

# Effects of *Glomus lamellosum* on Growth, Essential Oil Production and Nutrients Uptake in Selected Medicinal Plants

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## Abstract

The effect of *Glomus lamellosum* on root colonization, growth, essential oil production and composition and nutrient acquisition of *Santolina chamaecyparissus*, *Salvia officinalis*, *Lavandula angustifolia*, *Geranium dissectum*, and *Origanum dictamnus* was investigated. The results showed that mycorrhizal plants had significantly higher growth, essential oil production and nutrient contents compared to non-inoculated plants. The *S. officinalis* plants showed the highest percentage of colonization, while the *S. chamaecyparissus* the lowest. Similar percentage of colonization was found among the *G. dissectum*, *L. angustifolia* and *O. dictamnus* plants. Analysis of essential oil by GC and GC/MS showed that the effect of *G. lamellosum* on the main compounds in leaf essential oils was different on the plant species used.

It is concluded that the use of *Glomus lamellosum* may allow plant growth in low fertility soils, reduce fertilizer inputs and increase aromatic plant production of essential oils, while they indicate that it may be possible to use mycorrhizae to affect the quality of the essential oil produced.

**Keywords:** Arbuscular mycorrhizal fungus, Colonization, *Geranium dissectum*, *Lavandula angustifolia*, *Origanum dictamnus*, *Salvia officinalis*, *Santolina chamaecyparissus*

## 1. Introduction

Medicinal herbs are known as sources of phytochemicals, or active compounds that are widely sought after worldwide for their natural properties. They are useful source of essential oil and have been used for a long time in the perfumery, cosmetic, food and pharmaceutical industry. Essential oils 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).

The arbuscular mycorrhizal symbiosis is recognized for its multiple positive effects on plant growth and for its important contribution towards the maintenance of soil quality. In spite of these benefits to agriculture, at present, the realization of the full potential of this symbiosis has not yet been reached. The understanding of interactions existing among crops, fungal partners and environmental conditions must improve to allow for the efficient management of the mycorrhizal symbiosis through selected agronomic practices and inoculation of cultivated crops (Hamel, 1996).

Arbuscular mycorrhizal fungi (AMF) are a ubiquitous group of soil fungi colonizing the roots of plants belonging to more than 90% of plant families (Brundrett, 1991). Enhanced plant growth due to AMF association is well documented (Bagyaraj, 1984). In the past few decades, AMF have emerged as potential biofertilisers, a cheap, environmentally friendly alternative to expensive chemical fertilizers (Srivastava *et al.*, 1996). These fungi are known to improve the nutritional status of the host, particularly that of phosphorous, and thereby enhance their growth, development and yield (Bagyaraj and Varma, 1995). Many other aspects of arbuscular mycorrhizal interactions including 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 (Kapoor *et al.*, 2002; Copetta *et al.*, 2006; Morone-Fortunato & Avato, 2008).

During the establishment of the arbuscular mycorrhizal symbiosis, a range of chemical and biological parameters is affected in plants, including the pattern of secondary compounds. However, little is known about the effect of AMF upon either plant secondary metabolic pathways or the production and yield of secondary compounds of their hosts (Copetta *et al.*, 2006). For instance, studies have demonstrated that AMF can influence phytohormone levels of (Hause *et al.*, 2002) terpenoids and carotenoids (Fester *et al.*, 2002) and phenols (Zhu and Yao, 2004). In addition, the association with AMF has altered essential oil yield and quality of several plants (Kapoor *et al.*, 2002).

Though not host specific, earlier studies have indicated host preferences of mycorrhizal fungi (Miller *et al.*, 1987), thus suggesting the need for selecting efficient AMF for a particular host (Sailo and Bagyaraj, 2005).

The objective of this paper is a comparative analysis of the effects induced by the arbuscular mycorrhizal fungus, *G. lamellosum* on plant growth, nutrient uptake and essential oil production and composition of *Santolina chamaecyparissus*, *Salvia officinalis*, *L. angustifolia*, *Geranium dissectum*, and *O. dictamnus*.

## 2. Materials and Methods

### 2.1 Isolates

A strain of *G. lamellosum*, isolated from oregano plant collected from Elatochori, Pieria, Greece, was used in this study. Identification was made by using the molecular method described by Karagiannidis *et al.* (2011) and retained on mother maize plants.

