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Flavonoids, Essential Oil and Its Constituents of *Anethum graveolens* L. Herb Affected by Nitrogen and Bio-Fertilizers

Hussein A. H. Said-Al Ahl[1, *], Atef M. Sarhan[2], Abou Dahab M. Abou Dahab[2], El-Shahat N. Abou Zeid[1], Mohamed S. Ali[1], Nabila Y. Naguib[1]

[1]Department of Medicinal and Aromatic Plants, National Research Centre, 33 El Bohouth St. (former El Tahrir St.), Dokki, Giza, Egypt
[2]Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Cairo, Egypt

Abstract

This study was to investigate the response of *Anethumgraveolens* L. plants to the nitrogen fertilizer (0, 20, 40, 60 and 80kg N/fed.) with or without bio-fertilizers (at 2kg/fed.) on the flavonoids content and essential oil as well as its chemical constituents during the vegetative stage (90 days after sowing). The recorded results showed that in the two seasons, treatment of 60kgN/fed. with or without bio-fertilizers gave the best values of volatile oil percentage and oil yield in the herb, Also, the most effective fertilization treatment on flavonoids content was 40 or 60 Kg N/fed., with bio-fertilizers. In the essential oil constituents of dill herb, all treatments showed α-phellandrene (42.44 to 66.39%)as the major compound followed by p-cymene(11.97to 18.39%) and limonene(1.42 to 14.25%)then β-phellandrene (7.67 to 11.18%).

Keywords

Anethum graveolens L., Flavonoids, Essential Oil, Nitrogen, Bio Fertilizer

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1. Introduction

Dill (*Anethum graveolens* L.) is an annual herb, belonging to the family Apiaceae, native to South-West Asia or South-East Europe and was widely distributed in antiquity and is now grown worldwide. Dill is one of the first-known multi-purpose plant which has been used as a spice and medicine. It has been cultivated since ancient times (Bailer et al., 2001) as a vegetable, aromatic, carminative and antispasmodic (Simon et al., 1984) as well as an inhibitor of sprouting in stored potatoes (Score et al., 1997). Dill is one of the most popular seasonings both in our country and in the world. Fresh herb is very instable and after harvesting unsuitable for storage. Therefore, the sale of dill as well as other seasonings in containers is gaining popularity (Fras, 2009). Dill herb is marketed fresh or dried for culinary use; chopped green herbage is sprinkled on foods and may also be used to flavor pickles. Herb oil is mainly used in the food industry for flavoring and seasoning, perfumes, cosmetics, soaps, creams, lotions and in detergents. Also, herb and seed oil have also been widely investigated in respect of their antiseptic and exhibits anti carcinogenic activity, antimicrobial and antioxidant (Weiss, 2002).

The purpose of this work, which is carried out for the first time in Egypt, is to characterize chemically *Anethum graveolens* herb harvested under different nitrogen and bio-fertilizers through its essential oil and flavonoids as well as to evaluate their essential oil constituents.

* Corresponding author
E-mail address: hussein_saidalahl@yahoo.com (H. A. H. Said-Al Ahl)

2. Materials and Methods

2.1. Plant Material and Experimental Procedure

The experiments of this study were carried out at the Farm Station of National Research Centre, at Shalakan, Kalubia Governorate during the two successive seasons of 2000/2001 and 2001/2002.Seeds of dill (*Anethum graveolens* L.) were obtained from medicinal and aromatic plants Dep., National Research Centre, Dokki, Giza, Egypt.The soil was prepared and divided into plots 3x3.5m with five rows. The dill seeds were sown in 15thOctober in the two seasons. The distance between each row was 60cm a part and 20cm between the hills. The seedlings were thinned one month after sowing to leave two plants per hill.

