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Partial substitution of mineral nitrogen fertilizer by bio-fertilizer on (*Anethum graveolens* L.) plant

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ABSTRACT

A field experiment was conducted at the Experimental Farm of the Pharmaceutical Department, Agriculture college, Giza, Egypt to study the effect of partial substitution of mineral nitrogen fertilizer by biofertilizer and their effects on the growth, yield and chemical constituents of dill plant during the two successive seasons of 2007/2008 and 2008/2009. Because of the great importance of volatile oil, its composition was also investigated in this study. Five strains of bacteria (1- *Azotobacter chroococcum*, 2- *Azospirillum lipoferum*, 3- *Bacillus polymyxa,* 4- *Bacillus megatherium* and 5- *Pseudomonas fluorescens)* were mixed in equal parts and used as biofertilizer in this experiment. The biofertilizer treatment was applied alone or in combination with 1/3, 2/3 or full recommended dose of mineral nitrogen fertilizer. The results indicated that applying biofertilizer treatment alone or in combination with chemical N fertilizer increased the growth, yield and chemical constituents of dill plant compared to the untreated control. The highest values of vegetative growth, oil yield, chlorophyll content and NPK percentages were recorded by the treatment of bio-fertilizer plus two third of recommended dose of nitrogen fertilizer. The lowest values in this respect were obtained by control plants during two seasons. The GC analysis of volatile oil indicated that the main components were carvone, limonene and apiol. These components were affected by biofertilization and chemical N treatments. Partial substitution of mineral nitrogen fertilizer by bio-fertilizer was recommended to increase the yield as well as the quality of dill plant. By applying the treatment of bio-fertilizer plus two third of recommended dose of nitrogen fertilizer we can save the quantity of N chemical fertilizer used and obtain high quality product. The results of this study gave also the possibility of shifting toward clean agriculture.

Keywords: Chlorophyll, oil yield, nitrogen, bio-fertilizer, dill, GC analysis

INTRODUCTION

Dill (*Anethum graveolens* L.) plant is an annual herb, belonging to Family Umbelliferae (Apiaceae). Dill is one of the first known multipurpose plants which have been used as a spice and medicine. Dill fruits used as a carminative, antispasmodic, sedative, lactagogue, diuretic, stimulant and to treat heamorrhoids, bronchial asthma, neuralgio, remal colic, dysurea, genital, ulcers and dysmenorrheal (Mahran *et al*., 1992). The physiological effects of dill volatile oil were occurred by its active ingredients i.e. carvone, limonine and apiol (Yili *et al*., 2006). Production of medicinal and aromatic plants using bio-fertilizers became an essential process to ensure the safety, not only for human, but also for the environment in which we live. Recently, the production of chemical-free medicinal and aromatic plants has been the focus of interest of many researchers and producers in order to ensure the high quality and safety of the product. Therefore, it would be beneficial to use alternatives to chemical fertilizers or at least to minimize the levels of these chemical fertilizers.

It is well known that the chemical fertilizers promote plant growth through the role of nitrogen in protein synthesis and increasing the meristmatic activity. Various authors reported that increasing N rate significantly promoted vegetative growth, increased the seed yield and improved NPK content of *Anethum graveolens* (Singh, 1991, Randhawa *et al*., 1996 and Kewalanand *et al*., 2001). The mechanisms by which biofertilizers can exert a positive effect on plant growth can be through the synthesis of phytohormones, N_2 fixation, reduction of membrane potential of the root, synthesis of some enzymes (such as ACC deaminase) that modulate the level of plant hormones as well as the solubilization of inorganic phosphate and mineralization of organic phosphate, which make phosphorus available to the plants (Rodriguez and Fraga, 1999). Free living nitrogen fixing bacteria such as *Azotobacter* and *Azospirillum* have the ability not only to fix nitrogen but also to release certain phytohormons of GA_3 and IAA nature which could stimulate plant growth, absorption of nutrients and photosynthesis process (Fayez *et al*., 1985 and Abdel-Latif *et al*., 2001).