### 2.2 Effect of *Glomus lamellosum* on five medical plants

Plants of *Santolina chamaecyparissus*, *Salvia. officinalis*, *L. angustifolia*, *G. dissectum*, and *O. dictamnus* were propagated by stem cuttings (originated from the mother plantations established in the experimental field of Alexander Technological Education Institute of Thessaloniki) rooted in autoclaved perlite in the greenhouse under a misting system. The rooted cuttings were transplanted in April in pots containing 1.5 kg of soil substrate mixed with fine cut pieces of highly colonized roots of maize plants with a pure *G. lamellosum* strain following the method described by Karagiannidis (1980). For the mycorrhizal treatments, 10 cm<sup>3</sup> inoculum per pot (about 12g) was placed in the planting hole beneath the plants at transplanting. The inoculum consisted of colonized root fragments (roots were cut into 1cm pieces), hyphae and spores. Pots without inoculum were used as control. The soil used was a sandy loam with a pH 6.5; low salinity (0.82 mS conductivity), low organic matter (0.6%), low CaCO<sub>3</sub> (0.11%) with a Ca, Mg and P content at 152, 9.6, and 0.7 mg/kg respectively and it was autoclaved at 120 °C for 25 minutes. A fertilization treatment with 25.4 mg kg<sup>-1</sup> KCl, 29.7 mg kg<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub>, 162 mg kg<sup>-1</sup> Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>OH, 27.6 mg kg<sup>-1</sup> MgCl<sub>2</sub>.6H<sub>2</sub>O and 190 mg kg<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub> was added to the potting mix before planting.

A total of 200 pots were used with the following combinations: 2 inoculation treatments (inoculated plants with *G. lamellosum*; non-inoculated control) replicated 4 times of 5 pots for each of the plant host species evaluated. Plants were grown in a random design for 2.5 months in the greenhouse.

Shoots and roots were harvested separately to analyse nutrient and essential oil composition in the shoots and the establishment of the arbuscular mycorrhizal symbiosis in the roots. The shoots were air-dried for three days (10% moisture content) and nutrients were analysed after mineralization by calcination in a muffle furnace at 450°C for 8 hours, and extraction with HCl (35%). Na and K were determined using flame photometry. N was determined using the Kjeldahl method, P was determined using chromatography and Ca and Mg were determined volumetrically using the Versenate method. Concentration of trace elements was determined with atomic absorption spectrophotometry (Fe, Mn, Cu, Zn) and B with the azomethine. All methods are described in Jackson (1960) and Cottenie (1980).

Roots were stained with trypan blue (Sylvia, 1994) and mycorrhizal colonization was estimated according to McGonigle *et al.* (1990).

Leaves were separated from shoots, and the essential oils were obtained by hydrodistillation in 500 mL H<sub>2</sub>O in a Clevenger apparatus for 2 hours. The composition of the volatile constituents was established by GC and GC-MS analyses. GC analyses were performed on a Shimadzu GC-14A, with a FID (Flame Ionization Detector), using a DB5 column (30m x 0.25mm, film thickness: 0.25m), The temperature program was from 65° C for 10 min, to 160° C at a rate of 3 °C/min for 5 min. Helium was used as a carrier gas at a flow rate of 0.6 ml/min.

GC-MS analyses were performed on a Shimadzu GC-2010-GCMS-QP2010 system operating in EI mode (70eV) equipped with a split/splitless injector (230°C), a split ratio 1/30, using a fused silica HP-5 MS capillary column (30 m x 0.25 mm (i.d.), film thickness: 0.25 μm) and a polar column HP-Innowax. The analytical conditions were: for the HP-5MS column the temperature program was from 60°C (5min) to 280°C at a rate of 4°C /min and for the HP-Innowax column the temperature program was from 60°C (5min) to 260°C at a rate of 3°C /min.

Helium was used as a carrier gas at a flow rate of 0.8 ml/min. Injection volume of each sample was 1  $\mu\text{l}$ . Retention indices for all compounds were determined using n-alkanes as standards. The identification of the components was based on comparison of their MS with those of NIST21 and NIST107 and those described by Adams (2001). Quantitative determination was based on the total ion count detected by the GC-MS.

This experiment was repeated.

### 2.3 Statistical analysis

Statistical analysis of the data was carried out by using analysis of variance. Means were separated by using the Duncan's multiple range test at 0.05 significance level.

