

Growth and Chemical Composition of Dill Affected by Nitrogen and Bio-Fertilizers

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Abstract

A field experiment was carried out during two successive seasons to investigate the effect of nitrogen and bio-fertilization on growth, yield and chemical composition of *Anethum graveolens* L. plants. Plant height (cm), number of branches per plant, fresh and dry weights of herb g/plant and chlorophyll, carotenoids, flavonoids, carbohydrates and nitrogen contents of dill herb were studied. The effect of Nitrogen or bio-fertilizers was significantly increased plant height, number of branches, herb fresh and dry weights. The highest values of branches number, fresh and dry weights were recorded by using 60 kg N/feddan. In the same time, the combined treatment between bio-fertilizer and 80 kg N/feddan gave the largest values of plant height. Using nitrogen or bio-fertilizers increased chlorophyll a, b and total carotenoids compared unfertilized plant. The highest values of chlorophyll a, b; total carotenoids and total flavonoids were resulted by plants fertilized with 60 kg N/fed., with or without bio-fertilizer. The treatments of 40 kg N/fed., or 60 kg N/fed., combined with bio-fertilizer gave the highest total flavonoids content. Using bio-fertilizer or nitrogen fertilizer up to 60 kg N/fed., in fertilizing dill plants increased total carbohydrate content. The results of the used 60 kg N/fed., alone or 40 kg N/fed., combined with bio-fertilizer were the best affected. Using bio-fertilizer or nitrogen fertilizer increased the percentage values of N compared to control ones. Moreover, the combination treatments between 40 or 60 kg N/fed., with bio-fertilizer or 60 kg N/fed., alone more effective in increasing N content.

Keywords

Anethum graveolens L., Bio-Fertilizer, Growth Characters, Chlorophyll, Carotenoids, Chemical Composition, Nitrogen Fertilizer

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1. Introduction

The *Anethum graveolens* L. (Dill plant) species is cultivated extensively in many countries of Europe, Asia and USA, for its use as an aromatic herb and medical applications (Singh et al., 2005). Dill (*Anethum graveolens* L.) is an annual and sometimes biennial herb of the family Apiaceae, which is native to south-west Asia or south-east Europe, and has been cultivated since ancient times (Bailer et al., 2001). It is used as a vegetable, a carminative, an aromatic and an

antispasmodic (Hornok, 1992; Sharma, 2004), and as an inhibitor of sprouting in stored potatoes (Score et al., 1997). Herbs contain a wide variety of antioxidant phytochemicals or bioactive molecules that can neutralize the free radicals and thus retard the progress of many chronic diseases associated with oxidative stress and reactive oxygen species (Sun et al., 2002; Liu, 2003). *A. graveolens* has been reported to contain flavonoids, phenolic and essential oil (Delaquis et al., 2002). The increased consumption of vegetables and herbs containing high levels of phytochemicals has been

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recommended to prevent or reduce oxidative stress in the human body (Sun *et al.*, 2002; Liu, 2003; Chu *et al.*, 2002). Endogenous antioxidant defense mechanisms may be insufficient and hence dietary intake of antioxidant compounds is essential (Yakubu *et al.*, 2013). The intake of natural antioxidants has been associated with reduced risk for cancer, cardiovascular disease, diabetes, and diseases associated with aging (Ani *et al.*, 2006). The liver is associated with many important life functions; it has great capacity to detoxicate toxic substances and synthesize useful principles (Si-Tayeb *et al.*, 2010). The extract of *A. graveolens* could protect the liver against high-fat-diet-induced oxidative damage in rats (Bahramikia *et al.*, 2009). The aim of this study was to investigate the effect of bio-fertilizers, nitrogen fertilizer on growth, yield and chemical composition of *Anethum graveolens* L. plants.

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 Research Dep., Ministry of Agriculture, Egypt. The physical and chemical characteristics of the soil were presented in Table (1). The analysis of the soil was conducted using the methods described by Jackson (1967).

Table 1. The physical and chemical properties of the experimental soil.

