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Original Article

Quantity and Quality of Dill Essential Oil as Influenced by Organic Fertilizers

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Abstract

Increasing attention is being paid to use of organic fertilizers such as manure and vermicompost which can increased yield and protect the environment. Replacing chemical fertilizers with manures has the benefit of low production cost and imparts beneficial effects on soil. Then an experiment was conducted during 2013 to measure effects of organic fertilizers on essential oil of dill (*Anethum graveolens* L.). Use of organic fertilizers beneficially affected seed yield, percent of essential oil, and essential oil yield. Essential oil percent was highest due to treatment with compost tea applied to the soil compared to foliar application. The combination of 20 $t{\cdot}ha^{-1}$ of manure and 7 $t{\cdot}ha^{-1}$of vermicompost, without compost tea,producedthe maximum essential oil yield (23.85 $kg{\cdot}ha^{-1}$). The GC-MS analysis of dill essential oil indicated 94% of essential oil compounds were made up of: carvone, α-phellandrene, p-cymene, dillapiole and trans-dihydrocarvone. The maximum value of carvone (73.58%) was obtained by application of 20 $t{\cdot}ha^{-1}$ of manure and 15 $t{\cdot}ha^{-1}$of vermicompost and compost tea in form of soil application.

Keywords: *Anethum graveolens,* Composition, Foliar application, Manure, Vermicompost

Introduction

Increasing attention is being paid to use of organic fertilizers such as manure and vermicompost which can increased yield and protect the environment [1]. In addition to being profitable economically, application of different organic fertilizers leads to increased biomass, available nutrients, vitamins and secondary metabolites of medicinal and aromatic plants and improved crop health [2].

Replacing chemical fertilizers with manures has the benefit of low production cost and imparts beneficial effects on soil microorganisms, improving soil properties, increasing levels of soil organic carbon and results in positive effects on plant quality and quantity [3].

Application of compost tea increases biomass and crop quality and improves their health [4]. Compost tea contains useful organisms, including microbial communities, and when added to soil increases soil

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microorganism activity, makes soil elements absorbable, and allows useful bacteria to colonize the surface of plants, protecting them against pathogens and pests [5].

By increasing soil organic matter, vermicompost makes soil nutrients available to plants by causes gas movement in soilby increases soil capacity of cationic exchange and moisture availability [6]. Vermicompost increases economic yield in dill [7]. Being used in amount above critical threshold of soil, organic fertilizers inflict damage on crops by reducing fruit yield due to extreme vegetative growth [8].

Dill (*Anethum graveoles* L.) is used in traditional medicine [9] and contains essential oils, protein, fat, carbohydrates, flavonoid, phenolic acids, petroselinic acid as well as carotenoids and minerals [10].Essential oil produced varies in different tissues of dill [11].Levels of plant essence are affected by climatic conditions, harvest time and application of fertilizers making various food elements available to plants which increases production and changes the composition of essence [12].Vermicompost and organic fertilizers can increase amounts of essential oil in rosemary (*Rosemarinus officinalis* L.) [13]. They can alters the composition, quantity and quality of dill essential oil [14].Dill can produce the same essential oil with different chemical compounds used to treat different diseases. Dill has three chemo types, in each of which the highest essence compounds contain carvone, limonene and α-phellandrene [15]. Essential oil of the plant includes dillapiole, p-cymene and other chemical compounds, with the main combination being carvone and α-phellandrene [16]. Presence of α-phellandrene causes aroma of dill while carvone is main characteristic of dill [17,18]. Chemical compounds of dill essence are carvone, limonene, dillapiole and linalool [19].

The goals of this study were 1) determine the effect of organic fertilizers on dill (*Anethum graveolens* L.) grain yield and essential oil chemical composition and 2) determine the combination or single of proper organic fertilizer application that increased carvone (as one of the main chemical compound of dill essential oil) without reducing essential oil yield.