## 3. Results and Discussion

The results of mycorrhizal inoculation resulted in a significant increase in growth, nutrient contents and essential oil productions in all plant species used (Table 1). Improved plant growth, nutrients uptake and essential oil production was evident because arbuscular mycorrhizal fungal inoculation has previously been reported in mychorrizal aromatic plants (Boby and Bagyaraj, 2003; Tharun *et al.*, 2006; Geneva *et al.*, 2010; Karagiannidis *et al.*, 2010). In this study, plant biomass (shoot + root) was enhanced by 33.33 %, 44.35%, 46.72%, 24.9%, 25.27% in arbuscular mycorrhizal *Santolina chamaecyparissus*, *Salvia officinalis*, *L. angustifolia*, *G. dissectum* and *O. dictamnus* plants, respectively (Table 1). According to Geneva *et al.*, (2010) arbuscular mycorrhizal colonization on *Salvia officinalis* improved the growth of plants. Nowak (2004) found that mycorrhizal inoculation of geranium increased the growth of plants. Previous works showed that increased plant growth due to AMF inoculation is mainly through improved uptake of diffusion limited nutrients such as P (Lambert *et al.*, 1979; Sailo and Bagyaraj, 2005) and those findings are in good agreement with the results of this study in which all the arbuscular mycorrhizal plants had significant higher nutrient contents in comparison to uninoculated treatment. The contents of N, P, Ca, Mg, B, Zn, Fe, Cu and Mn were significantly higher in all mycorrhizae applied plants compared to control ones, while the *Santolina chamaecyparissus*, *Salvia. officinalis* and *O. dictamnus* mycorrhizal plants had significant higher K and Na contents than uninoculated treatments (Table 2). The mycorrhizal and non-mycorrhizal *G. dissectum* plants had similar K and Na contents. The content of K was significantly higher in mycorrhizal plants of *L. angustifolia*, while the Na content was similar in both mycorrhizal and nonmycorrhizal plants. Such higher nutrient content in AMF inoculated plants is attributed to higher influx of nutrients into the plant system through AMF which explores the soil volume beyond depletion zone as previous works showed for P (Sanders and Tinker, 1971; Bayaraj and Varma, 1995). According to Bagyaraj and Reddy (2000) the extramatrical hyphae produced by AMF act as extensions of roots and increase the surface area of the root system, making it more efficient for absorption of water and diffusion limited nutrients, this effect being more pronounced in P-deficient soils. Previous works have shown that AMF increase plant uptake of phosphate (Bolan, 1991), micronutrients (Burkert and Robson, 1994), nitrogen (Barea *et al.*, 1991). Moreover, it has been demonstrated that plants inoculated with AMF utilize more soluble phosphate from rock phosphate than noninoculated plants (Antunes & Cardoso, 1991).

The *Salvia officinalis* plants showed the highest percentage of colonization, while the *Santolina chamaecyparissus* the lowest (Table 1). Similar percentage of colonization was found among the *G. dissectum*, *L. angustifolia* and *O. dictamnus* plants. Karthikeyan *et al.* (2009) inoculated *Ocimum sanctum*, *Catharanthus roseus*, *Coleus forskholii* and *Cymbopogon flexuosus* plants with the mycorrhizal fungus *G. fasciculatum* and found similar situation in all the four medicinal plants, which recorded 58 to 75% vesicular arbuscular mycorrhizal association.

The essential oil production in arbuscular mycorrhizal plants was significantly increased in all plant species tested compared to the uninoculated treatment (Table 1). Specifically, the essential oil production was enhanced by 28.75 %, 55.56%, 56.95%, 53.63%, 55.24% in arbuscular mycorrhizal *Santolina. chamaecyparissus*, *Salvia officinalis*, *L. angustifolia*, *G. dissectum* and *O. dictamnus* plants respectively (Table 1). The favorable effect of root colonization of *Salvia officinalis* by *Glomus intraradices* was determined both on quantitative and qualitative pattern of sage essential oil (Geneva *et al.*, 2010). Venkateshwar *et al.* (2002) found that rose-scented geranium plants inoculated with one of *Acaulospora laevis*, *Gigaspora margarita*, *G. fasciculatum* or *G. mosseae* recorded significantly higher essential oil yield (21.1%) in comparison to non-mycorrhizal plants.

