Medicinal and Aromatic Plant Science and Biotechnology ©2011 Global Science Books

Responses of *Ocimum sanctum* to Inoculation with Arbuscular Mycorrhizal Fungi and Fertilization with Different Phosphate Sources

Mohammad J. Saharkhiz[1*] • Mohammad Merikhi[1] • Mehdi Zarei[2] • Jaime A. Teixeira da Silva[3]

[1] Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran
[2] Department of Soil Science, College of Agriculture, Shiraz University, Shiraz, Iran
[3] Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Kagawa 761-0795, Japan

Corresponding author: * **saharkhiz@shirazu.ac.ir**

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) can change some morphological and physiological characteristics of host plants. A pot experiment was conducted to evaluate the responses of holy basil (*Ocimum sanctum*) to the inoculation with AMF species (*Glomus mosseae* and *Glomus versiforme*) and the use of two different phosphate sources (superphosphate and rock phosphate) as phosphorus fertilizers in a calcareous soil. After a growth period of three months, morphological parameters, root colonization, phosphorus concentration and uptake, chlorophyll content, and the yield of essential oil were measured. AMF significantly ($P \leq 0.05$) increased shoot biomass, flower stem length, chlorophyll content, phosphorus concentration and uptake, and root colonization. Phosphorus fertilizers increased all factors except for root colonization compared with the control. Superphosphate was more effective than rock phosphate and control treatments. A synergistic relationship between AMF and phosphorus fertilizers for improving phosphorus uptake and morphological characteristics was observed. Maximum shoot biomass, phosphorus concentration and uptake, as well as essential oil yield were observed when *G. versiforme* was combined with superphosphate. Rock phosphate combined with inoculation with *G. versiforme* showed a more positively significant ($P \leq 0.05$) effect on all measured traits compared to a single application of rock phosphate. In conclusion, the introduction of AM biofertilizer together with the balanced application of P-fertilizers will be helpful in *O. sanctum* production.

Keywords: essential oil, *Glomus mosseae*, *Glomus versiforme*, growth parameters, holy basil, rock phosphate, superphosphate

INTRODUCTION

An organism's interaction with its environment is fundamental for its survival and for the establishment and function of natural ecosystems (Witzany 2008). Mutualistic symbiosis is one of the most abundant symbiotic activities in ecosystems. Arbuscular mycorrhizae are symbiotic associations formed between fungi, which belong to the phylum Glomeromycota (Schüßler *et al.* 2001) and the root system of many plant species. Host plants significantly supply photosynthetically fixed carbon to fungi, which enhance the uptake of plant nutrients, water, and exudate other growth-promoting factors such as siderophore, phytohormones and organic acids (Selosse *et al*. 2006). Mycorrhizae are beneficial for host plants, especially under low fertility conditions. Arbuscular mycorrhizal fungi (AMF) are known to play an important role in improvement of plant nutrition and growth of several ornamentals and vegetable crops (Rouphael *et al.* 2010; Koltai 2010). Nevertheless, little is known about their potential effect in increasing the production of secondary metabolites in medicinal and aromatic plants (Kapoor *et al*. 2002a, 2002b; Copetta *et al*. 2006; Khaosaad *et al*. 2006). Several studies have been performed on changing secondary compound patterns of medicinal plant symbioses with mycorrhizal fungi such as terpenoids (Akiyama and Hayashi 2002), phenols (Zhu and Yao 2004), phenylpropanoids (Weiss *et al*. 1997), glucosinolates (Vierheilig *et al*. 2000), carotenoids (Maier *et al*. 1995; Fester *et al*. 2002) and flavonoids (Larose *et al*. 2002). AMF also influence the quantity of essential oil (EO) in aromatic plants. In some plants such as *Carum* species, *Glomus macrocarpum* or *Glomus fasciculatum* significantly enhanced the concentration of EO in the fruits. Maximum EO yield was obtained from *Anethum graveolens* and *Trachyspermum ammi* inoculated with *G. macrocarpum* followed by *G. fasciculatum* (Kapoor *et al*. 2002a). Khaosaad *et al*. (2006) showed that *G. mosseae* increased the concentration of EO in two genotypes of *Origanum vulgare* in comparison with non mycorrhizal-infected plants. Consumption efficiency and recovery of chemical phosphorus (P) fertilizers in calcareous soils is low (10-30%) due to alkaline pH and the formation of calcium and magnesium phosphate. Therefore, attempting to utilize indigenous reactive ground rock phosphate as a cheap source and application of biological fertilizers as an environmental friendly and alternative method is essential. Among microorganisms, AMF are able to solubilize insoluble phosphates and improve plant P nutrition (Zarei *et al*. 2006; Smith and Read 2008; Sabannavar and Lakshman 2009). *Ocimum sanctum* (holy basil) is one of the important medicinal and aromatic plants belonging to the Lamiaceae family (Warrier 1995). Holy basil is grown in Southeastern Iran (Zargari 1990). This plant is cultivated and used extensively in India and South Eastern Asia. Different parts of holy basil plant such as leaves, flowers, stem, root, and seeds are known to possess therapeutic potential and have been used by traditional medical practitioners, as expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidaemic, and antistress agents (Zargari 1990). The foliage, flower, and fruits have volatile oil. The qualitative and quantitative improvement of EO in aromatic plants represents an area of high commercial interest. Holy basil can be colonized by AMF (Gupta *et al*. 2000; Karthikeyan *et al*. 2009). The current study is a comparative ana-

Received: 21 March, 2011. Accepted: 9 July, 2011.