The bio-fertilizers used in this study were Rhizobacterin (a mixture of *Azotobacter* and *Azospirillum*) and Microbein (a mixture of *Azotobacter*, *Azospirillum, Pseudomonas, Rhizobium* and *Bacillus*) (1kg Rhizobacterin + 1kg Microbein / feddan) added as inoculated the seeds and sowing. Bio-fertilizers produced by General Organization for Agriculture Equalization Fund (G.O.A.E.F.).The quantity of nitrogen fertilizer (urea 46.5%) at the rates of 20, 40, 60 and 80kgN / feddan was divided into two equal portions as side dressing, the first portion was added after thinning and the second one was added after one month later. The experimental design was factorial and planned in a complete randomized block design having three replications. This experiment included ten treatments as follows: Control; 20 kg N/ feddan; 40 kg N/ feddan; 60 kg N/ feddan; 80 Kg N/ feddan; Inoculation with bio-fertilizers; 20 kg N/ feddan + bio-fertilizers; 40 Kg N/ feddan + bio-fertilizers; 60 Kg N/ feddan + bio-fertilizers; 80 Kg N/ feddan + bio-fertilizers.

2.2. Data Recorded

Data for caroteniod, flavonoid and essential oil were estimated for the all treatments during vegetative stage as follows:

2.2.1. Total Flavonoids Content

Total flavonoids content was determined by using colorimetric method of Mabry et al. (1970).

2.2.2. Essential Oil Extraction, Determination and Analysis

The fresh herb was subjected to hydro distillation (100 g) for 3 hours using a Clevenger-type apparatus according to Gunther (1961). The oils were dried over anhydrous sodium sulphate before analysis. Gas liquid chromatographic (GLC) analysis.GLC analysis of the oil samples was carried out in the second season using Hewlett Packard gas chromatograph apparatus with the following specifications in Table 1:

Table 1. Hewlett Packard gas chromatograph apparatus specifications

Instrument:	**(HP) 6890 Series Hewlett Packard**
Column:	HP (Carbwax 20 M) (25m length X 0.32 mm I.D) Film thickness: 0.3 Mm
Sample size:	1 µl
Oven temperature:	60°C – 190 °C
Program:	60°C / 2min, 8°C/min, 190°C/ 25min.
Injection port temperature:	240°C
Carrier gas:	Nitrogen
Detector temperature(FID):	280°C
Flow rate:	N_2 30 mL/min; H_2 30 mL/min Air 300ml/min

Main compounds of the essential oil were identified by matching their retention times with those of the authentic samples injected under the same conditions. The relative percentage of each compound was calculated from the peak area of the peak corresponding to each compound.

2.2.3. Statistical Analysis

The data of the three experiments were statistically analyzed and the differences between the means of the treatments were considered significant when they were more than least significant differences (L.S.D) at 5% level according to Steel and Torrie (1980).

3. Results and Discussion

3.1. Essential Oil Content

The results reported in Table (1) indicate that using bio-fertilizer in fertilizing dill plants increased volatile oil percentage in fresh herb in both seasons. However, the increment in volatile oil percent in herb of dill plants treated by nitrogen application in all rates from 20 to 80 kg N/fed., but bio-fertilizer plus nitrogen application raised volatile oil percent in dill up to 40 kg N/fed., + bio-fertilizer in both seasons. The fertilizing dill plants with 60 kg N/fed., resulted in the largest volatile oil percent (0.076 and 0.076%) in both seasons.This increment of volatile oil might be due to the resultant of the increase in vegetative growth as the plant advanced towards maturity as well as the increase in the carbohydrate synthesis. Also, Singh et al. (1993) found that oil yield increased with up to 100 KgN/ha, but decreased at 125 kg/ha. The increase in oil percentage in plants fertilized with nitrogen and bio-fertilizer might be due to the beneficial effects of these fertilizers on the synthesis of carbohydrates in plant tissues, consequently used for oil production. The yield and quality of essential oil produced depends on various intrinsic and extrinsic factors. Intrinsic

factors comprise all internal hereditary characters. Extrinsic factors affecting the production of essential oil in plants are soil, nutrition and water supply, climate (light/temperature), maturity, post harvest treatment and distillation (Varshney, 1991). Nitrogen fertilization might enhance the essential oil biosynthesis processes through its direct or indirect role in plant metabolism resulting in more plant metabolites. However, Al-Humaid (2004) emphasized the beneficial role of the inoculation of fennel seeds with Rhizobacterin as a bio-fertilizer on the metabolic process that is responsible for the essential oil content. These results are in agreement with those obtained by Bhati and Shaktawat (1994); Venskutoniset al.(1999); Kewalanandet al.(2001); Rupamet al.(2001); Al-Humaid (2004); Al-Qadasi (2004); Kandeelet al.(2004 a and b) and Youssef et al.(2004);Said-Al Ahlet al. (2010).