Physical and chemical properties	2000/2001	2001/2002
Sand (%)	48.8	50.8
Silt (%)	28	26
Clay (%)	23.2	23.2
Soil texture	Sandy loam	Sandy loam
pH	8.08	8.22
E.C (m. mhos/cm)	0.68	0.89
Organic matter (%)	2.07	2.18
N (%)	0.11	0.12

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*). Bio-fertilizers produced by General Organization for Agriculture Equalization Fund (G.O.A.E.F.). Urea (46.5 % N) was used as sources of N. The soil was prepared and divided into plots 3x3.5m with five rows. The dill seeds were sown in 15th October in the two seasons. The distance between each row was 60cm apart and 20cm between the hills. The seedlings were thinned one month after sowing to leave two plants per hill.

The used bio-fertilizers (1kg Rhizobacterin + 1kg Microbein/feddan) added as inoculated the seeds and sowing. 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 of the three experiments was factorial and planned in a complete randomized block design having three replications and each replicate contained one plot, the plot contained 175 plants.

2.2. Data Recorded

Data for growth characters and chemical constituents were estimated for the all treatments during vegetative stage (90 days after sowing). as follows: plant height (cm), number of branches per plant, fresh and dry weights of herb g/plant.

2.3. Chemical Composition

Chlorophyll (a), (b) and total carotenoids content (mg/100 mg fresh leaves), Total flavonoids content in dried herb, Total carbohydrates content in dried herb and Nitrogen content in dried herb.

2.4. Determination of Chemical Constituents

2.4.1. Determination of Plant Pigments

Chlorophyll (a), chlorophyll (b) and carotenoids as (mg/100mg) of the fresh leaves were extracted from representative samples of the fresh leaves using acetone 85%. The concentrations of chlorophyll a, b and carotenoids were determined by using spectrophotometer and calculated by using Wettstein formula (Wettstein, 1957).

2.4.2. Determination of Total Flavonoids (%)

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

2.4.3. Determination of Total Carbohydrate (%)

The percentage of total carbohydrates was determined using the method described by Dubois *et al.*, (1956).

2.4.4. Determination of Nitrogen (%)

It was determined colorimetrically according to the method described by Naguib (1969).

2.5. 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. Plant Height

The data in Table (2) showed that all treatments led to increase in plant height compared to the control in both seasons. Difference between with and without bio-fertilizer treatments was significant in the two seasons. Also, the results indicated that the different nitrogen treatment had a significant effect on plant height stages in the two seasons. In the first season, the tallest plants (39.25 cm) were recorded by inoculation combined with 80kg N per feddan. In the second season, the same trend was observed as in the first one. Whereas, the tallest plants were (38.39 cm) resulted from the treatment of 80kg N per feddan and bio-fertilizer. Application of bio-fertilizer and nitrogen treatments to dill plants increased plant height. This may be due to the increase of N in the root zone and the synergistic effect of these microorganisms on the physiological and metabolic activities of the plant. This enhancing effect may induce exudate of some hormonal substances like cytokinins and auxins, which encourage plant height. The nutrients available in the soil increased with increasing nitrogen application and bio-fertilizers. This may be attributed due to more atmospheric

nitrogen fixed in the soil, which was probable due to mobilization of bacteria, providing favorable conditions (Rajput and Singh, 1996). The increase in nitrogen uptake enhanced physiological activities of plants and thereby increased the growth and yield (Rajput et al., 1995). The ability of Azotobacter to produce growth substances and antifungal substances in addition to fixed nitrogen made available to plants was probably the reason of higher yields (Mishutin and Shilnikova, 1971). Similarly, Azospirillum is reported to produce indole acetic acid, gibberellins and cytokinins like substances along with nitrogen fixation (Tein et al., 1979). Nitrogen stimulates the meristematic activity for producing more tissues and organs. Abd-El-Fattah and Sorial (2000) ensured that increasing nitrogen levels increased of cytokinins and gibberellins which enhance cell division and cell enlargement and thus increased vegetative growth. Meanwhile, (Subb-Rao, 1984) who stated that, the favourable effect of bio-fertilizers on growth parameters might be ascribed to its important role in fixing atmospheric N as well as increasing the secretion of natural hormones namely IAA, GA3 and cytokinins, antibiotics and possibly raising the availability of various nutrients. These findings are in agreement with those of (Hussein, 1995; Rupam et al., 2001; Rashed, 2002; Kandeel et al., 2004, a and b; Youssef et al., 2004).