Original Research Paper

lysis of the application of two AMF species (*Glomus mosseae* and *Glomus versiforme*) as well as soluble and insoluble P fertilizers on plant development and quantity of *O. sanctum* EO in a calcareous soil.

MATERIALS AND METHODS

Soil preparation and experimental design

A pot experiment was conducted at the Department of Horticulture Science, Shiraz University from May to August 2009. Each pot was filled with 5 kg of soil. A non-sterile compound soil sample was used for plant cultivation (depth of 0-30 cm, from a field located at the College of Agriculture, Shiraz University). An air-dried soil sample was passed through a 2-mm sieve and mixed uniformly. The soil was clay loam, with 1: 1 soil: water, pH of 7.3, electrical conductivity of 0.5 dS m^{-1}, organic matter content of 0.46%, field capacity moisture of 20%, total nitrogen content of 0.03%, Olsen P of 5.4 mg kg^{-1}, available K of 300 mg kg^{-1} and carbonate calcium equivalent (CCE) of 25% (Page *et al*. 1982), DTPA-extractable Fe of 4.4 mg kg^{-1}, DTPA-extractable Zn of 1.8 mg kg^{-1}, DTPA-extractable Cu of 1.4 mg kg^{-1}, DTPA-extractable Mn of 4.8 mg kg^{-1} (Lindsay and Norvell 1978). All treatments received mineral nutrients applied in solution to each compartment at rates of 100 mg N kg^{-1} of soil as urea (46%), and Fe, Zn, Cu, and Mn at a rate of 5 mg kg^{-1} of soil as $FeSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$ and $MnSO_4 \cdot H_2O$, respectively.

The experiment was a completely randomized design in a factorial arrangement with two factors: (1) AMF at three levels, G_0 (control), G_1 (*Glomus versiforme*) and G_2 (*Glomus mosseae*); (2) P sources at three levels, P_0 (control), P_1 (triple superphosphate) and P_2 (rock phosphate). The quantity of P applied in both fertilizers was 20 mg P kg^{-1} of soil. P fertilizers were completely incorporated into the soil before cultivation. Six replications were prepared for each treatment.

AMF preparation and inoculation

G. mosseae and *G. versiforme* spores were obtained from the Department of Soil Science, Shiraz University. The fungi were isolated from a non-contaminated area of Anguran Mine, Zanjan, Iran (Zarei 2008). These fungi are abundant in Iranian soils (Sadravi 2000; Aliasgharzadeh *et al*. 2001; Kariman *et al*. 2005). Mycorrhizal inocula were prepared through the trap culture of maize (*Zea mays* L.). Trap culture medium was composed of autoclaved soil/quartz-sand (< 1 mm) (1: 4, v/v). Simultaneously, some pots were kept without any spore inoculation for preserving the naturally-occurring microbial association and used for control treatments. After 4.5 months, at the beginning of the reproductive period, shoots were removed and the contents of pots (mycorrhizal roots plus soil possessing fungal spores and mycelia) were maintained in polyethylene bags at 4°C. Seeds of holy basil (*O. sanctum*) were provided by Zaravand Co., located in Shiraz, Iran. In mycorrhizal treatments, 40 g of AMF inoculants (with 440 spores and 85% root colonization) were added to pots at the time of sowing just below the seeds. The potential of inoculants was measured based on the described methods of Zarei *et al.* (2008), for spore extraction and counting, and for evaluating root colonization. After germination, seedlings were thinned to 5 plants pot^{-1}. Pots were irrigated every two days and maintained for three months in field conditions at the Research Station, College of Agriculture, Shiraz University in the suburb of Shiraz (Badjgah), Iran. The station is located at 1810 m above mean sea level, latitude 29° 36′ north and Altitude 52° 32′ east. The minimum and maximum temperatures of the field in last 10-year period were -10 and 38°C, respectively.

Morphological measurements

At the full flowering stage (75 days after seedling emergence) the following morphological parameters were determined: shoot and root length, shoot and root fresh and dry weight, leaf number and area. The value for total root length was obtained using a ruler before drying the roots. Leaf area was determined by an AM200 portable leaf area meter (AM 200, Analytical Development Co. (ADC), Hoddesdon, UK). The dry weight of roots and shoots was measured after washing and drying at 65°C for 72 h.

Mycorrhizal colonization

The percentage of root colonization was determined by using the grid-line intersect method, after clearing washed roots of debris in 10% KOH and staining with blue ink in lactoglycerol according to the Kormanik and McGraw (1982) method.

