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Oil constituents of European dill (*Anethum graveolens* L.) Affected by irrigation and FYM Levels and storage conditions

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Abstract

A field as well as laboratory experiment was conducted to investigate the effect of irrigation and farmyard manure as well as storage conditions on oil constituents of European dill (*Anethum graveolens* L.). At field level, treatments viz., 4 levels of irrigation scheduled at 50 (I₅₀), 100 (I₁₀₀), 150 (I₁₅₀) and 200 (I₂₀₀) mm CPE and 3 levels of farmyard manure like 0 (F₀), 15 (F₁₅) and 30 (F₃₀) t FYM ha⁻¹ were conducted for two seasons from 2005-06 and 2006-07 in completely randomized blocks with 3 replications. In laboratory, oil was extracted from seeds stored upto 90 days and kept at cloth bag at room temperature, cloth bag in deep fridge, poly bag at room temperature, poly bag in deep fridge and oil constituents were determined by using Gas-liquid chromatography. Findings suggested that European dill may be irrigated at 100 mm CPE depends on rainfall and fertilized with 15-30 t FYM ha⁻¹. Also, seeds may be stored for two months in polythene or polythene lined bags under room temperature without loss of oil quality.

Keywords: *Anethum graveolens*, Medicinal uses, Irrigation, FYM, Storage condition, Carvone, Dillapiole

Introduction

European dill (*Anethum graveolens*) is one of an annual aromatic and medicinal plant belonging to the *Apiaceae* (*Umbelliferae*) family, commonly known as *vilayati saunf* in India. European dill is well known for essential oil present in this having medicinal properties like mildly diuretic, galactogogue, stimulant and stomachic (Radulescu *et al.*, 2010). The oil of dill fruits and its emulsion in water (dill water) are considered to be an aromatic, carminative (useful in flatulence, especially for infants), colic pains, vomiting and hiccups (Singh, 2012). The seeds are common and very effective household remedy for a wide range of digestive problems (Kaur & Arora, 2010) and infusion of seeds is especially efficacious in treating gripe in babies and flatulence in young children. The seed oil is considered to be of good quality due to presence of more carvone and least dillapiole. The major constituents of European dill seed oil are carvone (34.5%), dihydrocarvone (12.0%), limonene (10.0%), terpinene (6.0%), carveol (4.0%), dillapiole (3.0%) and some trace compounds. Higher carvone and limonene contents and negligible dillapiole content in oil has been reported of good quality (Guenther, 1949). The dillapiole is considered to be toxic to human consumption at more than 5% in essential oil and thus, quality of essential oil is considered better if dillapiole content in the oil varies between 0 to 5%.

European dill has a good demand in the country and recently it has also developed great potential for export in the international market. Because of its diversified uses and good source of foreign exchange the present investigation was done to improve the quality of essential oil with judicious water management and good nutrient supply which play important role and also by providing favorable storage condition to store the seeds.

Materials and methods

The field experiment was conducted in two *rabi* seasons (December to May) during 2005-07 at Norman E. Borlaug Crop Research Centre, G.B.P.U.A.&T., Pantnagar, Uttarakhand, India then biochemical analysis of oil is carried out in lab. In field, 4 levels of irrigation scheduled at 50 (I₅₀), 100 (I₁₀₀), 150 (I₁₅₀) and 200 (I₂₀₀) mm CPE and 3 levels of soil fertility viz, 0 (F₀), 15 (F₁₅) and 30 (F₃₀) t FYM ha⁻¹, treatments were evaluated in factorial randomized block design with three replications. Post-sowing irrigations of 60 mm depth and well decomposed and pulverized farmyard manure were applied as per treatments two days before sowing and mixed uniformly and thoroughly by digging with the help of spade.

Irrigation treatments were executed by providing irrigation channels between the plots. One common irrigation was given in each plot after 25 days of sowing for proper crop establishment. Subsequent irrigation treatments were scheduled based on cumulative pan evaporation (CPE) data. Irrigation water was measured with the help of *Parshall* flume under free flow conditions fixed in the irrigation channel to provide water upto 6 cm depth of soil at each irrigation. Depending upon rainfall, number of irrigation needed in I₅₀, I₁₀₀, I₁₅₀, I₂₀₀ treatments during first and second year were 4, 2, 1, 1 and 7, 3, 2, 1, respectively. After proper sun drying, the umbels were threshed manually. Thereafter, the seeds were collected, cleaned and weighed. The crop quality was judged by extracting oil from seeds and active principles present in oil.