Table 2. Effect of bio-fertilizers, nitrogen fertilizer and their interaction treatments on plant height and number of branches of dill plants during the two seasons (2000/2001 and 2001 / 2002).

Nitrogen fertilizer (kg N/fed.)	Bio-fertilizers		First Season			
	Plant height(cm)		Number of branches / plant			
	Without bio-fert.	Bio-fert.	Mean	Without bio-fert.	Bio-fert.	Mean
0	24.92	28.41	26.66	2.60	3.33	2.96
20	29.41	29.98	29.69	2.73	3.70	3.21
40	32.34	34.08	33.21	3.66	4.20	3.93
60	35.99	37.83	36.91	4.30	4.40	4.35
80	38.02	39.25	38.63	4.30	4.53	4.41
Mean	32.13	33.91		3.51	4.03	
L.S.D. at 5%	Bio-fertilizers = 0.51 Nitrogen = 0.81 Interaction = 1.14			Bio-fertilizers = 0.19 Nitrogen = 0.31 Interaction = N.S		
				Second Season		
0	22.37	26.09	24.23	2.56	3.36	2.96
20	26.47	26.36	26.41	3.43	3.66	3.54
40	30.31	33.40	31.85	3.76	4.13	3.94
60	35.23	36.17	35.70	4.26	4.26	4.26
80	37.72	38.39	38.05	4.33	4.50	4.41
Mean	30.42	32.08		3.66	3.98	
L.S.D. at 5%	Bio-fertilizers = 0.48 Nitrogen = 0.77 Interaction = 1.08			Bio-fertilizers = 0.08 Nitrogen = 0.13 Interaction = 0.19		

3.2. Number of Branches

It was clear from data in Table (2) that all treatments

increased the number of branches per plant in both seasons compared to the untreated plants. In addition, the highest branch numbers (4.53 and 4.50 per plant) was obtained by

inoculation combined with 80kg N per feddan in both seasons, respectively. Regarding the effect of bio-fertilizer treatments on number of branches per plant, data in Table (2) clearly show that in both seasons, bio-fertilizer significantly increased the number of branches per plant compared to the treatments without bio-fertilizer. Increasing nitrogen doses up to 80kg N/fed., increased number of branches. These increments were significant during both stages in the two seasons comparing to unfertilized plants. The interaction between using bio-fertilizer and nitrogen fertilizer treatments on branches number were significant in the two seasons except those of in the first season. Thus, it can be concluded that treating dill plants with nitrogen and bio-fertilizer increased the formation of branches. The increase in number of branches may be due to the increase in nitrogen content in the soil and biological fixation nitrogen as well as growth promoting substances such as indole acetic acid, cytokinins and gibberellins produced by the organisms used. The increase in the level of nitrogen fertilizer and biological fixation nitrogen as well as growth promoting substances were responsible for increased number of branches, causing higher photosynthesis and assimilation rates, metabolic activity and cell division which were responsible for increase in the growth characters, yield attributes and yield of dill (Chauhan *et al.*, 1996). Similar results were obtained by (El-Kashlan, 2001; Rupam *et al.*, 2001; Al-Qadasi, 2004; Kandeel *et al.*, 2004, a and b).