Determination of P concentration and uptake in plants

Shoot P concentration was assessed by colorimetry using the vanado-molybdate method (Spectronic 20, Bausch and Lomb, Rochester, New York, USA) (Cottenie 1980). P-uptake was calculated by following formula:

P-uptake (mg pot^{-1}) = P concentration (g kg^{-1}) × dry weight of shoot (g pot^{-1}).

Determination of EO and chlorophyll content

At the full flowering stage, the aerial parts (50 g) of plants (including flowers, leaves and stems in equal amounts) were hydrodistilled for 2.5 h using an all-glass Clevenger-type apparatus according to the method outlined by the British Pharmacopoeia (1988). EO samples were weighed and yields were determined. EOs were dried over anhydrous sodium sulfate and stored in sealed vials (2-ml vials, Razi Co, Iran) at 4°C. Ten fresh leaves of each plant (four independent plants) were randomly used to measure the chlorophyll content with a chlorophyll meter (SPAD 502, Minolta, Japan).

Statistical analyses

Data was analyzed statistically with SPSS software (version 15) and means comparison was performed with Duncan's multiple range test (DMRT) at $P \leq 0.05$.

RESULTS

AMF significantly increased shoot length, shoot dry weight, flower length, chlorophyll content, P concentration and uptake, and root colonization. In this study, the application of *G. versiforme* singly and in combination with P sources was superior to *G. mosseae* and the control by increasing most of the parameters that were surveyed (*G. versiforme* > *G. mosseae* > control) (**Tables 1, 3**).

The effect of P source was significant for all the measured characteristics. Fertilizer treatments (P_1 and P_2) increased all factors – except for root colonization – more than the control (**Table 2**). Superphosphate was more effective than rock phosphate and control treatments. Moreover, the maximum EO content was obtained with superphosphate (**Tables 2, 3**).

Co-application of AMF and P source had a significant positive effect on the shoot and root dry weights, shoot length, leaf area, P concentration and uptake, and root colonization. The maximum shoot length and dry weight, flower stem length, P concentration and uptake, and EO yield were observed by co-application of *G. versiforme* and superphosphate (**Table 3**). The application of rock phosphate and inoculation of plants with *G. versiforme* showed a more positive significant effect on the shoot and root dry weights, shoot length, P concentration and uptake, EO content, and root colonization compared to a single application of rock phosphate (**Table 3**).

DISCUSSION

In the present study, inoculation of holy basil with AMF greatly increased plant growth parameters, P concentration and uptake, and root colonization. The response of holy basil to inoculation was greatly dependent on the fungal

Table 1 Effects of inoculation with AMF (G) on the measured parameters of *Ocimum sanctum*.

AMF	Shoot length (cm)	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1}))	Leaf area (cm^2)	Flower length (cm)	Chlorophyll	P		Essential oil (%)	Root colonization (%)
							Concentration (g kg^{-1})	Uptake (mg pot^{-1})		
G_0	14.8 ± 0.3 c*	1.6 ± 0.07 c	0.5 ± 0.03 b	25.0 ± 0.7 b	5.1 ± 0.2 b	35.3 ± 0.7 b	2.8 ± 0.1 b	4.7 ± 0.3 c	0.6 ± 0.03 a	17.2 ± 0.6 c
G_1	16.1 ± 0.3 a	3.0 ± 0.4 a	0.8 ± 0.09 a	28.6 ± 1.6 a	6.6 ± 0.38 a	40.6 ± 1.5 a	4.0 ± 0.3 a	11.9 ± 2.0 a	0.6 ± 0.02 a	41.1 ± 3.6 a
G_2	15.7 ± 0.2 b	2.0 ± 0.3 b	0.9 ± 0.06 a	29.9 ± 0.7 a	5.6 ± 0.32 b	36.3 ± 1.2 b	3.5 ± 0.2 a	7.4 ± 1.4 b	0.6 ± 0.02 a	34.5 ± 2.6 b

*Values followed by different letters in the same column are significantly different at $P \leq 0.05$. Data reported as average ± SE (n = 6)
G_0: control, G_1: *Glomus versiforme* and G_2: *Glomus mosseae*

Table 2 Effects of phosphate sources (P) on the measured parameters of *Ocimum sanctum*.

Phosphate sources	Shoot length (cm)	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})	Leaf area (cm^2)	Flower length (cm)	Chlorophyll	P		Essential oil (%)	Root colonization (%)
							Concentration (g kg^{-1})	Uptake (mg pot^{-1})		
P_0	14.7 ± 0.3 c*	1.5 ± 0.1 c	0.5 ± 0.03 c	24.7 ± 0.6 c	5.0 ± 0.3 c	33.3 ± 1.2 b	2.6 ± 0.2 b	3.9 ± 0.5 b	0.6 ± 0.01 b	38.4 ± 5.5 a
P_1	16.3 ± 0.3 a	2.8 ± 0.4 a	0.9 ± 0.07 a	31.3 ± 1.2 a	6.7 ± 0.4 a	38.5 ± 1.6 a	3.9 ± 0.3 a	11.3 ± 0.2 a	0.7 ± 0.04 a	24.7 ± 2.3 b
P_2	15.6 ± 0.2 b	2.3 ± 0.2 b	0.8 ± 0.09 b	27.5 ± 1.7 b	5.7 ± 0.4 b	40.4 ± 1.1 a	3.8 ± 0.2 a	8.9 ± 0.1 a	0.6 ± 0.03 b	28.7 ± 2.7 c

*Values followed by different letters in the same column are significantly different at $P \leq 0.05$. Data reported as average ± SE (n = 6)
P_0: Control, P_1: Super phosphate and P_2: Rock phosphate.