Material and Methods

The experiment was conducted at the Research Farm and Laboratories of the Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran (latitude 34°21' N, longitude 47°9' E, altitude 1319 m above sea level) during 2013. Based on USDA soil taxonomy the soil is classified as fine mixed thermic and Vertic Calcixerept, 2 m deep, with clay-loam texture, and the following characteristics: electrical conductivity 1.55 $mmos{\cdot}cm^{-1}$, nitrogen 0.129%, phosphor 23.8 $mg{\cdot}kg^{-1}$, potassium 590 $mg{\cdot}kg^{-1}$, organic carbon 1.29%, pH 7.89, Manganese 23.8 $mg{\cdot}kg^{-1}$, Iron3.26 $mg{\cdot}kg^{-1}$, Zinc 2.08 $mg{\cdot}kg^{-1}$, Copper 2.04 $mg{\cdot}kg^{-1}$, PH of Paste7/89, Total Neutralizing Value32/5, Field Capacity 34,Permanent wilting point (18 %), Bulk Density 1/30 $gr.cm^{3.-1}$,Sand 27%, Silt 39%, Clay 34%,Soil Textural Clay-Loam. The previous crop in rotation was wheat.

The experimental factors were type of organic fertilizers including cow manure as main plots, including: 0, 10, or 20 $t.ha^{-1}$, vermicompost as subplots: 0, 7, or 15 $t.ha^{-1}$ and compost tea as sub-subplots, including nonuse, foliar application and soil application.

The amounts of compost tea used were 1.65 L and 4.85 Lin foliar and soil application respevtively. The compost tea treatment was applied to leaves and shoots after sunset (due to the presence of useful microorganisms and microbial communities) using a sprayer which has not been used before.

It was applied to the soil in a mixture with well water after sunset. To do this, plastic containers with different volumes were used. Compost tea and water was mixed in these plastic containers. The resulting soluble was applied to the soil using different sprinklers to prevent entering other plots.

Each plot consisted of 5 rows, 2m long, spaced 30 cm apart; theplant density which used by local farmers. Seeds of dill were planted at12 June 2013 and were irrigatedimmediately after that. Additional irrigations were done every 7 days. At full physiological maturity, 3 middle rows of each plot were harvested and shade-dried to determine grain yield.

The seed samples were dried in shade to keep the quantity and quality of their essential oil. Then essential oils were isolated by hydro distillation of dried plant material for 4h (50 g of sample in 1000 mL of distilled water) using a Clevenger-type apparatus. The oils were centrifuged and the upper phase discarded to desiccate the oils[20]. The oils were stored in sealed glass vials covered with parafilm and aluminium foil at 4 °C for further use. Mixing essential oil in water is not desired because of the ester hydrolysis.

Essential oils were injected into chromatography gaseous system connected to Trace MS Model mass spectrometer (GC-MS) (Uest-finnigan Ö thermo Q) with Quadrupole-type mass spectrum with DB-5 column 30 m long with a0.25 mm internal diameter and 0.25 µm of statistic phase thickness. Initial and final oven temperatures were 60 ºC and 250 ºC, respectively. An FID[1]type detector was employed at 280 ºC. The temperature of the injection port was 250 ºC. Helium carrier gas had a flow rate of 1.1mm·min^{-1}. Voltage and temperature of ionization was 70 eV and 250 ºC, respectively. Oil constituents were identified by comparing linear retention indices with those of standard compounds and by comparison with literature and MS data obtained from Wiley and NIST libraries [21,22]. Relative percentwas calculated from TIC by the computer. It mentioned that the essential oil was measured in2 replicates.

Data were subjected to analysis of variance in SAS (ver 9.2 SAS, Inc., Cary, NC) software. If interactions were significant they were used to explain the results. If the interactions were significant, they were used to explain the results. Otherwise, the means of simple effects were compared by the Duncan’s Multiple Range test.

Result and Discussion

Grain yield was affected by vermicompost and compost tea and by the vermicompost×compost tea and manure×compost tea interactions (Table 1).Although use of compost tea alone,and/or with cow manure/vermicompost,affected essential oil yield, essential oil percent was only affected by compost tea application (Table 2).

