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The effect of organic and biological fertilizers on essential oil content of *Foeniculum vulgare* Mill. (Sweet Fennel)

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ABSTRACT : In order to evaluate the effect of different biological and organic fertilizers on essential oil content of sweet fennel (*Foeniculum vulgare* Mill), an experiment was conducted at Research field of Faculty of Agriculture, Shahrood University of Technology in 2010. The experiment was conducted in a completely randomized block design with 11 treatments and three replications. The experimental treatments were urea fertilizer (N), *Glomus mosseae* (M1), *Glomus intraradiceae* (M2), cow manure (F), M1+M2, N+M1, N+M2, F+M1, F+M2, F+N and control (C). There were no significant differences between different treatments in terms of seed essential oil percentage but there were significant differences between different treatments on seed essential oil yield. Results showed that the highest and the lowest percentage of essential oil contents were obtained in control (1.85%) and M1+ M2 (1.25%) treatments. The highest essential oil yield (24.6 l/ha) were obtained in cow manure treatment. Based on GC\MS analysis the main components of essential oil were Anethole, , Limonene and Estragole. The highest Anethole (81.3%) and the lowest (8.532%), Estragole (3.05%) and Limonene (3.231%) in essential oil content were obtained in M2 treatment.
Keywords*:* Anethole, Cow manure, Essence components, Mycorrhiza, Urea fertilizer

INTRODUCTION

Plants as a first part of food cycle have essential role in human life. The total dependency on plants has forced human to use science and the new methods to earn more information about plants (Amiri et al., 2006). During the history of medical cure the use of medical plants has been consistent. The potential of producing medical plants has been great in Iran for a long time. The side effects of chemical medicines make medical plants more popular among the other ways of treatment, and so this herbs worth a lot (Sharifiashoriabadi et al., 2002 and Akbarinia et al., 2003). The price of producing can be increased by using chemical fertilizers and they can cause some fatal ecological damages on environment (Ghost et al, 1998). For satisfying the current needs of herbs and stability of agriculture systems we must utilizes the new input and sources (Rigby et al., 2001). Sweet fennel is an important medicinal plant in Iran and different countries all over the world. Essential oil of sweet fennel has been used as pharmaceutical, food, cosmetic and hygienic agents since ancient time (Marotti et al., 1993 and Mallangulla, 1995). Since the cultivation of this plant was not usual, our information about its cultivation is not sufficient, one of the important aspects in producing a plant is how to feed it. Medicals are customary used to nutrition plants and their effects on plant growth have been proved. Organic agriculture is more trustable than the others because chemicals are not use in this kind of industry (Rigby et al., 2001). Hence, the organic agriculture of medical plants can reduces the negative effects on the quality of its medical purpose. Anethole is the major component in the essence of Sweet fennel (Foeniculum vulgare) that has a major role on it´s essence quality (Gross et al., 2002). There other components in the essence are, Limonene, Estragole and Methyl Cavicol (Darzi et al., 2008). Several experiments have shown that the using of biological fertilizers causes changes ion the essence´s components of medical plants (Darzi et al., 2008; Marotti et al., 1993; Gross et al., 2002). For example, using Biological Mycorrhiza, Vermicompost and Phosphate increase Sweet fennel essence and decrease it´s and Limonene because of increasing it´s Anethole, in turn (Darzi et al., 2009). Sweet fennel root symbiosis with two vesicular arbuscular mycorrhiza fungi species (VAM) enhances the amount and quality of it´s essence significantly, in a way that the amount of Anethole increases versus the decrease of and Limonene component in comparison with the controls(Kapoor et al., 2004). Using mycorrhiza significantly enhances the biological yield of *Cymbopogon* spp

in comparison with controls(Gupta and Jandrdhanan, 1991). Two separate researches that are done by (khaliq and Janardhanan, 1997) on *Mentha arvensis* showed that using mycorrhiza increase the amount and yield of it´s essence in comparison with controls. They reported that mineral feed increased as well as the essence . The components of structure (amount of essence and vegetative tissues) has improved by the yield of essence. Cow manure enhances the amount of essence and yield of seed in *Ammi copticum* L. So that 30 tons of cow manure per hectare increased the essence by 4% in comparison to controls(Akbarinia et al., 2003). By emphasizing on cow manure for medical plants, mentioned that the materials increase treated plants production by affecting their physical, chemical and biological properties of soil (Sharma and Chaterrjee et al., 2002). Several advantages of cow manure like holding water in soil and having nutritious soil food can increase the amount of plant´s essence by raising the vegetative growth that has a magnificent part in essence. Regarding to the importance of Sweet fennel as a medical plant, this experiment was designed to investigate the quality and quantity of its essence on the treatment of organic and biological fertilizers. Therefore our aim in this experiment is to introduce an alternative choice for chemicals in producing Sweet fennel.

