World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Impact of organic compost and humic acid on essential oil composition of sweet fennel (*Foeniculum vulgare* var. Dulce) under sandy soil conditions in Egypt

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Received: 08-01-2015 / Revised: 22-01-2015 / Accepted: 27-01-2015

ABSTRACT

Two field experiments were carried out at the Experimental Farm of National Research Centre (NRC), Egypt during two seasons (20**10**/20**11** and 20**11**/20**12**) to investigate the effect of compost and / or humic acid on fruit yield and essential oil [(%) and yield (ml plant^{-1})] of sweet fennel cultivated in sand soil. Humic acid or compost had a significant effect on fruit yield (g plant^{-1}) while the treatments interaction had more significant for the same character. Essential oil (%) and essential oil yield (ml plant^{-1}) were more significant for humic acid or compost. The interaction treatments between humic acid and compost were highly significant for both essential oil % and oil yield (ml plant $^{-1)}$. Trans-anethole, estragole and fenchone were found to be the major compounds in the essential oil of sweet fennel. The maximum mean values of trans-anethole (71.1 %), estragole (35.3%) and fenchone (8.6%) were resulted from 15 kg ha $^{-1}$ of humic acid, 0 kg ha $^{-1}$ of humic acid (with compost) and 5 kg ha $^{-1}$ of humic acid (with compost) treatments, respectively. The highest relative percentage of monoterpene hydrocarbons (2.9%) was obtained as a result of humic acid at 10 Kg ha^{-1} without compost. On the other hand control (without humic and compost) as well as humic at 5 Kg plant^{-1} without compost gave the maximum relative percentage for oxygenated monoterpene (97.5%).

Key words: Sweet fennel (*Foeniculum vulgare* var. Dulce), humic acid, compost, essential oil, trans-anethole and estragole.

INTRODUCTION

Foeniculum vulgare var. Dulce (Family *Apiaceae*) is cultivated in China, Egypt and India [1]. Fennel is traditionally used as a remedy for upper respiratory catarrh due to its calming effects on bronchitis and cough. It is also used as a diuretic and often added to purgatives to alleviate their tendency to cause gripe and improve their flavor [2]. It is also used to promote lactation, aid to weight loss and longevity [3]. Fennel oil showed bactericidal and antifungal properties. The flavor of fennel oil depends upon its main constitutes: fenchone, estragole and anethole. Eestragole is a bitter tasting element, while anethole has a sweet anise-like flavor [4]. Proportions of these ingredients vary according to strains and region [5]. Organic products based on concept of increasing market opportunity exclude or prohibit the use of conventional crop inputs common to modern farming. Chemical pesticides and fertilizers are not allowed in organic certification program [6]. To achieve optimal quality and economic returns, organic farming system rely upon crop rotation, crop residues, animal manures, legumes, green manures, off farm organic wastes and biological pest control [6]. These components maintain soil productivity, supply nutrients and help control insects, weeds, and other pests. Pollution with chemical fertilizers arose as an aim of health cure, thus attempts were done for solving problems of chemical fertilization, and the organic farming technique represents a move towards an alternative system of agriculture [6]. Organic material improve soil physical properties (structure and aggregation) and soil chemical properties (decrease soil pH, increase cation exchange capacity and enhance the most nutrient) that important for plant growth and essential oil production [7]. Application of organic fertilizer increased essential oil yields of davana (*Artemisia pallens* Wall. Ex. D.C.) and *Cymbopogen martinii* plants [8, 9]. Marculescu [10] revealed that soil with its content in macro and microelements enhanced by the use of organic

