doi:10.14720/aas.2018.111.2.06 Original research article / izvirni znanstveni članek

Glomus intraradices (N.C. Schenck & G.S. Sm.) C. Walker & A. Schuessle enhances nutrients uptake, chlorophyll and essential oil contents and composition in *Anethum graveolens* L.

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Received October 08, 2017; accepted September 30, 2018.
Delo je prispelo 08. oktobra 2017, sprejeto 30. septembra 2018.

ABSTRACT

Arbuscular mycorrhizal (AM) fungi are plant-root symbionts whose application in agriculture has been proven its efficiency. However, their application in medicinal plants and their impact on accumulation of essential oils (EO) is still limited. In order to investigate the effect of AM fungi (*Glomus intraradices* N.C. Schenck & G.S. Sm.) C. Walker & A. Schuessle) on nutrients uptake, biomass production, yield components, chlorophyll content, and EO content and composition in dill (*Anethum graveolens* L.), a field experiment was conducted as randomized complete block design with three replications. This medicinal plant was grown under AM fungi colonization and non-colonization treatments. Plant inoculation by mycorrhiza increased aerial tissues P and Fe concentrations. However, K, Ca, and Zn concentrations were not affected by AM colonization. The plants inoculated with AM significantly increased plant biomass, chlorophyll content, and EO content by 363 g m^{-2}, 11.83 SPAD and 0.683 % in comparison with non-inoculated plants, respectively. Changes in EO composition were found in AM-colonized dill plants. The contents of myristicin, dill-ether and N-dihydrocarvone increased in EO obtained from AM-colonized plants, while AM colonization resulted in a lesser content of α-pinene, α-phellandrene, limonene, and β-phellandrene.

Key words: arbuscular mycorrhizal fungi; dill; essential oil; medicinal plants; nutrient uptake

IZVLEČEK

MIKORIZACIJA Z ARBUSKULARNO GLIVO *Glomus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schuessle POVEČUJE PRIVZEM HRANIL, KONCENTRACIJO KLOROFILA IN VSEBNOSTI ETERIČNIH OLJ PRI NAVADNEM KOPRU (*Anethum graveolens* L.)

Arbuskularne mikorizne glive (AM) so glivni simbionti večine kopenskih rastlin, tudi mnogih kmetijskih rastlin. Njihov pomen za uspevanje rastlin je potrjen, malo pa je znanega o njihovem vplivu na tvorbo eteričnih olj v zdravilnih rastlinah. Z namenom analize vplivov okužbe koreninskega sistema z AM na tvorbo sekundarnih metabolitov smo analizirali navadni koper (*Anethum graveolens* L.) z glivo *Glomus intraradices* N.C. Schenck & G.S. Sm.) C. Walker & A. Schuessle v poljskem poskusu organiziranem kot naključno zasnovani komplet s tremi ponovitvami. Analizirali smo absorpcijo hranil, proizvodnjo biomase, deleže pridelka, vsebnost klorofila in vsebnost eteričnih olj ter kemijsko sestavo nadzemnih delo rastline. Inokulacija rastlin z AM je povečala koncentracije P in Fe v nadzemnih tkivih, nismo pa ugotovili značilnih sprememb v koncentracijah K, Ca in Zn. Rastline, inokulirane z AM, so imele bistveno bujnejšo rast, večjo vsebnost klorofila in eteričnih olj v primerjavi z neinokuliranimi rastlinami. Pri inokuliranih rastlinah smo ugotovili tudi spremembe v sestavi nabora eteričnih olj, povečana je bila količina miristicina, koprovega etra in N-dihidrokarvona, zmanjšala pa se je količina α-pinena, α-felandrena, limonena, in β-felandrena.

