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Effects of arbuscular mycorrhizal (AM) fungi on growth, essential oil production and nutrients uptake in basil

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The effect of arbuscular mycorrhizal (AM) fungi on root colonization, growth, essential oil content and composition and nutrient acquisition of basil (*Ocimum basilicum*) was investigated as complete randomized design with 4 treatments and 4 replications. Fungi inoculation treatments consisted: Gf (*Glomus fasciculatum*), Ge (*Glomus etuonicatum*), Gi (*Glomus intraradices*), and NM (non-mycorrhizal). The results showed mycorrhizal plants significantly had higher shoot and root dry weight, leaf area, plant height, numbers of lateral branches, as well as N, P, K, Ca, Fe, Cu and Mn concentration compared to non-inoculated plants. The effect of AM fungi inoculation on the root colonization, growth parameters and yield of basil are more pronounced with *G. fasciculatum* than other AM fungi. The *G. fasciculatum* inoculation significantly increased essential oil content and yield. Analysis of essential oil by GC and GC/MS showed that linalool formed the highest relative abundance of the main compounds in leaf essential oils of basil and methyl chavicol profile was considerably increased with AM fungi inoculation. Increased essential oil percent of AM fungi plants was correlated with root fungal colonization ($r = 0.997^{**}$) and leaf P content ($r = 0.994^{**}$). It is concluded that *G. fasciculatum* was more effective than other species, which may indicate effective symbiotic potential of this strain with basil roots.

Key words: Arbuscular mycorrhiza, *Ocimum basilicum*, root colonization, essential oil.

INTRODUCTION

Medicinal herbs are known as sources of phytochemicals, or active compounds that are widely sought after worldwide for their natural properties. Basil (*Ocimum basilicum*) known also as sweet basil, is an annual spicy herb of the lamiaceae family (Prakash, 1990). Basil is a useful source of essential oil and has been used for a long time in the perfumery, cosmetic, food and pharmaceutical industry (Javanmardi et al., 2003; Mahmoud, 1996). Essential oils

(EO) are volatile, lipophilic mixtures of secondary plant compounds, mostly consisting of monoterpenes, sesquiterpenes and phenylpropanoids. The qualitative and quantitative improvement of essential oil production presents an area of high commercial interest (Copetta et al., 2006; Khaosaad et al., 2006). In the past few decades, Arbuscular mycorrhizal (AM) fungi have emerged as potential biofertilisers, a cheap, environmentally friendly alternative to expensive chemical fertilizers (Srivastava et al., 1996).

The production of many agricultural and horticultural crops in soil is dependent on the formation AM, making this

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symbiosis an essential factor in low-input sustainable agriculture (Bethlenfalvai and Linderman, 1992). Many aspects of Arbuscular mycorrhizal (AM) interactions including growth effect, nutritional exchanges, biocontrol toward plant pathogens, tolerance to water stress and adverse environmental conditions were studied, but little is known about their potential effect on the quantitative and qualitative profile of the secondary metabolites (e.g., essential oils) in medicinal and aromatic plants (Copetta et al., 2006; Kapoor et al., 2002a; Morone-Fortunato and Avato, 2008). The objective of this paper is comparative analysis of the effects induced by three AM fungi *Glomus intraradices*, *Glomus etunicatum* and *Glomus fasciculatum* on plant growth and development, nutrient uptake and essential oil content and composition of sweet basil.

MATERIALS AND METHODS

Experimental design and plant culture

This study was performed on a loamy sandy soil, collected from outside the township of Salmas (38°11'N, 44°46'E), West Azerbaijan province located in North-West of Iran. Any discernible root pieces were removed and the samples were air dried for 48 h at 25°C, sieved (2 mm) and thoroughly mixed. Soil properties were: pH (1:5 H₂O) 7.2, EC 0.45 dS m⁻¹, CaCO₃ 7.2%, organic matter 13 g kg⁻¹, P 9.65 mg kg⁻¹, K 165 mg kg⁻¹ and the DTPA (Diethylenetriamine pentaacetate)-extractable Zn, Fe, Mn and Cu were 0.80, 1.80, 2.2 and 1.1 mg kg⁻¹, respectively.