Use of vermicompost (7 t·ha^{-1}) together with compost tea (in soil application) resulted in the highest grain yield. The cow manure × compost tea interaction indicated that soil application of compost tea without cow manure had the best effect on grain yield. Soil application of compost tea caused a higher increase of grain yield compared to foliar application (Table 3).

Results of this study are in agreement with a study which reported maximum yield of dill-seed grains with 8 t.ha^{-1} of vermicompost treatment[23], who reported maximum yield of dill-seed grains with8 t·ha^{-1} of vermicompost treatment. Vermicompost can reduce costs by reducing use of chemical fertilizers and reduces damage to the environment [7].During varied studies, utilization of vermicompost has increased growth and economic yield of various crops significantly, for example grain yield of parsley (*Petroselinum crispum*) [6], coriander (*Coriandrum sativum*) and caraway (*Carumcarvi*) [24].

The beneficial effects of compost tea on dry matter and yield of plants is likely due to nutrient resources and microbial communities present [25]. Compost tea is capable of increasing aeration and adjusting soil acidity [26]. Compost tea improves yield by improving soil structure, development of root system and preventing production of soil toxins [5]. Favorable bacteria and communities, present in compost tea, penetrate into plant tissues and do not pathogens access [5].

According to the results of a comparison of means (Table 4), Essential oil percent was improved when compost tea was applied to the soil, compared to foliar application. The combination of 20 t·ha^{-1}of manure and 7 t·ha^{-1}of vermicompost, without compost tea, improved essential oil yield (Table 5).

Various organic fertilizers increase growth, yield and quantity and quality of essential oil in dill [27]. Essential oil percent in dill fruit is between 0.2 and

[1] Flame ionization detector

4.6 [28]. Nitrogen and phosphorus are necessary to form terpenoid essential oil compounds [29]. Increased dill essential oil might be due to the role of compost tea in effective uptake of phosphorus and to some extent nitrogen, by dill roots. Compost tea increased vegetative growth and essential oil levels in marjoram (*Majorana hortensis Moench*) [26] as well as essential oil percent in coriander (*Corianderum sativum*) [30]. Therefore, it maybe that application of compost tea results in increased essential oil production [31].

In the present experiment, almost all of dill essential oil compounds contained carvone, α-phellandrene, p-cymene, dillapiole and trans-dihydrocarvone (Table 6). The level of carvone was less when no organic fertilizer was used. Its maximum value was obtained by application of 20 t·ha^{-1}of cow manure, 15 t·ha^{-1}of vermicompost and compost tea applied to the soil which was higher than the control. The lowest value of carvone was with the combination of 7 t·ha^{-1}of vermicompost together with the soil application of compost tea.

Table 1 Mean squares for effect of organic fertilizers on grain yield of dill

SOV	df	Grain yield
Replication	2	296684.636
Cow Manure (CM)	2	280544.486
Main plot error	4	213772.518
Vermicompost (V)	2	796684.50**
CM × V	4	206916.549
Sub plot error	12	277067.84
Compost tea (Ct)	2	576212**
(CM) × (Ct)	4	607789.78**
(Ct) × (V)	4	816606.5**
(CM) × (V) × (Ct)	8	129798.970
Sub-sub plot error	36	101117.447
CV (%)	-	19.12

*, **significant at 5%, 1% levels, respectively, ANOVA

Table 2 Mean squares for effect of organic fertilizers on essential oil of dill

SOV	df	Essential oil	Essential oil yield
Replication	1	0.047	6.407
Cow Manure (CM)	2	0.001	24.683
Main plot error	2	0.040	20.25
Vermicompost (V)	2	0.007	22.332
CM × V	4	0.016	15.347
Sub plot error	6	0.011	34.267
Compost tea (Ct)	2	0.068*	112.307**
(CM) × (C)	4	0.039	48.509**
(Ct) × (V)	4	0.017	70.218**
(CM) × (V) × (Ct)	8	0.017	24.8**
Sub-sub plot error	18	0.017	5.553
CV (%)	13.30	14.81	-

Table 3 Mean comparison of grain yield in different levels of vermicompost × compost tea and manure×compost tea