Inoculation of seeds with *Azotobacter* and *Azospirillum* in combination with chemical fertilizers resulted in improving both growth and yield of anise plants (Gomaa and Abo-Aly, 2001) and *Foeniculum vulgare* plants (Kandeel *et al*., 2001). Applying the commercial biofertilizers (Biogene, Netrobene and Serialene) improved the vegetative growth, seed yield and volatile oil yield of dill plant (Kandeel *et al*., 2004). Al-Qadasi (2004) reported that inoculation with (*Azotobacter* + *Azospirillum* + *Bacillus*) caused an increase in total carbohydrate content, chlorophyll (a, b), carotenoids and NPK contents of *Ocimum basilicum* herb. The combined application of 60 kg N/ fed with biofertilizer resulted in the highest fresh and dry weight of different plant parts, oil percentage, chlorophyll, total flavonoids and NPK contents of dill plant (Said Al Ahl, 2005). Seed inoculation with *Azotobacter* and *Azospirillum* in the presence of chemical fertilizers resulted in improving both growth and yield of *Foeniculum vulgare* plant and improved the carbohydrate content as well as NPK elements in the dried herb (Mahfouz and Sharaf-Eldin, 2007). Hassan (2009) found that bacteria inoculation separately or combined with chemical fertilizers significantly improved growth characters and increased sepal yield of roselle plant compared to the control. In addition, total carbohydrates, chlorophyll content and NPK percentages were increased as a result of applying biofertilizers alone or combined with chemical fertilizers.

Based on the limited information in the literature concerning inoculation dill seeds with N2 – fixing bacteria, the objective of the current study was to investigate the effect of partial substitution of mineral nitrogen fertilizer by biofertilizer and their effects on the growth, yield and chemical constituents of dill plant as a way for deceasing the environmental hazard from chemical nitrogenous fertilizers, facing the price jump of it and producing a high quality product under the experimental conditions. Because of the great importance of volatile oil, its composition was also investigated in this study.

MATERIALS AND METHODS

Two field experiments were carried out at the Experimental Farm of Pharmaceutical Dept., Agriculture college, Giza, Egypt during the two successive seasons of 2007/2008 and 2008/2009, to study the effect of partial substitution of mineral nitrogen fertilizer by biofertilizer and their effects on the growth, yield and chemical constituents of dill plant. Dill seeds were obtained from Medicinal and Aromatic plants Research Dept., A.R.C., Ministry of Agriculture, Egypt. Seeds were sown in the prepared nursery on 18th November in both seasons. The soil was prepared and divided into plots of 3 x 3.5 m, with five rows at 60 cm apart and 20 cm between hills. The seedlings were thinned one month after sowing to leave two plants per hill. The experimental soil was clay and its chemical properties were as follows:

PH = 8.12	EC = 2.81 (ds/m)
N = 180 ppm	P = 17.8 ppm
K = 35.1 ppm	OM = 0.23 %

The strains of bacteria used as a bio-fertilizer were: 1- *Azotobacter chroococcum*, 2- *Azospirillum lipoferum,* 3- *Bacillus polymyxa*, 4- *Bacillus megatherium* and 5- *Pseudomonas fluorescens*. Distilled water was put in the flask, then peptone (5 g/L) and beef extract (3 g/L) and all sterilized in autoclave at 121ºC for 20 minutes. The flask was left at room temperature for 2 hour till it cool. After that, the strain was inoculated in sterile room condition. The flask which inoculated was incubated at 28 ºC for 7-10 days to obtain the highest growth (10^9 per ml). Finally, the five strains were mixed in equal parts and used for seed inoculation before sowing. In addition, the bio-fertilizer (2.5 L mixture of 5 strains + 22.5 L tap water) was added after one month of sowing to the plots which treated with biofertilizer.

The recommended dose (RD) of NPK chemical fertilizers used in this experiment was 300 kg/fed ammonium sulphate (20.5 % N), 200 kg/fed calcium super phosphate (15.5 % P_2O_5) and 50 kg/fed potassium sulphate (48 % k_2O) according to Ministry of Agriculture, Egypt. Ammonium sulphate and potassium sulphate were added as a basal dressing, in two equal doses. The first one was added one month after sowing and the second was applied at flowering stage. While calcium super phosphate was added during soil preparation prior to sowing. The biofertilizer was used alone or in combination with 1/3 (100 kg/fed. ammonium sulphate), 2/3(200 kg/fed. ammonium sulphate) or complete recommended dose of mineral nitrogen fertilizer (300 kg/fed. ammonium sulphate).