An amount of 8 kg soil was placed into plastic pots (300 × 200 mm) of 10-L volume. Basal nutrients (in mg kg⁻¹ of dry soil) 145 K₂SO₄, 147 CaCl₂·2H₂O, 21 MgSO₄·7H₂O, 2 CuSO₄·5H₂O, 15 MnSO₄·H₂O, 0.7 H₃BO₃, 0.2 Na₂MoO₄·2H₂O, and 93 NH₄NO₃ were applied to soil surface as solution and allowed to dry. The nutrients were thoroughly mixed with soil, and the soil mixture was packed into the pots for planting.

Sweet basil (*O. basilicum* L.) seeds were surface sterilized by gently shaking in 1% NaClO solution for 3 min and rinsed six times for 5 min and four times for 20 min in sterile deionized water (Gamalero et al., 2004). Culture substrate loamy sand soil was sterilized at 180°C for 2 h (Copetta et al., 2006). The pot experiment was carried out in a complete randomized design with 4 replicates. Four treatments were considered: Control plants or non-mycorrhizal (NM), plants inoculated with *G. intraradices* (Gi), *G. etunicatum* (Ge) and *G. fasciculatum* (Gf). Seeds were planted into a hole in substrate where the inoculum (5 g per seed) had been previously added (Khaosaad et al., 2006).

Plants were kept in a glasshouse with a 16/18 h light/dark photoperiod, 26/22°C light/dark thermoperiod. After 84 days growth, the plants were harvested and the following parameters including shoot dry weight (SDW) and root dry weight (RDW), number of leaves, plant height, and stems diameter were measured by standard methods. Furthermore, chlorophyll index (by Minolta-502), leaf area (Leaf area-meter) and macro and microelement content in shoot dry matter were determined at the end of experiment.

Mycorrhizal colonization

Twelve weeks after inoculation, plant roots stained for observation of fungal structures and mycorrhizal colonization (Phillips and Hayman, 1970). Mycorrhizal fungi colonization was also measured by cutting root samples into 1 cm segments, put them in 10% KOH for 2 days at

room temperatures followed by rinsing them several times with tap water and staining with ink (black ink, Schaeffer) as well as household vinegar (equal to 5% acetic acid) solution 4 min. Then, colonization percent determined using modified intersection method proposed by McGonigle et al. (1990).

Isolation and analysis of the essential oils

After 84 days of growth (at full flowering stage), essential oil content was evaluated in aerial parts of host plants. For this purpose, 50 g of each treatment dry matter were hydro-distilled in a Clevenger-type apparatus for 2 h and then percentage and yield of essential oils were calculated. The essential oils were dried over anhydrous sodium sulfate, stored in a dark glass vials and kept at 4°C (Omidbaigi et al., 2003).

GC analysis was performed using an Ultra Fast Chromatograph (Thermo-UFM) equipped with a Ph-5 column (10 m × 0.1 mm, film thickness 0.4 µm). Oven temperature was kept at 60°C for 3 min and then programmed to 285°C at rate of 80°C/min. Injector and detector (Fid) temperature were 280°C and Helium (with 99.999% purity) was used as carrier gas with a linear velocity of 32 cm/s. Data were calculated by electronic integration of FID peak area without using of response correction factor. GC/MS analysis was also carried out on a Varian 3400GC/MS system equipped with a DB-1 fused silica column (60 × 0.25 mm, film thickness 0.25 µm). Oven temperature program was 50 - 280°C at a rate of 4°C/min, transfer line temperature 290°C, carrier gas was Helium (with 99.999% purity) with a linear velocity of 31.5 cm/s, split ratio 1/60, ionization energy 70 eV. The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literatures (Davies, 1990; Shibamoto, 1987).

