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ONION GROWTH AND YIELD AS AFFECTED BY BIO-FERTILIZATION

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ABSTRACT

The present investigation was conducted during the two successive seasons of 2003/2004 and 2004/2005 on onion (cv. Giza 6) grown at a private Farm, Etsa, Fayoum, Egypt, to study the possibility of replacing biofertilization instead of the mineral-N source in onion plants. Ammonium nitrate (33.5%N) was used as a mineral-N source at the rate of 0, 62.5, 125 and 250 Kg feddan^{-1} which represent; control, 25%, 50% and 100% from the recommended fertilizer rate. The mineral-N fertilizer was applied to the soil alone and in combination with a mixture of biofertilizer (namely, *Azotobacter chroococcum* and *Azospirillium brasilense,* 1:1) which represent eight treatments. The obtained results indicated that, growth characters; i.e., plant height, No. of leaves plant^{-1}, fresh and dry weight of leaves plant^{-1} fresh and dry weight of bulb plant^{-1} and the total fresh and dry weights plant^{-1} were positively affected by the application of mineral-N fertilizer either alone or in combination with the mixture of biofertilizer in the two successive seasons. The highest increase was obtained by the treatment of 250 Kg feddan^{-1} mineral-N fertilizer feddan^{-1} mineral-N when combined with biofertilizer as compared to the other treatments in both seasons. Also, total yield feddan^{-1} recorded the highest values with the same treatment which gave marked increase in the two seasons. The application of 125 and 250 Kg feddan^{-1} in combination with biofertilizer, gave a significant increase in leaf pigments (chlorophyll and total and carotenoids), N, P and K (in both leaves and bulb) as compared to 250 Kg feddan^{-1} alone. The concentration of nitrite (NO_2) and nitrate (NO_3) in leaves and bulbs was significantly decreased by biofertilizer singly or with any rate of mineral-N fertilizer as compared to mineral fertilizer in both seasons. The GLC analysis indicated that the treatment of biofertilizer and mineral-N treatment gave a possitive effect in the percentage of volatile oil compounds of onion. The major compounds of volatile oil were propyl allyl sulfide, methul allyl trisulfide and dipropyl trisulfide and the highest values of these compunds were recorded by the treatment of biofertilizer in the presence of 250 Kg feddan^{-1} followed by the treatment of 125 Kg feddan^{-1}. In general, the treatment of 250 and 125 Kg feddan^{-1} in combination with biofertilizer treatment gave the same effect on growth characters, yield and chemical constituents including volatile oil compounds. Thus, the application of biofertilizer (*Azotobacter* and *Azospirillium)* in combination with 125 kg feddan^{-1}; ammonium nitrate is recommended for improving growth, yield capacity (appr. 20.30% as compared to the recommended dose) and some chemical constituents including

the major compounds of volatile oils which are responsible for the flavour and pungency of onion plants and to lower production cost by reducing mineral-N fertilizer used (appr. 50%).

INTRODUCTION

In Egypt, onion (*Allium cepa* L.) is considered one of the important crops growing for local consumption and exportation. Onion crop is normally fertilized with a high amount of mineral-N fertilizers which, may have hazardous effect on the environment and may produce poor quality. Mineral-N fertilizers are expensive and is considered a source of environmentally pollution. Recently,research work is oriented to evaluate biofertilizer as a new method for plant nutrition. Several reports indicated that the inoculation of some plants with biofertilizers or in combination with mineral fertilizers improved plant growth, yield and chemical composition (Abdel-Mouty *et al.*, 2002; and Gadallah *et al.*, 2004, El-Assiouty and Abou-sedera, 2005). Therefore, it seems that biological fertilization is a virgin field and more research work should be carried out to evaluate biofertilizers as a source for plant nutrition. The main goal of this research is to study the relation between biofertilizer and mineral-N fertilizer application in the soil and their effect on onion plant growth, yield and volatile oil compounds.

MATERIAL AND METHODS

Two field experiments were conducted at a private Farm, Etsa, Fayoum, Egypt on onion plants (cv. Giza 6), during the two growing seasons of 2003/2004 and 2004/2005. The most important physical and chemical characters of the selected soil were determined (Table 1) according to Wilde *et al.*, (1985).

Eight treatments were arranged as 0, 62.5, 125 and 250 Kg ammonium nitrate (33.5%N) fed.$^{-1}$ as a source of mineral-N fertilizer (which represents, control, 25%, 50% and 100% from the recommended fertilization rate) either alone or with the mixture of biofertilizer (*Azotobacter chroococcum* (AT) and *Azospirillium brasilense* (AZ) [1:1]) beside biofertilizer alone.

