contents of phenol and flavonoids decreased. In fact, terpenoids of essential oils (by mevalonate pathway) and carotenoids (by methylerythritol phosphate pathway) in plants treated with magnetic solution and M were responsible for improvement of growth and quality.

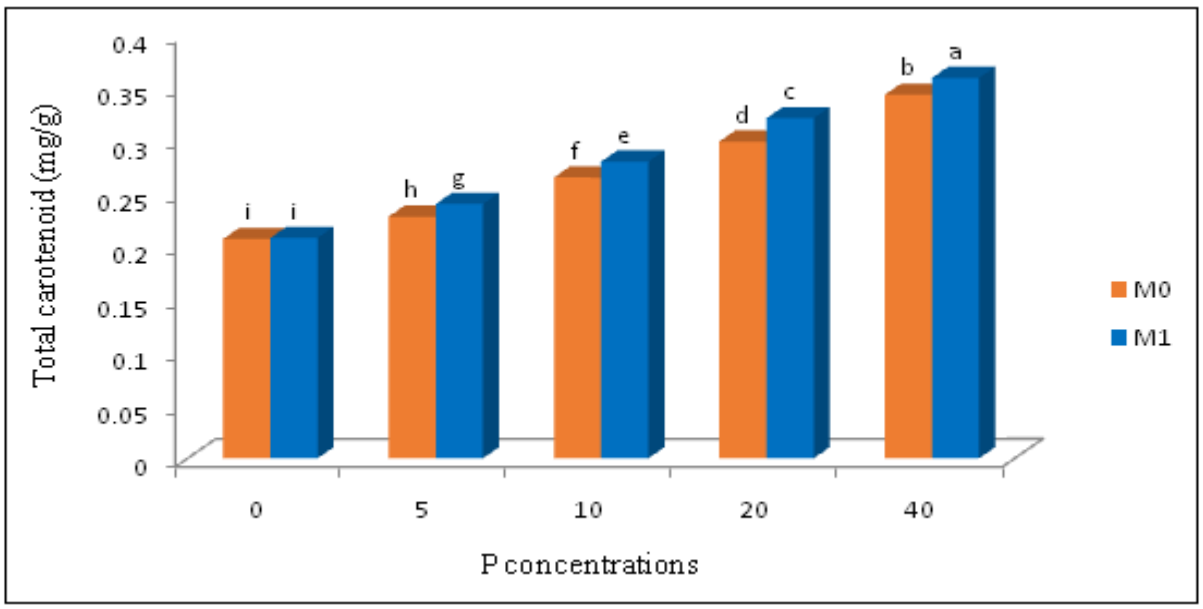

Fig. 3. Total carotenoid contents as affected by arbuscular mycorrhizal fungi (M) and phosphorus concentrations in the nutrient solution. Different letters indicate significant differences according to Duncan's multiple-range test (P = 0.05).

Application of M led to decrease linearly in monoterpenes such as methyl chavicol, lemonal, neral and geranial while sesquiterpenes such as caryophyllene, α-humulene, ß-cubebene, α-bergamotene, ß-farnesene and germacrene-D increased linearly (Table 4). As well as interaction between magnetic P solution and M showed an increasing in sesquiterpen contents.

The last reports in literature indicating that using of G increased levels of caryophyllene oxide and caused the decrease of basil plants growth (Ghanati *et al.,* 2007), as well as, plants colonized by *G. margarita* showed a significant decrease in the yield of eucalyptol, linalool, and caryophyllene (Copetta *et al.,* 2006). We found that applying magnetic P solution and M not only increased the caryophyllene yield; but also caused to decrease of caryophyllene oxide, eventually increased plant growth and major compounds of essential oil in basil plants. The generation of ROS occurs in response to caryophyllene oxide (Park *et al.,* 2011). Reducing of this compound shows that using a combination of magnetic P solution and M inhibited the possible combinations of ROS by terpenoids such as caryophyllene, α-humulene, ß-cubebene, α-bergamotene, ß-farnesene, germacrene-D, carotenoid and enzymatic antioxidants such as CAT and POD, as a result were caused increase of quantity and quality of basil plants. Although the exact mechanism of this modifications were not studied in this research, but it seems that, modification in biosynthesis of plant from production of phenol and flavonoid compounds to terpenoids and antioxidant enzymes are possible mechanisms.

Phenylpropanoids and sesquiterpenes present in essential oils contribute to their antiviral activity against Herpes simplex virus type 1 (HSV-1) (Astani *et al.,* 2011) and other maladies. Therefore, treatment of medicinal plants such as basil with magnetic P solution and M can be suggested as a tool in order to discovery of novel effective antiherpetic drugs without adverse effects and produce more desired medicinal compounds.

Conclusions

Our findings showed that basil plants under treatments of magnetic P solution and M have shifted their metabolism from biosynthesis of phenolic compounds to production of essential oils. Therefore, magnetic P solution and M potentially represent natural ways of promoting growth and motivate the production of pharmaceutical compounds in this important medicinal herb.

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