Statistical analysis

Treatment effects were determined by one-way analysis of variance (ANOVA) and differences between treatments were determined using Duncan multiple range test (p < 0.05) using MSTAT software.

RESULTS AND DISCUSSION

Results showed that mycorrhizal plants had significantly higher shoot and root dry weight, plant height and chlorophyll index (Table 1). Moreover, all mycorrhizal plants showed a higher degree of shoot branching, while root and shoot weight was lower in control plants (Table 1).

Increased growth and development in AM plants, compared to non-mycorrhizal ones, was reported for many different species (Gupta and Janardhanan, 1991; Smith and Read, 1997). Among mycorrhizal fungal species, inoculation with Gf (*G. fasciculatum*) resulted in significant differences in growth parameter (Table 1)

For instance, shoot dry weight in plants inoculated with Gf was increased 1.2, 1.8 and 4.2 times more than Ge, Gi and non-mycorrhizal treatments, respectively. The similar results were observed in other growth indices when inoculated with *G. fasciculatum* (Table 1). Plants inoculated with different mycorrhizal fungal species showed higher

Table 1. Effect of mycorrhizal fungi on growth parameters of basil.

Treatments	Stem diameter (Cm)	No. of leaves	leaf area index (Cm ²)	Plant height (Cm)	Chlorophyll index (spad)	SDW (g/pot)	RDW (g/pot)
<i>Glomus fasciculatum</i>	4.14a*	71.75a	359.24a	52.25a	38.4a	3.9a	14.05a
<i>Glomus etunicatum</i>	3.59 ab	54.75ab	248.67b	45.27b	38.9a	3.35a	11.49a
<i>Glomus intraradices</i>	3.18b	52ab	195.48b	41b	37.9a	2.41b	7.82b
Control	3.14b	42.25b	176.5b	33.05c	34.3b	0.95c	3.34c

*means in each column followed by the same letter are not significantly different at $p < 0.05$ according to Duncan's multiple range tests.

Table 2. Effect of mycorrhizal fungi on total macro and micronutrient content in basil shoot.

Treatments	N	P	K	Ca	Mg	Cu	Mn	Fe
	(mg/pot)			(µg/pot)				
<i>Glomus fasciculatum</i>	39.8a*	2.8a	53.7a	50.4a	20.1a	340.7a	3032.3a	1705.1a
<i>Glomus etunicatum</i>	28.8b	1.9b	35.3b	42.7a	8.1b	227.9b	1677.4b	1356.7b
<i>Glomus intraradices</i>	20.5c	1.1bc	26.9bc	28.5b	7.5b	160.8b	1300.2b	907.1bc
Control	9.4d	0.5c	14.7c	10.5c	3.5b	67.5c	304.9c	640.1c

*means in each column followed by the same letter are not significantly different at $p < 0.05$ according to Duncan's multiple range tests.

nutrient acquisition. The contents of N, P, K, Ca, Mg, Fe, Cu and Mn in shoots of host plant were higher in mycorrhizal treatments compared to control ones (Table 2). The mycorrhiza *G. fasciculatum* induced significant increase in shoot nutrient contents rather than other fungal species. Phosphorus content in non-mycorrhizal, Gi and Ge treatments was 82.3, 61.1 and 32.5%, respectively, lower than that of Gf inoculated plants (Table 2). A similar finding was reported for white ash and black walnut plants inoculated with *G. fasciculatum* (Ponder, 1984). This higher nutrient uptake in mycorrhizal plants might be attributed to the contribution of fungal external mycelia which explore a large volume of soil and thus absorb more nutrients (Gupta and Janardhanan, 1991). Previous works have shown that arbuscular mycorrhizal fungi increase plant uptake of phosphate (Bolan, 1991), micronutrients (Burkert and Robson, 1994), nitrogen (Barea et al., 1991) and act as antagonists against some plant pathogens (Duponnois et al., 2005). Moreover, it has been demonstrated that plants inoculated with Arbuscular mycorrhizal fungi utilize more soluble phosphate from rock phosphate than non-inoculated plants (Antunes and Cardoso, 1991). The main explanation is that mycorrhizas developed an extramatrical mycelium, which increased the root phosphate absorbing sites (Bolan, 1991).

