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Effects of organic and biological fertilizers on fruit yield and essential oil of sweet fennel (*Foeniculum vulgare* var. dulce)

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Abstract

In order to evaluate the effects of different organic and biological fertilizers on quantity and quality of fennel essential oil, an experiment was conducted in a completely randomized block design with three replications. The experimental treatments included two organic (compost and vermicompost) and two biological (*Pseudomonas putida* and *Azotobacter chroococcum*) fertilizers, their all twin combinations (*Ps. putida* + *A. chroococcum, Ps. putida* + compost, *Ps. putida* + vermicompost, *A. chroococcum* + compost, *A. chroococcum* + vermicompost and compost + vermicompost) and control (non fertilized). There were significant differences between treatments in terms of seed essential oil percentage, essential oil yield; anethole, fenchone, limonene and estragole content in seed essential oil. Results showed that the highest and the lowest percentages of essential oil were obtained in control (2.9%) and *A. chroococcum* + vermicompost (2.2%) treatments, respectively. The highest essential oil yield (29.9 L ha^{-1}) and anethole content of essential oil (69.7%) and the lowest contents of fenchone (6.14%), limonene (4.84%) and estragole (2.78%) in essential oil were obtained in compost + vermicompost treatment. It seems that compost + vermicompost treatment compared to other treatments supplied the highest equilibrium of nutrients and water in the root zone of sweet fennel which is led to increasing the anethole content, there upon, decreasing other compounds. Essential oil yield and percentage of anethole content in essential oil were significantly higher in all organic and biological treatments compared with control.

Additional key words: anethole; *Azotobacter chroococcum*; compost; *Pseudomonas putida*; vermicompost.

Resumen

Efecto del abonado orgánico y biológico sobre el rendimiento de los frutos y los aceites esenciales del hinojo (*Foeniculum vulgare* var. dulce)

Con el fin de evaluar los efectos de diferentes fertilizantes orgánicos y biológicos sobre la cantidad y calidad del aceite esencial de hinojo, se realizó un experimento en un diseño aleatorizado de bloques completos con tres repeticiones. Los tratamientos experimentales incluyeron todas las combinaciones dobles de dos fertilizantes orgánicos y dos biológicos (*Pseudomonas putida* + *Azotobacter chroococcum*, *Ps. putida* + compost, *Ps. putida* + humus de lombriz, *A. chroococcum* + compost, *A. chroococcum* + humus de lombriz, y compost + humus de lombriz) y un control (sin fertilizar). Hubo diferencias significativas entre tratamientos en términos de porcentaje del aceite esencial de las semillas, rendimiento de aceite esencial y contenido en anetol, fenchone, limoneno y estragol en el aceite esencial. Los resultados mostraron que el mayor y el menor porcentaje de aceites esenciales se obtuvieron en los tratamientos control (2,9%) y *A. chroococcum* + humus de lombriz (2,2%), respectivamente. En el tratamiento compost + humus de lombriz se obtuvo la mayor producción de aceite esencial (29,9 L ha^{-1}) y contenido en anetol (69,7%), así como el menor contenido de fenchone (6,14%), limoneno (4,84%) y estragol (2,78%). Parece que el tratamiento de compost + humus de lombriz suministró el mayor equilibrio de nutrientes y agua en la zona de la raíz del hinojo en comparación con otros tratamientos, lo que aumenta el contenido de anetol, y por tanto disminuye el de otros compuestos. La producción de aceite esencial y el porcentaje de contenido en anetol en el aceite esencial fue significativamente mayor en todos los tratamientos orgánicos y biológicos que en el control.

Palabras clave adicionales: anetol; *Azotobacter chroococcum*; compost; humus de lombriz; *Pseudomonas putida*.