Although analysis of essential oil by GC and GC/MS showed that mycorrhizal inoculation influenced the plant species differently, the results show only very small differences in volatile composition between mycorrhizal and non-mycorrhizal plants in all species. The major volatile constituents in each species did not differ significantly between mycorrhizal and non-mycorrhizal plants. The two volatiles that are found in all plant species show either no change between mycorrhizal treatments ( $\beta$ -myrcene), or no change or decrease in abundance (a-pinene)

with mycorrhizal inoculation. In *Salvia officinalis*, the compound Carvacrol + Geranyl formate was detected only in arbuscular mycorrhizal plants, while the compounds Lemonene and Geranyl acetate only in noninoculated plants. The compounds  $\beta$ -Pinene, and 1,8-Cineole were significantly higher in noninoculated than inoculated plants. In contrast the compounds Camphor and Bornyl acetate were significantly higher in inoculated plants (Table 3). In *G. dissectum*, the concentration of the compound Spathulenol was significantly higher in noninoculated than inoculated plants. Only trace of Terpinen-4-ol was detected in AM plants, while only trace of the  $\alpha$ -Caryophyllene, *allo*-Aromadendrene,  $\beta$ -Bisabolene, Geranyl isobutanoate, 1,10-di-*epi*-Cubenol, 1-*epi*-Cubenol,  $\gamma$ -Eudesmol were detected in noninoculated plants. In *L. angustifolia*, the concentration of *trans*-Pinocarveol was significantly higher in noninoculated than inoculated plants. The compounds Octen-3-ol, Geranyl acetate, Terpinolene were detected only in trace in noninoculated *L. angustifolia* plants. In *S. chamaecyparissus*, the concentration of the compounds Bornoel and Caryophyllene oxide were significantly higher in noninoculated than inoculated plants. In *O. dictamnus*, only the AM plants had some amounts of the compound  $\beta$ -Phellandrene, while the compounds  $\beta$ -Cubebene,  $\beta$ -Bisabolene,  $\delta$ -Cadinene were detected only in noninoculated plants. Information about the effects of AMF 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 AMF inoculation on basil and found that AMF induced various modifications in the considered parameters. Geneva *et al.* (2010) found that the *G. intraradices* inoculation of *Salvia officinalis* in combination with fertilization promoted the production of 1,8-cineole and alpha-thujone, mycorrhizal colonization enhanced bornyl acetate, 1,8-cineole, alpha- and beta-thujones. Kapoor *et al.* (2002) observed that inoculation with AMF *G. macrocarpum* and *G. fasciculatum* increased significantly the concentration of limonene and  $\alpha$ -phellandrene, respectively, relative to non-mycorrhizal control plants of *Anethum graveolens* L. Kapoor *et al.* (2004) also observed enhanced concentration and quality of essential oils on mycorrhizal *Coriandrum sativum* L (coriander) and *Foeniculum vulgare*. Mill. (fennel). For *Mentha arvensis* L. (mint) mycorrhizal colonization significantly increases oil content and yield relative to non-mycorrhizal plants (Gupta *et al.*, 2002). Freitas *et al.* (2004) also observed that inoculation with AMF resulted in increments of 89% in the essential oil and menthol contents of mint.

#### 4. Conclusions

Generally, *Santolina chamaecyparissus*, *Salvia officinalis*, *L. angustifolia*, *dissectum*, *G. dissectum*, and *O. dictamnus* plants resulted in bigger plants with greater yield, had a higher essential oils production and nutrient elements when mycorrhizal, but mycorrhizal inoculation did not exert a significant effect on volatile composition. These results indicate the importance of the AM symbiosis, that should be managed to help in the reduction of fertiliser and other agrochemical inputs, thus enhancing the sustainability of the commercial cultivation of aromatic plants, even in low fertility, mountainous soils.