Ključne besede: arbuskularna mikoriza; navadni koper; eterična olja; zdravilne rastline; privzem hranil

1 INTRODUCTION

Medicinal plants play major roles in human health services worldwide (Weisany et al., 2015), and herbal medicine is gaining importance at global level (Wondimu et al., 2007). Essential oils (EO) constituents of the medicinal and aromatic plants are most frequently used as a source of new bioactive molecules. The EOs

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are abundant in flowers, leaves, seeds, and are usually isolated via hydro-distillation, cold pressing methods (Edris, 2007). Their main active components are: carvone, carvacrol, eugenol, myristicin and apiole (Duke, 2001), although their mechanism of action is still poorly understood (Burt, 2004; Calo et al., 2015).

Dill (*Anethum graveolens* L.) is important essential oils producing plant. Myristicin and apiole in *A. graveolens* EO components are applied as a toxin and repellent to growing larvae and adults of *Tribolium castaneum* (Chaubey, 2007). Furthermore, the essential oils from its fruits and shoots are used in pharmacology as well as food and in soap industries.

It is well accepted that suitable use of irrigation and chemical fertilizers improve yield and quality of oil in aromatic plants (Singh and Randhawa, 1990; Tiwari and Banafar, 1995). However, for sustainable agriculture, traditional agriculture practices, including heavy fertilizer input are adverse owing to their long-lasting impact on the ground water quality (Yao et al., 2001). The alternative for sustainable plant production system to ensure increased productivity is to limit chemical input along with bio-inoculants so as to augment nutrient uptake by plants (Bethlenfalvay and Linderman, 1992).

Arbuscular mycorrhizal (AM) fungi can result in beneficial effects on soil and plant ecosystem such as improving soil structure (Rillig and Mummey, 2006; Bedini et al., 2009) and influencing plant nutrient uptake (Smith and Read, 2008; Clark and Zeto, 2000) and influencing major element cycles (for example, carbon, phosphorus and nitrogen) (Fitter et al., 2011). It is well accepted that AM fungi can improve the uptake of micronutrients and other mineral nutrients with low mobility including Fe (Clark and Zeto, 2000), Zn (Weisany et al., 2016a) and Mn (Weisany et al., 2016b). Utilizing management practices of AM fungi associated with promote effects of AM fungi on phosphorus uptake, growth, and grain yield of crops (Sohrabi et al., 2012b; Arihara and Karasawa, 2000; Karasawa et al., 2002). Previous studies have shown that different species and isolates of *Glomus* had various effects on mycorrhizal plants (Sohrabi et al., 2012a,b).

AM fungi promote the accumulation of effective ingredients of medicinal plants, which has become a hot area of research lately (Raei and Weisany, 2013). AM fungi application may provide a natural regulation mechanisms and ecological method to increase the accumulation of secondary metabolites in medicinal plants, because it contributes to a reduction of need to apply chemical fertilizers and development of sustainable agriculture (Pedone-Bonfim et al., 2013). AM fungi can affect the production of active ingredients in medicinal and aromatic plants (Karagiannidis et al., 2011), resulting from a better nutritional condition or by means of protecting the host from the pathogenic fungi (Volpin et al., 1994). Symbiosis between plants and AM fungi can increase the accumulation of several secondary metabolites in medicinal plants which plays important roles in treating human diseases (Weisany et al., 2015). The influence degree of different AM fungi varies among the medicinal plants. The scientific reports suggest the improved production of EO in coriander and dill inoculated by *Glomus fasciculatum* (Taxt.) Gerd.ec Trappe or *G. macrocarpum* Tul. & C. Tul. (Kapoor et al., 2002a, b), in mint inoculated by *G. fasciculatum* or a suite of AM fungi (Freitas et al., 2004), in oregano and dill inoculated by *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe 1974 (Khaosaad et al., 2006; Weisany et al., 2015), and in annual wormwood colonized by *G. faciculatum* (Kappoor et al., 2007; Chaudhary et al., 2008).