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Introduction

Chemical fertilizers are key components for providing crop nutrients needs in recent years (Sharifi-Ashori-Abadi, 1998). In many cases, using chemical fertilizers have different negative environmental effects such as soil, water and air pollution, which increase environmental production cost (Ghost and Bhat, 1998). To avoid the risk of these negative effects of chemical fertilizers, it is necessary to use organic or biological fertilizers which provide plant nutrients and also increase long term sustainability of agroecosystems (Murty and Ladha, 1988; Mehnaz and Lazarovits, 2006). There is a strong relation between soil organic matter content and soil fertility, widely and universally accepted (Melero *et al.,* 2007).

Biofertilizers can be defined as not only the organic materials, manures and residual of plants but also as bacteria and fungi, especially plant growth promoting bacteria (PGPB), which release phytohormones (Akbarinia *et al.,* 2003). Previous studies have shown that the PGPB can fix the atmospheric nitrogen, dissolve the phosphorus and potassium of the soil and control the pathogen via producing plant growth regulators (Sturz and Christie, 2003). These bacteria are known as «yield promoting bacteria» because they improve increasingly growth and development of plants (Sturz and Christie, 2003). Among the PGPBs, *Azotobacter chroococcum* and *Pseudomonas putida* are the greatest prevalent species, founding in the soil, that fix nitrogen and phosphorus, respectively (Kumar *et al*., 2001; Seilsepour *et al.,* 2002; Vessey, 2003).

Vermicompost is a kind of biofertilizer which has a high porosity and a high ability for absorption and conservation of mineral nutrients (Marinari *et al.,* 2000). Marinari *et al.* (2000) showed that in soil treated with vermicompost, total porosities were 24% higher than in the unfertilized soil. Vermicompost is enriched with several beneficial soil microbes and also contains many essential plant nutrients like N, P and K (Sinha *et al.,* 2010). Sinha *et al.* (2010) reported that soil from the plot in which vermicompost was added, showed the highest number of bacterial colonies per gram of soil.

Compost is an organic fertilizer, which is produced by different microorganisms from organic materials (Lazcano *et al.,* 2009). The addition of compost to agricultural soils supplies nutrients and organic matter, decreases leaching of mineral elements from the soil and also improves soil physical structure (Rantala *et al.,* 1999). Compost also used to provide biological control against various plant pathogens (Hoitink and Grebus, 1994). Compost has already been established as a suitable fertilizer for improving the productivity of several medicinal and aromatic plants, such as *Dracocephalum moldavica* (Hussein *et al.,* 2006), peppermint (O'Brien and Barker, 1996) and *Tagetes erecta* (Khalil *et al.,* 2002).

Sweet fennel (*Foeniculum vulgare* var. dulce) is a well known aromatic medicinal plant which is used in traditional medicine as spice and substrate for different industrial purpose (Telci *et al.,* 2009). It is cultivated and also widespread in many parts of Mediterranean and midlist countries such as Italy, Turkey and Iran (Zargari, 1986; Marino *et al.,* 2007). In Iran, sweet fennel cultivation area was approximately 1,250 ha in 2008 (Jihad-Agricultural Ministry of Iran, 2008). The fruits of sweet fennel contain essential oil which is rich source of anethole, limonene, fenchone, estragole and camphene among them the anethole is the most important constituent with determinant role in quality of the essential oil of seeds (Gross *et al.,* 2002). These depend upon internal and external factors affecting the plant such as genetic structures and ecological conditions (Telci *et al.,* 2009).

Previous studies have shown that using organic and biofertilizers lead to a change in the composition of essential oil in the different plant species (Atiyeh *et al.,* 2000; Tanu *et al.,* 2004; Darzi *et al.,* 2009). It is demonstrated that using vermicompost and compost was led to an increase in the relative content of linalool and methyl chavicol in basil (*Ocimum basilium*) (Anwar *et al*., 2005). Darzi *et al.* (2009) stated that mycorrhiza, vermicompost and phosphatic biofertilizer increased the percentage of anethole and decreased the percentage of limonene and fenchone in essential oil of fennel fruits.