First oven temperature	240°C	Second oven temperature	230°C
Injector temperature	245°C	Oil temperature	235°C
Detector temperature	250°C	Hydrogen flow rate	40 ml/ minute
Oxygen flow rate	400 ml/ minute	Nitrogen flow rate	18 ml/ minute
Column material	Carbo-bax 10%	Column dimension	1/8 inch × 6 feet

The area percentage obtained in chromatographs was taken as percentage of the constituents. The percentage of major constituents *viz.*, carvone, limonene and dillapiole obtained from 3 readings were used for statistical analysis for treatments comparison.

Results and discussion

Limonene content (%)

Fresh seeds

Limonene content in oil obtained from fresh seeds was found to increase significantly when collected from 50 mm CPE irrigation treatment compared to remaining irrigation treatments during both the years. A significant increase in limonene contained in oil obtained from the seeds collected from increasing levels of FYM was noticed during both the years.

Essential oil was distilled from seeds after threshing and stored for 30 days in different conditions like cloth bag at room temperature (CBRT), cloth bag in deep fridge (CBDF), polythene bag at room temperature (PBRT) and polythene bag in deep fridge (PBDF). For obtaining seed oil, water distillation method using Clevenger's type glass distillation apparatus was used. Essential oil samples were analyzed for determination of active principles in oil *i.e.* carvone using Gas-liquid chromatography. The filtered dehydrated oil was used for analysis. The RF value of pure carvone was obtained by injecting 0.2 μ ml of pure liquid carvone (standard) with micro syringe, which was diluted 4 times with ethanol. After getting constant RF value, 0.2 μ ml of each oil sample was injected three times. The standard conditions for running the GLC were:

Thirty days stored seeds

The oil obtained from the seeds collected from 200 mm CPE irrigation treatment and stored in CBRT and PBRT had significantly more limonene content compared to remaining irrigation treatments during both the years. When seeds were kept in CBDF the limonene content in oil was found to be significantly more in seeds collected from 150 mm CPE irrigation treatment in both the years. Limonene content in oil obtained from the seeds collected from 100 mm CPE irrigation level and kept in PBDF was found to be significantly more compared to the oil obtained from seeds collected from remaining irrigation treatments during both the years.

The oil in the seeds stored in CBRT or CBDF showed significant increase in limonene content with increasing levels of FYM. Limonene content showed reverse trend to this when stored in PBRT or PBDF during both the years.

Table 1: Limonene content (%) in fresh and 30 days stored seeds under different conditions as influenced by the treatments

Treatments	Fresh seeds		30 days stored seeds							
			CBRT		CBDF		PBRT		PBDF	
	I	II	I	II	I	II	I	II	I	II
Irrigation levels (mm CPE)										
50	7.250	7.395	2.684	2.732	2.075	2.127	0.612	0.625	3.278	3.340
100	6.517	6.647	1.190	1.211	2.642	2.708	1.371	1.401	5.316	4.279
150	6.564	6.695	1.026	1.044	3.173	3.252	5.323	5.440	1.202	1.225
200	6.156	6.279	4.430	4.510	1.042	1.068	6.506	6.649	1.448	1.176
C.D. at 5 %	0.005	0.005	0.004	0.004	0.003	0.003	0.003	0.003	0.002	0.002
FYM levels (t ha ⁻¹)										
0	6.738	6.873	2.300	2.341	2.222	2.278	4.604	4.705	4.375	4.458
15	6.273	6.398	2.233	2.263	2.227	2.283	3.447	3.523	2.852	2.906
30	6.854	6.991	2.466	2.510	2.249	2.305	2.309	2.360	1.207	1.230
C.D. at 5 %	0.004	0.004	0.003	0.003	0.002	0.002	0.002	0.002	0.002	0.002

Carvone content (%)

Fresh seeds

Oil obtained from fresh seeds contained significantly more carvone content when irrigation was scheduled at 200 mm CPE level and application of 15 t FYM ha⁻¹ resulted in significantly more carvone content in oil compared to remaining FYM levels during both the years.