3.3. Plant Fresh Weight

From data obtained in Table (3) it may be remarked that all treatments of bio-fertilizer and nitrogen rates application alone or together tended an increases in the fresh weight of herb in both successive seasons when compared with that of untreated plants. In general, this parameter was significantly increased by increasing nitrogen rates when compared with that of unfertilized plants in both seasons. Using bio-fertilizer significantly increased plant fresh weight in both seasons. The interaction between bio-fertilizer and nitrogen fertilizer had significant on fresh weight of herb in the second season. Parallely, the heaviest fresh weights of dill (g/plant) during vegetative and flowering stages in both seasons were obtained by the treatment of nitrogen at rate of 60kg N/fed., alone or by inoculation combined with 60 kg N/fed., that evaluated by (49.48 and 52.32 g/plant) of herb in both seasons, respectively. In conclusion the increment in plant fresh weight may be attributed to a greater proliferation of root biomass resulting in the higher absorption of nutrients and water from the soil leading to production of higher vegetative biomass (Taylor and Klepper, 1978; Hamblin, 1985). The increase in plant fresh weight may be due to the increase of N in the root zone as a result of nitrogen application and fixed N by bacteria. Also, the solubilization

of mineral nutrients, synthesis of vitamins, amino acids and gibberellins, which stimulate growth and yield (Sprenat, 1990). The stimulation effects of applying nitrogen on vegetative growth characters may be due attributed to the well known functions of nitrogen in plant life, being a part of protein, it is an important constituent of protoplasm. Also, enzymes, the biological catalytic agents, which speed up life processes, have N as their major constituents. Moreover, nitrogen involves in many organic compounds of plant system. A sufficient supply of various nitrogenous compounds is therefore, required in each plant cell for its proper functioning (Mengel and Kirkby, 1987). Generally, the enhancing effect of N-fertilization and bio-fertilizers on plant growth may be due to the positive effects of N-element on activation of photosynthesis and metabolic processes of organic compounds in plants which in turn, encourage the plant vegetative growth (Gardener *et al.*, 1985). The increase in plant growth ascribed to contributing some hormone substances, such as gibberellins, auxins and cytokinins (Tien *et al.*, 1979, Bouton *et al.*, 1985 and Cacciari *et al.*, 1989). These phytohormones may stimulate the cell elongation and division and hence plant growth (Paleg, 1985). The obtained results are in line with those of (Garrabrants and Craker, 1987; Halva and Puukka, 1987; Halva *et al.*, 1987; Abou-Hadid *et al.*, 1993; Rashed, 2002; Hamed, 2004; Singh *et al.*, 2005).

3.4. Plant Dry Weight

Plant dry weight (g/plant) of *Anethum graveolens* as affected by different nitrogen rates and bio-fertilizer are presented in Table (3). It is clear that all used treatments of inoculation and nitrogen rates application alone or together caused increases in herb in both seasons as compared with that of untreated ones. The gradual increases in dry weights were resulted from treatments of bio-fertilization interacted with increasing nitrogen rates from 20 to 60 kg N/fed. singly or collectively, then suddenly reduced by nitrogen level of 80kg N/fed., with or without inoculation. In this regard, the largest values of dry weights were obtained by the nitrogen rate of 60 kg/fed., alone or combined with bio-fertilization which recorded by (8.22 and 8.37 g/plant) in both seasons, respectively. Regarding the effect of nitrogen application treatments on plant dry weight (g/plant), data in Table (3) clearly show that in both seasons significantly differences between all treatments and the control. Using bio-fertilizer significantly increased plant dry weight in the first season. The results of the interaction between using bio-fertilizer and nitrogen fertilizer treatments on dry weights were significant in the two seasons. The superiority of bio-fertilizers and nitrogen application alone or together for stimulating plant dry weight exhibited the same trend owing to The favorable effect of nitrogen fertilizer and bio-fertilizers on plant growth

and yield attributes might be due to the improved nutrition and production of growth-promoting substances by micro-organisms (Dhillon et al., 1980). The superiority of the application of nitrogen + bio-fertilizers than the other treatments may be due to the increase in available amounts of nutrients (Salem, 1986), also, microbiological processes can change unavailable forms of nutrients into available ones that can be easily assimilated by plants (Subb-Rao, 1981; Alaa El-Din, 1982). In other words, the positive interactions between the applied N-fertilizer levels and biofertilizers on plant

vegetative growth may be due to the promoting effects of both N-element and biofertilizers together on the established plant roots and nutrient uptake (Wange, 1995). Increasing N fertilizer increase the population of bacteria and this in turn increase nitrogen fixation and release of phytohormones and trace elements for that interaction increase plant growth. This is confirmed by similar findings of (Shalaby et al.,1997; EL-Gengaihi et al., 2000; Ibrahim, 2000; Rashed, 2002; El-Seifi, 2004; Kandeel et al., 2004, a and b; Youssef et al., 2004; Garcia-Gonzalez et al., 2005; Lakhana and Gupta, 2005).