Gharib *et al.* (2008) reported that inoculation of marjoram (*Majorana hortensis*) plants with each of *Azospirillum brasilienses*, *Azotobacter chroococcum*, *Bacillus polymyxa* and *B. circulans* increased the essential oil yield and percentage of this species. Essential oil yield of *Origanum majorana* L. also was significantly increased in biofertilizer treatments relative to non-inoculated plants, without alteration of oil composition (Banchio *et al.,* 2008). Therefore, they

Abbreviations used: GC (gas chromatography), MS (mass spectrometry), PGPB (plant growth promoting bacteria), RI (retention index).

demonstrated that plants inoculated with *Pseudomonas fluorescens* or *Bradyrhizobium* showed significant increase in total essential oil yield by 24- and 10-fold, respectively. Kandeel *et al*. (2001) with application of biofertilizers on *Foeniculum vulgare*, reported that the highest percentage of essential oil obtained from plants inoculated with *Azotobacter* + *Azospirilium* in the presence of a full dose of nitrogen, phosphorus, and potassium (714 kg ammonium sulphate + 714 kg calcium super phosphate + 190 kg potassium sulphate ha^{-1}).

The aim of this study was to determine fruit yield and quantity and quality of sweet fennel essential oil as affected by the application of organic and biofertilizers.

Material and methods

Experimental layout

This experiment was carried out in a complete randomized block design with three replications at Ferdowsi University of Mashhad, Mashhad, Iran, in 2008. The Research Station (36° 16' N, 59° 36' E), located at about 985 m a.s.l, in the east of Iran. The experimental treatments were two organic fertilizers (compost and vermicompost) and two biological fertilizers (*Pseudomonas putida* and *Azotobacter chroococcum*), their all twin combinations (*Ps. putida* + *A. chroococcum; Ps. putida* + compost; *Ps. putida* + vermicompost; *A. chroococcum* + compost*; A. chroococcum* + vermicompost and compost + vermicompost) and control (non fertilized). Before sowing, physical and chemical characteristics of the field soil, compost and vermicompost were determined (Table 1). Based on soil information and sweet fennel needs, compost and vermicompost were prepared at the rates of 10 and 7.5 t ha^{-1}, respectively. The mixed organic treatment was prepared at 50% of compost and vermicompost. All organic treatments were applied before sowing and well mixed with the soil. *A. chroococcum* and *P. putida* (at least 10^{7} CFU mL^{-1}) were supplied from Mehr Asia Biotechnology Company (MABCO), Semnan, Iran. Before inoculations of sweet fennel seeds with *Azotobacter* and *Pseudomonas*, seeds were treated with sugar solution. Then *Azotobacter chroococcum* and *Pseudomonas putida* were added to the solution and shaken thoroughly to facilitate uniform coating of seeds with the inocula. The inoculated seeds were kept in shade for about one hour for drying before sowing so that *Azotobacter* and *Pseudomonas* inocula adhere to seed properly.

Sweet fennel seeds were hand sown in 3 × 4 m plots so as given 50 cm inter- and 10 cm intra-row spacing in six rows on 24 Feb. 2008. As soon as the seeds were sown, irrigation continued every 10 days. Hand thinning (one plant hill^{-1}) was performed when seedlings reached 4-6 leaf-stage. During growth season, hand weeding was conducted at three times. Seeds were harvested 170 days after sowing.

Isolation of essential oil and gas chromatography/mass spectrometry analysis

From each plot, fruits were crushed at 50 g by electric grinder and suspended in 750 mL distilled water. Ground mass was subjected to hydro-distillation using Clevenger's apparatus (Clevenger, 1928). After 4 h, the essential oils were collected and dehydrated with sodium sulphate (Na_2SO_4) using the method of Guenther (1961). Then essential oil yield and percentage was determined. Dehydrated extracts were stored at 2°C in dark until GC/MS (gas chromatography/mass spectrometry) analysis.

