



Effect of arbuscular mycorrhizal fungi on plant growth and essential oil content and composition of *Ocimum basilicum* L.

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Abstract

The potential of three arbuscular mycorrhizal fungi (AMF) - *Glomus mosseae*, *G. fasciculatum* and *G. intraradices*- to enhance the production of essential oil and plant growth parameter was investigated in sweet basil (*Ocimum basilicum*). The AMF inoculation significantly increased plant height, fresh and dry matter yield, oil content and oil yield as compared to non-inoculated basil plants. Essential oil content in plants inoculated with *G. mosseae* and *G. fasciculatum* was significantly higher than other treatments. Shoot fresh weight significantly increased by all three mycorrhiza fungi species, but only inoculation with *G. intraradices* and *G. fasciculatum* increased root dry weight. Also oil composition improved with AMF inoculation and linalool and methyl chavicol that enhance the essence quality, increased in inoculated plant compared to control plants. These results demonstrate that AMF concomitantly increase essential oil production and biomass in an herbaceous species rich in commercially valued essential oils. Therefore, AMF potentially represent an alternative way of promoting growth of this important medicinal herb, as natural ways of growing such crops are currently highly sought after in the herbal industry.

Keywords: *Ocimum basilicum*; arbuscular mycorrhizal fungi; essential oil

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Introduction

Traditional systems of medicine are popular in developing countries, and up to 80% of the population relies on traditional medicines or folk remedies for primary health care needs. A great number of scientists and organizations turn their attention to traditional therapies in order to

find and conserve important resources (Akerle, 1990). Medicinal herbs are known as sources of phytochemicals or active compounds that are widely sought after worldwide for their natural properties. Members of the Lamiaceae family have been used since ancient times as sources of spices and flavorings (Hirasa and Takemasa, 1998) and for their pharmaceutical properties (Bais et al., 2002). Plants show enormous versatility in synthesizing complex materials which have no immediate obvious growth or

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metabolic functions. These complex materials are referred to as secondary metabolites. Plants secondary metabolites have recently been referred to as phytochemicals. Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities. It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect (Halliwell and Gutteridge, 1992).

Antioxidants protect other molecules (*in vivo*) from oxidation when they are exposed to free radicals and reactive oxygen species which have been implicated in the etiology of many diseases and in food deterioration and spoilage (Halliwell and Gutteridge, 1992; Farombi, 2000).

The genus *Ocimum* comprises more than 150 species and is considered as one of the largest genera of the Lamiaceae family (Evans, 1996). Several *Ocimum* species (Lamiaceae) are used to treat central nervous system disorders in various parts of the world and its antidepressive activities are frequently reported (Corrêa, 1984). Leaves from *Ocimum* species release a pleasing odor when squashed between the fingers and could be used as a culinary condiment and for insect control (Holm, 1999). *Ocimum basilicum* L. (sweet basil) is an annual herb which grows in several regions all over the world. The plant is widely used in food and oral care products. The essential oil of the plant is also used as perfumery (Bauer et al., 1997). The leaves and flowering tops of sweet basil are used as carminative, stomachic and antispasmodic medicinal plant in folk medicine (Chiej, 1998; Duke, 1989). Antiviral and antimicrobial activities of this plant have also been reported (Chiang, 2005). *Ocimum* spp. contains a wide range of essential oils rich in phenolic compounds and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins. The chemical composition of basil oil has been the subject of considerable studies. There is extensive diversity in the constituents of the basil oils and several chemotypes have been established from various phytochemical investigations. However, linalool, methyl chavicol, methyl cinnamate, methyl eugenol, eugenol and geraniol are reported as major components of the oils of different chemotypes of *O. basilicum*. Sweet basil (*Ocimum*

basilicum) has been traditionally used for the treatment of many ailments, such as headaches, coughs and diarrhea and it is generally recognized as safe and is a rich source of phenolic antioxidant compounds and flavonoids (Juliani and Simon, 2002). Recent scientific research has investigated the health benefits associated with basil essential oils. Studies reveal the anti-viral, anti-microbial, antioxidant, and anti-cancer properties of the oils.