While AM colonization can increase the EO contents of medicinal plants, it is not clear whether the composition of the EO could be affected. Therefore, the overall aim of the present study is to investigate the fact that whether AM fungi can provide an effective and natural way of improving the growth, chlorophyll content, nutrient uptake, and EO content and composition in dill plants. More specifically, through testing two hypotheses we attempted to evaluate whether the composition of the secondary metabolites in dill are affected by AM fungi inoculation. The first hypothesis is that AM fungi enhance chlorophyll content and nutrient uptake in dill and this, in turn, increase the plant production. The second hypothesis is that symbiosis between plant and AM fungi boosts the accumulation of several secondary metabolites in dill.

2 MATERIALS AND METHODS

2.1 Experimental design

A field experiment was conducted in the Agriculture and Natural Resources Research Center of Kurdistan Province in 2014. Soil samples were taken from depths of 0–10 cm and 10–25 cm and mixed, using a soil auger. These samples were collected in spring from 8 points of experimental area. All soil samples were air dried at laboratory for 7 days and then crushed and sieved through a 2 mm sieve to determine the chemical composition (Rao, 1993). The texture of the soil was sandy clay loam. Different chemical and physical

properties of soils are presented in Table 1. The experiment carried out as randomized complete block design with three replications. The medicinal plant studied in this research was dill (*Anethum graveolens* L.). It was applied as a colonized and non-colonized plant by arbuscular mycorrhizal fungi. The medicinal plant was managed according to the organic farming practices without using pesticides or fertilisers. Seeds were sown in plots (4 × 5 m), each with 8 rows. Three seeds were sown by hand on the eastern side of the ridges in each hole being at a 10-cm distance from another hole. After emergence, the seedlings were thinned and one plants was kept in each hole. Plots of non-colonized seeds were sown first in order to avoid AM cross contamination.

Table 1: Some physical and chemical properties of the soil of experimental area

Texture	Organic carbon %	pH (1:2.5)	K	P	Ca	Na	Zn	Mn	Fe	Cu
			(mg kg^{-1}soil)							
Sandy clay loam	1.14	7.12	131	12.2	1150.1	450.2	0.476	7.054	6.97	0.826

Thirty grams of soil inoculum (100 endomycorrhizal spore/10 g soil) along with 300 mg of chopped *G. intraradices* -colonized *Zea mays* L. roots were added to each plot at sowing time just below the seeds. The AM fungus (*G. intraradices*) was obtained from the culture collection of Tabriz University, Tabriz, Iran (Weisany et al., 2015).

2.2 Arbuscular mycorrhizal fungi colonization

Five plants from each plot were randomly collected at 95 days after AM fungi inoculation. The root samples were extracted by using a cylindrical corer (10 mm). The roots were washed, cut into about 1 cm long pieces and mixed thoroughly. The staining procedure was applied according to Phillips and Hayman (1970) with the modified parameters for the present study. The roots were cut into small pieces (1 cm) and placed in a beaker (10 % KOH) for 60 min in a water bath at 65 °C. The roots were then rinsed with tap water and acidified with 5 % lactic acid at room temperature for 12 h. Finally, they were stained by a solution containing 875 ml of lactic acid, 63 ml of glycerin, 63 ml of tap water, and 0.1 g of fuchsine acid for 30 min at 70 °C and were then de-stained by lactic acid for 15 min. Afterwards, root segments were mounted onto slides and examined at 100-400 magnification under a Nikon YS100 microscope. Beneath the glass slide an acetate film with 10 thin lines was adapted. At crossing points between roots and lines, each point that had an infection was recorded and the number of infections was expressed as percentage (Weisany et al., 2015). The percentage of AM root colonization was calculated by the following equation (McGonigle et al., 1990):

Root colonization (%) = (number of root segments colonized/number of root segments studied) × 100

2.3 Mineral nutrient analysis

The dry ash method was used (Jones and Case, 1990) to measure different elements in dill. In this way, the aerial tissues of plants were dried in an oven at 70°C. Five plants from the each plot were randomly harvested. Subsequently, 1 g of dry matter was transferred into ceramic vessels and was slowly subjected to 500 °C in the oven. The final product was a white ash. White ash was cooled in room temperature and then 20 ml 1N HCl was added to each sample, followed by the sand bath for 30 minutes. The samples were elutriated in a 100 ml volumetric balloon (Cottenie, 1980). Having provided plant extracts, the concentrations of calcium (Ca) and potassium (K) were measured via flame photometer (Model 410, Corning, Halstead, UK). Iron (Fe) and zinc (Zn) concentrations were measured by atomic absorption spectrometer (Shimadzu AA6600) (Jones 1972). Plant phosphorous was gauged through the yellow method, in which, vanadate-molybdate (Tandon et al., 1968) was employed as an indicator. P concentration was determined at 430 nm, using a spectrophotometer apparatus (Shimadzu, UV3100).

