Middle East Journal of Agriculture Research
ISSN 2077-4605
Volume : 04 | Issue : 02 | April-June | 2015
Pages: 291-296

Effect of Nitrogen and/or Bio-fertilizer on the Yield, Total Flavonoids, Carbohydrate Contents, Essential Oil Quantity and Constituents of Dill Plants

[1]Mohammed S. Aly, [1]Abou-Zeid N. EL-Shahat, [1]Nabila.Y.Naguib, [1]Hussein A. Said-Al Ahl, [2]Atef M. Zakaria and [2]Mohamed A. Abou Dahab

[1] Medicinal and Aromatic Plants Researches Department, National Research Centre, 33 El-Bohouth St., (former El- Tahrir St.,) Dokki, Giza, Egypt. Postal Code: 12622.
[2] Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Cairo, Egypt.

ABSTRACT

This study was conducted during two successive seasons of 2000/2001 and 2001/2002 to evaluate the response of *Anethum graveolens L.* plants to nitrogen fertilizer with or without bio-fertilizer on fruits yield, total flavonoids and carbohydrates content, essential oil yield and constituents. Results cleared that the treatment of 60Kg N/feddan without and with bio-fertilizer gave the highest umbels number per plant through the two seasons and these increments were significant in comparison with control plants. Meanwhile fruits weight (g/plant) and yield(kg/Fed) showed significant increasing with the treatments of nitrogen fertilizers alone and combined with bio-feritlizers compared with control plants in the first and second season and the maximum increments were resulted with the treatment (60Kg N/fed) ..

Total flavonoids contents were significantly increased with all of used treatments, and the maximum increase was noticed with bio-fertilizers combined with 60Kg N/fed. during the two seasons. Total carbohydrate contents showed significant increase with applied Nitrogen fertilizers treatments alone, meanwhile the total carbohydrate contents were increased non-significantly with the other used treatments during the two seasons in comparison with control plants content. Applying of bio-fertilizer and nitrogen fertilizer levels significantly increased essential oil percentage, content and yield. However the treatment of 60 Kg N/fed. with or without bio-fertilizer gave the best values.

All identified compounds were observed in the essential oil of all treatments. The major compounds were limonene, carvone and dillapiole. The most effective fertilization on limonene content was 40 Kg N/fed. and/or bio-fertilizers. Meanwhile applying 20 Kg N/fed. with or without bio- treatments increased carvone content, the most of fertilization treatments (except those of bio-fertlizers and 40 Kg N/fed.) caused an increase in dillapiole content.

Key words: Carbohydrates, dill, essential oil, fertilizer, flavonoids

Introduction

Bio-fertilizers are complex of some microorganisms that mobilize main nutrients from unavailable to available one, could improve seed germination and root system, they are considered as a replacement for chemical fertilizers for improving soil fertility and crop production. Regarding the importance of medicinal plants and their role in human health, it is very important to increase their biomass without application of harmful chemical fertilizers. The most important advantages of growth promotion bacteria inducing and regulation hormones, development of root system and improving water and nutrients uptake (Fatma *et al.,* 2006)

Dill (*Anethum graveolens* L.) is a medicinal annual grassy plant belonging to the Umbelliferae family that originally comes from Eastern Mediterranean, planted for medicinal purposes and as spice in most parts of the world, Europeans have used this plant as vegetative organ and seeds to treat headache and hemato-vascular diseases. *Anethum graveolens* L. is laxative and carminative agent and used too to treat stomachache and digestion problems, (Hellal *et al.,* 2011).

Material and Methods

Field experiment

The experiment of this study were conducted at the farm station of National Research Centre, at Shalakan Kalubia Governorate during two successive seasons of 2000/2001 and 2001/2002. The aim of this study was to

Corresponding Author: Mohammed S. Aly, Medicinal and Aromatic Plants Researches Department, National Research Centre, 33 El-Bohouth St., (former El- Tahrir St.,) Dokki, Giza, Egypt. Postal Code: 12622.
E-mail: mohasam53@gmail.com

evaluate the effect of bio-fertilizers, nitrogen fertilizer and their interaction on the growth characters and yield of *Anethum graveolens L.* plants. The used seeds were brought from Medicinal and Aromatic Research Dep., A.R.C., Ministry of Agriculture. Soil analysis was done according to the method of Jackson (1967). Cleared sandy loam, and physical composition of 48.80% sand, 21.00% silt, 23.20% clay and 2.07% organic matter. Meanwhile the chemical analysis showed PH= 8.08; E.C (mmohs/cm) = 0.68 and total niterogen = 0.11%. Dill seeds were sown in 15th October through the two seasons. The distance between each row was 60 cm apart and 20cm between the hills. The seedlings were thinned one month after sowing to leave two plants per hill.