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Table 1. Dry weight, essential oil production, and percentage of root colonization of *Santolina chamaecyparissus*, *Salvia officinalis*, *Lavandula angustifolia*, *Geranium dissectum*, and *Origanum dictamnus* inoculated with the indigenous *Glomus lamellosum*

Plant Species		Dry Weight (g)		Essential Oil Production (%)		Colonization (%)	
<i>S. chamaecyparissus</i>	M <sup>x</sup>	42.49 <sup>y</sup>	a <sup>z</sup>	0.80	a	38	C
	NM	14.16	b	0.23	b		
<i>S. officinalis</i>	M	25.57	a	1.17	a	83	A
	NM	11.34	b	0.65	b		
<i>L. angustifolia</i>	M	39.62	a	2.23	a	52	B
	NM	18.51	b	1.27	b		
<i>G. dissectum</i>	M	51.72	a	4.27	a	64	B
	NM	12.88	b	2.29	b		
<i>O. dictamnus</i>	M	33.72	a	5.25	a	68	B
	NM	8.52	b	2.90	b		

<sup>x</sup>M = Mycorrhizal Plants, NM = Non-mycorrhizal plants

<sup>y</sup>Values are the means of 2 experiments, each with 4 replicates; results were similar according to the Bartletts's test of homogeneity of variance, so data were combined

<sup>z</sup>Values in the same column followed by different letters are significantly different ( $P = 0.05$ ) according to Duncan's Multiple Range Test.

Table 2. Nutrient concentration in *Santolina chamaecyparissus*, *Salvia officinalis*, *Lavandula angustifolia*, *Geranium dissectum*, and *Origanum dictamnus* plants inoculated with the indigenous *Glomus lamellosum*

Plant Species		N (%)		P (%)		K (%)		Na (%)		Ca (%)		Mg (%)		B (mg l <sup>-1</sup> )	Zn (mg l <sup>-1</sup> )	Mn (mg l <sup>-1</sup> )	Fe (mg l <sup>-1</sup> )	Cu (mg l <sup>-1</sup> )					
<i>S. chamaecyparissus</i>	M <sup>a</sup>	2.68 <sup>y</sup>	a <sup>z</sup>	0.86	a	3.48	a	3.96	a	1.53	a	0.65	a	57.9	a	122.4	a	167.5	a	560.5	a	59.17	a
	NM	0.66	b	0.11	b	1.04	b	1.56	b	0.65	b	0.26	b	21.9	b	26.4	b	19.0	b	104.8	b	10.20	b
<i>S. officinalis</i>	M	2.74	a	0.80	a	3.54	a	3.06	a	1.88	a	0.75	a	62.6	a	128.8	a	157.1	a	499.8	a	56.77	a
	NM	0.56	b	0.11	b	1.12	b	2.06	b	0.68	b	0.37	b	20.5	b	18.5	b	23.8	b	107.6	b	9.23	b
<i>L. angustifolia</i>	M	2.46	a	0.75	a	1.87	a	2.87	a	1.46	a	0.59	a	76.4	a	110.1	a	167.2	a	837.7	a	46.50	a
	NM	0.63	b	0.10	b	0.96	b	2.17	a	0.50	b	0.22	b	18.1	b	20.2	b	20.5	b	88.93	b	8.57	b
<i>G. dissectum</i>	M	2.74	a	0.63	a	1.76	a	2.95	a	1.90	a	0.67	a	70.2	a	121.2	a	176.4	a	568.0	a	56.43	a
	NM	0.69	b	0.11	b	1.19	a	2.24	a	0.68	b	0.22	b	21.8	b	18.0	b	17.03	b	121.3	b	9.47	b
<i>O. dictamnus</i>	M	2.43	a	0.81	a	2.59	a	2.49	a	1.55	a	0.64	a	87.7	a	131.5	a	176.5	a	378.6	a	52.53	a
	NM	0.63	b	0.1	b	0.9	b	1.11	b	0.76	b	0.31	b	26.63	b	24.03	b	15.7	b	126.7	b	13.27	b

<sup>a</sup>M=mycorrhizal plants, NM=non-mycorrhizal plants

<sup>y</sup>Values are the means of 2 experiments, each with 4 replicates; results were similar according to the Bartletts's test of homogeneity of variance, so data were combined

<sup>z</sup>Values in the same column followed by different letters are significantly different ( $P = 0.05$ ) according to Duncan's Multiple Range Test.