Furthermore, the mycorrhizal responses relative to nutrient uptake and plant growth is related to the size of macro and micronutrients depletion zone that develops around a root. Under nutrient-deficient conditions the extramatrical hyphae of mycorrhizal fungi bridge the nutrient depletion zone and increase nutrient uptake with the

addition of soil nutrients. Gupta et al. (2002) found that *G. fasciculatum* inoculated mint plants depleted the available N, P and K in the rhizosphere soil as compared to non-inoculated control plants, however the extent of nutrient depletion was greater for P than N and K. Moreover, *G. fasciculatum* inoculation significantly increased the root colonization, growth, essential oil yield and nutrient acquisition of mint. Therefore, in present study G_f treatment plants extensively reduced depletion zone extent and consequently basil roots absorbed higher amount of nutrients compared to other treatments. This might be attributed to the higher root colonization of *G. fasciculatum* rather than other fungal species.

After root staining, different fungal structures could be observed in host plant roots including extraradical hyphae, vesicles as well as Arbuscules (Figure 1). The most abundant structures were fungal mycelia and vesicles. Root colonization percent was significantly different between mycorrhizal plants and non-inoculated ones as well as among different fungal species (Table 3). The highest colonization rate (85%) as well as abundant rate of Arbuscules was observed in roots inoculated with *G. fasciculatum*. In other hand, the least rate of colonization (32%) recorded in plants inoculated with *G. intraradices* (Table 3).

Differences in colonization of *O. basilicum* by *Glomus mosseae*, *Gigaspora margarita* and *Gigaspora rosea* were reported by Dickson (2004). There are significant differences in essential oil percent as well as essential oil yield between inoculated and non-inoculated plants and among different fungal species (Table 3). Plants inoculated