The bio-fertilizers that used in the present study were Rhizobactrerin (a mixture of *Azobacter* and *Azospirillum*) and Microbein (a mixture of *Azobacter*, *Azospirillum*, *Pseudomans*, *Rhizobium* and *Bacillus*) which produced by General Organization for Agriculture Equalization Fund (G.O.A.E.F.). One Kg Rhizobactererin plus one Kg Microbein/feddan were added as inoculated the seeds and sowing. Urea (46.5%N) was used as a source of nitrogen at rates of 20, 40 60 and 80KgN/feddan, divided into two equal portions . The first one were added after thinning and the second one were added after one month later.

The experimental design of present experiment was factorial and planned in a complete randomized block design with three replications. The fruits yield (umbels number per plant, fruits weight(g/plant) and fruits yield(Kg/feddan) were recorded. The data of all measurements were statistically analyzed and the different 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).

Flavonoids and carbohydrates

Total flavonoids content was determined calorimetrically according to the method of Mabry *et al.,* (1970), while the percentage of total carbohydrates was carried out using the method described by Dubois *et al.,* (1956).

Essential oil extraction

The extraction of essential oil was carried out at the laboratory of Medicinal and Aromatic plants research (NRC), Dokki, Cairo, to extract and quantify the volatile oil, a weight of 100gm of air dried fruits of each treatment during the fruiting stage in both seasons was separately subjected to hydro distillation for 3 hours using a modified Clevenger apparatus according to Guenther (1961). The percentage of essential oils was calculated, as will as the essential oil per plant and yield/Fed.

GLC analysis

Gas liquid chromatographic (GLC) was done out at the Central laboratory of NRC. of the oil samples were carried out in the second season using Hewlett Packard gas chromatograph apparatus type of (HP) 6890 Series Hewlett Packard, Column HP(Carbwax 20 M), 25 ml length X 0.32mm I.D and film thickness of 0.3Mm, sample size (1µl), oven temperature was 60-190 ^{0}C, program (60^{0}C/ 2min, 8^{0}C/min, 190^{0}C/25min.), injection port temperature was 240^{0}C, Nitrogen was the carrier gas, detector temperature (FID) was280^{0}C and flow rate was N_2 30mL/min.

Results

The finding are displayed in Table (1), comparison of the means for various treatments suggests that bio-fertilizers, nitrogen and their interaction cleared significant differences in the umbels number per plant, and the highest number were observed with 60Kg N/fed. as alone or combined with bio-fertilizer (34.80) and (34.70) respectively in the first season, (33.26) and (32.46) in the second season. The same previously treatments showed the same promotion results on fruits weight (g/plant) in the two seasons, but the bio-fertilizers as alone gave a non-significant increase in comparison with untreated plants. Fruits yield (Kg/feddan) cleared significant increments with all treatments of nitrogen fertilizer as alone and combined with bio-fertilizer, meanwhile bio-fertilizers alone caused non-significant increases of flowering stages through the first and second seasons.

Data in Table (2) revealed that all used treatments caused a significantly increments of total flavonoids content, and the highest increments were (0.77%) and (0.70%) in the first and second seasons respectively, recorded with applied the combined of 60N Kg/fed. and bio-fertilizers.

Total carbohydrate content gave a significantly increases with various Nitrogen fertilizers treatments as alone, while the other fertilizer treatments caused a non-significant increases through first and second seasons, and the maximum increases were observed with interaction treatment of bio-fertilizers and 40NKg/ feddan. Which recorded (33.16 and 33.61% at first and second season.

Concerning with Table (3), noticed that essential oil percentages at first season were significantly increased with both of bio-fertilizers and nitrogen fertilizers, but the interaction treatments resulted a non-significant increments. The highest accumulation was observed with treatment of bio-fertilizer that produced (3.40%) and (3.15%) in the first and second seasons respectively. On the other hand the increments of essential

oil% the second season were significant with nitrogen fertilizers as alone. Oil yield (ml/plant) revealed a significant increases with all used treatments in the first season, and the highest (1.1165ml/plant). was noticed with 60Nkg/fed. In second season oil yield accumulation were showed also with60Kg N/fed. which recorded1.0193 ml/plant, meanwhile the bio-fertilizer showed a non-significant increment. The oil yield (Litre/fed.) gave the same trend of oil yield (ml/plant), and the greatest contents were observed with 60NKg/fed. as alone in both two seasons.