3.2. Essential Oil Constituents

The quantities of the compounds identified by GLC in the volatile oils distilled from the fresh herb of dill are presented in Table (2). Altogether 19 compounds were identified from each treatment. In the essential oil of dill herb, α-phellandrene, limonene, β-phellandrene and p-cymene were found as the main compounds (more than 10%); besides two compunds such as α-pinene and carvone were identified as minor compounds (less than 10% and more than 1%); in lower concentrations, the other compounds were present in trace amounts (less than 1%), all treatments showed α-phellandrene as the major compound followed by p-cymeneand limonene then β-phellandrene.α-phellandrene ranged from 42.44to 66.39% while p-cymene ranged from 11.97 to 18.39%. All treatments except of 20 kg N/fed., increased α-phellandrene compared to control, but decreased P-cymene. Treating the plants with 80 kg N/fed., resulted in the highest α-phellandrene (66.39%) compared to the other treatments. P-cymene content (18.39%) increased with the treatment of 20 kg N/fed. compared to other treatments.

Limonene was found to be the third main compound and ranged from 1.42 to 14.25%. Fertilizing the plants with 20 and 60 kg N/fed., increased limonene and inoculating the plants alone resulted in the highest limonene (14.25%) compared to the other treatments. β-phellandrene content increased with the treatment of 20 kg N/fed., +bio-fertilizers (11.18%), it is relative percentage ranged from 7.67 to 11.18%. However, Houpalahti and Linko (1983) found a rapid decrease of α-phellandrene after flowering stage. On the other hand, a high α-phellandrene level and low carovone content in the dill herb. Halva et al. (1987) on dill found that α-phellandrene, β-phellandrene and limonene were the major components of herb in Finland. β-phellandrene and limonene contents varied slightly in relation to nitrogen fertilizer.

3.3. Total Flavonoids Percentage

Table 2. Effect of bio-fertilizers, nitrogen fertilizer and their interaction treatments on volatile oil % and flavonoids % of dill

Bio-fertilizers Nitrogen fertilizer (kg N/fed.)	First Season					
	Volatile oil (%)			Flavonoids content (%)		
	Without bio-fert.	Bio-fert.	Mean	Without bio-fert.	Bio-fert.	Mean
0	0.0560	0.0660	0.0610	0.4060	0.4130	0.4095
20	0.0730	0.0730	0.0730	0.4162	0.5530	0.4846
40	0.0730	0.0760	0.0745	0.4209	0.5950	0.5079
60	0.0760	0.0700	0.0730	0.4816	0.5051	0.4933
80	0.0600	0.0560	0.0580	0.4414	0.4414	0.4414
Mean	0.0676	0.0682		0.4332	0.5015	
L.S.D. at 5%	Bio-fertilizers = N.S Nitrogen = 0.0074 Interaction = N.S			Bio-fertilizers = 0.0163 Nitrogen = 0.0258 Interaction = 0.0366		
	Second Season					
0	0.0630	0.0830	0.0730	0.3999	0.4083	0.4041
20	0.0730	0.0760	0.0745	0.4092	0.5541	0.4816
40	0.0660	0.0660	0.0660	0.4186	0.5891	0.5038
60	0.0760	0.0660	0.0710	0.4781	0.4962	0.4871
80	0.0630	0.0630	0.0630	0.4363	0.4400	0.4381
Mean	0.0682	0.0708		0.4284	0.4975	
L.S.D. at 5%	Bio-fertilizers = N.S Nitrogen = N.S Interaction = N.S			Bio-fertilizers = 0.0143 Nitrogen = 0.0227 Interaction = 0.0321		