The experimental treatments were arranged as follows:

1- Control: Untreated plants. 2- 62.5 kg fed.$^{-1}$ mineral-N fertilizer
3- 125 kg fed.$^{-1}$ mineral-N fertilizer. 4- 250 kg fed.$^{-1}$ mineral-N fertilizer
5- (AT + AZ): The mixture of *Azotobacter chroococcum* (AT) and *Azospirillium brasilense* (AZ) [1:1]
6-62.5 Kg fed.$^{-1}$ mineral-N fertilizer + (AT+AZ). 7- 125 Kg fed.$^{-1}$ mineral-N fertilizer + (AT+AZ). 8- 250 Kg fed.$^{-1}$ mineral-N fertilizer + (AT+AZ).

Preparation of inocula:

Modified Ashby's medium (Heggazi and Niemela, 1976) was used to grow the *Azotobacter chroococcum* and Dobereiner medium for *Azospirillium brasilense* (Dobereiner *et al.*, 1976). The strains (*A. chroococcum* FN 33 and *A.*

brasilense FN 17) were isolated and identified in the microbiology laboratory, Faculty of Agriculture, Fayoum University from the soil in which the experiments were performed. Isolates and inoculates were prepared immediately before inoculation. At the logarithmic growth phase, the cultures were centrifuged at 1000 rpm and the cell pellets were washed three times with sterile phosphate buffer (100 mM, pH=7.0).

The washed cells were resuspended in the same buffer to the final concentration of about 4×10^8 cfu/ml and used for transplants inoculation.

Transplants inoculation:

Onion transplants were immersed in Arabic Gum solution (16%) as a sticking agent and then inoculated with *Azotobacter chroococcum* FN 33 and *Azospirillium brasilense* FN 17 mixed in equal quantities (1:1). Inoculated transplants were allowed to air dry in shade before transplanting according to Allen (1971).

Table (1): Physical and chemical characters of the selected soil before planting in both seasons

Property	2003/2004	2004/2005
particle size distribution		
Clay%	58.2	57.1
Silt%	27.4	28.9
Sand%	14.4	13.0
Soil texture	Clay	Clay
Chemical:		
PH (1:2.5 suspension)	7.32	7.39
ECe (dS m^{-1})	2.14	2.23
Organic matter%	1.21	1.32
$CaCO_3$%	5.69	5.13
Macro-and microelements (ppm):		
N	440.00	470.00
K	375.00	382.00
P	15.30	19.80
Fe	74.00	82.00
Zn	28.90	32.10
Mn	8.08	7.91
Cu	0.68	0.72

The different treatments were arranged in randomized complete blocks design with three replications. The experimental plot was 10.5 m^2. Each plot was planned to consist of 6 rows; 3.5m long and 0.5m width. Fifty days old onion transplants were planted 10 cm apart in both sides of each row on 23rd of November in both seasons. The rates of ammonium nitrate were applied in two equal doses, the first dose was added after 30 days from transplanting and the second dose a month later. The general agricultural practices were applied as recommended for commercial onion production. The following data were recorded: 1- After 90 days from transplanting, fresh samples from mature fully expanded leaves were randomly collected from each treatment for determination of leaf pigments; chlorophyll a, b and total and carotenoids concentration (mg g^{-1} fresh weight of leaf). 2- At 150 days from transplanting, random samples of ten plants from each plot were used to record the following growth parameters: plant height (the length of plant from the soil surface till the highest point of plant, cm), No. of leaves $plant^{-1}$ and fresh weight of leaves $plant^{-1}$ (g). Also, mean weight $bulb^{-1}$ (g), and bulbing ratio (the largest leaf bases diameter divided by the minimum diameter of the neck) were estimated. Bulbs were recorded were collected from the four inner rows from each plot and weighted and used for estimating total bulb yield (ton $feddan^{-1}$). Samples of the leaves and bulbs of plants which were randomly taken, washed and dried at 70°C until the constant of weight for recording the dry weight of leaves and bulb $plant^{-1}$ (g). The dried samples were grounded and kept for the chemical analysis. The whole fresh and dry weights $plant^{-1}$ (leaves and bulb) were estimated as well.