Table 3 The results of interaction between AMF (G) and phosphate sources (P) on the measured parameters of *O. sanctum*.

G ×P	Shoot length (cm)	Shoot dry weight (g)	Root dry weight (g)	Leaf area (cm^2)	Flower length (cm)
$G_0 \times P_0$	13.7 ± 0.2 d*	1.3 ± 0.1 e	0.4 ± 0.02 e	23.1 ± 0.8 d	4.5 ± 0.2 d
$G_0 \times P_1$	15.4 ± 0.2 c	1.8 ± 0.02 cde	0.5 ± 0.03 de	25.4 ± 0.4 cd	5.9 ± 0.5 bcd
$G_0 \times P_2$	15.3 ± 0.2 c	1.7 ± 0.1 de	0.6 ± 0.05 d	26.5 ± 1.5 cd	5.0 ± 0.2 bcd
$G_1 \times P_0$	14.9 ± 0.3 c	1.7 ± 0.2 de	0.6 ± 0.03 d	25.7 ± 0.7 cd	5.6 ± 0.4 bcd
$G_1 \times P_1$	17.3 ± 0.2 a	4.3 ± 0.2 a	1.1 ± 0.04 ab	32.3 ± 1.7 ab	7.9 ± 0.1 a
$G_1 \times P_2$	16.2 ± 0.06 b	3.2 ± 0.2 b	0.8 ± 0.07 c	27.7 ± 1.7c	6.4 ± 0.6 b
$G_2 \times P_0$	15.3 ± 0.2 c	1.5 ± 0.2 e	0.5 ± 0.0 de	25.3 ± 0.4 cd	4.8 ± 0.3 cd
$G_2 \times P_1$	16.3 ± 0.1 b	2.4 ± 0.1 c	1.2 ± 0.04 a	36.2 ± 2.0 a	6.3 ± 0.1 bc
$G_2 \times P_2$	15.4 ± 0.1 c	2.1 ± 0.4 cd	1 ± 0.03 bc	28.2 ± 0.8 bc	5.8 ± 0.8 bcd

G ×P	Chlorophyll	P		Essential oil (%)	Root colonization (%)
		Concentration (g kg^{-1})	Uptake (mg pot^{-1})		
$G_0 \times P_0$	32.4 ± 0.8 d	2.4 ± 0.1 e	3.1 ± 0.1 e	0.50 ± 0.01d	18.6 ± 1.4 f
$G_0 \times P_1$	34.9 ± 0.4 cd	3.0 ± 0.3 de	5.4 ± 0.4 de	0.63 ± 0.03 bcd	15.9 ± 0.9 f
$G_0 \times P_2$	38.7 ± 1.4 bc	3.2 ± 0.2 cd	5.6 ± 0.5 de	0.58 ± 0.07 bc	17.1 ± 0.7 f
$G_1 \times P_0$	35.1 ± 2.4 cd	2.7 ± 0.3 de	4.4 ± 0.6 de	0.53 ± 0.01c	53.8 ± 0.7 a
$G_1 \times P_1$	42.4 ± 2.5 ab	4.9 ± 0.2 a	18.3 ± 2.1 a	0.74 ± 0.01a	30.3 ± 1.0 de
$G_1 \times P_2$	44.0 ± 0.5 a	4.3 ± 0.3 ab	13.0 ± 1.3 b	0.56 ± 0.08 bc	35.7 ± 0.8 c
$G_2 \times P_0$	32.4 ± 1.4 d	2.8 ± 0.3 de	4.1 ± 1.0 de	0.70 ± 0.03 ab	44.1 ± 2.3 b
$G_2 \times P_1$	38.3 ± 1.2 bc	3.9 ± 0.2 b	10.3 ± 2.1bc	0.64 ± 0.06 abc	27.8 ± 1.1e
$G_2 \times P_2$	38.4 ± 1.6 bc	3.8 ± 0.2 bc	8.1 ± 1.7 cd	0.59 ± 0.04 bc	32.3 ± 0.5 cd