MATERIALS AND METHODS

This experiment was done at Research field of Faculty of Agriculture, Shahrood University of Technology in 2010. The research field (36° 25' N, 54° 58' E), located 1380 m above sea level, in the east of Iran. Regional meteorological are shown in table 1.(Refrence : meteorological, Islamic Republic of Iran)

Table1. atmospheric status of shahrood (2010)

Month	Relative humidity (%)		Pericipation (mm)	Temperature (c˚)	
	Absolute Maximum	Absolute Minimum		Maximum Average	Minimum Average
May	19	88	14/2	25/2	12/9
June	7	75	2/3	31/9	8/7
July	16	47	2/7	35/5	22/9
August	12	70	0	31/8	20/3
September	14	88	11/9	28/6	16/6

The experiment was designed at complete randomized block with 3 replications in 11 treatments. Treatments in include: 1- urea (N) (100 kg net Nitrogen per hectare) 2- Mycorrhiza (*Glomus moseae*) (M1) (The component of root: soil, spore and fungi) 3- Mycorrhiza (*Glomus intraradices*) (The component of root: soil, spore and fungi) 4- cow manure (F) (8 Tons per hectare) 5-(M1 + M2) 6- (M1 + N) 7- (M2 + N) 8- (F+M1) 9- (F + M2) 10- (F + N) 11- Control (c) (no treatment).* Each treatment was located separately in each plot. Before the beginning some samples from farm soil and the cow manure were selected and their PH and nutrients were determined (Table 2 and 3).

Table 2. Physical and chemical properties of the soil

Total Nitrogen (%)	Available Phosphorus (ppm)	Available Potassium (ppm)	EC (dS/m)	pH
0.06	14.7	149	3.68	7.94

Table 3. Physical and chemical properties of the cow manure

Total Nitrogen (%)	Total Potassium(%)	Total Phosphorus (%)	C/N	EC (dS/m)	pH
2.56	0.37	0.08	10.6	5.06	8

Each plot had 5 cultivating line with 5 meter space from each other, plant space 10cm on each line, plot length 5meter, plot distance 100 meter and block distance 3 meter. The amount of chemical was determined on soil experiment results. Regarding the refrences , sweet fennel needs 100kg nitrogen per hectare (Omid beigi, 2005). 217kg urea with 46% nitrogen per hectare was calculated and regarding the area of each plot it´s urea requirement was determined. Then according to lines in each plot the required chemical for each line was calculated. The amount of cow manure was determined according to the it´s nitrogen amount. Therefore because 40-50% of its nutrition are released at the first year, 8ton per hectare was used. Every stage for land preparation including plough, disk, etc, was done. The used seeds were prepared from Esfahan.

Cultivation was done at 2010/5/10 in stack form. For inducing mycorrhiza to the seeds after putting the seeds in each hole 20 of mycorrhiza was poured on it and then was covered with a layer of soil. It is worth mentioning that the seed seeds were not treated with any chemical including fungicide. Watering began exactly after cultivation in periods of 7 days. The plants began to sprout after 20 years .Spreading was conducted to

get the optimum density on the stage of 4 levels appearing on the plant. Handy weeding was done. No pest or disease was observed at the plant growing period, hence no toxic or pesticide was used. Before harvesting, 5 plants were randomly selected to evaluate the amount of seed essence and its components. To measure seeds essence amount, a 50 g sample from each (plot) was prepared and after being grinded it essence was extracted by the use of Clevenger set and water vaporing method for four hours. Essence yield (%) after un moisturizing by the use of dried sodium sulphate was calculated (Sephidkan, 2002). And essence yield was determined. For analyzing essence samples and exact measurement of some essence components including Anethole, , Limonene and Estragole, the prepared essence was injected to Gas Chromatograph set (/ms) the used set was Agilent 6890 with 30 meters column, inter diameter 0/25 (mm) and layer thickness 0/25 (mm) (HP-5ms). The temperature of the columas set as the following: initial oven temperature 50 co for 5 (min), temperature gradient 3 $^{\circ}C$ in each (min), temperature increase up to 240 $^{\circ}C$ with the ratio of 15 degrees per minute, temperature increase up to 300 $^{\circ}C$ in each minute and stopping in this temperature for 30 minutes .The injection room temperature was 290 $^{\circ}C$ and Helium was used as the transit gas with flow velocity of 0/8mm/min used mass spectrophotometer was Agilent 5973 with ionization voltage of 220 $^{\circ}C$. Spectrum determination was done by the of their prevention index and their comparison with existing indices in the refrences as well as the use of mass spectrums of standard components and computational library data bases.
For analyzing data, SAS software was used and dun can was used to compare treatment averages at significant level of 5%