Chemical analysis:

In both seasons, leaf pigments; chlorophyll a, b and total and carotenoids were extracted by acetone 80% then determined using colorimetric method as described by Welburn and Lichtenthaler(1984). In fresh bulbs, total soluble solids% (TSS%) were estimated in homogenized portion of fresh bulbs using a handle refractometer model PZO Nr. 19877 (A.O.A.C., 1990). Nitrogen was colorimetrically determined by using the Orange G dye according to the method of Hafez and Mikkelsen (1981). For the nitrite and nitrate determination (mg kg^{-1} dry matter of leaves and bulbs), 0.5g dried material was shaken in 20 ml distilled water for 30 min., then filtered (Bar-Akiva, 1974). An aliquot of the same extract was also analyzed for nitrite using sulphanilic acid and α-naphthylamine method (Chapman and Pratt, 1961). Another aliquot of the extract was analyzed for nitrate using phenol disulfonic acid method (Page *et al.*, 1982). Phosphorus (mg g^{-1} dry matter of leaves and bulbs) was colorimetrically estimated by using chlorostannous molybdophosphoric blue colour method in sulfuric acid system as described by Jackson (1967). Potassium (mg g^{-1} dry matter of leaves and bulbs) was determined by using a Perkin Flame Photometer (Page *et al.*, 1982).

Volatile oil determination:

At the maturity stage, volatile oil components were determined in the oil samples which were extracted from the fresh onion bulbs (in the second season, at harvesting time).

Extraction of onion oil:

The crushed bulbs of onion were extracted by steam distillation of fresh bulbs according to Fenaroli (1990), as follows. Five hundred grams of the crushed bulbs of each onion sample were blended with distilled water then evaporated under vacuum at 45°C for 2 hours. The distillate was extracted with diethyl ether. The extract was dried over anhydrous sodium sulphate, filterd and concentrated to 1.0-0.5 ml under vacuum at 45°C. The collected oils were kept in brown sealed bottles at −15°C for the required analysis.

Gas chromatography (GC) analysis of the volatile oil:

Oil samples of each treatment was subjected to GC analysis by using a varian 3400 GC equipped with DH5 capillary column CO. 32 mm idx30 m and coupled to a finnigen-Mat 559-4000. Analysis was carried out by using helium as the carrier gas, flow rate 1 ml min^{-1}. The column temperature was maintained initially at 50^{0}C for 6 min. and programmed from 50 to 260^{0}C at a rate of 6^{0}C min.$^{-1}$ The injection port temperature was maintained at 250^{0}C and detector at 280^{0}C. Sample size was 0.3 μL. The relative percent of each compound was determined by Varian 4270 integrator.

Statistical analysis:

All data of the two seasons were subjected to the statistical analysis according to the used design. The least significant difference test (LSD) at p=0.05 level was used to verify the differences between treatments as mentioned by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Vegetative growth characters:

Data in Table (2) clarified that all the evaluated growth characters were affected by both mineral-N and biofertilizer treatments in both seasons.. Concerning the effect of biofertilizer, the results show that, the inoculation of onion transplants with the mixture of biofertilizer singly showed a significant increase in the recorded growth characters in both seasons as compared to the control treatment. The increases in plant height, No. of leaves plant^{-1}, fresh and dry weights of leaves plant^{-1}, fresh and dry weights bulb and total fresh and dry weights plant^{-1} (leaves and bulb) were: 23.73%, 75%, 59.84%, 123.76%, 53.20%, 28.81%. 55.86% and 53.49%, respectively in the first season and 14.38%, 56.58%. 58.92%, 86.70%, 23.00%, 14.94%, 34.56% and 35.14%, respectively in the second one.

Data in Table (2) showed that, during both seasons, the inoculation of onion transplants with the mixture of biofertilizer and any used level of mineral-N fertilizer (62.5, 125 and 250 Kg fed.$^{-1}$), caused a significant increase in the studied growth characters comparing with the un inoculated treatments only supplied with corresponding mineral-N. The treatment of 62.5 Kg fed.$^{-1}$ in combination with biofertilizer recorded a significant increase by 5.36%, 49.05%, 24.77%, 42.12%. 39.86%, 21.95% 33.44% and 27.64%, respectively in the first season. While the increments, in the second one, reached: 5.39%, 41.81%, 42.75%, 35.59%. 46.42%, 24.07%, 44.98% and 11.71%, respectively more than the treatment of 62.5 Kg fed.$^{-1}$ only for the mentioned growth characters as stated before. The treatment of 125 Kg fed.$^{-1}$ in combination with biofertilizer showed a significant increase by 7.09%, 50.57%, 38.37%, 31.43%, 35.19%, 36.15% 36.44% and 34.22%, respectively in the first season and 4.98%, 30.34%, 28.94%, 47.82%. 37.29%, 47.63%, 33.92% and 47.42%, respectively in the second one as compared with 125 Kg fed.$^{-1}$ treatment alone. The increase in the recorded growth characters which obtained by the treatment of 250 Kg fed.$^{-1}$ combined with biofertilizer as compared to the treatment of 250 Kg fed.$^{-1}$ alone in both seasons reached 5.89%, 19.59%, 8.25%, 16.75%, 23.03%, 32.08%, 16.67% and 25.58%, respectively in the first season and 2.30%, 2.00%, 2.82%, 44.20%, 16.00%, 44.00%, 10.48% and 44.09%, respectively in the second season. In general, the differences between the combined treatment of biofertilizer with 125 Kg fed.$^{-1}$ was superior or equal with 250 Kg fed.$^{-1}$ mineral -N in both seasons. However, the plants fertilized with 250 Kg fed.$^{-1}$ combined with biofertilizer were insignificantly different from those fertilized by 125 Kg fed.$^{-1}$ combined with biofertilizer in all studied growth characters in both seasons which, indicate that the bio-fertilization could be compensated to the reduction of the mineral-N level. This insignificance could be referred to higher efficiency of biofertilizer under lower nitrogen rate.