The experiments were planned in a split plot design with three replicates and the treatments were as follows:

T1: Control, without N applied (N_0) without biofertilizer (-Bio)
T2: 1/3 RD (N_1) without biofertilizer (-Bio)
T3: 2/3 RD (N_2) without biofertilizer (-Bio)
T4: Full RD (N_3) without biofertilizer (-Bio)
T5: without N applied (N_0) with biofertilizer (+Bio)
T6: 1/3 RD (N_1) with biofertilizer (+Bio)
T7: 2/3 RD (N_2) with biofertilizer (+Bio)
T8: Full RD (N_3) with biofertilizer (+Bio)

The following measurements were recorded at maturity of fruits: plant height, number of main branches, fresh as well as dry weight of herb and fruit yield per feddan. The obtained data were statistically analyzed according to Snedecor and Cochran (1980). Means were compared by using LSD test at 0.05 level. Nitrogen, Phosphorus and potassium were determined according to Cottenie *et al*. (1982). Chlorophyll content in fresh leaves was determined according to Wettstein (1957). The volatile oil from air-dried fruits of dill plant was isolated by hydro distillation for 3 hr in order to extract the essential oils according to Guenther (1961) and the oil yield per feddan was calculated. The isolated volatile oil was dehydrated over anhydrous sodium sulphate and stored in refrigerator until GC analysis.

The GC analysis was carried out at the Central Laboratory of National Research Center, Giza. Essential oil samples were performed using a Hewlett–Packard 5890 A series 11 instrument equipped with flame ionization detector (FID) and a carbon wax fused silica column (50 m x 0.25 mm. i. d., film thickness 0.32 µm). The oven temperature was programmed from 60 to 200 ^oC, respectively. Percentages of peak area were calculated with a Hewlett–Packard 3396 integrator.

RESULTS AND DISCUSSION

Growth parameters

Plant height: Data of Table (1) clearly indicate that the plant height was increased as a result of using the biofertilizer, chemical N treatments or the combined treatments of both in the two experimental seasons. The plant height was gradually increased with increasing the N level and reached its maximum value by applying the full recommended dose in both seasons. In addition, using biofertilizer with different levels of N chemical fertilizer increased plant height compared to the treatment of each of them alone. The best treatment in this respect was using biofertilizer + 2/3 RD of N chemical fertilizer in both seasons. The untreated control resulted in the shortest plants in both seasons. Similar findings were obtained by Mahfouz and Sharaf-Eldin, (2007) on fennel plant and Hassan (2009) on roselle plant.

Number of branches /plant: It is evident from data in (Table 1) that the treatment of biofertilizer enhanced the number of branches compared to the control in both seasons. In addition, the effect of biofertilizer was more appeared when combined with different levels of N chemical fertilizer. Otherwise, increasing the level of N chemical fertilizer led to increase the number of branches of dill plant during the two experimental seasons. The number of branches was (8.8 and 9.5) when the treatment of biofertilizer + 2/3 RD of N chemical fertilizer was applied in comparison with (8.2 and 9.6) by the full RD of N chemical fertilizer in both seasons, respectively. However, there were no significant differences between the two treatments. These results are in agreement with those of Gomaa and Abo-Aly (2001), Kandeel *et al*. (2001) and Hassan (2009).

Table 1. Effect of biofertilization treatment, N chemical treatments and their combination on the growth parameters of dill plant during 007/2008 and 2008/2009 seasons

Treatments: Biofertilizer	Treatments: Chemical N	Plant height (cm)	Number of branches	Fresh weight of herb (g/plant)	Dry weight of herb (g/plant)
		First season 2007/2008			
- Bio[1]	N_0	138.4	6.8	37.2	10.8
	N_1	145.2	7.3	46.3	14.3
	N_2	148.8	8.4	58.1	19.4
	N_3	156.7	8.2	57.8	19.1
+ Bio[2]	N_0	145.3	6.9	38.6	11.6
	N_1	150.4	7.5	47.1	15.7
	N_2	163.3	8.8	63.6	21.8
	N_3	165	8.3	65.7	22.6
LSD at 0.5% for Bio		6.83	NS	NS	NS
for N		4.39	0.995	16.04	1.6
for Bio x N		NS	NS	NS	NS
		Second season 2008/2009			
- Bio[1]	N_0	140.6	6.9	34.4	11.6
	N_1	148	7.8	49.6	17.9
	N_2	156.4	9.1	59.3	23.6
	N_3	159.1	9.6	59.1	21.3
+ Bio[2]	N_0	143.2	7.1	39.8	12.7
	N_1	151.6	8.6	49.2	18.9
	N_2	166.4	9.5	68.6	22.6
	N_3	169.6	8.9	69.9	24.1
LSD at 0.5% for Bio		NS	NS	NS	NS
for N		16.42	0.628	12.8	2.22
for Bio x N		NS	NS	NS	NS