Arbuscular mycorrhizal symbiosis is formed by approximately 80% of the vascular plant species in all terrestrial biomes (Smith et al., 2010). Arbuscular mycorrhizal fungi (AMF) are of great ecological importance, since arbuscular mycorrhizae is the most widespread plant symbiosis that often improves plant productivity (Fedderman et al., 2010). The main advantage of mycorrhiza to the host plants is the extension of the penetration zone of the root fungus system. The interconnected networks of external hyphae act as an additional catchment and absorbing surface in the soil (Sharma, 2004). The increased efficiency of mycorrhizal roots versus non mycorrhizal roots is caused by the active uptake and transport of nutrients especially immobile minerals like P, Zn and Cu (Phiri et al., 2003). There are few reports on AM studies of family Lamiaceae including *Ajuga pyramidalis* (Erikson et al., 2002), *Betonica officinale* (Fuchs and Hasselwandter, 2004), *Clinopodium gracile* (Yamato, 2004), *Lavandula angustifolia* (Linderman and Davis, 2003), *Thymus polytrichus* (Whitefield et al., 2004), *Ocimum basilicum* (Dickson, 2004), *Salvia azurea* (Wilson et al., 2001) and *Mentha species* (Gupta et al., 2002; Freitas et al., 2004). Mycorrhizal inoculation not only promoted the growth of medicinal plants but also improved the productivity and quantity of chemicals. Hence, there is an upcoming demand for research in improving the quality and quantity of drugs produced from native medicinal plants in relatively less time with application of AM fungi (Karthikeyan et al., 2009).

Many species belonging to the Lamiaceae, including sweet basil, form arbuscular mycorrhizas (Wang and Qiu, 2006). In addition to increasing uptake of poorly available nutrients such as phosphorus and nitrogen (Smith and Read, 1997; Toussaint et al., 2004) or conferring

protection against pathogens (Fillion et al., 1999), arbuscular mycorrhizal fungi can also induce changes in the accumulation of secondary metabolites, including phenolic compounds, in host plant roots (Devi and Reddy, 2002). Relatively little is known about the effects of AM colonization on the accumulation of active phytochemicals in shoots of medicinal plants, which are often the harvest products. However, it was reported that *Glomus mosseae* directly increases the essential oil content in shoots of *Origanum* sp. (Khaosaad et al., 2006) as well as sweet basil (Copetta et al., 2006).

The overall aim of the present study was to see if different AMF species can provide an efficient and natural way of improving the growth of this medicinal herb and increase essential oil content of basil plants.

Materials and Methods

In order to assess the effect of Arbuscular Mycorrhiza Fungi (AMF) on growth parameters and essential oil content of sweet basil plant, three different species of AMF were used in this study. *Glomus fasciculatum*, *Glomus intraradices* and *Glomus mosseae* were used for the inoculation of *Ocimum basilicum* seeds.

The experiment was done in the greenhouse of College of Agriculture in University of Tehran, in a randomized complete block design with four treatments (three AMF and one control) and four replications. Seeds of sweet basil were sown directly into pots. The pots contained 5 kg of sand and soil mix (50:50 %V) with or without (control) AMF inoculum. Mycorrhizal inoculum was obtained from Soil and Water Research Institute, Karaj, Iran.

Basil plants grew in the greenhouse at 25 °C day/18 °C night temperatures. Plants were

watered every day during the first days and after germination, when needed.

The essential oil of basil plant is the highest level in the flowering stage, so after about 90 day growth when plants were in flowering stage, they were harvested and the following parameters were recorded. These included shoot and root fresh and dry weight, plant height, number of leaves, inflorescence length and essential oil content.

For the essential oil extraction, basil plants were dried under shade, pulverized in a mechanical grinder. The essential oil was obtained by hydro distillation in a Clevenger-type apparatus using 20 g of dried leaves over 1 hour. The obtained oil was measured by direct weighing. The oil content was calculated as the amount (g) of oil per weight (g) of dry basil tissue.

The essential oil was analyzed with gas chromatography/mass spectrophotometer (GC/Mass) for chemical analysis of oil compounds. Gas Chromatography/Mass spectrometry analyses were performed on a Varian 3400 and the analytical conditions were as follows. Helium was the carrier gas; 1 ml of sample was injected at 250 °C with a column flow of 1.2 ml/min in a DB-1 column (60 m×0.25 mm, 0.25 µm film thicknesses). Components were identified according to databases and quantified by comparison with certified standards for 28 oils.

Statistical analyses

Treatment effects were determined by one-way analyses of variance (ANOVA) by SPSS software and differences between treatments were determined using Duncan's multiple range tests at a significance level of 95%.