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fertilizers plays an essential role in the plant growing and development. It can be noted that the vegetative mass is rich and the amount of essential oil is high in *Chrysanthemum balsamita* L. plant with application of organic fertilizer. Different organic manures/composts applied increased the essential oil content of *Cymbopogon winterianus* significantly [11]. Treatments with organic fertilizers had a positive effect on essential oil content (%) essential oil yield (ml plant^{-1}) and essential oil composition of Celery (*Apium graveolens* L.) fruits [12]. Humates have long been used as a soil conditioner; fertilizer and soil supplement [13]. Humic acid (HA) is one of the major components of humus and can be used as growth regulate-hormone level improve plant growth and enhance stress tolerance [13, 14, 15]. Fortun [16, 17] reported that humic acid improve soil structure and change physical properties of soil, promote the chelation of many elements and make these available to plants, aid in correcting plant chlorisis, enhancement of photosynthesis density and plant root respiration has resulted in greater plant growth with humate application [18, 19. Increase the permeability of plant membranes due to humate application resulted in improve growth of various groups of beneficial microorganisms, accelerate cell division, increased root growth and all plant organs for a number of horticultural crops and turf - grasses, as well as, the growth of some trees [20, 21, 22]. Oregano essential oil production increased significantly with K-humate application [23] .Sandy soils in Egypt are characterized with poor nutrients (including macro and micronutrients) and unfavorable environmental conditions which negatively affect growth, productivity and active principal's essential oil of aromatic plants including *Foeniculum vulgare* var. Dulce plants [6]. This study is focused on the evaluation of fruit yield, essential oil content [(%) and yield (ml plant^{-1})] as well as chemical constituents of essential oil extracted from sweet fennel fruits adjusted to organic compost, humic acid and their interactions under sandy soil conditions in Egypt.

EXPERIMENTAL

Two field experiments were carried out at the Experimental Farm of National Research Centre (NRC), Nubaria, Menofya Governorate, Egypt, during two seasons (2010/2011 and 2011/2012). The experimental plots (4m^2, 2mx2m per each) arranged and then divided into two main groups. The first group was subjected to different levels of humic acid. Amounts of 0, 5, 10 and 15 kg ha^{-1} were added to the soil one week before sowing. The second group was subjected to the same treatments but compost was added to the soil at the rate of 30 ton ha^{-1} 4 weeks before sowing date. Sweet fennel seeds were obtained SEKM Company, Egypt. In the first week of November in both seasons, seeds were sown in the soil at 25cm between hills on rows at 50 cm apart), 5 seeds per hill. The viability of the seeds was approximately 92%. After 4 weeks from sowing, the seedlings were thinned to three plants per hill. Physical and chemical properties of soil, humic and compost used in this study were determined according to Jackson [24, 25] are presented in Tables 1, 2 and 3, respectively. All agricultural practices were conducted according to the recommendations by the Egyptian Ministry of Agriculture as follows: 1). No used any chemical fertilizers. 2). Plants were subjected to sprinkler irrigation system in an amount that corresponded to field water capacity (63.2%) of soil used in this experiment. 3). Weeds were removed by hand and only natural pesticides were used for any plant diseases.

Harvesting: At the fruit ripening stage, all plants of each plot (96 plants) were harvested, and then air dried and weighted to determine the biological yield [fruit yield] (g plant^{-1}).

Essential oil isolation: Fruits were collected from each replicate for all treatments in both seasons, air dried, then crushes the fruits by electric grinder and weighted to extract the essential oil and to determine the essential oil percentage. Dry fruits (100 g) from each replicate were hydro- distilled for 3 h using a Clevenger-type apparatus [26]. The essential oil as ml plant^{-1} was calculated according to the dry weight of fruits (g plant^{-1}) of each replicate.

Gas Chromatography-Mass Spectrophotometric (GC-MS): The GC-MS analysis of the essential oil samples was carried out in the first season using gas chromatography – mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 40 C for 1 min; rising at 4.0 C/min to 160 C and held for 6 min; rising at 6 C/min to 210 C and held for 1min. The injector and detector were held at 210 ^{o}C. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using two different analytical methods: (a) KI, Kovats indices in reference to n-alkanes (C_9-C_{22}) [27]; and (b) mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library). The separated components of the essential oil were identified by matching with the National Institute of Standards and Technology (NIST) published data [28].