Table (2): Influence of bio-and mineral-N fertilization on growth characters of onion plants in both seasons of 2003/2004 and 2004/2005.

Treatment		Plant height (cm)	NO. of leaves plant^{-1}	Leaves weight plant^{-1} (g)		Bulb weight plant^{-1} (g)		Total weight plant^{-1} (g)	
Bio -F	Mineral-N (kg fed.$^{-1}$)			Fresh	Dry	Fresh	Dry	Fresh	Dry
				2003/2004					
Control		51.00	8.00	60.08	5.33	89.64	15.03	149.72	20.36
-	62.5	62.17	11.07	91.69	10.09	123.67	16.94	215.36	27.03
-	125	63.50	13.17	100.28	13.14	154.30	19.06	254.58	32.20
-	250	65.17	17.00	132.07	16.48	174.53	20.43	306.60	36.91
AT+AZ	-	63.10	14.00	96.03	11.89	137.33	19.36	233.36	31.25
	62.5	65.50	16.50	114.40	14.34	172.97	20.06	287.37	34.40
AT+AZ	125	68.00	19.83	138.76	17.27	208.60	25.95	347.36	43.22
	250	69.01	20.33	142.97	19.24	214.73	27.11	357.70	46.35
$LSD_{(0.05)}$		1.11	2.03	5.37	2.24	8.52	1.26	21.07	3.14
				2004/2005					
Control		54.17	7.67	54.45	6.24	106.39	15.93	160.84	22.17
-	62.5	61.17	10.93	77.88	10.20	121.40	16.62	199.28	26.82
-	125	63.83	14.50	105.87	12.46	156.60	18.16	262.47	30.62
-	250	66.50	17.97	135.30	13.28	187.60	19.11	322.90	32.39
AT+AZ	-	61.96	12.01	86.53	11.65	129.90	18.31	216.43	29.96
AT+AZ	62.5	64.47	15.50	111.17	13.83	177.74	20.62	288.91	34.05
AT+AZ	125	67.01	18.90	136.51	18.33	215.00	26.81	351.51	45.14
AT+AZ	250	68.13	21.57	139.11	19.15	217.63	27.52	356.74	46.67
$LSD_{(0.05)}$		1.17	3.15	3.01	2.01	11.34	1.54	15.37	3.04

AT = *Azotobacter chroococcum* **AZ** = *Azospirillium brasilense*
Bio-F = biofertilizer **Control** = Untreated plants

The positive effect of mineral-N fertilization on growth parameters of onion plants may be attributed to the role of nitrogen in protoplasm formation and all proteins, amino acids, nucleic acid, many enzymes and energy transfer materials; ADP and ATP (Russel, 1973), and stimulating nutritional status of the plant (Medani *et al.*, 2000).The beneficial effect of inoculation of onion transplants with the mixture of biofertilizer on growth parameters may due to its ability to release plant promoting substances, mainly IAA, gibberellic acid and cytokinin like substances which might stimulate plant growth (Fayez *et al.*, 1985 and El-Merich *et al.*, 1997), increasing the water mineral uptake from the soil, which led to increases in root surface area, root hairs and root elongation as affected by Azotobacter (Sundaravelu and Muthukrishinan, 1993), and increasing the ability to convert N_2 to NH_4 and thus make it available to plants (Hanafy *et al.*, 1997). The positive effects on growth might be attributed to the enhancing effect of biofertilizers on the nutritional availability and its uptake. The obtained result from growth characters are in harmony with those by Alkaff *et al.*(2002), Sabry (2003), El-Assiouty and Abou-Sedera (2005).