2.4 Plant growth measurements and chlorophyll content

Ten plants from each plot were randomly harvested after seed maturation. In the sampling plots, the randomly chosen plants from the each treatment were harvested along with complete roots, and the plant biomass (dry mass) was recorded (Weisany et al., 2015). Ten indiscriminately selected plants at full maturity stage in each plot were cut, and the plant height and yield components were defined.

Chlorophyll status of dill plants was evaluated in each plot by SPAD analysis (SPAD 502, Minolta Ltd. Osaka, Japan). SPAD measurements were performed at flowering stage, and the mean of three random SPAD measurements on the middle part of the leaf blade was recorded (Weisany et al., 2015).

2.5 Essential oil extraction

At the beginning of flowering, aerial tissues of dill were harvested from 1 m^2. The EOs were extracted by hydrodistillation in 500 ml of water, using a Clevenger apparatus for 2 h. The distillate was extracted using diethyl–ether as solvent (1/1, v/v) and drying the sample over anhydrous sodium sulphate. The organic layer was then concentrated at 35 °C by a Vigreux column and the EO was stored at 4 °C prior to analysis (Weisany et al., 2016b). The percentage of EO content was measured in volume/100 g dry mass basis.

2.6 Gas chromatography–mass spectrometry

Gas chromatography (GC) analysis was performed by means of a trace GC ultra-gas chromatograph coupled with a TSQ quantum tandem mass spectrometer upgraded to the XLS configuration. A DuraBrite IRIS ion source with pre-filter was installed so as to improve the performance of the spectrometer. The system was equipped with a triplus autosampler (Thermo Electron Corporation, Waltham, MA). The injection volume was 1 µl, post injection dwell time 4 sec, and tray temperature 7 °C. GC separation was done on a 30-m VF-WAXms capillary column with an internal diameter of 0.25 mm and a film thickness of 0.25 µm (Varian, Inc. USA). Temperature programming was as follows: 40 °C held for 4 min after injection and 6 °C min^{-1} up to 250 °C held for 5 min. Injection parameters were as follows: split injection, split ratio of 100:1, inlet temperature of 250 °C, carrier gas being helium 5.5, and constant flow: 1.2 ml min^{-1}. The mass spectrometry was used in scan mode in the range of 40-400 m/z with a scan time of 0.2 sec. The ionization mode was electron impact (EI), and the source temperature was kept at 250 °C (Perini et al., 2014).

2.7 Statistical Analysis

Analysis of variance was carried out, using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) (SAS Institute Inc. 1988). Means of the treatments were compared through orthogonal comparisons. The data showed normal distribution and no transformation has ben done.

3 RESULTS AND DISCUSSION

3.1 AM colonization

Arbuscular mycorrhiza colonization was observed in inoculated plants and root samples. The percentage of mycorrhizal root colonization was significantly greater in all the treatments of plant colonization with mycorrhiza in comparison with non-inoculated control plants (Table 2).