[1] - Bio means without using biofertilizer
[2] + Bio means five bacterial strains of (1- *Azotobacter chroococcum*, 2- *Azospirillum lipoferum*, 3- *Bacillus polymyxa,* 4- *Bacillus megatherium* and 5- *Pseudomonas fluorescens)* were mixed in equal parts and used as biofertilizer

Fresh and dry weight of herb: The fresh and dry weights of dill herb was increased with increasing the chemical N level and the maximum values were obtained by using 2/3 RD of chemical n fertilizer in both seasons (Table 1). Applying biofertilizer treatment also increased the fresh and dry weights however it was insignificant compared to the untreated control in both seasons. The interaction between biofertilizer and different levels of chemical N fertilizer resulted in heaviest fresh and dry weights in comparison with applying each of them separately. The treatment of biofertilizer + 2/3 RD of N chemical fertilizer resulted in heaviest fresh and dry weights compared to the treatment of full RD of chemical N fertilizer (Table 1).

The promotion of vegetative growth of dill plant could be attributed to the effect of non symbiotic N_2-Fixing bacteria (*Azospirillum)* and phosphate solubilizing (*Bacillus polymyxa* and *Pseudomonas fluorescens*) in exerting a positive effect on plant growth through the synthesis of phytohormones, N_2 fixation, reduction of membrane potential of the root, synthesis of some enzymes (such as ACC deaminase) that modulate the level of plant hormones as well as the solubilization of inorganic phosphate and mineralization of organic phosphate, which make phosphorus available to the plants (Rodriguez and Fraga, 1999). It is well known that the chemical fertilizers promote plant growth through the role of nitrogen in protein synthesis and increasing the meristmatic activity. In addition, mineral-P is an essential component of the energy compounds (ATP and ADP) and phosphoproteins. Similar results have been reported when roselle plants treated with NPK fertilizers (Harridy and Amara, 1998 and Hassan, 2009). Therefore, plant height and number of branches of dill plant were increased and consequently reflected in increasing the plant fresh and dry weights.

Fruit yield: Data recorded in Table (2) revealed that using bio-fertilizer treatment significantly increased the fruit yield of dill plant compared to the control. The fruit yield was (1355 and 1420.4 kg/fed) by applying the biofertilizer treatment, however it was (986.5 and 1040 kg/fed) for the control in the first and second seasons. Increasing the chemical N level significantly increased the fruit yield compared to the untreated control. The highest values in this respect (1539.2 and 1592.7 kg/fed) were obtained by full RD treatment in both seasons, respectively. Concerning the interaction between chemical N levels and biofertilization treatment, data showed that the fruit yield of dill plant was increased as a result of this interaction in comparison with any treatment alone. The maximum yield (1759.1 kg/fed) was obtained by the treatment biofertilizer + full RD of N chemical fertilizer in the first season, while the treatment of biofertilizer + 2/3 RD of N chemical fertilizer resulted in the maximum yield (1860.1 kg/fed) in the second one (Table 2). However, there were no significant differences between the two treatments in this respect.

The increment in fruit yield of dill plant could be explained through the effect of chemical N or biofertilization treatments in promoting the vegetative growth of dill plant and this promotion was reflected in increasing the fruit yield (Shaalan, 2005, Mahfouz and Sharaf-Eldin, 2007 and Hassan, 2009). Therefore, adding 1/3 or 2/3 RD of chemical N fertilizer to biofertilizer improved the plant growth and increased the fruit yield. However, the 1/3 RD of chemical N fertilizer may be not enough for maximum promotion of microorganisms therefore, 2/3 RD of chemical fertilizers resulted in maximum increment of growth and yield of dill plants when combined with biofertilizer. This improvement may be due to the direct effect of chemical fertilizer or indirect through the microbial propagation activation since the free living nitrogen fixing bacteria have the ability not only to fix nitrogen but also to release certain phytohormons of GA_3 and IAA nature which could stimulate plant growth, absorption of nutrients and photosynthesis process (Fayez *et al*., 1985 and Abdel-Latif *et al*., 2001). These results are in accordance with those obtained by Harridy and Amara, (1998) and Shaalan *et al.* (2001).