Concerning to essential oil constituents, Table (4), it could noticed that treating the plants with bio-fertilizers, 40 Kg N/fed. and 80 Kg N/fed. + bio-fertilizers increased limonene content compared to the other treatments .At the main times fertilizing the plants with 20 Kg N/fed. alone or plus bio-fertilizers increased carvone content compared to the other treatments. Moreover all used treatments except those of bio-fertilizers and 40 Kg N/fed. caused increasing in dillapiole content.

Table 1: Effect of bio-fertilizers, nitrogen and their interaction treatments on the yield of dill plants during the two seasons (2000/2001 and 2001/2002).

Bio-fertilizers	First Season								
	Umbels number per plant			Fruits weight (g/plant)			Fruits yield (kg/feddan)		
Nitrogen fertilizer (kg N/fed.)	Without bio-fert.	Bio-fert.	Mean	Without bio-fert.	Bio-fert.	Mean	Without bio-fert.	Bio-fert.	Mean
0	14.00	20.26	17.13	12.72	20.35	16.53	848.43	1356.64	1102.53
20	25.40	28.30	26.85	27.00	31.96	29.48	1799.97	2131.08	1965.52
40	30.30	30.83	30.56	31.33	34.40	32.86	2088.86	2293.30	2191.08
60	34.80	34.60	34.70	38.33	28.66	33.49	2555.52	1911.08	2233.30
80	29.26	26.23	27.74	26.68	23.00	24.84	1778.86	1533.31	1656.08
Mean	26.75	28.04		27.21	27.67		1814.32	1845.08	
L.S.D. at 5%	Bio-fertilizers = 0.89 Nitrogen = 1.41 Interaction =2.00			Bio-fertilizers = N.S Nitrogen = 1.74 Interaction = 2.47			Bio-fertilizers = N.S Nitrogen = 109.02 Interaction =154.18		
	Second Season								
0	13.33	21.53	17.43	13.54	19.61	16.57	902.87	1307.76	1105.31
20	27.86	31.00	29.43	28.78	32.49	30.63	1919.08	2166.19	2042.63
40	30.93	31.26	31.09	30.87	34.80	32.83	2058.41	2319.97	2189.19
60	33.26	31.66	32.46	37.06	29.03	33.04	2470.63	1935.75	2203.19
80	30.90	28.16	29.53	28.81	24.53	26.67	1920.86	1635.53	1778.19
Mean	27.25	28.72		27.81	28.09		1854.37	1873.04	
L.S.D. at 5%	Bio-fertilizers = 1.11 Nitrogen = 1.76 Interaction =2.49			Bio-fertilizers = N.S Nitrogen = 4.74 Interaction =6.71			Bio-fertilizers = N.S Nitrogen = 120.63 Interaction =170.60		

Table 2: Effect of biofertilizers, nitrogen fertilizer and their interaction treatments on the total flavonoids and carbohydrate contents (%) in the fruits of dill plants during the two seasons (2000/2001` and 2001/2002).

Bio-fertilizers	First Season					
Nitrogen fertilizer (kg N/fed.)	Total flavonoids percentage			Total carbohydrate percentage		
	Without bio-fert.	Bio-fert.	Mean	Without bio-fert.	Bio-fert.	Mean
0	0.4506	0.4690	0.4598	28.63	29.90	29.26
20	0.4778	0.5723	0.5250	30.10	31.05	30.57
40	0.4888	0.4953	0.4920	30.76	33.16	31.96
60	0.5100	0.7711	0.6405	30.88	30.37	30.62
80	0.5266	0.4928	0.5097	29.20	28.97	29.08
Mean	0.4907	0.5601		29.91	30.69	
L.S.D. at 5%	Bio-fertilizers = 0.032 Nitrogen = 0.0507 Interaction =0.0717			Bio-fertilizers = N.S Nitrogen = 1.86 Interaction = N.S		
	Second Season					
0	0.4509	0.4669	0.4589	30.05	30.85	30.45
20	0.4741	0.5600	0.5170	30.14	33.43	31.78
40	0.4881	0.5993	0.5437	31.57	33.61	32.59
60	0.5128	0.7000	0.6064	31.61	30.93	31.27
80	0.5236	0.4853	0.5044	28.24	28.14	28.19
Mean	0.4899	0.5623		30.32	31.39	
L.S.D. at 5%	Bio-fertilizers = 0.0130 Nitrogen = 0.0205 Interaction =0.0291			Bio-fertilizers = N.S Nitrogen = 1.93 Interaction = N.S		

Table 3: Effect of bio-fertilizers, nitrogen fertilizer and their interaction treatments on the essential oil yield of dill Plant fruits during the two seasons (2000/2001 and 2001/2002).