Table 2: Root AM colonization, growth and yield components, chlorophyll, mineral nutrient and essential oil contents of dill inoculated (+AM) and non-inoculated with arbuscular mycorrhiza (-AM)

Parameters	Dill		
	-AM	+AM	Pr > F
AM colonization (%)	0.00±0.00	80.6±6.568	0.0056**
Phosphorous (mg kg^{-1}DM)	13.78±0.016	14.29±0.025	0.0003**
Zinc (mg kg^{-1}DM)	0.449±0..009	0.458±0.001	1.0000ns
Iron (mg kg^{-1}DM)	1.131±0.338	1.946±0.499	0.0367*
Potassium (mg kg^{-1}DM)	255.45±33.80	320.05±21.33	0.3620ns
Calcium (mg kg^{-1}DM)	622.0±20.78	685.5±53.98	0.1959ns
Plant height (cm)	43.00±5.291	50.66±7.310	0.3995ns
Number of branches/plant	6.66±0.333	7.660±0.666	0.4226ns
Number of umbels/plant	6.67±0.333	7.33±0.881	0.4226ns
Number of umbelet/plant	94.66±3.179	109.33±5.456	0.3570ns
Root length (cm)	10.01±0.577	14.50±0.866	0.0701ns
Plant biomass (g m^{-2})	205.33±3.289	363.33±26.193	0.0204*
Chlorophyll (SPAD)	6.90±0.2645	11.83±0.2962	0.0121*
Total essential oil (%)	0.377±0.000	0.683±0.000	0.0251*

+AM, -AM: inoculated and non-inoculated with arbuscular mycorrhiza, respectively.
Results are the mean of three replications±SD. NS, * and **, non-significant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

3.2 Mineral nutrient uptake

Results of this study indicated that plant colonization with AM enhanced the aerial tissues P concentration of dill, compared with control plants (Table 2). So, the inoculated dill with *G. intraradices* had the most (14.29 mg kg^{-1}DM), but non-inoculated plants had the least (13.78 mg kg^{-1}DM) P concentrations (Table 2). Mycorrhization of target plant provides a better nutrient

status by the wide extra-radical mycelium of AM that equips the plant roots with a more surface area for uptake of nutrients and water (Jefferies et al., 2003).
AM fungal hyphae can transfer immobile P resources to the roots from a long distance (over centimeters) and therefore, play an even more important role in P uptake by their host plants than what was previously taken for granted (Richardson et al,. 2011, Weisany et al., 2016a).

The colonization of dill with *G. intraradices* increased Fe concentration to 1.946 mg kg^{-1}DM, whereas the non-inoculated plants had the least Fe concentration (1.131 mg kg^{-1}DM). However, there was no significant difference between inoculated and non- inoculated plants in terms of K, Ca and Zn concentration (Table 2). The increased utilization of soil volume is especially important in the uptake of less mobile nutrients such as P and Fe (Smith and Read, 2008). Increased uptake of Fe by mycorrhizal fungi may be in part due to production of siderophores that specifically chelate Fe (Shende and Rai, 2010). AM fungi moderate the ion balance in the plant that affecting the availability of mineral nutrients in plant tissues (Bermudez and Azcon, 1996).

3.3 Growth and yield components

Dill plant biomass was effected by AM colonization, so that the inoculated plants with *G. intraradices* had more (363.3 g m^{-2}) compared with non-inoculated plants (205.3 g m^{-2}) plant biomass. However, plant height, number of branches per plant, number of umbels per plant, number of umbelets per plant and root length of dill were not substantially affected by AM inoculation (Table 2).

Our previous studies have revealed that different species and isolates of *Glomus* increased plant height, total dry mass and root and aerial tissues dry mass of chickpea (Sohrabi et al., 2012a,b). The improvement of plant growth was also observed in coriander (*Coriandrum sativum* L.) by colonization of AM and application of phosphorus (Farahani et al., 2008). The results of the present study are consistent with these reports. The increase in growth can be attributed to improved P and Fe nutrition in these treatments (Table 2). In the current research, plant inoculation with AM augmented the growth of dill, supporting the observation that mycorrhizal plants obtain more nutrients. Furthermore, AM are able to share nutrients via an underground network of hyphal connections linking individuals within and between species (Simard et al., 2003).