Table1.
Effect of different AMF on growth parameter of *Ocimum basilicum* plants

Treatments	Shoot fresh weight(g)	Shoot dry weight(g)	Root fresh weight(g)	Root dry weight(g)	Leaves number	Plant height(cm)	Inflorescent height(cm)
<i>G.mosseae</i>	22 a	4 a	9 b	1 b	88 a	52 a	9 a
<i>G.fasciculatum</i>	18 ab	3 ab	10 b	1.2 ab	90 a	50 a	8.5 ab
<i>G.intraradiceces</i>	11 c	2 c	12 a	1.3 a	75 b	45 b	7.8 b
Control	8 d	1.5 d	6 c	0.8 c	70 c	35 c	7.6 b

Within each column means followed by the same letter are not significantly different based on Duncan's Multiple Range Test at $P \leq 0.05$.

Results

Effects of three AMF inoculations on *Ocimum basilicum* development differed for the *Glomus* species (*G. intraradices*, *G. fasciculatum* and *G. mosseae*). Overall, *G. mosseae* performed better than *G. fasciculatum* and *G. intraradices*. Maximum shoot fresh and dry weights were obtained in plants treated with *G. mosseae* inoculation followed by *G. fasciculatum*, *G. intraradices* and control (no inoculation) (Table 1).

Numbers of leaves and leaflets did not differ significantly, in basil plant inoculated with *G. fasciculatum* and *G. mosseae* treatments even though were they higher than in other treatments. Shoot length and inflorescent length were lower in basil plants inoculated with *G. intraradices* compared to the other mycorrhizal fungi, but no significant difference was observed in inflorescent length in all treatments.

Maximum root fresh and dry weight were obtained in plants inoculated with *G. intraradices* and *G. fasciculatum* followed by *G. mosseae* and control.

The concentration of essential oil of basil plants increased significantly with mycorrhization (Fig. 1). Although no significant differences were observed between *G. fasciculatum* and *G. mosseae*, *G. fasciculatum* was more efficient than the other two mycorrhiza fungi on essential oil

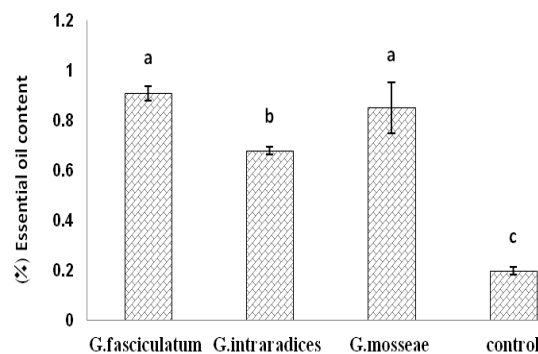


Fig. 1. Effect of different AMF on essential oil content of *Ocimum basilicum* plants; columns with the same letter are not significantly different based on Duncan's Multiple Range Test at the 0.05 level.

content of basil plant and this was followed by *G. mosseae* and *G. intraradices*. In comparison with control plants, essential oil was increased significantly in all arbuscular mycorrhizal fungi treatments.

Chemical characterization of essential oil by GC/Mass revealed that quality of oil improved on mycorrhization with significant increase in the concentration of linalool and Methyl chavicol concentration (Table 2).

Discussion

Enhanced growth and development following inoculation with AMF has been

Table 2

Percentage composition of oil extracts from *O. basilicum* according to quantitative chemical analyses

oil component %	<i>G. fasciculatum</i>	<i>G. mosseae</i>	<i>G. intraradices</i>	control
Linalool	45	41.27	31.05	21.75
Methyl chavicol	6.61	5	2.32	1.45
Neral	9.92	4.39	15.72	2.92
Nerol	0.8	0	2.28	1.14
Geraniol	0.64	1.97	2.17	1.02
Geranial	14.9	6.6	10	0.85
trans- α -bergamotene	4.83	10.75	9.33	3.59
Muurola4,5diene(cis)	0.47	0.85	0	2.42
Muurola4,5diene(Trans)	2.49	2.75	2.14	1.04
germacrene A	0.98	1.62	1.31	2.26
Cadinene	1.73	4.14	4.86	0
elemol	0.85	0.63	1.05	5.65
Caryophyllene oxide	0.92	1.4	3.14	0.69
1,10-diepi-cubenol	0	1.5	4.33	2.17
δ -cadinol	0	1.21	0	3.6
Epi- α -cadinol	3.83	0	3.81	23.26

reported for a number of plant species (Freitas et al., 2004; Copetta et al., 2006; Smith and Read, 1997). Possible causes are increases in growth hormone production, phosphate solubilization, sulfur oxidation, nitrate availability, extracellular production of antibiotics, lytic enzymes, hydrocyanic acid, root permeability, competition for available nutrients from root sites, and/or induction of plant systemic resistance (Fillion et al., 1999).