Statistical analysis: In this experiment, 2 factors were considered: 4 concentrations of humic acid and the same 4 concentrations of humic acid X compost. For each treatment there were 4 replicates. The number of experimental units was 24 plots. The experimental design was a complete randomization block design according to Snedecor [20]. The data from each season were statistically analyzed using two-way analysis of variance (ANOVA-2). Significant differences was considered at the levels of P values ($P \leq 0.05$ = significant, P and < 0.01 = more significant) and LSD at 0.05 and 0.01 by using the STAT-ITCF program [30]. Pearson correlation (r) and regression coefficients were estimated among these variables as suggested by Steel [31].

RESULTS

It is clear from the data presented in Table (4) that more significant increment was recorded in the treatment of humic acid at 15 Kg ha^{-1} to fruit yield (58.8 g $plant^{-1}$). Regarding the effect of compost application, it is clear that, compost was more significant for fruit yield (50.0 g $plant^{-1}$). Compost and humic acid treatments resulted in more significant effect for fruit yield, where the interaction between compost with humic acid at 30 ton ha^{-1} gave the highest mean values of fruit yield (66.7 g $plant^{-1}$). The increment in essential oil (%) and yield (ml $plant^{-1}$) were more significant for humic acid or compost. Comparing the mean values of humic acid treatments indicated that, application of humic acid at 10 Kg ha^{-1} increased essential oil (%) with 100% from the mean values of essential oil (%) without application. Moreover, increment of essential oil yield (ml $plant^{-1}$) as a result of humic acid at 10 or 15 Kg ha^{-1} reached to 233.3% than that of untreated plants. Compost application caused more significant effect on essential oil (%) and oil yield (ml $plant^{-1}$) which recorded 1.6% and 0.8 ml $plant^{-1}$ respectively. The combination between compost and humic acid treatments had no significant effect on essential oil (%). On the other hand, the combination treatments caused significant effect on essential oil yield (ml $plant^{-1}$) while the combination between compost and humic acid at 10 Kg ha^{-1} or compost X humic acid at 15 Kg ha^{-1} gave the highest mean value of essential oil yield (1.2 ml $plant^{-1}$). Twenty two constituents were identified in essential oil of the fruits of all treatments and accounted for 97.0% to 99.1 of total constituents (Table, 5). The major constituents of sweet fennel essential oil were trans-anethole (63.4 – 71.1 %), estragole (16.9 – 35.3%) and fenchone (4.3 – 8.6%). Monoterpene hydrocarbons ranged from 0.6 to 2.9%, while sesquiterpene hydrocarbons ranged from traces to 0.1%. Oxygenated monoterpene compounds ranged from 95.2% to 97.5%. The maximum mean values of monoterpene hydrocarbons compounds (2.9%), sesquiterpene hydrocarbons (0.1%) and oxygenated monoterpene (97.5%) resulted from the treatments of 10 kg ha^{-1} of humic acid, 0 kg ha^{-1} of humic acid (with or without compost) and 0 & 5 kg ha^{-1} of humic acid, respectively. The maximum mean values of trans-anethole (71.1 %), estragole (35.3%) and fenchone (8.6%) were resulted from 15 kg ha^{-1} of humic acid, 0 kg ha^{-1} of humic acid (with compost) and 5 kg ha^{-1} of humic acid (with compost) treatments, respectively. Correlation was carried out to determine the relationship between interaction treatments and essential oil percentage as well as its main constituents (Table 6). Essential oil %, monoterpene hydrocarbons, Oxygenated monoterpene, Fenchone, Estragole and Trans Anethole which were present in all treatments were used for analysis. The highest positive correlation was between treatments and essential oil percentage (0.8) while, the highest negative relationship was observed between interaction treatments and oxygenated monoterpenes. Data presented in Table (7) clear that, essential oil percentage had a positive relationship with monoterpene hydrocarbons, oxygenated monoterpene and trans-anethole where the significant relationship with oxygenated monoterpene (0.8). Fenchone had a negative significant relationship with estragole (-0.7) while estragole had a high significant negative correlation with trans-anethole (- 0.8). The results indicated that, trans- anethole consists of 57.9 to 71.1 % of the sweet fennel essential oil; it is predictable that increasing anethole content will decrease estragole. This means that estragole content are strongly depended on anethole content in essential oil of sweet fennel. In this respect,