*Values followed by different letters in the same column are significantly different at $P \leq 0.05$. Data reported as average ± SE (n = 6)
G_0: Control, G_1: *Glomus versiforme*, G_2: *Glomus mosseae*, P_0: Control, P_1: Super phosphate and P_2: Rock phosphate

species. An increase in the growth rate and development of plant-AMF symbiosis compared to non-inoculated plants was also reported by other researchers (Kapoor *et al*. 2007; Ultra *et al*. 2007; Smith and Read 2008; Karthikeyan *et al*. 2009; Koltai 2010). AMF improve plant growth by increasing P uptake (Duponnois *et al*. 2005), micronutrients (Bürkert and Robson 1994), nitrogen (Barea *et al*. 1991; Gogoi and Singh 2011), and by enhancing water absorption (George *et al*. 1992). Inoculation of plants with mycorrhizal fungus creates a thinner root system, influencing small soil pores, and improves water and macronutrient efficiency, thus increasing the dry weight, shoot length, root colonization, and N, P, K concentration of *Eucalyptus globulus* (Arriagada *et al*. 2007). This study showed a significant effect of AMF on morphological and growth characteristic of holy basil, in agreement with reports of other aromatic species of the Lamiaceae family, such as *Origanum vulgare* var. Conaand, *O. vulgare* ssp. *hirtum* var. Kalitera and *O. basilicum* (Copetta *et al*. 2006; Khaosaad *et al*. 2006). However, the greatest effect on growth was observed with *G. versiforme* compared to *G. mosseae*. This may be the resulted of higher root colonization of plants by *G. versiforme*. High functional diversity among AM fungal species results in variations in plant responses (Munkrold *et al.* 2004).

P-fertilization increased all measured parameters except for root colonization. It is well documented that P is an essential element in the reproductive and vegetative growth of plants (Marschner 1995); therefore, vegetative growth and flower number increased after the application of P. P is also known to have multifarious cellular functions in plants, including signaling and transmembrane metabolic flux; secondary metabolism is thus modulated by these mechanisms (Ram *et al*. 2003). P significantly increased essential oil content and dry matter in *Tanacetum parthenium* L. (Saharkhiz and Omidbaigi 2008). Increasing P as a fertilizer also promoted flowering (Egle *et al*. 1999; Besmer and Koide 1999) particularly when P is a limiting factor in natural systems (Feller 1995).

Our results show that the infectivity of AMF is dependent on the solubility of P-fertilizers. The root colonization of AMF was high in soil without added P and decreased when the concentration of available P in the soil was high. The results in this study related to root colonization are similar to those of other studies with lentil (*Lens culinaris*), faba bean (*Vicia faba*), soybean (*Glycine max*) (Bader El-Din and Moawad 1988), clover (*Trifolium repens*) (Powell and Daniel 1978; Liu *et al*. 2003), *Trifolium pratense* L. (Takács *et al*. 2006), lentil *Lens culinaris* (Zarei *et al*. 2006) and *Piper longum* L. (Gogoi and Singh 2011), which showed that the application of P influences the lipid metabolism of AM fungi. Plant roots may reduce the flow of C to the fungus under high P conditions and thus decrease the fungal neutral lipid/phospholipid ratio in the extra radical mycelium or allocate C to fungal storage struc-

tures (Olsson *et al*. 2002). Moreover, high contents of P could increase phospholipid levels of plants and decrease root exudates and membrane permeability, which would not favor spore germination and hyphal development (Muthukumar *et al*. 1994).

The results of the interaction between mycorrhizal fungi and P source showed that biomass and the P content of holy basil improved due to a synergistic effect of these two factors. There is a synergistic relationship between AMF and P fertilizers for improving P uptake, morphological and physiological characteristics of *Lens culinaris* L. and *Artemisia annua* L. (Zarei *et al*. 2006; Kapoor *et al*. 2007). These positive effects may be attributed to an increase in contact zones between plant roots and P fertilizer through hyphal extension in soil (Bader El-Din and Moawad 1988). Mycorrhizal roots exploit the soil profile, with fungal hyphae extending beyond the depletion zone surrounding the absorbing root and its root hairs. The hyphae take up P and transport it to the host (Smith and Read 2008).

Positive significant effects of simultaneous application of *G. versiforme* and rock phosphate on plant biomass and P content may be attributed to the ability of AMF to exude organic acids and chelating agents and/or excrete H^+ ion (Harley 1989; Tawaraya *et al*. 2006; Zarei *et al*. 2006; Sabannavar and Lakshman 2009). Rock phosphate may be solubilized under the influence of water, acids, chelating agents, and oxygen (Rivas *et al*. 2002; Duponnois *et al*. 2005). Hyphal exudates of AMF have the ability to solubilize insoluble inorganic P (Tawaraya *et al*. 2006). An improvement in the bioavailability of insoluble phosphate and high concentrations of P in mycorrhizal symbiotic plants has been shown in *Cymbopogon martini* L. (Ratti *et al*. 2001), *Plantago lanceolata* L. (Lang *et al*. 2003), *Artemisia annua* L. (Kapoor *et al*. 2007), *Ocimum basilicum* L. (Toussaint *et al*. 2007), and *Sesamum indicum* L. (Sabannavar and Lakshman 2009).