AMF typically alter root morphology. More specifically, they increase root surface area and thereby enhance soil nutrient uptake potential (Copetta et al., 2006).

All three AMF strains evaluated in this experiment produced a significant increase in shoot fresh and dry weight and plant height of sweet basil plants. A similar result was observed in *Origanum majoranum* inoculated with *G. fasciculatum* (Devi and Reddy, 2002). Consistent with our findings, *Glomus fasciculatum* were reported to improve overall growth of various crops (Gupta et al., 2002).

Concentration and composition of essential oils in plants serve important ecological roles. Increased synthesis of essential oils provides a defensive response to colonization by microorganisms, since several essential oils have antimicrobial properties (Sangwan et al., 2001). There have been few attempts to elucidate relative quantitative and qualitative contributions of AMF to formation of plant secondary compounds.

In this study, inoculation with *G. fasciculatum* or *G. mosseae* caused a 4-fold increase of essential oil accumulation compared to non-inoculated plants. This finding suggests increased terpene biosynthesis, although direct measurements were not made. The systemic induction of monoterpenes in *O. basilicum* is consistent with previous reports in other aromatic plant species (Devi and Reddy, 2002; Banchio et al., 2009).

Our findings are in agreement with those of other experiments. In a study on basil plant by Rasouli-Sadaghiani, et al. (2010) showed that mycorrhizal plants had significantly higher shoot and root dry weight and plant height and *G. fasciculatum* were more effective than other mycorrhizal fungi to increase essential oil content

and yield in basil. Treatment of *Ocimum basilicum* with *G. mosseae* increased total essential oil yield and content of eugenol and terpineol (Banchio et al., 2009). Induction of secondary metabolite responses has been reported in other beneficial microbe-plant interactions involving arbuscular mycorrhizal (AM) fungi. Gupta et al. (2002) inoculated the *Glomus fasciculatum* in cultivars of wild mint (*Mentha arvensis*) and observed increased plant height, shoot growth, and oil content. Khaosaad et al., (2006) observed changes of essential oil concentration (but not composition) following mycorrhizal inoculation of *Origanum* sp. Copetta et al. (2006) found increased abundance of glandular hairs, and essential oil yield in mycorrhizal inoculated *Ocimum basilicum*.

The increased oil yield was associated with a larger number of peltate glandular trichomes, the main site of essential oil synthesis. AM fungi increase plant growth and essential oil production because mycorrhization allows the root system to exploit a greater volume of soil by (a) extending the root zone; (b) reaching smaller soil pores not accessible by root hairs; (c) acquiring organic phosphate through production of extracellular acid phosphatases (Bouwmeester et al., 2007).

Factors that increase dry matter production may influence the interrelationship between primary and secondary metabolism, leading to increased biosynthesis of secondary products (Shukla et al., 1992). Increased plant biomass appears to result in greater availability of substrate for monoterpene biosynthesis (Harrewijn et al., 2001). The increased concentration of monoterpenes in inoculated plants may be attributed to growth-promoting substances, produced by the inoculated microorganisms that affect plant metabolic processes.

Knowledge of the adaptive mechanisms of plants is of interest from an ecophysiological point of view, but these mechanisms also constitute an important starting point, probably the key to improving plant production, including optimization of secondary metabolite production.

The use of fungal and bacterial inoculants is an efficient biotechnological alternative for stimulating secondary metabolism in plants, and

may also lead to relevant information on certain adaptive processes which are poorly understood.

The root colonization of *Ocimum basilicum* by AMF may bear considerable economic significance, because such colonization has been demonstrated to dramatically improve biomass yield of other medicinal and aromatic plants. Thus, the addition of suitable AMF to the rhizosphere could be expected to significantly improve the growth and productivity of commercial *Ocimum* spp. planting (Gupta et al., 2000).

In the present study it was found that inoculation of arbuscular mycorrhizal fungi causes systemic induction of monoterpene pathways in basil, suggesting that inoculation with *G. fasciculatum* and/or *G. mosseae* can significantly increase productivity and reduce the amount of fertilizer required for economically viable *Ocimum basilicum* crops. Carefully designed and controlled field trials are essential before the ability of *G. fasciculatum* and *G. mosseae* to promote growth of *Ocimum basilicum* or other aromatic crops can be commercially exploited.

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