Thirty days stored seeds

Oil from CBRT stored seeds contained similar carvone when

seeds were collected from irrigation treatment at 100 and 150 mm CPE level but significantly more than in the seeds collected from remaining irrigation levels during both the years. Oil obtained from the seeds stored in CBDF contained similar carvone content when seeds were collected from 50 and 100 mm CPE irrigation levels but significantly more than in oil of seeds collected from remaining irrigation treatments. Carvone content in the oil obtained from seeds stored in PBRT was found to be significantly more when collected from irrigation scheduled at 100 mm CPE compared to

remaining irrigation treatments during both the years. Carvone content in the oil obtained from the seeds stored in PBDF increased significantly when collected from 100 and 200 mm CPE irrigation level over 50 and 100 mm CPE levels

of irrigation during both the years. In all the seed storage conditions, carvone content in oil from 30 t FYM ha⁻¹ was significantly highest during both the years.

Table 2: Carvone content (%) in fresh and 30 days stored seeds under different conditions as influenced by the different treatments

Treatments	Fresh seeds		30 days stored seeds							
			CBRT		CBDF		PBRT		PBDF	
	I	II	I	II	I	II	I	II	I	II
Irrigation levels (mm CPE)										
50	83.202	84.866	84.765	86.291	87.544	89.933	88.344	90.286	86.699	88.346
100	83.493	85.163	87.050	88.617	87.766	89.960	89.370	91.336	85.126	86.743
150	82.949	84.608	87.138	88.706	86.822	88.993	84.285	86.139	88.623	90.307
200	83.964	85.643	81.782	83.254	86.317	88.475	78.556	80.284	88.594	90.278
C.D. at 5 %	0.021	0.021	0.486	0.495	0.060	0.061	0.099	0.101	0.030	0.031
FYM levels (t ha ⁻¹)										
0	82.084	83.726	84.450	85.970	85.445	87.581	83.888	85.733	85.895	87.527
15	84.211	85.895	85.090	86.623	87.335	89.518	85.055	86.926	87.150	89.565
30	83.911	85.589	86.010	87.558	88.556	90.770	86.474	88.376	88.759	90.445
C.D. at 5 %	0.018	0.017	0.421	0.429	0.052	0.052	0.085	0.087	0.026	0.027

Dillapiole content (%)

Fresh seeds

In fresh seed oil, dillapiole content was found to increase significantly with increase in CPE levels upto 150 mm CPE and decreased significantly with further increase in CPE levels also increasing levels of FYM caused significant decrease in dillapiole content in fresh seeds oil during both the years.

Thirty days stored seeds

Dillapiole content in CBRT, CBDF and PBRT stored seeds

oil increased significantly when seeds were collected from increasing levels of CPE for scheduling irrigation during both the years. In PBDF stored seeds oil, dillapiole content increased significantly when seeds were collected from increasing levels CPE upto 100 mm CPE irrigation treatment during both the years. Increasing levels of FYM caused significant decrease in dillapiole content in oil from the seeds stored in different conditions during both the years except seeds stored in PBRT, where application of 30 t FYM ha⁻¹ caused significant increase in dillapiole content compared to remaining FYM treatments.

Table 3: Dillapiole (%) in fresh and 30 days stored seeds under different conditions as influenced by the treatments

Treatments	Fresh seeds		30 days stored seeds							
			CBRT		CBDF		PBRT		PBDF	
	I	II	I	II	I	II	I	II	I	II
Irrigation levels (mm CPE)										
50	4.201	4.285	4.282	4.359	5.052	5.178	4.810	4.916	4.935	5.029
100	4.638	4.731	5.182	5.275	3.929	4.027	4.136	4.270	5.203	5.302
150	5.014	5.114	5.445	5.543	4.776	4.895	5.170	5.284	4.134	4.203
200	4.838	4.935	7.168	7.297	7.176	7.355	8.898	9.094	4.090	4.168
C.D. at 5 %	0.003	0.003	0.004	0.004	0.005	0.005	0.003	0.003	0.013	0.013
FYM levels (t ha ⁻¹)										
0	5.370	5.477	6.072	6.181	6.229	6.385	5.701	5.826	5.092	5.189
15	4.539	4.630	5.516	5.615	5.196	5.326	5.744	5.870	4.684	4.773
30	4.109	4.191	4.970	5.059	4.204	4.309	5.817	5.945	3.997	4.073
C.D. at 5 %	0.003	0.003	0.003	0.003	0.005	0.005	0.003	0.003	0.010	0.010