A maximum amount of EO was obtained by the combined application of *G. versiforme* and rock phosphate followed by the single application of *G. mosseae*. Studies carried out on Lamiaceae species indicated a significant relation between the presence of AMF and EO accumulation in mints (Khaliq and Janardhanan 1997), *Mentha arvensis* (Gupta *et al*. 2002; Freitas *et al*. 2004), and *Ocimum basilicum* L. var. *genovese* (Coppeta *et al*. 2006). For example, it has been shown that inoculation of *Mentha viridis* and *Origanum onites* with AMF increased the EO content in both species up to 100% more than non-mycorrhizal plants (Karagiannidisa *et al*. 2011).

Mycorrhizal plants had significantly higher P concentration and EO content than non-mycorrhizal plants. This indicates that the increase in EO concentration in mycorrhizal plants might be attributed to enhanced P content.

CONCLUSION

Inoculation of holy basil with a suitable AMF species together with the application of P-fertilizers could increase morphological characteristics, P-concentration and uptake, and EO content. However, field trials to test the performance of the AM inoculants under real conditions are advisable since the efficiency of the inoculation varies with the soil type, P content of the soils and other environmental variables (Khan and Zaidi 2006). The introduction of AM biofertilizer in conjunction with P-fertilizers, especially rock phosphate, will be helpful in the cultivation of *O. sanctum*.

ACKNOWLEDGEMENTS

The authors wish to extend their thanks and appreciation to Prof. Dr. Najaf Ali Karimian for his valuable comments and scientific editing of the manuscript and to Shiraz University Research and Technology Council for their financial support (Grant 87-GR-AGRST-98).

REFERENCES

Akiyama K, Hayashi H (2002) Arbuscular mycorrhizal fungus promoted accumulation of two new triterpenoids in cucumber roots. *Bioscience, Biotechnology and Biochemistry* **66**, 762-769

Aliasgharzadeh N, Rastin NS, Towfighi H, Alizadeh A (2001) Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. *Mycorrhiza* **11**, 119-122

Bader El-Din SMS, Moawad HA (1988) Enhancement of nitrogen fixation in lentil, faba bean and soybean by dual inoculation with rhizobia and mycorrhizae. *Plant and Soil* **108**, 117-124

Barea JM, El-Atrach F, Azcon R (1991) The role of VA mycorrhizas in improving plant N acquisition from soil as assessed with ^{15}N. In: Fitton C (Ed) *The Use of Stable Isotopes in Plant Nutrition, Soil Fertility and Environmental Studies*, Joint IAEA, FAO Division, Vienna, pp 677-808

Besmer YL, Koide RT (1999) Effect of mycorrhizal colonization and phosphorus on ethylene production by snapdragon (*Antirrhinum majus* L.) flowers. *Mycorrhiza* **9**, 161-166

Bürkert B, Robson A (1994) ^{65}Zn uptake in subterranean clover (*Trifolium subterraneum* L.) by three vesicular-arbuscular mycorrhizal fungi in a root free sandy soil. *Soil Biology and Biochemistry* **26**, 1117-1124

Copetta A, Língua G, Berta G (2006) Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. *genovese*. *Mycorrhiza* **16**, 485-494

Cottenie A (1980) *Soil and Plant Testing as a Basis of Fertilizer Recommendations*, FAO Soil Bulletin 38/2, 115 pp

Duponnois R, Colombet A, Hien V, Thioulouse J (2005) The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericea*. *Soil Biology and Biochemistry* **37**, 1460-1468

Egle K, Manske G, Römer W, Vlek PLG (1999) Improved phosphorus efficiency of three new wheat genotypes from CIMMYT in comparison with an older Mexican variety. *Journal of Plant Nutrition and Soil Science* **162**, 353-358

Feller IC (1995) Effects of nutrient enrichment on growth and herbivory of dwarf red mangrove (*Rhizophora mangle*). *Ecological Monographs* **65**, 477-505

Fester T, Schmidt D, Lohse S, Walter M, Giuliano G, Bramley P, Fraser P, Hause B, Strack D (2002) Stimulation of carotenoid metabolism in arbuscular mycorrhizal roots. *Planta* **216**, 148-154

Freitas MSM, Martins MA, Curcino Vieira IJ (2004) Yield and quality of essential oils of *Mentha arvensis* in response to inoculation with arbuscular mycorrhizal fungi. *Pesquisa Agropecuária Brasileira* **39**, 887-894

George E, Haussler KU, Vetterlein D, Gorgus E, Marschner H (1992) Water and nutrient translocation by hyphae of *Glomus mosseae*. *Canadian Journal of Botany* **70**, 2130-2137

Gupta ML, Khali A, Pandey R, Shukla RS, Singh HN, Kumar S (2000) Vesicular-arbuscular mycorrhizal fungi associated with *Ocimum* spp. *Journal of Herbs Spices and Medicinal Plants* **7 (2)**, 57-63

Gogoi P, Singh RK (2011) Differential effect of some arbuscular mycorrhizal fungi on growth of *Piper longum* L. (Piperaceae). *Indian Journal of Science and Technology* **4 (2)**, 119-125