GC/MS machine (Shimadzu GC/MS model QP5050) was used to specify the percentage of fennel essential oil components such as fenchone, limonene, estragole, and anethole. The capillary column was DB-5 (30 × 0.2 mm, film thickness 0.32 µm). The operating conditions were as follows, carrier gas, helium with a flow rate at 1.7 mL min^{-1}, column injector and detector temperatures were both kept at 260°C with ionization potential of 70 ev. The initial temperature of column was 60°C (held 1 min) and then heated to 160°C with

Table 1. Physical and chemical properties of the soil, compost and vermicompost

	Texture	Nitrogen (%)	Phosphorus (%)	Potassium (%)	EC (dS m^{-1})	Carbon (%)	pH
Compost	—	1.5	1.2	1.1	0.72	34.4	7.4
Vermicompost	—	1.4	1.7	1.2	0.89	32.1	8.1
Field soil	Silt loamy	0.15	0.14	0.12	0.12	1.21	7.47

Table 2. Analysis of variance (mean squares) of measured traits of sweet fennel

	df[1]	Percentage of essence	Essence yield	Anethole in essence	Fenchone in essence	Limonene in essence	Estragole in essence
Block	2	0.033^{ns}	9.3^{ns}	60.99**	7.70**	2.14**	1.07**
Treatment	10	0.132^{ns}	22.6*	98.75**	3.44**	8.16**	0.12**
Error	20	0.066	9.4	3.23	0.030	0.020	0.001

[1] Degree of freedom. ns: non significant. *: Significant at 5% level. **: Significant at 1% level.

a 3°C min^{-1} rate and then heated to 250°C with 20°C min^{-1} and kept constant for a 3 min period. Identification of components in the sample was based on the retention index (RI), National Institute of Standards and Technology (NIST) MS spectral library and literature survey. The relative percentage of the oil constituents was calculated from GC peak areas.

Statistical analysis

Data were subjected to two-way analysis of variance (ANOVA) and the difference between treatments was confirmed by least significant difference test (LSD) using SAS software (SAS Institute, 2003). A significance level of 95% was applied according to Little and Hills (1978).

Results

Effect of organic and biological fertilizers on essential oil content

The results showed that different treatments had no significant effect on essential oil percentage. However, the essential oil yield was significantly affected by different treatments (Table 2). Control and vermicompost + *Azotobacter* had the highest and the lowest essential oil percentage, respectively (Fig. 1a). The essential oil yield was higher in organic and biological fertilizer treatments compared with control. The essential oil yield was increased in vermicompost plus compost by 49% compared with control treatment (Fig. 1b). There was a negative and significant correlation between essential oil percentage and seed yield (Fig. 2). The control treatment had the lowest seed yield and the highest essential oil percentage (Fig. 2).

Analysis of the essential oils in sweet fennel (Table 3) showed the presence of 12 compounds. The major compounds were anethole, fenchone, limonene and estragole, as were detected in all the treatments (Table 3). Therefore, we will mention just them, hereinafter.

Effect of organic and biological fertilizers on anethole

There was a significant difference between all treatments in proportion of anethole in essential oil (Table 2). The anethole content was increased in all organic and