Volatile oil content

Volatile oil percentage: It is evident from data in Table (2) that the volatile oil percentage of dill fruit was increased as a result of applying the biofertilization treatments, however this increment was insignificant in both seasons. The volatile oil percentage was gradually increased with increasing N level and reached its maximum value by applying the full RD in both seasons. The effect of biofertilization and chemical N levels was more effective when the two treatments were combined. The highest values in this respect were (4.26 and 3.99 %) obtained by applying biofertilizer + 2/3 RD of N chemical fertilizer in both seasons, respectively.

Table 2. Effect of biofertilization treatment, N chemical treatments and their combination on the fruit and oil yield of dill plant during 2007/2008 and 2008/2009 seasons

Treatments		Fruit yield Kg/fed	Volatile oil percentage %	Volatile oil yield L/fed	Fruit yield Kg/fed	Volatile oil percentage %	Volatile oil yield L/fed
Biofertilizer	Chemical N						
		First season 2007/2008			Second season 2008/2009		
- Bio[1]	N_0	986.5	1.87	18.48	1040.0	1.67	17.37
	N_1	1135.1	3.53	40.06	1212.4	3.35	40.65
	N_2	1414.4	3.84	54.32	1521.4	3.61	54.97
	N_3	1539.2	4.28	65.85	1592.7	4.15	66.03
+ Bio[2]	N_0	1355.0	2.23	30.19	1420.4	2.26	32.09
	N_1	1574.9	3.03	47.66	1670.0	2.89	48.26
	N_2	1729.4	4.26	73.75	1860.1	3.99	74.17
	N_3	1759.1	3.74	65.73	1771.0	3.74	66.26
LSD at 0.5%	for Bio	132.2	1.23	3.72	62.34	1.42	3.29
	for N	152	0.81	1.99	40.29	0.84	2.3
	for Bio x N	NS	NS	NS	56.98	NS	3.25

[1] - Bio means without using biofertilizer
[2] + Bio means five bacterial strains of (1- *Azotobacter chroococcum*, 2- *Azospirillum lipoferum*, 3- *Bacillus polymyxa,* 4- *Bacillus megatherium* and 5- *Pseudomonas fluorescens)* were mixed in equal parts and used as biofertilizer

Volatile oil yield

The volatile oil yield of dill fruit was significantly increased the volatile oil yield of dill fruit compared to the control in the two seasons. The volatile oil yield was (30.19 and 32.09 L/fed) by applying the biofertilizer treatment, however it was (18.48 and 17.37 L/fed) for the control in the first and second seasons, respectively (Table 2). The volatile oil yield was gradually increased with increasing the N level and reached its maximum value by applying the full recommended dose in both seasons. The volatile oil yield of dill fruit was significantly increased as a result of the interaction between biofertilization and chemical N levels treatments in the two experimental seasons. The highest volatile oil yield (73.75 and 74.17 L/fed) were obtained by the treatment biofertilizer + 2/3 RD of N chemical fertilizer in the first and second seasons, respectively (Table 2). These results support the other results obtained by Randhawa and singh (1991), Gomaa and Abo-Aly, (2001), Kandeel *et al*., (2004) and Mahfouz and Sharaf-Eldin (2007).

3. 3 Volatile oil constituents

The GC analysis of volatile oil of dill fruit indicates that the main components of volatile oil were Limonene, Carvone and Apiol (Table 3). The results clearly indicate that the volatile oil composition was affected as a result of applying various treatments compared to the control. However, there was no constant trend for the main components of volatile oil. The highest value of limonene (50.87%) was recorded by applying 1/3 N chemical fertilizer without biofertilizer. However, the treatment of 2/3 N chemical fertilizer without biofertilizer resulted in the highest value of carvone component (48%). Concerning the apiol component, the highest value (31.01%) was obtained by using biofertilizer + 2/3 N chemical fertilizer treatment.