The positive influence of P and Fe on growth and yield has been reported for many plants, including those with medicinal value (Naguib et al., 2007). The Fe element plays roles either as functional or structural co-factors or as the metal components of different enzymes (Marschner, 1995). Inoculation of plant by AM fungi results in higher growth and yield, since it offers an opportunity to optimize the rate of photosynthesis via improved uptake of P and Fe nutrients. Therefore, AM fungi provide balanced nutrition to the host plants, leading to increased growth and yield.

3.4 Chlorophyll content

Chlorophyll content in dill leaves was markedly influenced by inoculation with AM. In general, inoculated plants with *G. intraradices* had significantly more chlorophyll (11.83 SPAD) than the non-inoculated plants (6.90 SPAD) (Table 2). The association of AM fungi with the roots of dill plants influence Fe uptake (Table 2). The Fe plays an important role in various biochemical and physiological processes, such as chlorophyll synthesis, photosynthetic transport, respiration, nitrate reduction and N_2 fixation (Robinson and Postgate, 1980). The obtained AM-mediated higher chlorophyll content in dill leaves may be due to improved nutrient uptake by this plant, especially that of Fe. These findings are in agreement with those previously found by Mathur and Vyas (2000). They discovered that root colonization with AM increased chlorophyll synthesis. The contribution of AM fungi and the roots of dill plant influences Fe acquisition that has poor mobility rates. Fe is an integral component of the chlorophyll molecule (Taiz and Zeiger, 2004).

3.5 Essential oil yield and composition

Inoculation of plant with AM fungi considerably influenced EO yield. AM inoculation noticeably enhanced the total EO yield in dill aerial tissues (Table 2). The effect of AM fungi in increasing the production of EO has been reported in some of medicinal plant species (Khaosaad et al., 2006; Copetta et al., 2006; Chaudhary et al., 2008, Weisany et al., 2015). Karagiannidis et al., (2011) obtained similar results in their study on three AM fungi colonization that increased the nutrient concentration, plant growth and EO yield of oregano and mint plants. The boosted EO production is the outcome of enhanced shoot fresh mass (Subrahmanyam et al., 1992; Piccaglia et al., 1993). Kapoor et al. (2007) also noticed that plant inoculation with AM enhances the number of glandular trichomes of *Artemisia annua* L. and, as a consequence, increases artemisinin content in leaves. This bigger number of glands could be related to variation in the hormonal profile of plants due to enhanced amounts of auxins, cytokinins and gibberellins in plants inoculated with AM (Torelli et al., 2000). Additionally, inorganic P concentration can influence the biosynthesis of EO in the plants (Loomis and Corteau, 1972). In the present research, AM fungi improved the absorption of P in plants. P element may play a direct role in increasing

the content of secondary metabolites (Abu-Zeyad et al., 1999). The same results were found by Kapoor et al. (2004) in their study on the accumulation of EO in fennel. The present findings concur with these conclusions, because a significant positive correlation between EO synthesis and shoot P concentrations was found for dill.

Photosynthesis of mycorrhizal plants can increase due to an increased plant chlorophyll content and by the drain of carbon, as a consequence of Calvin cycle activation and higher production of primary metabolites that act as precursors for secondary metabolism (Kaschuk et al., 2009). Gas chromatography (GC) analysis of EO composition showed that dill-apiole was the main component in all treatments and its amount varied among the treatments (Table 3, Fig 1). The second main component of the EO was carvone. The results indicated that inoculation of dill with AM increased myristicin, dill-ether and N-dihydrocarvone contents in plant (Table 3). These findings are similar with those of Karagiannidis et al. (2011). Variation in EO composition due to AM colonization (Table 3) maight be related to the nutrition of plants. The accumulation of flavonoids (Larose et al., 2002), phytoalexins (Yao et al., 2003), cyclohexanone derivatives and apocarotenoids (Fester et al., 2002; Vierheilig et al., 2000a,b), triterpenoids (Akiyama and Hayashi, 2002) and phenolic compounds (Devi and Reddy, 2002) in plants inoculated by AM fungi has been previously reported. AM fungi colonization of *Salvia officinalis* L. changes EO composition, and improves the relative amounts of 1,8-cineole, bornylacetate, α-thujones and β-thujones (Geneva et al., 2010). The mechanisms by which AM fungi changes the production of EO are not clear, but they are possibly associated with improved nutrition.