Table 3. Effect of bio-fertilizers, nitrogen fertilizer and their interaction treatments on the fresh and dry weights of dill plants during the two seasons (2000/2001 and 2001 / 2002).

Bio-fertilizers Nitrogen fertilizer (kg N/fed.)	First Season					
	fresh weight / plant (g)			dry weight / plant (g)		
	Without bio-fert.	Bio-fert.	Mean	Without bio-fert.	Bio-fert.	Mean
0	18.42	21.95	20.18	4.11	4.22	4.16
20	24.70	29.62	27.16	4.95	6.32	5.63
40	35.71	42.48	39.09	4.79	7.08	6.43
60	49.41	49.48	49.44	8.22	7.83	8.02
80	46.61	49.13	47.87	7.55	7.44	7.49
Mean	34.97	38.53		6.12	6.57	
L.S.D. at 5%	Bio-fertilizers = 2.06 Nitrogen = 3.26 Interaction = N.S			Bio-fertilizers = 0.35 Nitrogen = 0.56 Interaction = 0.80		
	Second Season					
0	17.11	23.13	20.12	3.93	4.28	4.10
20	25.25	31.25	28.25	4.54	6.15	5.34
40	34.47	40.99	37.73	5.79	6.90	6.34
60	52.32	49.69	51.00	8.37	7.85	8.11
80	46.29	45.87	46.08	7.81	7.38	7.59
Mean	35.08	38.18		6.08	6.51	
L.S.D. at 5%	Bio-fertilizers = 1.71 Nitrogen = 2.71 Interaction = 3.83			Bio-fertilizers = N.S Nitrogen = 0.69 Interaction = 0.98		

Table 4. Effect of bio-fertilizers, nitrogen fertilizer and their interaction treatments on the chlorophyll (a) and (b) contents (mg/100mg fresh leaves) of dill plants during the two seasons (2000/2001 and 2001/2002).

Bio-fertilizers Nitrogen fertilizer (kg N/fed.)	First Season					
	chlorophyll (a) content (mg/100mg fresh leaves)			chlorophyll (b) content (mg/100mg fresh leaves)		
	Without bio-fert.	Bio-fert.	Mean	Without bio-fert.	Bio-fert.	Mean
0	0.0663	0.0701	0.0682	0.0466	0.0686	0.0576
20	0.0709	0.0723	0.0716	0.0529	0.0640	0.0584
40	0.0752	0.0900	0.0826	0.0601	0.0727	0.0664
60	0.0869	0.0929	0.0899	0.0780	0.0789	0.0784
80	0.0849	0.0856	0.0852	0.0751	0.0896	0.0823
Mean	0.0768	0.0821		0.0625	0.0747	
L.S.D. at 5%	Bio-fertilizers = N.S Nitrogen = 0.0098 Interaction = N.S			Bio-fertilizers = 0.0069 Nitrogen = 0.0110 Interaction = N.S		
	Second Season					
0	0.0681	0.0733	0.0707	0.0466	0.0671	0.0568
20	0.0705	0.0749	0.0727	0.0504	0.0728	0.0616
40	0.0759	0.0817	0.0788	0.0735	0.0635	0.0685
60	0.0943	0.1003	0.0973	0.0698	0.0924	0.0811
80	0.0749	0.0784	0.0766	0.0574	0.0823	0.0698
Mean	0.0767	0.0817		0.0595	0.0756	
L.S.D. at 5%	Bio-fertilizers = N.S Nitrogen = 0.0089 Interaction = N.S			Bio-fertilizers = 0.0102 Nitrogen = N.S Interaction = N.S		