RESULTS

Essence Percentage and yield

According to Anova, There were no difference among treatments about evaluate of essence.(P<0/05) (table 4 and figure 1). Nevertheless we had max and min essence percentage for control (c) and M1+M2 respectively. essence yield was evaluated or affected from treatment (p<0/01)(table 4), showing that use of organic and biological fertilizers causes of had significant difference in essence yield on some treatments as compared to control (figure2). Analysis of the essential oils in sweet sweet fennel (Table 5) showed the presence of 10 compounds, The major compounds were Anethole, , Limonene and Estragole, as were detected in all treatments (Table 5).

Table 4. Analysis of variance (mean square) of measured traits of sweet fennel

	df[1]	Percentage of essence	Essence yield
Block	2	0.01^{ns}	1.22^{ns}
Treatment	10	0.09^{ns}	47.20**
Error	20	0.18	5.6

[1] Degree of freedom. ^{ns}: no significant. **: Significant at 1% level

Anethole amount in essence

Sweet fennel essence gas chromatographical results showed that the existing Anethole had a more proportion in comparison to other component in add treatments in comparison to controls even having minimum amount by 5.5% more than controls (table 6) among treatment m2 and c had the max and min Anethole respectively (table 6).

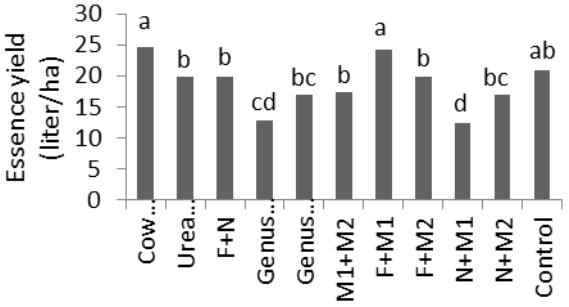

Figure 1. Effect of different treatment on essential oil yield of sweet fennel. M1: (*Glomus mosseae*). M2: (*Glomus intraradices*)

Table 5. Essential oil contituents of sweet fennel fruit

No.	Comounds	RI[1]
1	α-Pinene	933
2	Camphene	948
3	Sabinene	969
4	α-Phellandrene	1,003
5	Limonene	1,022
6	α-Terpinene	1,041
7	Fenchone	1,071
8	Camphore	1,162
9	Estragole	1,194
10	Anethole	1,298

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

Essence Estragole amount

Add treatments decreased Estragole amount in comparison to control (table 6). Amount treatments, (F+N) and m2, showed the max and min amount of Estragole respectively in comparison to controls (table 6).

Table 6. Effect of biological and organic fertilizers on concentration (%) of various constituents in sweet fennel essential oil

Treatment[1]	Limonene	Fenchone	Estragole	Anethole
C	4.28	9.26	6.91	69.98
F	3.37	8.85	3.44	80.40
F+N	3.39	10.53	3.53	79.93
F+M1	4.91	10.41	3.39	75.33
N	4.51	12.25	3.13	75.01
N+M2	3.30	10.45	3.09	76.35
M1	4.32	10.63	3.34	76.05
N+M1	4.06	14.12	3.20	76.16
M2	3.23	8.53	3.05	81.30
F+M2	3.35	10.39	3.30	79.99
M1+M2	4.34	9.78	3.10	80.65

[1] C: Control. F: Cow manure. N: Urea fertilizer. M1: Glomus mosseae. M2: Glomus intraradices.
* Each of the average of table have obtained from mixed of 3 replication

Essence Fenchone amount

Among treatments (N+m1) and M2 showed the max and min amount of Fenchone respectively (table 6). This amount in m2 and F decreased in comparison to control.

Essence Limonene amount

Among treatments (F+M1) and M2 showed the max and min amount of Limonene respectively (table 6). essence Limonene amount decreased in M2 and F in comparison to control resulting in increased urea and M1 use in comparison to control (table 6).