Table 3. Effect of biofertilization treatment, N chemical treatments and their combination on the main components of volatile oil of dill plant during 2008/2009 season

Treatments		Main components of volatile oil %		
Biofertilizer	Chemical N	Limonene	Carvone	Apiol
- Bio[1]	N_0	50.06	40.58	3.01
	N_1	50.87	24.69	18.43
	N_2	40.17	48.00	3.23
	N_3	43.36	36.11	6.22
+ Bio[2]	N_0	33.62	28.24	3.01
	N_1	40.12	34.62	2.46
	N_2	29.78	33.89	31.01
	N_3	36.21	32.22	3.84

[1] - Bio means without using biofertilizer
[2] + Bio means five bacterial strains of (1- *Azotobacter chroococcum*, 2- *Azospirillum lipoferum*, 3- *Bacillus polymyxa,* 4- *Bacillus megatherium* and 5- *Pseudomonas fluorescens)* were mixed in equal parts and used as biofertilizer

Chemical analysis

Chlorophyll content: Data concerning the effect of biofertilizer and N chemical fertilizer on chlorophyll content of dill herb were presented in Table (4). The chlorophyll content was gradually increased with increasing the N level and reached its maximum value by applying the full recommended dose in both seasons. The highest chlorophyll content (1.78 and 1.71 mg/g F.W) was obtained by using biofertilizer + 2/3 RD of N chemical fertilizer in both seasons. The untreated control resulted in the lowest values in this respect in both seasons. These results may be due to the effect of microorganisms in biofertilizer or the role of N nutrition in producing growth promoting substances resulting in more efficient absorption of nutrients, which main components of photosynthetic pigments and consequently the chlorophyll content was increased (Gomaa and Abou-Aly, 2001). Similar results were obtained by Hassan (2009).

Nutrients content: The percentages of total N, P and K in dried herb of dill plant were increased with increasing the chemical N level and reached its maximum values by full RD of chemical N in both seasons. In addition, applying the biofertilizer treatment led to increase the previous percentages compared to untreated control in both seasons (Table 4). The combination between biofertilizer and chemical N treatments enhanced N, P and K percentages in comparison with separate application with each of them. The highest N, P and K percentages (2.07, 0.379 and 3.18 %) and (1.71, 0.371 and 3.22 %) were obtained by using biofertilizer + 2/3 RD of N chemical fertilizer in both seasons, respectively.

NO_3 content in herb followed the same trend observed in case of nitrogen percentage. The treatment of biofertilizer + 2/3 RD of N chemical fertilizer resulted in the highest NO_3 content in the two experimental seasons (Table 4). Concerning the total soluble N in soil, the results of chemical analysis clearly indicate that the chemical N, biofertilizer or combination treatments enhanced the total soluble N in soil. The highest values in this respect (118.4 and 119.1 ppm) were obtained by applying the treatment of biofertilizer + 2/3 RD of N chemical fertilizer in both seasons, respectively (Table 4).

Table 4. Effect of biofertilization treatment, N chemical treatments and their combination on the fruit and oil yield of dill plant during 2007/2008 and 2008/2009 seasons

Treatments		Total chlorophyll mg/g FW	Total N %	NO_3 ppm	Total soluble N in soil ppm	Total P %	Total K %
Biofertilizer	Chemical N						
		First season 2007/2008					
- Bio[1]	N_0	1.27	1.37	460	31.6	0.256	2.5
	N_1	1.48	1.43	503	62.5	0.273	2.73
	N_2	1.65	1.55	618	72.9	0.287	2.81
	N_3	1.69	1.62	734	118.6	0.314	2.85
+ Bio[2]	N_0	1.28	1.51	511	64.4	0.288	2.61
	N_1	1.51	1.56	742	78.3	0.293	2.85
	N_2	1.78	2.07	861	119.1	0.379	3.18
	N_3	1.65	1.83	796	96.5	0.356	2.88
LSD at 0.5%	for Bio	NS	0.073	13.1	1.26	0.037	0.311
	for N	0.089	0.061	6.63	2.59	0.025	0.285
	for Bio x N	NS	0.086	9.37	3.67	NS	NS
		Second season 2008/2009					
- Bio[1]	N_0	1.28	1.38	473	33.5	0.278	2.56
	N_1	1.52	1.46	515	66.3	0.288	2.84
	N_2	1.68	1.63	639	75.4	0.295	2.89
	N_3	1.70	1.68	755	113.2	0.326	2.96
+ Bio[2]	N_0	1.29	1.55	524	54.1	0.291	2.63
	N_1	1.54	1.58	758	76.7	0.298	2.93
	N_2	1.71	2.11	827	118.4	0.371	3.22
	N_3	1.69	1.94	814	91.9	0.313	2.95
LSD at 0.5%	for Bio	0.0082	0.025	7.45	5.28	0.041	0.185
	for N	0.0054	0.052	4.64	2.79	0.027	0.179
	for Bio x N	0.0075	0.073	6.56	3.95	NS	NS