Vermicompost×Compost tea			Manure×Compost tea		
Vermicompost (t·ha^{-1})	Compost tea application type	Grain yield (kg·ha^{-1})	Manure (t·ha^{-1})	Compost tea application type	Grain yield (kg·ha^{-1})
0	nonuse	1036 e[a]	0	nonuse	1418 cd
0	foliar	1670bcd	0	foliar	1941 a
0	in soil	1699 abcd	0	in soil	1981 a
7	nonuse	1901 ab	10	nonuse	1584 bcd
7	foliar	1469 d	10	foliar	1486 bcd
7	in soil	2016 a	10	in soil	1767ab
15	nonuse	1838 abc	20	nonuse	1774 ab
15	foliar	1556 cd	20	foliar	1270 d
15	in soil	1776 abcd	20	in soil	1744 abc

Table 4 Mean comparison of essential oil percent in compost tea

Compost tea application type	Essential oil percent
none	0.9889 ab[a]
foliar	0.9167 b
in soil	1.039 a

[a]values in columnfollowed by the same letter are not significantly different,p≤0.01

Table 5 Mean comparison of essential oil yield in Manure×Vermicompost × Compost tea

Manure (t·ha^{-1})	Vermicompost (t·ha^{-1})	Compost tea application method	Essential oil yield (kg·ha^{-1})
0	0	0	8.705 h
0	0	foliar	22.72 abc
0	0	in soil	19.88 abcd
0	7	0	13.03 efgh
0	7	foliar	12.81 efgh
0	7	in soil	23.7 ab
0	15	0	17.74 cde
0	15	foliar	15.07 defg
0	15	in soil	19.22 abcd
10	0	0	11.8 fgh
10	0	foliar	10.26 gh
10	0	in soil	18.17 bcde
10	7	0	15 defg
10	7	foliar	11.82 fgh
10	7	in soil	22.65 abc
10	15	0	22.05 abc
10	15	foliar	14.74 defg
10	15	in soil	18.19 bcde
20	0	0	10.21 gh
20	0	foliar	14.89 defg
20	0	in soil	14.99 defg
20	7	0	23.85 a
20	7	foliar	10.75 fgh
20	7	in soil	14.64 defg
20	15	0	16.26 def
20	15	foliar	10.24 gh
20	15	in soil	16.11 def

Data in the interaction analyzed with Least Squares Means and means separated with Least Significant Difference.

[a]values in columnsfollowed by the same letter are not significantly different,p≤0.01

Table 6 The four main components of dill essential oil based on chemical composition

Treatment	RT[a]	α-phellandrene	RT	P-cymene	RT	Carvone	RT	Dillapiole
1[b]	7.68	3.9293	8.31	18.8076	14.81	61.3872	23.92	10.0704
2	7.68	1.8969	8.31	16.2144	14.82	62.3366	23.95	13.7635
3	7.67	2.5383	8.32	16.4261	14.84	61.9596	23.97	13.6089
4	7.66	1.9055	8.26	22.9137	14.64	54.9886	23.85	14.4874
5	7.67	2.4754	8.29	13.2998	14.80	63.8026	23.93	14.4387
6	7.67	3.4753	8.31	19.8557	14.78	54.7502	23.94	14.7733
7	7.73	3.1997	8.37	19.9647	14.88	58.869	24.01	12.4419
8	7.67	2.19	8.31	15.3009	14.83	64.8374	23.94	12.2107
9	7.69	2.6907	8.31	13.0399	14.84	66.0377	23.94	11.9748
10	7.67	0.9484	8.31	19.1781	14.8	61.5583	23.94	14.0112
11	7.69	1.0908	8.34	12.8371	14.9	70.6497	23.99	9.9018
12	7.68	2.4239	8.32	18.4894	14.81	59.7405	23.95	14.1795
13	7.68	2.1697	8.31	14.4451	14.84	68.0996	23.91	7.8551
14	7.66	0.8791	8.29	14.6144	14.81	65.9634	23.93	13.5656
15	7.69	4.1999	8.31	15.8231	14.84	58.8917	23.97	14.6874
16	7.71	2.479	8.34	15.247	14.88	64.2959	23.99	12.4148
17	7.67	2.635	8.31	17.5343	14.80	59.659	23.95	14.428
18	7.67	2.5871	8.31	18.0713	14.79	59.3341	23.93	13.8086
19	7.68	2.7751	8.32	19.0739	14.82	62.6117	23.91	9.6084
20	7.68	3.1191	8.30	15.3896	14.80	63.0666	23.91	10.8621
21	7.69	3.5387	8.32	14.3812	14.87	65.89	23.96	9.4252
22	7.68	2.6062	8.31	15.9588	14.82	64.2565	23.92	11.6951
23	7.74	1.8426	8.37	16.0645	14.89	66.4631	23.99	10.4039
24	7.68	3.6312	8.30	15.7293	14.81	62.2237	23.93	12.2628
25	7.67	2.6463	8.30	12.6723	14.83	70.2426	23.90	8.361
26	7.69	1.749	8.33	16.3919	14.88	65.5278	23.95	10.6616
27	7.71	2.4508	8.34	17.3845	14.88	73.5866	-	tr