Bio-fertilizers	First Season								
	Oil Yield (%)			Oil Yield (ml/plant)			Oil Yield (Litre/feddan)		
Nitrogen fertilizer (kg N/fed.)	Without bio-fert.	Bio-fert.	Mean	Without bio-fert.	Bio-fert.	Mean	Without bio-fert.	Bio-fert.	Mean
0	2.3660	2.7160	2.5410	0.3018	0.5527	0.4272	6.0360	11.0540	9.4540
20	2.7660	2.8160	2.7910	0.7448	0.9004	0.8226	14.8960	18.0080	16.4520
40	2.9830	3.1500	3.0665	0.9349	1.0840	1.0094	18.6980	21.6800	20.1880
60	3.2830	3.4000	3.3415	1.2585	09746	1.1165	25.1700	19.4920	22.3300
80	2.8500	2.8330	2.8415	0.7591	0.6513	0.7052	15.1182	13.0260	14.1040
Mean	2.8496	2.9830		0.7998	0.8326		15.9960	19.6520	
L.S.D. at 5%	Bio-fertilizers =0.0745 Nitrogen =0.1177 Interaction =N.S			Bio-fertilizers =0.0317 Nitrogen = 0.0502 Interaction = 0.0710			Bio-fertilizers = 0.634 Nitrogen = 1.004 Interaction =1.420		
	Second Season								
0	2.5660	2.6000	2.5830	0.3482	0.5095	0.4288	6.9640	10.1900	8.5760
20	2.7000	2.7000	2.7000	0.7768	0.8776	0.8272	15.5360	17.5520	16.5440
40	2.7500	2.9500	2.8500	0.8492	1.0272	0.9382	16.9840	20.5440	18.7640
60	3.0330	3.1500	3.0195	1.1240	0.9146	1.0193	22.4800	18.2920	20.3860
80	2.8660	2.7500	2.8080	0.8253	0.6744	0.7498	16.5060	13.4880	14.9660
Mean	2.7830	2.8300		0.7847	0.8006		15.6940	16.0120	
L.S.D. at 5%	Bio-fertilizers = N.S Nitrogen = 0.1242 Interaction =N.S			Bio-fertilizers = N.S Nitrogen = 0.0609 Interaction =0.0862			Bio-fertilizers = N.S Nitrogen = 1.218 Interaction =1.724		

Table 4: Effect of bio-fertilizers, nitrogen fertilizer and their interaction treatments on constituents of dill volatile oil " fruits" (210 days after sowing) in the second season (2001/2002).

Treatments Identified compounds	Control	20kg N/fed.	40kg N/fed.	60kg N/fed.	80kg N/fed.	Bio-fert.	20kg N/fed. +Bio-fert.	80kg N/fed. +Bio-fert.
Limonene	14.6156	10.8468	17.5903	13.4504	trace	20.0850	12.3035	16.6281
P-cymene	0.3043	0.2136	0.2840	0.2214	trace	0.2560	0.1091	0.1332
Linalool	0.0173	0.0145	0.0419	0.0347	trace	0.0377	0.0111	0.1011
Dillether	1.6490	1.6042	2.0832	2.1820	1.6247	1.7832	1.1172	1.1191
Dihydrocarvone	0.0740	0.1218	0.0836	0.1194	0.1686	0.0146	0.2113	0.1282
Sabinol	0.0301	0.0367	0.0288	trace	trace	trace	0.0121	0.0894
Carvone	62.4883	63.1138	61.0239	59.0117	53.1757	57.9056	65.3751	56.3311
Piperitone	0.2106	0.2551	0.2159	0.2280	trace	0.1945	0.2213	0.2311
Carveol	0.0072	0.0249	trace	trace	trace	trace	0.0174	0.0131
Nerolidol	0.0165	0.0334	trace	0.0141	trace	trace	0.0351	0.0271
Eugenol	0.0053	0.0099	trace	trace	0.0315	trace	0.0118	0.0311
Thymol	0.0144	0.0090	0.0254	0.0396	0.0504	0.0237	0.1211	0.0441
Carvacrol	0.0299	0.0317	0.0248	0.0453	0.0712	0.0302	0.0331	0.0773
Myristicin	0.0555	0.0921	0.0541	0.0592	0.1131	0.1002	0.0858	0.0918
Dillapiole	19.5113	22.2993	17.7159	23.8016	41.5406	18.7487	20.3162	22.1739
Total	99.0293	98.7068	99.1718	99.2074	96.7758	99.1794	99.9812	97.2197