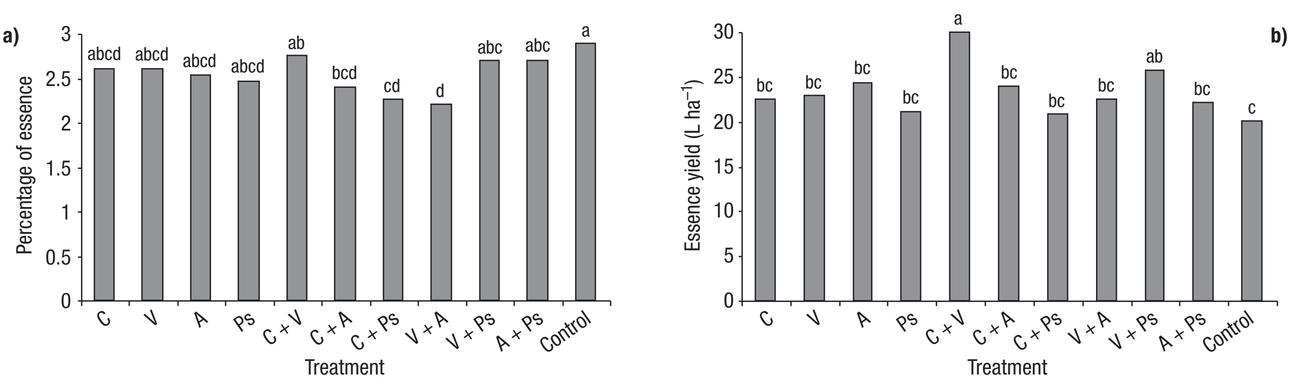

Figure 1. Effect of different treatments on a) essential oil percentage and b) essential oil yield of sweet fennel. C: compost. V: vermicompost. A: *Azotobacter chroococcum*. Ps: *Pseudomonas putida*. Treatments with a differing letter are significantly different by LSD test ($p < 0.05$).

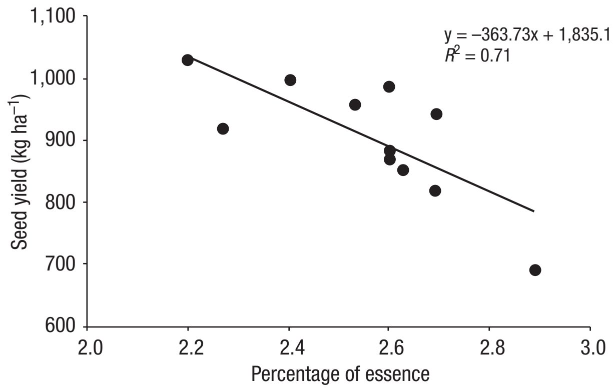

Figure 2. Linear relation between essential oil percentage and seed yield of sweet fennel.

Table 4. Effect of biological and organic fertilizers on concentration (%) of various constituents in sweet fennel essential oil

Treatment[1]	Anethole	Fenchone	Limonene	Estragole
C	55.69^{f}	8.94^{b}	7.82^{c}	3.19^{d}
V	56.19^{f}	8.18^{c}	7.23^{d}	3.17^{d}
A	64.01^{b}	7.46^{e}	5.79^{g}	3.02^{g}
Ps	53.18^{g}	9.06^{b}	8.65^{b}	3.27^{c}
C + V	69.73^{a}	6.14^{f}	4.84^{i}	2.78^{i}
C + A	64.75^{b}	7.36^{e}	5.55^{h}	2.93^{h}
C + Ps	58.19^{d}	8.04^{c}	6.83^{Ef}	3.12^{e}
V + A	64.88^{b}	7.57^{e}	5.47^{h}	2.80^{i}
V + Ps	59.20^{c}	7.90^{e}	6.63^{f}	3.08^{f}
A + Ps	57.19^{e}	8.80^{b}	6.93^{e}	3.31^{b}
Control	50.83^{h}	10.25^{a}	10.61^{a}	3.38^{a}

[1] C: compost. V: vermicompost. A: *Azotobacter chroococcum*. Ps: *Pseudomonas putida*. Within a column, means with the same letter are not significantly different by LSD test ($p < 0.05$).

biological fertilizers treatments compared with control (Table 4). Vermicompost + compost and Pseudomonas treatments had the highest and the lowest anethole content, respectively. Anethole content in vermicompost + compost treatment was 27% higher than control (Table 4).

Effect of organic and biological fertilizers on fenchone

Fenchone content was significantly affected by treatments (Table 2). Organic and biological fertilizers decreased the fenchone percentage compared to control (Table 4). The highest (10.25%) and the lowest (6.14%) fenchone content of essential oil were obtained in control and vermicompost + compost treatments, respectively (Table 4). There was a negative correlation between anethole and fenchone contents of essential oil (Fig. 3). Individual organic and biological fertilizers compared with combined treatments significantly increased fenchone content of essential oil (Table 4).