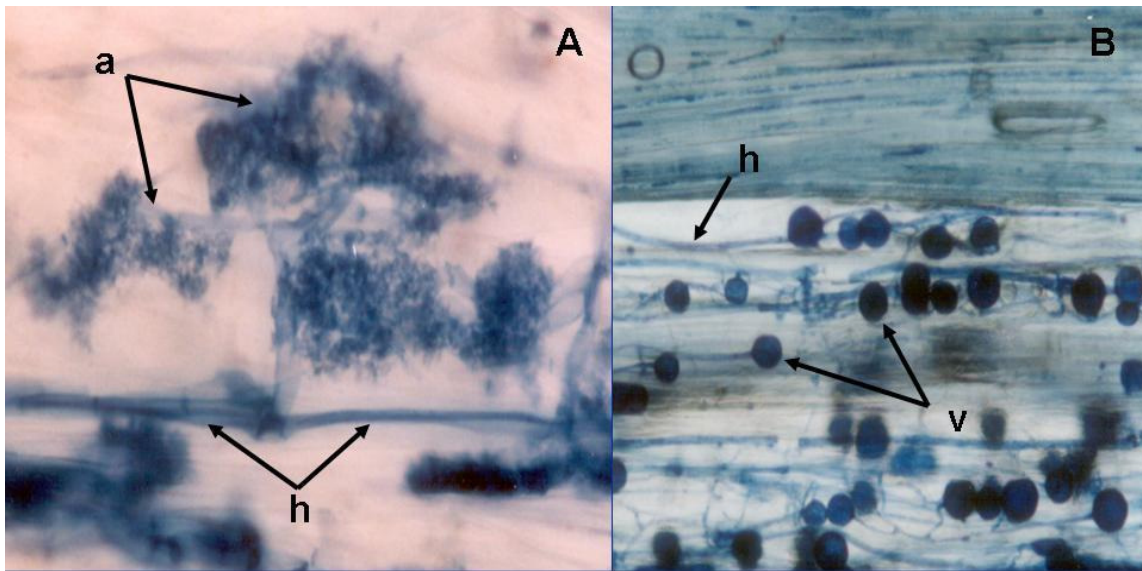


Figure 1. Arbuscular mycorrhizal fungal structures in plant roots. A. arbuscules (a) and fungal hyphae (h) in root cortex region of *O. basilicum* (100 ×). B. vesicles (v) and fungal hyphae in root cortex region of *O. basilicum* (40 ×).

Table 3. Effect of mycorrhizal fungi on root colonization, essential oil percent and yield of basil.

Treatments	Root colonization (%)	Essential oils (EO) (%)	Essential oils yield (ml pot ⁻¹)
Glomus fasciculatum	85a*	1.23a	0.172a
Glomus etunicatum	50b	1.04b	0.12b
Glomus intraradices	27.5c	1b	0.077c
Control	0d	0.75c	0.023d

*means in each column followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range tests.

with Gf showed significant increase in essential oil percent and yield in comparison with other treatments.

There was relatively difference in the composition of essential oils in leaves among treatments (Table 4). Linalool was the most abundant component of the essential oils, followed by Eugenol, Ocimene, Methyl Chavicol, Farnesol, Humulene and other compounds (Table 4). Linalool profiles were 66.30, 65.26, 66.74 and 63.85% in NM, Gi, Ge and Gf treatments, respectively. The greatest difference in essential oil compounds was seen in Methyl Chavicol which in inoculated plants was increased significantly compared to non-inoculated plants (3.32, 8.08, 6.83 and 7.82% in NM, Gi, Ge and Gf, respectively). It was shown that the contents of Eugenol were high in all the treatments rather than other essential oil components (Table 4). Results of correlation coefficient revealed that shoot dry weight had a significant positive correlation with essential oil yield ($r = 0.994^{**}$) and leaf area index ($r = 0.996^{**}$) in inoculated plants but had no correlation with other parameters (Table 5).

Correlation coefficient between essential oil percent and selected parameters was indicated in Table 6. Positive significant relationships were observed in inoculated plants between EO percent and colonization rate ($r = 0.997^{**}$) as well as leaf P content ($r = 0.994^{**}$). However, these relationships were not significant in non-mycorrhizal plants. Correlation coefficient between absorbed P and selected parameters (data not shown) showed that leaf P content of inoculated plants had significant relationships with colonization rate ($r = 0.946^{**}$), essential oil percent ($r = 0.992^{**}$) and essential oil yield ($r = 0.995^{*}$). In non-mycorrhizal plants, significant positive relationship was observed only between leaf P content and essential oil yield ($r = 0.999^{***}$).

Information about the effects of AM fungi on the production of essential oils is scarce and only a few papers concerning a limited choice of species have been published up to now. Copetta et al. (2006) compared different AM fungi inoculation on basil and found that AM fungi induced various modifications in the considered

Table 4. Relative abundance of the main compounds in leaf essential oils of *Ocimum basilicum* L. inoculated with different AM fungi.