Gupta ML, Prasad A, Ram M, Kumar S (2002) Effect of the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* on the essential oil yield related characters and nutrient acquisition in the crops of different cultivars of menthol mint (*Mentha arvensis*) under field conditions. *Bioresource Technology* **81**, 77-79

Kapoor R, Chaudhry V, Bhatnagar AK (2007) Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in *Artemisia annua* L. *Mycorrhiza* **17**, 581-587

Kapoor R, Giri B, Mukerji KG (2002a) *Glomus macrocarpum*: A potential bioinoculant to improve essential oil quality and concentration in dill (*Anethum graveolens* L.) and carum (*Trachyspermum ammi* Sprague). *World Journal of Microbiology and Biotechnology* **18 (5)**, 459-463

Kapoor R, Giri B, Mukerji KG (2002b) Mycorrhization of coriander (*Coriandrum sativum*) to enhance the concentration and quality of essential oil. *Journal of the Science of Food and Agriculture* **82 (4)**, 339-342

Karagiannidisa N, Thomidisa T, Lazarib D, Panou-Filotheoua E, Karagiannidoua C (2011) Effect of three Greek arbuscular mycorrhizal fungi in improving the growth, nutrient concentration, and production of essential oils of oregano and mint plants. *Scientia Horticulturae* **129**, 329-334

Kariman KH, Goltapeh EM, Minassian V (2005) Arbuscular mycorrhizal fungi from Iran. *Journal of Agriculture and Technology* **1 (2)**, 301-313

Karthikeyan B, Joe MM, Jaleel CA (2009) Response of some medicinal plants to vesicular arbuscular mycorrhizal inoculations. *Journal of Scientific Research* **1 (1)**, 381-386

Khaliq A, Janardhanan KK (1997) Influence of vesicular arbuscular mycorrhizal fungi on the productivity of cultivated mints. *Journal of Medicinal and Aromatic Plant Science* **19**, 7-10

Khan MS, Zaidi A (2006) Influence of composite inoculations of phosphate solubilizing organisms and an arbuscular mycorrhizal fungus on yield, grain protein and phosphorus and nitrogen uptake by greengram. *Archives of*

Agronomy and Soil Science **52 (5)**, 579-590

Khaosaad T, Vierheilig H, Nell N, Zitterl-Eglseer K, Novak J (2006) Arbuscular mycorrhiza alter the concentration of essential oils in oregano (*Origanum* sp., Lamiaceae). *Mycorrhiza* **16**, 443-446

Kormanik PP, McGraw AC (1982) Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: Schenck NC (Ed) *Methods and Principles of Mycorrhizal Research*, American Phytopathological Society, St. Paul, Minneapolis, pp 37-45

Koltai H (2010) Mycorrhiza in floriculture: Difficulties and opportunities. *Symbiosis* **52**, 55-63

Lang F, Bratek Z, Paradi I (2003) Influence of arbuscular mycorrhiza and phosphorus supply on polyamine content, growth and photosynthesis of *Plantago lanceolata*. *Biologia Plantarum* **46 (4)**, 563-569

Larose G, Chênevert R, Moutoglis P, Gagné S, Piché Y, Vierheilig H (2002) Flavonoid levels in roots of *Medicago sativa* are modulated by the developmental stage of the symbiosis and the root colonizing arbuscular mycorrhizal fungus. *Journal of Plant Physiology* **159**, 1329-1339

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal* **42**, 421-428

Liu A, Hamel C, Elmi AA, Zhang T, Smith DL (2003) Reduction of the available phosphorus pool in field soils growing maize genotypes with extensive mycorrhizal development. *Canadian Journal of Plant Science* **83**, 737-744

Maier W, Peipp H, Schmidt J, Wray V, Strack D (1995) Levels of a terpenoid glycoside (blumenin) and cell wall-bound phenolics in some cereal mycorrhizas. *Plant Physiology* **109**, 465-470

Marschner H (1995) *Mineral Nutrition of Higher Plants*, Academic Press, London, 889 pp

Munkrold L, Kjoller R, Vestberg M, Rosendahl S, Jakobsen I (2004) High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytologist* **164**, 357-364

Muthukumar T, Udaiyan K, Manian S (1994) Role of edaphic factors on VAM fungal colonization and spore populations in certain tropical wild legumes. *Pertanika Journal of Tropical Agricultural Science* **7 (1)**, 33-42

Olsson PA, van Aarle IM, Allaway WG, Ashford AE, Rouhier H (2002) Phosphorus effects on metabolic processes in monoxenic arbuscular mycorrhiza cultures. *Plant Physiology* **130**, 1162-1171

Page AL, Miller HR, Keeney RD (1982) *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties* (2nd Edn), Monograph No. 9, ASA-SSSA, Agronomy 9, Madison, WI, 1159 pp

Powell CL, Daniel J (1978) Mycorrhizal fungi stimulate uptake of soluble and insoluble phosphate fertilizer from a phosphate deficient soil. *New Phytologist* **80**, 351-358