Effect of organic and biological fertilizers on limonene

Limonene content was significantly decreased in all fertilizer treatments compared with control (Tables 2 and 4). Vermicompost + compost and *Pseudomonas* treatments decreased limonene contents by 54% and 18%, respectively compared with control (Table 4).

Table 3. Essential oil constituents of sweet fennel fruit

No.	Compounds	RI[1]
1	α-Thujene	924
2	α-Pinene	933
3	Camphene	948
4	Sabinene	969
5	β-Myrcene	986
6	α-Phellandrene	1,003
7	Limonene[2]	1,022
8	α-Terpinene	1,041
9	Fenchone[2]	1,071
10	Camphor	1,162
11	Estragole[2]	1,194
12	Anethole[2]	1,298

[1] Retention index (Adams, 2007). [2] Compounds found in all treatments.

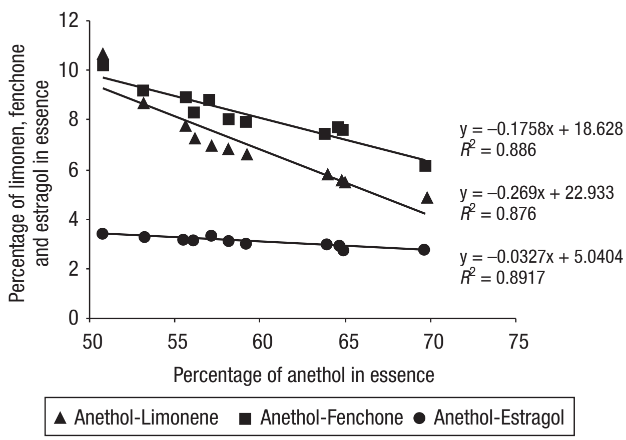

Figure 3. Fitted regression lines between percentage of anethole and percentage of limonene, fenchone and estragole in essential oil.

Effect of organic and biological fertilizers on estragole

Estragole content was significantly decreased in organic and biological fertilizers treatments compared with control (Table 4). The highest and the lowest reducing effect on estragole content were observed in *Pseudomonas* + *Azotobacter* and vermicompost + compost.

Discussion

Based on our results, sweet fennel was not demonstrated highly responsiveness to different organic and biological fertilizers in terms of percentage of essential oil, but organic and biological fertilizers had more significant effect on essential oil yield. While there were no significant differences between different treatments in terms of essential oil percentage but because of the higher fruit yield in vermicompost plus compost treatment, (Moradi *et al*., 2010) the essential oil yield was higher in this treatment compared with others. It has been reported that fennel essential oil yield increased by different organic fertilizers (Amin, 1997; Badran and Safwat, 2004; Kapoor *et al.,* 2004). Hussein *et al.* (2006) demonstrated that organic matters such as compost can improve soil structure, improving root development, providing plant nutrients and enhancing nutrient uptake by plants. Moreover, compost facilitates water absorption and retention by the soil, which has a favorable effect on growth and essential oil components of plants. In the other hand, Mahfouz and Sharaf-Eldin (2007) reported that free-living nitrogen-fixing bacteria *e.g. Azotobacter chroococcum* have not only the ability to fix nitrogen but also release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis with subsequent improvement of yield and essential components of medicinal plants. Darzi *et al.* (2009) reported that essential oil yield of fennel seeds increased significantly by increasing the vermicompost. They demonstrated that vermicompost supplied phosphate and nitrogen to the soil, giving a more balanced nutritional status than mineral fertilizers. Abdelaziz *et al.* (2007) showed that the highest essential oil yield of rosemary (*Rosmarinus officinalis* L.) was obtained from plants treated by the mixture of microorganisms and compost. They suggested that the stimulative effect of this treatment on increasing essential oil yield might be attributed to their enhancing effect on vegetative growth characteristics and plant chemical composition such as nitrogen, phosphorus, potassium and total carbohydrates of rosemary which can influence the yield and components of essential oil.