Table 3: Chemical composition (% of essential oil) of essential oils of dill shoots inoculated (+AM) and uninoculated with arbuscular mycorrhiza (-AM)

Compounds (Synonymous)	Dill			Confirmed by
	-AM	+AM	Pr > F	
α-pinene	0.15	0.11	0.2458ns	STD, MS
α-phellandrene	2.45	2.14	0.0011**	STD, MS
Limonene	4.22	3.18	0.0001**	STD, MS
β-phellandrene	0.47	0.39	0.0034**	RI, MS
Dill_ether (3,9-epoxy-1-p-menthene; anethofuran)	0.60	0.70	0.2879ns	RI, MS
N-dihydrocarvone (trans-dihydrocarvone)	4.80	5.99	0.0011**	STD, MS
Iso-dihydrocarvone (cis-dihydrocarvone)	10.07	11.66	0.0002**	STD, MS
Carvone	26.05	23.17	0.0001**	STD, MS
Neoiso-dihydrocarveol	0.41	0.43	0.3349ns	STD, MS
Iso-dihydrocarveol	0.73	0.69	0.2381ns	STD, MS
Trans-carveol	0.11	0.10	1.0000ns	STD, MS
Isopiperitenone	0.05	0.05	1.0000ns	RI, MS
Cis-carveol	0.08	0.07	0.2879ns	STD, MS
Thymol	0.02	0.09	0.0056**	STD, MS
Carvacrol	0.15	0.14	0.6560ns	STD, MS
Elemicin	0.24	0.24	1.0000ns	RI, MS
Myristicin	0.74	1.05	0.0006**	STD, MS
Dill_apiole	48.57	49.75	0.0001**	RI, MS

STD, MS = confirmed by injection of Standard and by Mass Spectra library; RI, MS = confirmed by n-alkanes Retention Index by Mass Spectra library;

+AM, -AM: inoculated and non-inoculated with arbuscular mycorrhiza, respectively.

NS, * and **, non-significant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively. Five plants from each plot were randomly collected.

The results of the present research showed that inoculation of plant with AM decreased contents of α-pinene, α-phellandrene, limonene, and β-phellandrene in dill plant (Table 3). Most presumably these variations are due to changes in the synthesis pathways and the role of EO in plant physiology. AM colonization significantly increased activities of enzymes related to secondary metabolism. Among enzymes such as chalcone synthase and chalcone isomerase, the key enzymes in the synthesis of flavonoids, and phenylalanine ammonia-lyase (PAL) that catalyzing the deamination of phenylalanine (Ibrahim and Jaafar, 2011) provide precursors for the synthesis of secondary metabolites. The AM fungi also effects cytological changes in the host plant, such as an increase in the number of plastids and mitochondria, leading to the activation of the tricarboxylic acid cycle and the plastid biosynthetic pathways and the increase in the production of primary and secondary metabolites (Lohse et al., 2005, Strack and Fester, 2006).