Table 3. Chemical composition of essential oils of non-mycorrhizal and mycorrhizal *Salvia officinalis*, *Santolina chamaecyparissus*, *Lavandula angustifolia*, *Origanum dictamnus* and *Geranium dissectum* plants, inoculated with the indigenous *Glomus lamellosum*

Compounds <sup>a</sup>	RI <sup>b</sup>	<i>G. dissectum</i>		<i>S. chamaecyparissus</i>		<i>S. officinalis</i>		<i>O. dictamnus</i>		<i>L. officinalis</i>		Identification <sup>c</sup>		
		M	NM	M	NM	M	NM	M	NM	M	NM	I	MS	
$\alpha$ -Thujene	925									1.3	1.05	0.18	0.07	
$\alpha$ -Pinene	931	0.09	0.01	0.15	0.13	0.22	1.03	0.7	0.55	1.32	0.90	I, MS, Co-GC		
Camphepane	945			0.56	0.42	0.52	1.45				0.70	0.96	I, MS	
Sabinene	970			4.55	3.94					1.1	1.0	0.37	0.55	I, MS
$\beta$ -Pinene	972			3.71	4.06	<b>0.77<sup>d</sup></b>	<b>2.62</b>	0.1	0.1	1.02	0.86	I, MS, Co-GC		
Octen-3-ol	978									0.3	0.3	0.09	trace	I, MS
$\beta$ -Myrcene	990	0.10	0.06	9.60	9.84	1.56	1.52	1.7	1.55	0.78	1.03	I, MS		
$\alpha$ -Phellandrene	1001			0.21	0.14			0.3	0.25	0.14	0.05	I, MS		
p-Mentha-1(7),8-diene	1007										1.12	0.64	I, MS	
$\alpha$ -Terpinene	1014							2.8	2.5	0.32	0.89	I, MS		
p-Cymene	1022			0.15	0.18			16.4	16.25	1.81	2.62	I, MS, Co-GC		
sylvestren	1025								0.6			I, MS		
$\beta$ -Phellandrene	1026	0.07	0.02	15.05	14.33	1.42	0.55	0.6				I, MS		
Lemonene	1027						1.51					I, MS		
1,8-Cineole	1028			1.19	1.03	<b>16.52</b>	<b>19.22</b>			36.22	35.91	I, MS		
cis-Ocimene	1037									0.21	0.27	I, MS		
trans-Ocimene	1047									0.07	0.08	I, MS		
$\gamma$ -Terpinene	1056			0.09	0.01	0.55	0.98	17.5	16.15	2.09	1.94	I, MS, Co-GC		
Artemisia ketone	1060	0.17	0.11	36.88	35.46					0.08	0.06	I, MS		
cis-Sabinene hydrate	1064			0.23	0.19	0.27	0.17	0.7	0.65	0.50	0.44	I, MS		
Terpinolene	1085			2.05	1.95			0.1	0.1	0.10	trace	I, MS		
6-Camphenone	1093									trace	trace	I, MS		
trans-Sabinene hydrate	1094							0.1	0.1	0.15	0.05	I, MS		
Linalool	1097	3.82	3.91					1.5	1.45	0.37	0.19	I, MS		
$\alpha$ -Thujone	1102					24.82	25.69					I, MS		
cis-Rose oxide	1110	0.19	0.15									I, MS		
$\beta$ -Thujone						4.48	4.62					I, MS		
trans-Rose oxide	1127	0.08	0.02									I, MS		
$\alpha$ -Campholenal	1123									0.17	0.03	I, MS		
trans-Pinocarveol	1134									0.37	1.27	I, MS		
Camphor	1140			2.33	1.67	<b>25.77</b>	<b>17.57</b>			16.07	16.94	I, MS		
Citronellal	1151	0.12	0.14									I, MS		
Pinocarvone	1159									0.10	0.08	I, MS		
Borneol	1161			<b>1.08</b>	<b>2.94</b>	3.32	3.29	0.1	0.1	4.53	4.06	I, MS		