3.5. Chlorophyll (a) Content

Data on chlorophyll (a) content of dill leaves are presented in Table (4). The data indicated that the treatments of inoculation and nitrogen application used singly or collectively increased the chlorophyll (a) content during vegetative stage in both seasons comparison to control plants. The highest content of chlorophyll (a) was found by inoculation combined with treatment of 60 kg N/fed., giving (0.0929 and 0.1003 mg/100mg leaf fresh weight) in both seasons, respectively. Regarding the effect of nitrogen fertilizer on chlorophyll (a) the data in Table (4) showed that, application of nitrogen at 60 Kg N/fed. significantly increased this character in both seasons. The increment in plant growth parameters reflect the vital importance role of nitrogen as a plant nutrient, since it is an integral part of the chlorophyll molecule, nucleic acids, proteins, and other important substances (Delvin and Witham, 1983). The increase in chlorophyll (a) content may be due to available nitrogen but also the increase in trace elements in the soil caused by the organic acids produced by microorganisms leading to a decrease in the pH of the soil (Subb-Rao, 1981). Similar results were obtained by (Abd-El-Salam, 1994; Jacoub, 1995; Gomaa and Abou-Aly, 2001; Rashed, 2002; Mahfouz, 2003; Al-Qadasi, 2004).

3.6. Chlorophyll (b) Content

As shown in Table (4) it is clear that chlorophyll (b) content (mg/100 mg leaf fresh weight) in the two seasons was increased significantly by the treatment of bio-fertilization. Also, all treatments of nitrogen fertilizer alone or combined with inoculation increased chlorophyll (b) content in the two seasons when comparison to control plants. The highest content of chlorophyll (b) was resulted by 80 Kg N/fed., plus bio-fertilizer (0.0896 mg/100 mg leaf fresh weight) in the first season, and by 60 kg N/fed., + inoculation (0.0924 mg/100 mg leaf fresh weight) in the second season. In this regard, the treatment of 20 kg N/fed. resulted in the highest chlorophyll (b) content (0.4970 and 0.5394 mg/100mg leaf fresh weight) in the two seasons, respectively, followed by the treatments of 60 kg N/fed. alone or with inoculation in both seasons, respectively. While, the least content of chlorophyll (b) (0.0466, 0.0466 and 0.3943, 0.3891 mg/100mg leaf fresh weight) in both seasons, respectively. Generally, the highest content of chlorophyll (b) was produced by nitrogen fertilization with or without bio-fertilizer. These results are in agreement with those of (Abd-El-Salam, 1994; Jacoub, 1995; Gomaa and Abou-Aly, 2001; Rashed, 2002; Mahfouz, 2003; Al-Qadasi, 2004).

3.7. Total Carotenoids Content

Data in Table (5) showed that, in the two seasons, plants supplied with nitrogen fertilization treatments had considerably higher carotenoids contents than those of control plants (with least values of 0.0717; 0.0756 mg/100 mg leaf fresh weight) in the two seasons, respectively. It is also clear from the data in Table (4) that in the two seasons, application of nitrogen at 60 kg N/fed. significantly increased carotenoids content, except those of in the first season comparison to control. Moreover, the data in Table (5) showed that, in most cases, the favourable effect of inoculation combined with nitrogen fertilization on the synthesis and accumulation of carotenoids in dill leaves was more pronounced when nitrogen fertilization was combined with bio-fertilizer i.e plants supplied with nitrogen fertilizer + bio-fertilization had a higher carotenoids content than those supplied with the same rate of nitrogen fertilizer, but without bio-fertilization. Among the different fertilization treatments, 60 kg N/fed., plus bio-fertilization was the most effective one for promoting the synthesis and accumulation of carotenoids in dill leaves. This treatment (60 kg N/fed. + bio-fertilization) gave the highest carotenoids content (0.1143 and 0.1215 mg/100mg leaf fresh weight) in the two seasons, respectively. Generally, the leaves of dill treated with bio-fertilizer contained higher carotenoids than the untreated plants. Similar data was obtained by those of (Abd-El-Salam, 1994; Jacoub, 1995, Al-Qadasi, 2004).