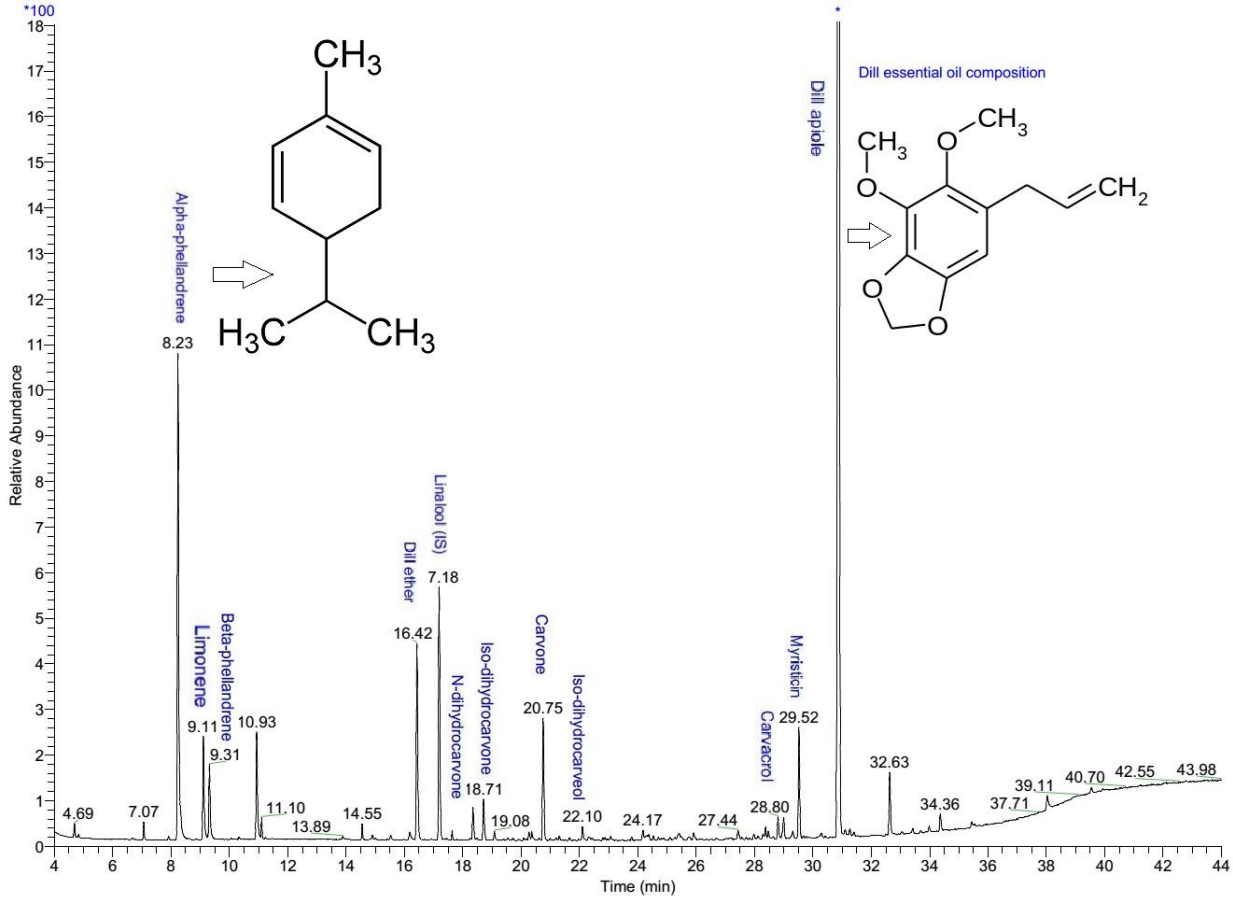

Figure 1: *Anethum graveolens* L. essential oil chromatogram carried out using a gas chromatograph mass spectrometry. Essential oils were obtained from inoculated with arbuscular mycorrhiza (*Glomus intraradices*) plant.

4 DISCUSSIONS

The study showed that inoculation of dill plant with AM improved mineral nutrition of plant in comparison with non- AM inoculated plants. Increases in growth and chlorophyll content were observed in AM colonized plants. When plant species are inoculated with AM fungi, it is concluded that yield benefits occur as a result of complementary use of resources by the plants. *A. graveolens* L. EO yield was enhanced in inoculated plants with AM fungi. Aerial tissues EO composition of dill was affected by colonization with AM. Our contribute to the expansion of effective organic and sustainable methods for the cultivation of medicinal plants. Further study in mycorrhizal technology is needed to develop the sustainability of the commercial cultivation of medicinal plants.

5 ACKNOWLEDGEMENTS

We would like to thank the research administrators of the University of Tabriz for their financial support. Thanks also to Fondazione Edmund Mach di San Michele all'Adige, Dr. Urška Vrhovšek, Cesare Lotti and Hamed Hajee for their scientific help.

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