Menthol	1170						0.2	0.15			I, MS	
<i>iso</i> -Menthone	1161	4.21	3.92								I, MS	
Terpinen-4-ol	1174	Tr	1.01	0.22	0.31		0.6	0.6	0.53	0.63	I, MS	
<i>p</i> -Cymen-8-ol	1181								0.16	0.27	I, MS	
<i>iso</i> -Menthol	1183	0.08	0.07								I, MS	
Cryptone	1184								0.92	0.94	I, MS	
$\alpha$ -Terpineol	1187	0.17	0.11				0.1	0.1	0.49	0.61	I, MS	
Myrtenal	1192								0.44	0.37	I, MS	
<i>trans</i> -carveol	1218								0.12	0.11	I, MS	
Citronellol	1230	29.73	28.35						1.37	1.48	I, MS	
Cumin aldehyde	1238								0.59	0.24	I, MS	
Neral	1238	0.59	0.71								I, MS	
Carvone	1241								0.32	0.62	I, MS	
Geraniol	1252	23.63	22.49						0.87	0.93	I, MS	
Geranial	1268	1.01	1.02								I, MS	
Citronellyl formate	1275	9.87	8.64						0.43	0.33	I, MS	
Neryl formate	1282	0.06	0.02								I, MS	
Bornyl acetate	1283					<b>9.22</b>	<b>2.68</b>		0.08	0.15	I, MS	
Thymol	1289	0.07	0.01					0.1	0.1	13.31	12.38	I, MS
Carvacrol	1298			0.95	0.34			<b>48.3</b>	<b>51.05</b>	5.32	4.68	I, MS
Carvacrol + Geranyl formate	1298	11.30	12.03			1.06					I, MS	
$\alpha$ -Cubebene	1348							0.2	0.2			I, MS
$\alpha$ -Longipinene	1349			0.22	0.47							I, MS
Citronellyl acetate	1351	0.17	0.15									I, MS
$\alpha$ -Ylangene	1373	0.06	0.09					1.0	1.15			I, MS
Geranyl acetate	1380	1.03	1.02				6.21		0.08	trace		I, MS
$\beta$ -Cubebene	1388								0.25			I, MS
Phenyl ethyl isobutanoate	1394	0.09	0.01									I, MS
$\alpha$ -Gurjunene	1409								0.09	0.12		I, MS
$\beta$ -Caryophyllene	1417	0.28	0.22			2.37	2.95	0.6	0.7	0.46	0.35	I, MS, Co-GC
Citronellyl propanoate	1445	0.14	0.13									I, MS
$\alpha$ -Caryophyllene	1451	0.08	trace			3.42	3.62					I, MS, Co-GC
<i>allo</i> -Aromadendrene	1459	0.08	trace									I, MS
Geranyl propanoate	1475	0.64	0.12									I, MS
Germacrene D	1479	0.80	0.76									I, MS
Viridiflorene	1496	0.50	0.09									I, MS
$\beta$ -Bisabolene	1506	0.12	trace						0.1	0.18	0.13	I, MS
$\gamma$ -Cadinene	1512									0.83	0.77	I, MS
Geranyl isobutanoate	1515	0.21	trace									I, MS
$\delta$ -Cadinene	1522	0.16	0.13						0.1			I, MS
Geranyl butanoate	1562	0.28	0.35									I, MS
Spathulenol	1577	<b>0.09</b>	<b>2.83</b>	2.09	1.99							I, MS
Caryophyllene oxide	1583			<b>0.41</b>	<b>2.23</b>					0.97	1.31	I, MS
2-Phenyl ethyl tiglate	1587	1.28	1.32									I, MS
Viridiflorol	1592					1.89	1.52					I, MS
1,10- <i>di-epi</i> -Cubenol	1617	0.14	trace							0.10	0.18	I, MS
10- <i>epi</i> - $\gamma$ -Eudesmol	1622	4.50	5.78									I, MS
1- <i>epi</i> -Cubenol	1630	0.10	trace									I, MS
$\gamma$ -Eudesmol	1634	0.07	trace									I, MS
$\tau$ -Cadinol	1640									1.36	0.63	I, MS
Vulgarone B	1651			11.41	12.60							I, MS
$\alpha$ -Eudesmol	1653	0.40	0.35									I, MS
Valerianol	1655	0.56	0.74									I, MS
(E)-Citronellyl tiglate	1668	0.10	0.02									I, MS
Geranyl tiglate	1701	1.01	0.95									I, MS
Total		98.25	98.07	93.88	95.46	98.18	97.2	96.7	97.2	97.90	97.12	

<sup>a</sup>Compounds listed in order of elution from an HP-5 MS capillary column<sup>b</sup>RI: Retention indices as determined on a HP-5 MS capillary column using a homologous series of n-alkanes (C9-C23) <sup>c</sup>Identification method: I=retention index, MS=mass spectrum, Co-GC=coinjection with authentic compound.<sup>c</sup>M = mycorrhizal, NM = non-mycorrhizal<sup>d</sup>Bold and underlined values are significantly different ( $P = 0.05$ ) according to T Test.