DISCUSSION

The increase in essential oil yield may be due to enhancement of yield production or / and essential oil content (%). The valuable effect of humic acid or/and compost may be due to its role in accelerating metabolic reactions as well as stimulating enzymatic systems responsible for the biosynthesis of essential oil and its compounds as the role of organic fertilizers [32]. The increase in essential oil content (%) under compost could be explained by the positive effect of compost on improving nutritional status of the soil, and due to rapid mineralization of organic matter that important for plant growth and essential oil production [33]. Obtained results agreed with those of Parakasa [8] who reported that application of organic fertilizer increased total essential oil yields of davana (*Artemisia pallens* Wall. ExD.C.) plant and Marculescu [10]. They revealed that the soil with its content in macro and microelements enhanced by the use of organic fertilizers, which plays an essential role in the plants development, in the biosynthesis of the organic substances. It had been noted that when using compost increased the amount of active principal (essential oil) in chrysanthemum (*Chrysanthemum balsamita* L.) plant. On the other hand the effect of compost agreed with the results of Khalid [12] and Said-Al Ahl [34], they indicated that organic fertilizers resulted in a significant increase essential oil and the main components of *Ocimum basilicum* L. (sweet basil) and coriander (*Coriandrum sativum*) plants. The positive effect of humic acid may be due to humic acid improve soil structure, changed physical properties of soil, promote the chelation of many elements and made them available to plants, aid in correcting plant chlorisis, enhancement of photosynthesis density. Greater plant growth and essential oil with humate application [18, 19]. In this respect, these results agree with those obtained by Said-Al Ahl [34], who reported that oregano essential oil production increased significantly with K-humate application. Moradi [35] indicated that anethole consists 50 to 70% of sweet fennel essential oil; it is predictable that increasing anethole content will decrease other essential oil components such as fenchone, estragole and limonene. Compost and humic acid were significantly affected essential oil of coriander fruits, fennel, mint and linalool constituent [36, 37, 38]. The specific effect of the mode of action of the compost and humic acid on the production of these compounds could be explained on the basis of different primary metabolic pathways of carbon. d-α-pinene, d-fenchone, and camphene are monoterpenes that are biosynthesized from acetyl-CoA via the mevalonate pathway. d- α-Pinene and d-fenchone are derived from the pinyl branch of this pathway, while camphene is derived from the closely related bornyl branch of the pathway. This explains why changes in their content in the essential oil correlate positively. In contrast, anethole and methylchavical are phenylpropanoids biosynthesised from phosphoenolpyruvate (PEP) and d-erythrose-4-phosphate via the shikimic acid pathway. They differ chemically only in the position of their instaurations in the side chain. Thus, we can anticipate that changes in their concentrations might also be parallel. The common biosynthetic point of the two pathways is that PEP is a precursor via pyruvate for the synthesis of acetyl-CoA. This explains why increased production of the phenylpropanoids is at the expense of biosynthesis of the monoterpenes, since the precursor required for monoterpenes is being diverted into the shikimate pathway [39, 40]. Although there are a few reports on fruit yield, essential oil content and yield, harvesting time, etc. [36, 37, 38, 39, 40], information on the effect of compost and humic acid on their constituents is meager.