[1] - Bio means without using biofertilizer

[2] + Bio means five bacterial strains of (1- *Azotobacter chroococcum*, 2- *Azospirillum lipoferum*, 3- *Bacillus polymyxa,* 4- *Bacillus megatherium* and 5- *Pseudomonas fluorescens)* were mixed in equal parts and used as biofertilizer

The increase of nitrogen percentage in soil may be due to not only nitrogen fixation, but also to phosphate dissolving bacteria which transfer unavailable phosphate into available phosphate leading to more (ATP) which is important to N_2-fixers bacteria. The present results are in agreement with those of Shahaby (1997) who found that nitrogen fixation bacteria increased total nitrogen content in the Nile valley and reclaimed soil.

These results may be due to the converting of the unavailable forms of nutrient elements to available forms by the microorganisms in biofertilizer. The microorganisms also produce growth promoting substances resulting in more efficient absorption of nutrients, which main components of photosynthetic pigments and consequently the chlorophyll content as well as N, P and K percentages were increased (Gomaa and Abou-Aly, 2001). In addition, the non symbiotic N_2-fixing bacteria (*Azospirillum)* produced adequate amounts of IAA and cytokinins with increasing the surface area per unit root length and enhanced the root hair branching with an eventual increase on the uptake of nutrients from the soil (Rodriguez and Fraga, 1999). Phosphate solubilizing bacteria (*Bacillus polymyxa* and *Pseudomonas fluorescens*) release organic and inorganic acids which reduce soil pH leading to change of phosphorus and other nutrients to available forms ready for uptake by plants (Singh and Kapoor, 1999). In addition, some kinds of bacteria belonging to genera *Pseudomonas* and *Bacillus* able to analyze aluminum silicates in clay minerals such as biotite, muscovite, microcline and orthoclase and release of potassium from them (El-Hadad *et al.,* 1993). Similar results have been reported on different medicinal and aromatic plants (Kandeel *et al.* 2001, Mahfouz and Sharaf-Eldin, 2007 and Hassan, 2009).

From the results of this study, it could be concluded that partial substitution of mineral nitrogen fertilizer by bio-fertilizer was recommended to increase the yield as well as the quality of dill plant. By applying the treatment of bio-fertilizer plus two third of recommended dose of nitrogen fertilizer we can save the quantity of N chemical fertilizer used and obtain high quality product. The results of this study gave also the possibility of shifting toward clean agriculture.

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(Anethum graveolens L.) plant. F. A. Hellal1, S. A. Mahfouz2 and F. A. S. Hassan3. 1Plant Nutrition Dept., National Research Center, Dokki, Egypt.Â study the effect of partial substitution of mineral. nitrogen fertilizer by biofertilizer and their effects on. the growth, yield and chemical constituents of dill. plant.Â the bio-fertilizer (2.5 L mixture of 5 strains + 22.5 L. tap water) was added after one month of sowing to. the plots which treated with biofertilizer. Bio-fertilizers fix atmospheric nitrogen in the soil and root nodules of legume crops and makes them available to the plants. They solubilize the insoluble forms of phosphate like tricalcium, iron, and aluminum phosphates into available forms, scavenge phosphates from soil layers and they also produce hormones and anti-metabolites which promote root growth. Bio-fertilizers decompose organic matter and help in mineralization of soil. After application to the soils or seeds, they increase the availability of the nutrients and improves the yield by 10 to 20 % without adversely affecting the soil and environment [37]. Photosynthetic rate of a plant is greatly influenced by the green pigment chlorophyll which is also considered as an index of the metabolic efficacy of plants. A slight fluctuation in chlorophyll content is sufficient to trigger changes in physiological processes of plants, particularly photosynthesis.Â 2011. Partial substitution of mineral nitrogen fertilizer by biofertilizer on (Anethum graveolens L.) plant. Agriculture and Biology J. of North America, 2(4): 652-660. Johnson, B. C. 2005.