The pharmaceutical value of medicinal plants depends on their secondary metabolite components (Khan *et al.,* 1992; Gross *et al.,* 2002). The observed increases in synthesis of essential oil can be considered as a defensive response to colonization by microorganisms, since several essential oil have antimicrobial properties (Banchio *et al*., 2008). Based on our results, application of different organic and biological fertilizers increased the anethole content which is the most important components of the sweet fennel essence (Gross *et al.,* 2002; Anwar *et al.,* 2005) and consequently improved the quality of the sweet fennel essence. Darzi *et al.* (2009) stated that biofertilizers increased the anethole content of essential oil, leading to higher quality of fennel essential oil. They reported that promoting effect of biofertilizers on this characteristic may be due to their ability to enhance the physical, chemical and biological properties of the soil. Furthermore, this may be related to the good balance of nutrients and water in the root zone (Gharib *et al.,* 2008).

It seems that compost and vermicompost supply plant nutrient requirements by gradual releasing the elements which increase the anethole content of sweet fennel essential oil, resulting in improve plant essential oils quality (Darzi *et al.,* 2009). These increases might be related to the positive effect of compost and vermicompost in increasing the root surface area per unit of soil volume, water-use efficiency and photosynthetic activity (Abdelaziz *et al.,* 2007). Anwar *et al.* (2005) reported that using vermicompost on basil (*Ocimum basilicum*) was led to increase in limonene and methyl chavicol content which improve the quality of essential oil.

The results indicated that anethole consists 50 to 70% of the sweet fennel essential oil; it is predictable that increasing anethole content will decrease other essential oils components such as fenchone, estragole and limonene. The highest anethole and the lowest fenchone, estragole and limonene content in essential oil were obtained in compost + vermicompost treatment. This means that the fenchone, estragole and limonene contents are strongly depended on anethole content in essential oil of sweet fennel. It has been confirmed that using biofertilizers decreased fenchone content in fennel essential oil (Kapoor *et al.,* 2004; Darzi *et al.,* 2009). Akbarinia *et al.* (2003) stated that applying cattle manure increased thymol and decreased ρ-cymene contents of essential oil in *Trachyspermum copticum*. It has been reported that application of mycorrhizae, vermicompost and their combination increased anethole and decreased limonene contents of fennel essential oil (Darzi *et al.,* 2009). In addition, Ratti *et al.* (2001) stated that phosphate solubilizing bacterium of *Bacillus polymyxa* increased the zhranyvl content and decreased the several components of essential oil in *Cymbopogon martinii*. Phosphate solubilizing bacterium such as *Pseudomonas* sp. improved plant growth through production of growth-promoting substances such as indole acetic acid and cytokinins (Banchio *et al.,* 2008). They demonstrated that increases in total essential oil yield of sweet marjoram in response to inoculation with *Pseudomonas fluorescens* was not due to increased biomass, and therefore may have resulted from increased biosynthesis of terpenes.

Biofertilizers improved growth conditions of sweet fennel, increased anethole content of essential oils and decreased the estragole content. It can be concluded that the biofertilizers increased nitrogen and phosphorus contents in the soil leading to an increase the anethole rate, subsequently, a decrease of other compounds such as estragole. It seems that due to the competition of *Pseudomonas* and *Azotobacter* bacteria over food resources and also secretion of specific inhibitor substances by each one, there were least differences between combination of *Pseudomonas* + *Azotobacter* compared with control treatment in terms of quantity of anethole, fenchone and estragole content in sweet fennel essential oil (Table 4). Apparently using organic and biological fertilizers in medicinal plants not only maintain stability and health of the production systems but also increase availability of different mineral nutrients for the crops.

This research revealed that compost, vermicompost and plant growth promoting bacteria had positive effects on quantity and quality of the sweet fennel essential oil. In addition, combination of vermicompost and compost had a higher effect on qualitative characteristics, however, combination of *Pseudomonas* and *Azotobacter* was less effective. Generally, it seems that organic and biological fertilizers can be considered as a suitable substitution for chemical fertilizers in developing sustainable medicinal plant production systems.

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