Conclusion

Adding compost and humic acid had a positive effect on fruit yield and essential oil contents compared with control treatment. For various major constituents (trans-anethole, estragole and fenchone) of essential oil extracted from sweet fennel fruits as well as mono and sesquiterpenes increased under compost and humic acid treatments. So we advise the farmers to applied these treatments (compost with humic) for produce the sweet fennel fruits and essential oil especially under organic farming.

Acknowledgement:

The authors would to thank the National Research Centre (NRC), Egypt for its finical support of this work through the project No. 9050101.

Table 1. Physical and chemical properties of the experimental soil.

Texture	Sand	K (%)	1.6
pH	7.4	Ca (%)	0.4
EC (%)	8.3	Mg (%)	0.2
Sand (%)	85.8	Na (%)	0.5
Silt (%)	11.8	Cl (%)	0.6
Clay (%)	2.23	SO4 (%)	0.3
N (%)	4.5	CO3 (%)	-
P (%)	0.9	HCO 3 (%)	0.3

Table 2. Chemical analysis of the used compost

Bulk density (kg/m³)	510.0	Total potassium (%)	2.3
Moisture content (%)	18.1	Available potassium (mg/kg)	0.6
EC (%)	9.1	Fe (%)	0.9
pH	8.1	Zn (%)	0.3
Total organic carbon (%)	25.2	Mn (%)	0.1
Total organic matter (%)	42.1	Cu (%)	0.1
Total nitrogen (%)	1.5	Nematodes	Nil
C/N Ratio	18.2	Weeds germination	Nil
NH4 (%)	8,8	Parasites	Nil
NO3 (%)	4.5	Radish germination test %	98
Total phosphorous (%)	0.2	Pathogenic	Nil
Available phosphorous (%)	4.1	Humus value	5.0

Table 3. Guaranteed analysis and physical data of humic total

Guaranteed analysis	
Humic acid	80 %
K2O	10-12%
Zn, Fe, Mn, etc.,	100 ppm
Physical Data	
Appearance	Black powder
pH	9-10
Water solubility	< 98%

Table 4. Effect of compost, humic acid and their interactions on essential oil content

Treatments		Fruit yield	Essential oil	
Compost	Humic acid (kg ha $^{-1}$)	g plant $^{-1}$	%	ml plant $^{-1}$
Without compost	0	25.0	0.8	0.2
	5	25.0	1.2	0.3
	10	36.8	1.9	0.7
	15	43.8	1.6	0.7
Overall without compost		35.7	1.4	0.5
With compost	0	27.3	1.1	0.3
	5	40.0	1.5	0.6
	10	57.1	2.1	1.2
	15	66.7	1.8	1.2
Overall with compost		50.0	1.6	0.8
Overall humic acid	0	30.0	1.0	0.3
	5	35.7	1.4	0.5
	10	50.0	2.0	1.0
	15	58.8	1.7	1.0
F ratio:				
Humic acid		236.2**	49.2**	46.8**
Compost		218.1**	15.0**	45.2**
Humic acid * compost		67.7*	0.2	3.4*
LSD at 0.05				
Humic acid		1.9	0.1	0.1
Compost		2.9	0.2	0.2
Humic acid * compost		3.1	NS	0.3
LSD at 0.01				
Humic acid		2,1	0.2	0.2
Compost		3.4	0.3	0.3
Humic acid * compost		4.8	NS	0.4

Table 5: Effect of humic acid and/or compost on the relative percentage of the main constituents of the essential oil of fennel