Table 3. Effect of bio-fertilizers, nitrogen fertilizer and their interaction treatments on the constituents % of dill volatile oil "herb"

Treatments compounds	1	2	3	4	5	6	7	8
α-pinene	1.63	1.90	1.96	2.02	1.60	1.80	2.08	1.64
β-pinene	0.27	trace	0.26	0.25	0.22	0.30	0.25	0.35
α-phellandrene	46.33	42.26	48.44	49.50	66.39	61.04	51.48	56.29
Limonene	13.72	11.81	13.78	13.80	1.42	14.25	11.01	10.67
β-phellandrene	11.01	10.89	10.89	8.49	8.31	7.67	11.18	9.81
γ-terpinene	0.36	trace	0.31	0.28	0.29	0.31	0.21	0.21
P-cymene	17.88	18.22	15.38	17.15	15.73	11.97	15.15	13.27
Dillether	0.45	0.48	0.36	0.38	0.35	0.29	0.39	0.36
Dihydrocarvone	0.22	0.50	0.24	0.31	trace	trace	0.43	0.31
Sabinol	1.04	1.26	1.23	0.67	0.66	0.48	0.95	0.64
Carvone	2.11	3.35	1.89	1.93	1.20	0.58	2.12	2.16
Piperitone	0.23	0.42	0.29	0.47	0.42	0.11	0.11	0.16
Carveol	0.66	1.69	0.87	0.94	0.34	trace	0.48	0.33
Nerolidol	0.71	1.27	0.78	0.61	0.52	0.20	0.87	0.78
Eugenol	0.79	0.83	0.97	0.50	0.72	0.49	0.84	0.64
Thymol	0.5909	0.50	0.43	0.2671	0.23	trace	0.21	0.21
Carvacrol	0.24	0.55	0.26	0.28	0.25	trace	0.21	0.21
Myristicin	1.07	0.47	0.2597	0.2201	0.3529	0.3575	0.2186	0.21
Dillapiole	0.59	0.63	0.21	0.23	0.55	0.08	0.37	0.29

Since: 1=control; 2=20kg N/fed.,;3=40kg N/fed.,;4=60kg N/fed.,;5=80kg N/fed.,; 6=Bio-fert.; 7= 20kgN/fed.+Bio-fert.; 8= 80kgN/fed.+Bio-fert.

Results presented in Table (2) is obviously noticed that total flavonoids percentage in dill plants in both seasons was increased comparing to untreated plants. Difference between with and without bio-fertilizer treatments was significant in both seasons. Application of nitrogen doses significantly increased total flavonoids percentage in both seasons. In this concern, the largest values of total flavonoids percentage was found by 40 kg N/fed., + bio-fertilizer (0.5950 and 0.5891%) in the in the two seasons, respectively. Volkhonskaya (1984) on *Bupleurumaureum* found that a direct relationship between flavonoid accumulation and flavonoid-splitting enzyme activity which was associated with ecological nature and its habitat. Variability was found in glycosidase and peroxidase activity of *B. aureum*stems, leaves and flowers. The distribution of flavonoid was changed in the different organs and growing conditions. These results are in accordance with those of Letchamo (1992); Kolar et al. (1998)*;* Tang et al.(2000)*;* Borellaet al. (2001); Barbara (2002) and Kovac-Besovic and Duric (2003).

4. Conclusion

The treatment of bio-fertilizer and in most of nitrogen fertilizer levels significantly increased essential oil content, and the treatment of 60 kg N/fed., with or without bio-fertilizer gave the best value. All identified compounds were observed in the essential oil of all treatments. The major compounds was found to be α-phellandrene, limoneen, β-phellandrene and p-cymene in dill herb and generally, fertilization treatments increased α-phellandrene and decreased limonene, β-phellandreneand p-cymene contents. The treatment of bio-fertilizer or nitrogen fertilizer significantly increased total flavonoids content. The treatments of 40 kg N/fed.,or 60 kg N/fed., combined with bio-fertilizer gave the highest total flavonoids content.

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