3.8. Total Flavonoids Percentage

Results presented in Table (5) is obviously noticed that total flavonoids percentage in dill plants in both seasons was increased comparing to control ones. 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 two seasons, respectively. While, in the first and second season, the least values were due to untreated plants. Volkhonskaya (1984) on *Bupleurum aureum* 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. Total flavonoid content was highest in the flowers. The distribution of flavonoid was changed in the different organs and growing conditions. Lemberkovics *et al.* (1996) on *Ocimum basilicum* showed that flavonoid glycosides began to appear from the bud development stage and increased during flowering, reaching a peak at the late stage of flowering. These results are in accordance with those of (Letchamo, 1992; Kolar *et al.*,

1998; Tang et al., 2000; Borella et al., 2001; Barbara, 2002; Kovac-Besovic and Duric, 2003).

3.9. Total Carbohydrate Percentage

Regarding the effect of different fertilization, the data in Table (6) showed that bio-fertilizer alone or combined with nitrogen fertilizer up to 40 kg N/fed., led to an increase in the total carbohydrates percentage in the dried herb of dill comparison to control plants, also increasing nitrogen fertilizer up to 60 kg N/fed. led to a steady increase in the total carbohydrates percentage then decreased during the two seasons. As a result, the treatment of 20 kg N/fed., plus bio-

fertilizer gave higher values (27.91 and 27.85%) in both seasons, respectively. The increment in total carbohydrates may be due to the increase of photosynthesis as a result of the increase in photosynthetic pigments content in the leaves. The necessity of N as a plant nutrient is emphasized by the fact that it is a main constituent of many organic compounds in plant (Tyler et al., 1988). These results are similar to those obtained by (Meawad and El-Deeb, 1991; Abd-El-Salam, 1994; Sharangi and Paria, 1995; Hammam, 1996; Khater, 2001; Mahfouz, 2003; Al-Humaid, 2004; Al-Qadasi, 2004; Hamed, 2004; Youssef et al., 2004).

Table 5. Effect of bio-fertilizers, nitrogen fertilizer and their interaction treatments on the total carotenoids and total flavonoids of dill plants during the two seasons (2000/2001 and 2001/2002)

Nitrogen fertilizer (kg N/fed.)	Bio-fertilizers		First Season			
	carotenoids content (mg/100mg fresh leaves)			flavonoids content (%)		
	Without bio-fert.	Bio-fert.	Mean	Without bio-fert.	Bio-fert.	Mean
0	0.0717	0.0866	0.0791	0.4060	0.4130	0.4095
20	0.0827	0.0888	0.0857	0.4162	0.5530	0.4846
40	0.0817	0.0996	0.0906	0.4209	0.5950	0.5079
60	0.1007	0.1143	0.1075	0.4816	0.5051	0.4933
80	0.0951	0.0981	0.0966	0.4414	0.4414	0.4414
Mean	0.0863	0.0974		0.4332	0.5015	
	Bio-fertilizers = 0.0090			Bio-fertilizers = 0.0163		
L.S.D. at 5%	Nitrogen = N.S			Nitrogen = 0.0258		
	Interaction = N.S			Interaction = 0.0366		
	Second Season					
0	0.0756	0.0878	0.0817	0.3999	0.4083	0.4041
20	0.0818	0.0940	0.0879	0.4092	0.5541	0.4816
40	0.0885	0.0919	0.0902	0.4186	0.5891	0.5038
60	0.0919	0.1215	0.1067	0.4781	0.4962	0.4871
80	0.0844	0.0857	0.0850	0.4363	0.4400	0.4381
Mean	0.0844	0.0961		0.4284	0.4975	
	Bio-fertilizers = 0.0076			Bio-fertilizers = 0.0143		
L.S.D. at 5%	Nitrogen = 0.0121			Nitrogen = 0.0227		
	Interaction = N.S			Interaction = 0.0321		

Table 6. Effect of bio-fertilizers, nitrogen fertilizer and their interaction treatments on the total carbohydrate and nitrogen content of dill plants during the two seasons (2000/2001 and 2001/2002).