Among oil constituents, fresh seed oil contained significantly higher limonene when seeds were collected from 50 mm CPE (Table 1), carvone when seeds were collected from 200 mm CPE (Table 2) and dillapiole when seeds were collected from 150 mm CPE irrigation treatments (Table 3). In addition to oil content, oil composition is also affected by water stress. Excessive irrigations (6 in the treatment IW: CPE ratio 1.5) were found detrimental for the major oil constituents. Carvone accumulation was highest under 'no-irrigation' treatment followed by irrigation at 40 and 60 per cent depletion of ASW. Active principles of oil increased under dry conditions (Langenheim *et al.*, 1979). An inverse relationship has been found in carvone and dillapiole content which suggests that the carvone content increase at the expense of dillapiole. Dillapiole is an undesirable compound, as it is toxic to human being. Therefore, moisture stress conditions are more favourable for reducing this component.

The present investigation has resulted in 83.9 to 85.6 % carvone in fresh seeds oil due to irrigating the crop at 200 mm CPE level. In this treatment, limonene and dillapiole were found least. It is indicative of more conversion of limonene and dillapiole into carvone. Rate of development conversion to oxygenated compounds has been found to get enhanced under moisture stress (Langenheim *et al.*, 1979). Higher carvone in 200 mm CPE irrigation treatment may also be due to higher oil content. In the present investigation dillapiole content decreased with decreasing CPE levels of irrigation and increasing levels of farmyard manure. It suggested that judicious application of irrigation water and organic manures not only increase the yield but also enhance the quality of oil by reducing dillapiole and increasing limonene and carvone content.

Forouzandeh *et al.*, 2012 revealed that drought stress led to increased essential oil and oil constituents percentage,

whereas the greatest percentage obtained when 60% FC was applied. The oil constituents showed varying trend with storage conditions for 30 days. Seeds stored in CBRT and PBRT had significantly higher limonene content when collected from 200 mm CPE irrigation treatment while oil from CBDF stored seeds had highest limonene at 150 mm CPE irrigation treatment. In case of oil from PBDF stored seeds, the limonene content was found significant higher when seeds were collected from 100 mm CPE level. These variations suggest that certain changes in oil constituents took place under different storage conditions. Gupta (1982) has suggested to store the dill seeds in polythene lined cloth bag to save the losses of terpenoids in oil. Reverse trend was noticed for dillapiole during both the years. It seems that limonene and dillapiole conversion was enhanced towards carvone during the storage conditions where carvone was found higher. Gupta (1982) observed higher oxygenated compounds during storage at the expense of terpenes. In the present investigation oxygenated fraction (carvone content) also increased under storage compared in the oil of fresh seeds.

Farmyard manure at its higher levels (15 to 30 t ha⁻¹) is expected to provide about 75, 37.5 and 75 Kg NPK ha⁻¹ (15 t FYM ha⁻¹) and 150, 75 and 150 Kg NPK ha⁻¹ (30 t FYM ha⁻¹), thus meeting the total nutrients requirement of the crop. In addition, it provides micronutrients which are essential for plant growth and secondary metabolites synthesis (Gendy *et al.*, 2012). Present study also supported this as the oil constituents like limonene and carvone increased with increase in farmyard manure levels in fresh seeds. However, in 30 days stored seeds limonene content decreased. When stored in CBRT and CBDF, it was found more due to application of 30 t FYM ha⁻¹ but reverse was the trend when seeds were stored in PBRT and PBDF. It shows that during storage the limonene underwent conversion to carvone (Jinesh *et al.*, 2010). Due to increasing levels of farmyard manure significant enhancement in carvone percentage was noticed in stored seeds compared to fresh seeds. This may be because of more increase in total oxygenated compounds during storage (Gupta, 1982). Dillapiole has been found to increase with storage at the expense of terpenoids. Unstability of dillapiole in oil of PBDF stored seeds was observed as it was found to reduce compared to fresh seeds. It may be due to more carvone content in PBDF stored seeds as carvone content has inverse relationship with dillapiole content.

Conclusion

On the basis of present study, it can be concluded that for good oil quality of European dill, crop must be irrigated at 100 mm CPE and fertilized with 15 to 30 t ha⁻¹ FYM. Also seeds may be stored in polythene or polythene lined bags under room temperature for two months without loss of oil quality.

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