Compounds	Treatments*				
	RI	NM	Gi	Ge	Gf
β-pinene	979	0.93	0.64	0.90	0.98
Myrcene	990	-	0.19	-	0.28
1,8-Cineole	1035	1.92	1.66	1.91	1.68
(E)- β-Ocimene	1050	4.33	4.17	4.17	4.95
Linalool	1101	66.30	65.26	66.74	63.85
Camphor	1142	0.09	0.08	0.13	0.16
Terpinen-4-OL	1177	0.71	0.73	0.65	0.72
α-Terpineol	1192	0.13	0.10	0.06	0.13
λ-Terpineol	1199	0.31	0.34	0.19	0.27
Methyl Chavicol	1200	3.32	8.08	6.83	7.82
Geraniol	1253	1.13	0.99	0.30	0.33
Bornyl Acetate	1292	0.42	0.39	0.36	0.48
Eugenol	1362	8.73	6.16	7.95	6.61
Methyl Eugenol	1408	0.40	0.23	0.08	0.19
E-caryophyllene	1420	0.14	0.12	0.12	0.13
Trans-α-Bergamotene	1432	1.44	1.40	1.47	1.60
α-Humulene	1458	2.78	2.13	1.85	2.38
Germacrene D	1485	0.60	0.61	0.66	0.70
Bicyclogermacrene	1500	0.08	0.10	-	0.12
α-Bisabolene	1510	0.27	0.26	0.20	0.30
Germacrene A	1513	0.19	0.18	0.06	0.19
Cadinene	1517	0.10	0.06	-	0.10
Trans-Calamenene	1531	0.41	0.47	0.42	0.51
Spathulenol	1576	1.03	1.02	0.91	1.05
α-Cadinol	1654	0.15	0.23	-	0.27
(Z,E)-Farnesol	1701	2.49	2.77	2.78	2.70

NM, Gi, Ge and Gf: non-mycorrhizal, *G. intraradices*, *G. etunicatum* and *G. fasciculatum*-inoculated plants, respectively. RI: Retention.

parameters, but among different AM fungi, only *Gigaspora rosea* significantly affected all of parameters in comparison to other fungal treatments. It significantly increased biomass, root branching, root length and total amounts of essential oil. Increased oil yield was associated with a significantly large number of peltate glandular trichomes (main sites of essential oil synthesis) in the basal and central leaf zones. Their results showed that different fungi can induce different effects in the same plant and that the essential oil yield can be modulated according to the colonizing AM fungus.

Nemec and Lund (1990) also reported that *G. intraradices* induces significant variations in the proportion and composition of leaf volatiles in *Citrus Jambhiri*. Studies on *Mentha arvensis* have shown a relation between presence of AM fungi, increased growth and essential oils accumulation as well as improved mineral uptake (Freitas et al., 2004; Khaliq and Janardhanan, 2002). Similar results were published about *Corianderum sativum*

(Kapoor et al., 2002b). Kapoor et al. (2002a; 2004) conducted experiment on three different plant species (*Anethum graveolens* L., *Trachy spermum ammi* L. and *Foeniculum vulgare* Mill.), and two fungal species (*Glomus macrocarpum* and *G. fasciculatum*) and showed that both fungi increased plant growth, phosphate content and concentration of essential oils in the fruits (Gang et al., 2001).

Conclusion

Nutrients uptake were high in mycorrhizal plants particularly in *G. fasciculatum* treatments. Among studied fungus species, inoculation of basil with Gf resulted in significant increase in growth indices and essential oil production in comparison with other AM fungal species. Colonization rate of roots with *G. fasciculatum* was higher than other species, which may indicate efficient symbiotic

Table 5. Correlation coefficient between SDW and selected growth parameters.

Treatment	Leaf area	EO yield	Chlorophyll	Leaf P content	Colonization %
Non-mycorrhizal	0.358	0.896	-0.902	0.896	0
mycorrhizal	0.996**	0.994**	-0.661	0.918	0.842

*, **: Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 6. Correlation coefficient between EO % and selected growth parameter.

Treatment	Colonization %	Leaf P content	Chlorophyll	Leaf area	Shoot dry weight
Non-mycorrhizal	0	-0.497	0.964*	-0.284	-0.829
mycorrhizal	0.977**	0.994**	-0.240	0.903	0.887

*, **: Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

potential of this species with basil roots. It is concluded that *G. fasciculatum* extensively colonized *O. basilicum* roots and considerably improved growth parameters as well as nutrient uptake.

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