Ram M, Singh R, Ram D, Sangwan RS (2003) Foliar application of phosphate increased the yield of essential oil in menthol mint (*Mentha arvensis*). *Australian Journal of Experimental Agriculture* **43 (10)**, 1263-1268

Ratti N, Kumar S, Verma HN, Gautam SP (2001) Improvement in bioavailability of tricalcium phosphate to *Cymbopogon martinii* var. *motia* by rhizobacteria, AMF, and *Azospirillum* inoculation. *Microbiological Research* **156**, 145-149

Rivas R, Peix A, Mateos PF, Trujillo ME, Martínez-Molina E, Velázquez E (2007) Biodiversity of populations of phosphate solubilizing rhizobia that nodulates chickpea in different Spanish soils. *Developments in Plant and Soil Sciences* **102**, 23-33

Rouphael Y, Cardarelli M, Mattia ED, Tullio M, Rea E, Colla G (2010) Enhancement of alkalinity tolerance in two cucumber genotypes inoculated with an arbuscular mycorrhizal biofertilizer containing *Glomus intraradices*. *Biology and Fertility of Soils* **46**, 499-509

Sabannavar SJ, Lakshman HC (2009) Effect of rock phosphate solubilization using mycorrhizal fungi and phosphobacteria on two high yielding varieties of *Sesamum indicum* L. *World Journal of Agricultural Sciences* **5 (4)**, 470-479

Sadravi M (2000) Identification of arbuscular mycorrhizal fungi symbiosis with wheat, barley, maize and sorghum in Tehran and Khuzestan provinces and possibility to propagation within *in vitro* culture. PhD thesis, Agricultural Faculty, University of Tarbiate Modares, Tehran, Iran, 185 pp

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: Phylogeny and evolution. *Mycological Research* **105 (12)**, 1413-1421

Selosse MA, Richard F, He X, Simard SW (2006) Mycorrhizal networks: Des liaisons dangereuses? *Trends in Ecology and Evolution* **2**, 621-628

Smith SE, Read DJ (2008) *Mycorrhizal Symbiosis* (3rd Edn), Academic Press, London, UK, 815 pp

Takács T, Osztoics E, Csathó P, Csillag J, Rajkai-Végh K, Magyar M, Lukács A (2006) Comparative effects of rock phosphates on arbuscular mycorrhizal colonization of *Trifolium prat*ense L. *Communications in Soil Science and Plant Analysis* **37 (15)**, 2779-2790

Tawaraya K, Naito M, Wagatsuma T (2006) Solubilization of insoluble inorganic Phosphate by hyphal exudates of arbuscular mycorrhizal fungi. *Journal of Plant Nutrition* **29 (4)**, 657-665

Toussaint JP, Smith FA, Smith SE (2007) Arbuscular mycorrhizal fungi can induce the production of phytochemicals in sweet basil irrespective of phosphorus nutrition. *Mycorrhiza* **17 (4)**, 291-297

Ultra VU, Tanaka S, Sakurai K, Lwasaki K (2007) Effects of arbuscular mycorrhizal and phosphorus application on arsenic toxicity in sunflower (*Helianthus annuus* l.) and on the transformation of arsenic in the rhizosphere. *Plant and Soil* **290**, 29-41

Vierheilig H, Bennett R, Kiddle G, Kaldorf M, Ludwig-Müller J (2000) Differences in glucosinolate patterns and arbuscular mycorrhizal status of glucosinolate-containing plant species. *New Phytologist* **146**, 343-352

Warrier PK (1995) *Indian Medicinal Plants*, Orient Longman, Madras, 444 pp

Weiss M, Mikolajewski S, Peipp H, Schmidt U, Schmidt J, Wray V, Strack D (1997) Tissue-specific and development-dependent accumulation of phenylpropanoids in larch mycorrhizas. *Plant Physiology* **114**, 15-27

Witzany G (2008) Biocommunication of unicellular and multicellular organisms. *Triple C* **6 (1)**, 24-53

Zarei M (2008) Diversity of arbuscular mycorrhizal fungi in heavy metal polluted soils and their effectiveness in phytoremediation, PhD thesis, College of Agriculture and Natural Resource, University of Tehran, Tehran, Iran, 220 pp

Zarei M, Saleh-Rastin N, Ali Alikhani H, Aliasgharzadeh N (2006) Responses of lentil to co-inoculation with phosphate-solubilizing rhizobial strains and arbuscular mycorrhizal fungi. *Journal of Plant Nutrition* **29**, 1509-1522

Zarei M, Saleh-Rastin N, Salehi Jouzani GH, Savaghebi GH, Buscot F (2008) Arbuscular mycorrhizal abundance in contaminated soils around a zinc and lead deposit. *European Journal of Soil Biology* **44**, 381-391

Zargari A (1990) *Medicinal Plants* (Vol 4, 4th Edn), Tehran University Publishing Co., 850 pp

Zhu HH, Yao Q (2004) Localized and systematic increase of phenols in tomato roots induced by *Glomus versiforme* inhibits *Ralstonia solanacearum*. *Journal of Phytopathology* **152**, 537-542