Discussion

Fatma *et al*. (2006) concluded favorable results for the effect of Azospirillum and Azotobacter on *Majorana hortensis*, Krishna *et al*. (2008) reported improvement in germination indexes such as percentage and speed of germination, viability and also the lenth of roots and steems of *Ocimum sanctum* and *Withania somniferum* when treated with Azospirillum and Azobacter bio-fertilizers and a combination of these fertilizers.

Many research studies have mentioned that microorganisms caused positive effect for improving the growth and performance of medicinal plants, Azospirillum improved root growth through generation of stimulating compounds that resulted in increment of water and nutrients uptake and soi on the general performance of the plant (Tilak *et al.,* 2005). Subba Rao *et al*. (1979) cited that the most important growth stimulating bacteria are Azospirillum and Azobacter, they stimulated biological fixation of nitrogen and solubilizing the soil phosphate, considerably affect plant growth regulators especially auxin, gibberellins and cytokinin and hence improved the plant performance. In addition Azotobacter is able to produce antifungal compounds that fight plant diseases and improve viability and germination of the plant, and as a result it improve overall plant growth (Chen, 2006).

The increments of dill fruit yield could be repacked to the effect of chemical N or bio-fertilizer treatments in promotion the vegetative growth, which was reflected in increasing the fruits yield (Shaalan, 2005, Mahfouz and Shraf-Eldin, 2007 and Hassan, 2009). The present results are in agreement with this obtained by Harridy and Amara, (1998) and Shaalan *et al*. (2001). Finding results are in harmony with those of Sanches Govin *et al*. (2005) on *Calendula officinallis L.* and *Matricaria recutita L.* Mona (2006), on *Plantago afra L*. El-Shafie *et al*. (2010) on khella plants, Hellal *et al*. (2011) on dill plants indicated that applying bio-fertilizers treatments alone or combined with chemical N fertilizer increased yield and carbohydrates and flavonoids content.

Ahmed *et al.,* (2013) on Guar plants cited that nitrogen or bio-fertilizers and their interactions increased significantly carbohydrates and flavonoids content, those results are in the same trend of present results. Essential oil was enhancing as a result of using N fertilizers or bio-fertilizers and their interaction, presented results are in agreement with those obtained by Randhawa *et al*. (1996). Gomaa and Abo-Aly (2001). Kandel *et al*. (2004). Mahfouz and Sharaf-Eldin (2007). Hellal *et al*. (2011) on dill plants essential oil, and Ahmed *et al*. (2013) on Guar essential oil.

Mahran *et al*. (1992) mentioned that seventeen components were identified from dill fruits, grown in Egypt, limonene (30 3%), dillapiole (26.8%), carvone (22.0%) and piperitone (8.2%). As well as appeared that limonene, dillapiole and carvon were the major components, their amount reached to 79%. Present results are in harmony with those obtained by Wander and Bouwmeester (1998) on *Anethum graveolens*, found that seed carvone yield was largest when N was applied at 30-60 Kg/ha. Khater (2001) on *Carum carvi* found that limonene and carvone as main components in the essential oil was increased by the treatments of bio-fertilizers. Hellal *et al*. (2011) indicated that the main components of volatile oil were limonene, carvone and apiol, the volatile oil composition was affected as a result of applying of N fertilizers or bio-fertilizers and their interaction, limonene and carvone contents were increased with application of N fertilizers as alone, meanwhile apiol content was increased according to the application of N and Bio-fertilizers.

Conclusion

From the results of present study, it could be concluded that substituting of mineral nitrogen fertilizer by bio-fertilizer is recommended for increasing the yield of fruits dill plant, and so on the contents of carbohydrates, flavonoids and essential oil yield. According to reported results of conducted study show the possibility of save the quantity of N chemical fertilizers and deal to more clean products.

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