Nitrogen fertilizer (kg N/fed.)	Bio-fertilizers		First Season			
	total carbohydrate content (%)			nitrogen content (%)		
	Without bio-fert.	Bio-fert.	Mean	Without bio-fert.	Bio-fert.	Mean
0	26.19	26.50	26.34	3.2832	3.3318	3.3075
20	27.64	27.91	27.77	3.3353	3.3918	3.3635
40	27.68	27.55	27.61	3.4642	3.4697	3.4669
60	27.81	24.92	26.36	3.5083	3.5178	3.5130
80	23.30	25.34	24.32	3.4254	3.2446	3.3350
Mean	26.52	26.44		3.4032	3.3911	
	Bio-fertilizers = N.S			Bio-fertilizers = N.S		
L.S.D. at 5%	Nitrogen = 1.29			Nitrogen = N.S		
	Interaction = 1.83			Interaction = N.S		
	Second Season					
0	26.14	26.74	26.44	3.1601	3.2808	3.2204
20	27.23	27.85	27.54	3.3441	3.3555	3.3498
40	27.26	27.42	27.34	3.3888	3.3987	3.3937
60	27.52	24.85	26.18	3.4036	3.5369	3.4702
80	24.63	25.04	24.83	3.1997	3.3320	3.2658
Mean	26.55	26.38		3.2992	3.3807	
	Bio-fertilizers = N.S			Bio-fertilizers = N.S		
L.S.D. at 5%	Nitrogen = 1.22			Nitrogen = 0.1360		
	Interaction = N.S			Interaction = N.S		

3.10. Nitrogen Percentage

From the Table (6) it was noticed that all fertilization treatments caused a positive effect on the nitrogen percentage at both seasons. The largest value of nitrogen percentage was resulted from combined treatment of 60 kg N/fed., with bio-fertilizer (3.5178; 3.5369) in the both seasons, respectively. The least values of nitrogen percentages of in the two seasons were determined in untreated plants. The increment in nitrogen percentage in both seasons may be due to fertilized with nitrogen and fixed nitrogen by fixers used as a source of nitrogen. As well as the increase of root size, which led to augmentations in nitrogen uptake from the soil. The application of nitrogen increased the concentration of N in plant tissue and also the total fresh herbage yield, which ultimately led to the increased uptake of N (Singh *et al.*, 1997). These results are in agreement with those of (Meawad and El-Deeb, 1991; Singh, 1991; Ughreja and Chundawat, 1992; Abd-El-Salam, 1994; Hammam, 1996; Kandeel *et al.*, 2001; Khater, 2001; Nofal *et al.*, 2001; Rashed, 2002; Mahfouz, 2003; Al-Humaid, 2004; Al-Qadasi, 2004; Hamed, 2004; Kandeel *et al.*, 2004, a and b; Youssef *et al.*, 2004).

4. Conclusion

The effect of Nitrogen or bio-fertilizers was significantly increased plant height, number of branches, herb fresh and dry weights. The highest values of branches number, fresh and dry weights were recorded by using 60 kg N/feddan. In the same time, the combined treatment between bio-fertilizer and 80 kg N/feddan gave the largest values of plant height. Using nitrogen or bio-fertilizers increased chlorophyll a, b and total carotenoids compared unfertilized plant. The highest values of chlorophyll a, b; total carotenoids and total flavonoids were resulted by plants fertilized with 60 kg N/fed., with or without bio-fertilizer. The treatments of 40 kg N/fed., or 60 kg N/fed., combined with bio-fertilizer gave the highest total flavonoids content. Using bio-fertilizer or nitrogen fertilizer up to 60 kg N/fed., in fertilizing dill plants increased total carbohydrate content. The results of the used 60 kg N/fed., alone or 40 kg N/fed., combined with bio-fertilizer were the best affected. Using bio-fertilizer or nitrogen fertilizer increased the percentage values of N compared to control ones. Moreover, the combination treatments between 40 or 60 kg N/fed., with bio-fertilizer or 60 kg N/fed., alone more effective in increasing N content.

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