[a] RT=Retention time

[b]1=control; 2=compost tea (foliar application); 3=compost tea (soil application); 4=vermicompost (7 t·ha^{-1}); 5=vermicompost (7 t·ha^{-1})×compost tea (foliar application); 6=vermicompost (7 t·ha^{-1})×compost tea (soil application); 7=vermicompost (15 t·ha^{-1}); 8=vermicompost (15 t·ha^{-1})×compost tea (foliar application); 9=vermicompost (15 t·ha^{-1})×compost tea (soil application); 10=cow manure (10 t/ha); 11=cow manure (10 t/ha)×compost tea (foliar application); 12=cow manure (10 t·ha^{-1})×compost tea (soil application); 13=cow manure (10 t·ha^{-1})×vermicompost (7 t·ha^{-1}); 14=cow manure (10 t·ha^{-1}) ×vermicompost (7 t·ha^{-1})×compost tea (foliar application); 15= cow manure (10 t·ha^{-1}) ×vermicompost (7 t·ha^{-1}) × compost tea (soil application); 16= cow manure (10 t·ha^{-1}) × vermicompost (15 t/ha); 17=cow manure (10 t·ha^{-1}) ×vermicompost (15 t·ha^{-1}) × compost tea (foliar application); 18=cow manure (10 t·ha^{-1}) ×vermicompost (15 t·ha^{-1})×compost tea (soil application); 19= cow manure (20 t·ha^{-1}); 20=cow manure (20 t·ha^{-1}) ×compost tea (foliar application); 21=cow manure (20 t·ha^{-1}) ×compost tea (soil application); 22=cow manure (20 t/ha) ×vermicompost (7 t/ha); 23= cow manure (20 t·ha^{-1}) ×vermicompost (7 t·ha^{-1}) × compost tea (foliar application); 24= cow manure (20 t·ha^{-1}) ×vermicompost (7 t·ha^{-1})×compost tea (soil application); 25=cow manure (20 t·ha^{-1}) ×vermicompost (15 t·ha^{-1}); 26=cow manure (20 t·ha^{-1})×vermicompost (15 t·ha^{-1})×compost tea(foliar application); 27=cow manure (20 t·ha^{-1}) ×vermicompost (15 t·ha^{-1}) ×compost tea (soil application).

Data in interaction analyzed with Least Squares Means and means separated with Least Significant Difference.

[a]values in columns followed by the same letter are not significantly different, p≤0.01.

Foliar and soil applications of compost tea, in conjunction with cow manure, increased carvone level; application of vermicompost resulted in lower levels of carvone (Table 6). The highest level of α-phellandrene was obtained by using 10 t·ha^{-1}of cow manure and 7 tonnes of vermicompost along with compost tea soil application; compared to lower levels when no organic fertilizer was used (Table 6). The highest, and lowest, p-cymene levels were obtained by using cow manure (20 t·ha^{-1}) and a combination of cow manure (20 t·ha^{-1})+vermicompost (15 t·ha^{-1}), respectively; the level of p-cymene for the control was intermediate. Compost tea lowered p-cymene compared with the control, while manure and vermicompost increased it (Table 6). The dillapiole value was low for the control treatment; maximum and minimum values were obtained by using 7 t·ha^{-1}vermicompost together with compost tea soil application and 20 t·ha^{-1}of cow manure with 15 t·ha^{-1}of vermicompost along with compost tea soil application, respectively (Table 6).