No	Components	KI*	Humic acid treatments (kg ha $^{-1}$)							
			Without compost				With compost (30 ton ha $^{-1}$)			
			0.0	5.0	10.0	15.0	0.0	5.0	10.0	15.0
1	Camphene	1151	tr.	tr.	tr.	tr.	tr.	tr.	0.1	tr.
2	β-Pinene	1193	tr.	tr.	0.2	tr.	0.1	0.1	tr.	tr.
3	Sabinene	1209	tr.	tr.	tr.	tr.	tr.	tr.	0.1	tr.
4	α-Phellandrene	1248	0.2	0.1	0.3	tr.	tr.	0.2	0.1	tr.
5	Myrcene	1251	tr.	0.3	0.2	tr.	0.4	tr.	tr.	tr.
6	Limonene	1281	0.7	0.6	0.9	0.5	0.7	0.7	1.7	1.6
7	β-phellandrene	1290	0.1	tr.	0.1	tr.	tr.	0.1	tr.	tr.
8	1,8-Cineole	1295	0.1	0.1	0.1	tr.	0.1	0.1	0.1	0.1
9	(E) – b-Ocimene	1322	0.1	tr.	tr.	tr.	tr.	0.1	tr.	tr.
10	γ-Terpinene	1327	0.1	0.4	0.1	tr.	0.9	0.8	tr.	0.4
11	p-cymene	1354	0.1	0.1	0.3	tr.	0.1	0.1	tr.	0.1
12	Fenchone	1488	7.0	4.9	5.7	8.0	5.8	8.6	4.3	4.4
13	limonene oxide	1533	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
14	Menthone	1581	tr.	0.1	0.1	tr.	0.1	tr.	tr.	tr.
15	Camphor	1605	0.4	0.1	0.2	0.2	0.3	0.5	0.1	0.1
16	Linalool	1643	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
17	Fenchol	1660	1.0	tr.	tr.	tr.	tr.	tr.	tr.	tr.
18	E-β-Farnesene	1650	0.1	tr.	tr.	tr.	0.1	tr.	tr.	tr.
19	Estragole	1665	21.0	32.8	18.6	16.9	35.3	23.5	33.4	30.0
20	α- Terpineol	1694	tr.	0.2	0.2	tr.	0.1	tr.	tr.	tr.
21	Carveol	1733	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1
22	trans-Anethole	1827	58.0	59.2	70.3	71.1	54.3	63.4	57.9	60.6
	Monoterpene hydrocarbons		1.3	1.5	2.9	0.6	2.2	2.2	1.3	2.3
	Oxygenated Monoterpene		97.5	97.5	95.2	96.4	96.1	96.2	95.8	95.2
	Sesquiterpene hydrocarbons		0.1	tr.	tr.	tr.	0.1	tr.	tr.	tr.
	Total identified		99.0	99.1	98.1	97.0	98.4	98.4	97.2	97.4

*KI = Confirmed by comparison with Kovats Indices on TG WAX MS column [27]
tr. = less than 0.1

Table 6. Simple correlation coefficients and regression equations between interaction treatments (compost + humic acid) and some essential oil constituents of sweet fennel

Traits	Correlation	Regression equation
Essential oil %	0.8^{**}	Y=0.116x+0.975
Monoterpene hydrocarbons	0.2^{ns}	Y=0.065x+ 1.492
Oxygenated monoterpene	-0.7^{*}	Y= - 0.260x+ 97.41
Fenchone	-0.3^{ns}	Y=-0.175x + 6.875
Estragole	0.4^{ns}	Y= 1.179x + 21.12
trans- Anethole	0.1^{ns}	Y=- 0.307 x + 63.23

ns = not significant *=Significant at 0.05 probability **= Significant at 0.01 probability

Table 7. Simple correlation coefficient between essential oil percentage and its main constituents of sweet fennel

	X1	X2	X3	X4	X5	X6
Essential oil % x1	---					
Monoterpene hydrocarbons x2	0.2^{ns}	----				
Oxygenated monoterpene x3	0.8^{*}	-0.6^{ns}	----			
Fenchone x4	-0.3^{ns}	-0.2^{ns}	0.3^{ns}	----		
Estragole x5	-0.0^{ns}	0.1^{ns}	0.0^{ns}	-0.7^{*}	----	
trans- Anethole x6	0.4^{ns}	-0.0^{ns}	-0.3^{ns}	0.4^{ns}	-0.8^{**}	----

ns = not significant *=Significant at 0.05 probability **= Significant at 0.01 probability

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