DICCUSION

Essence percentage and yield

biologic and organic chemicals couldn`t significantly affect the essence amount in Sweet fennel (Moradi, 2010). Organic fertilizers could increase essence yield in Sweet fennel (Kapoor et al., 2004). (Darzi, 2008) also reported same results about positive effects of manure fertilizers on essence yield in Sweet fennel. in this experiment controls showed the maximum essence percentage (figure 1). It seems that because of nutrient stress in controls, seed essence increased as a metabolical response (Moradi, 2010). Metabolicals, although genetically constructed, but are quantitatively and qualitatively affected by environmental factors including moisture, climate, light and soil, so that environmental factors cause some changes in growth and development of chemical plants and their metabolite qualitative and quantitative (Omid beigi, 2000). Essence are components of plant secondary metabolites that generally increase under environmental stresses (Badran et al., 2004). There are so many reports showing that using manure fertilizer can increase essence percentage in chemical plants (Amin, 1997; Atiyeh et al., 2000; Badran et al., 2004).In a study investigated the effect of fertilizer treatments on essence percentage of mentha piperita L. and reported that the yield reached by vermicompost and cow manure plus azotobacter and azosperilium are significantly equal to that of customary agriculture (Karla, 2003).

Essence Anethole amount

Using biological fertilizer could enhance essence Anethole amount resulting in more essence qualification in Sweet fennel (Darzi et al., 2008). They reported that treatment with biological fertilizers in comparison with chemicals provide much more suitable condition for useful microbial activation in soil and in addition to providing much suitable minerals for Sweet fennel cause more essence qualification in it in medical plant production. Medicinal product in discussion, the actual value of the product depends on the quality of essence (gross et al., 2002; khan et al., 1992). Thus, according to the results of this experiment we can conclude that fertilizers could enhance essence qualification in Sweet fennel by increasing Anethole amount one its most important metabolite (darzi et al., 2008).

Essence Estragole amount

Since essence one some terpenoid component and their structured units (isotropnoids) including isotropnetil pyrophosphate (IPP) and dimethylollyl pyrophosphate (DMAPP) essence need ATP and NADPH and regarding that the presence of nitrogen and phosphor is essential for producing the last component (Darzy et al., 2009).

We can conclude that fertilizer could increase essence Anethole amount in comparison to controls by increasing nitrogen and phosphor amount further more increasing these components result in decreasing others e.g. Estragole.

Essence Fenchone amount

(Kapoor et al., 2004) reported match our results. they reported that mycorrhiza symbiosis decreased essence amount so that this amount significantly fell down below controls (6.18%) under treatment by glomus fasciculatum (4.69%) and glomus macrocarpom (5.46%). furthermore, in another study in order to investigating the effect of mycorrhiza on essence quality on, (gupta et al., 2006) observed that the amount of linalool in essence under symbiosis of root and mycorrhiza in with Zygospore rozea (26.72%). In comparison with controls (39.39%) significantly decreased. results of some researches show that using biological and organic fertilizer cause a decrease in essence amount is Sweet fennel (sharifiashorabadi, 2001; Kapoor et al., 2009). In addition (Marotti et al., 1993) reported that chemicals affect essence amount in Sweet fennel increasing it.

Essence Limonene amount

In many studies related with sustainable agriculture it is obviously seen that organic and biological fertilizer decrease some components while increase others. (Akbarinia, 2003., Adams et al., 2004; Freitas et al., 2004; Anwar et al., 2005; Gupta et al., 2006). Sweet fennel is not exception in this aspect (Sharifiashoriabadi, 2002; Kapoor et al., 2004) and this subject is obviously seen in this study when Limonene amount decrease while using biological fertilizers. In a study done by (Kapoor et al., 2004) Limonene amount increased by utilizing chemicals. it is worth mentioning that according to plant species, various mycorrhiza species can variously affect the quality of essence. (Copetta et al., 2006) also observed that mycorrhiza species caused various behaviors in their host plant. So that only Zygospore rozea showed an increase in essence quality in comparison to controls.

CONCLUSION

Results show that utilizing biological fertilizers can provide essential minerals (elements) for Sweet fennel and cause an increase in essence quality meaning the amount of Anethole. other researchers also prove the enhancement of essence in medical plants under treatment of organic and biological fertilizers. (Sharifiashoriabadi, 2002; Akbarnia, 2003; Freitas et al., 2004; Kapoor et al., 2004; Anwar et al., 2005).

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