When no fertilizers were used, the value of trans-dihydrocarvone was lowest. The highest value of this compound was obtained vermicompost (15 t·ha^{-1}) and cow manure (20 t·ha^{-1}) together with compost tea (soil application).

Content, and composition, of dill essential oil depends on plant part from which theoil is extracted, time of harvest, extraction method, cultivar, growth conditions, geographical origins, maturity status and conditions of storage of the oil [32]. Application of organic fertilizers increased amounts of some essential oil compounds while decreasing others [33]. Use of 20 t·ha^{-1}of manure resulted in an increase in carvone, but decreases in dillapiole and α-phellandrene. Results from using compost tea agreed with [30], who reported that compost tea application reduced amounts of limonene and p-cymene and increased the amount of carvone in coriander (*Corianderum sativum*). Levels of carvone, apiole, limonene and dihydrocarvon in dill essential oil have been reported to be 38.89, 30.81, 15.93 and 10.99%, respectively [34]. Carvone (50.1%) and limonene (44.1%) were identified as main compounds of dill essential oil [32]. Carvone is the most abundant compound, its level varying at flowering and grain filling stages [35]. The value of carvone has been reported to be 73.61% [36]. Others reported very low amount of carvone 1.68% of compounds[36].

Planting site affects components present in dill fruit[36]. The aroma and taste of dill are due to α-phellandrene, limonene and dill ether, but carvone is not important for flavor [25]. Values of trans-dihydrocarvone in dill fruit in this work agree with those obtained by Radulescu *et al*.[37]. High levels of α-phellandrene occur in dill leaves, lower levels in flowersand the lowest levels in fruit [37], indicated that α-phellandrene values increase during vegetative growth and begins to lower in productive phase. This work reported very little limonene in dill fruit. The most limonene was obtained when 20 $t{\cdot}ha^{-1}$ of manure and 7 $t{\cdot}ha^{-1}$ of vermicompost were used along with soil application of compost tea (0.63%). However limonene in dill essential oilhas been reported to0.2% [11].

Environmental factors and the cultivars used at the cultivation site, may affect levels of limonene**.** In an experiment, analysis of essences of 18 local dill masses (in Iran) showed that values of dill essence vary between 0.3% in Maragheh and 2% in Khoramabad [38]. Values of carvone and Dillapiole vary between 31.3% (Tehran) and 60.8% (taghmaie) and between 0.2% (brigand) and 31.9% (Tehran), respectively. Values of trans-Dihydrocarvone vary between 3.6% for Khomein paeenband and 14.5% for brigand. Values of α-Phellandrene vary between 0.2% for Zarand and 6.6% for Khoramabad and, finally, values of Dihydrocarvon vary between 0.3% for Khuzestan and 4.3% for Qom[38]. In addition, genetic factors, climatic conditions, cultural practices and extraction methods can be possible causes for diversity observed in essence compounds of dill grains [38]. Compost tea had one of the sources for introduced or resident soil micro organisms. Physico-chemical properties of compost tea improved nutritional status, and can available food source for plant when mixed with other organic fertilizers, the degree of efficacy of compost tea depend on many production, physical, chemical and biological characteristics of the soil [39].

Conclusion

Considering that, increasing carvone is an aim of crop production and breeding in dill, use of 20 $t.ha^{-1}$ of cow manure with 15 $t.ha^{-1}$ of vermicompost, along with soil applied compost tea was the best organic fertilizer combination based on the present study. Use of vermicompost with soil applied compost tea resulted in the highest grain yield and consequently increased essential oil yield in dill. Therefore, it can generally be said that vermicompost and compost tea can increase the yield and also the amount of main chemical compounds dill essential oil.

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