Table (3): Influence of bio-and mineral-N fertilization on bulbing ratio and yield of onion plants in both seasons of 2003/2004 and 2004/2005.

Treatment		Bulbing ratio		Total bulb yield (ton fed.$^{-1}$)	
Bio-F	Mineral-N (Kg fed.$^{-1}$)	2003/2004	2004/2005	2003/2004	2004/2005
Control		3.13	3.10	8.47	8.84
-	62.5	3.27	3.31	9.64	10.16
-	125	3.30	3.56	12.82	12.21
-	250	3.35	3.96	13.60	13.02
AT+AZ	-	3.29	3.48	10.71	10.13
AT+AZ	62.5	3.45	3.55	13.48	13.85
AT+AZ	125	3.52	3.86	16.26	15.76
AT+AZ	250	3.62	4.15	16.74	16.96
$LSD_{(0.05)}$		N.S	N.S	0.81	1.24

AT = *Azotobacter chroococcum* **AZ** = *Azospirillium brasilense*
Bio-F = biofertilizer **Control** = Untreated plants

Total yield:

The results in Table (3) clarify that total bulb yield was positively affected by both mineral-N and biofertilizer treatments in both seasons, while, bulbing ratio was not affected by such treatments. Biofertilizer alone treatment showed a significant increase in total bulb yield in both seasons as compared to the control treatment. In the first season, the difference was: 26.45%, and was 14.59% in the second season. Data in Table (3) clearly show that the inoculated plants with the mixture of biofertilizer and fertilized with any level of ammonium nitrate; 62.5, 125 and 250 Kg fed.$^{-1}$ resulted in a significant increase in the total bulb yield as compared to plants given only a corresponding mineral-N fertilizer. In this respect, the treatment of 62.5 Kg fed.$^{-1}$ in combination with biofertilizer showed a significant increase by 39.83% and by 33.66% in the first and second seasons, respectively, as compared to the treatment of 62.5 Kg fed.$^{-1}$ alone. The treatment of 125 Kg fed.$^{-1}$ in combination with the mixture of biofertilizer recorded a significant increase in the first season by 26.83% and by 29.07% in the second one as compared to the treatment of 125 Kg fed.$^{-1}$ mineral N. The significant increase in the total bulb yield which obtained by the treatment of 250 Kg fed.$^{-1}$ combined with the mixture of biofertilizer as compared to the treatment of 250 Kg fed.$^{-1}$ alone was: 23.08% in the first season while, in the second one, the increase reached; 30.26%. The treatment of 125 Kg fed.$^{-1}$ in combination with the mixture of biofertilizer recorded a significant increase which reached: 19.56% and 21.04% for total bulb yield in both seasons, respectively as compared to the treatment of 250 Kg fed.$^{-1}$ alone. Thus, the combination between the treatments of mineral-N and biofertilizer had a greatly positive effect on total bulb yield as compared to the treatments of mineral-N alone. However, the treated plants with 250 Kg fed.$^{-1}$ combined with biofertilizer caused an insignificant increase in total bulb yield in both seasons when compared with the treatment of 125 Kg fed.$^{-1}$ combined with the biofertilizer.

The increase in total yield of onion is expected with mineral-N fertilizer when combined with biofertilizer which could be explained from the major functions of nitrogen on chlorophyll, enzymes, photosynthesis and endogenous hormones synthesis, which consequently affect plant growth and yield (Marschner, 1995 and Hanafy *et al.*, 1997). The favourable effect of biofertilizer treatments could be attributed to its enhancing effect on plant growth characters, i.e. plant height, No. of leaves plant^{-1} as well as dry matter of leaves which could be reflected on onion yield (El-Sawah *et al.*, 2004) . The results of the current study was coped with the results of several investigators who mentioned that, using 50% of required mineral nitrogen combined with biofertilizer inoculation caused marked increases in yield by 9.2% to 30% of tomato (Tantawy, 2000) and by 59% of okra (Mishra and Patjoshi, 1995).

III- Chemical constituents:

Table (4) presents the effects of mineral-N and biofertilization on leaf pigments (chlorophyll a, b and total, carotenoids) of onion leaves in both seasons. The data clearly show that the inoculated plants with the mixture of biofertilizer alone (AT+AZ) caused a significant increase in the leaf pigments concentration as compared to the control treatment (0.0 N level).

Regarding the effect of combination between mineral-N and biofertilizer, the data in Table (4) indicate that, by enhancing mineral-N fertilizer rates in combination with (AT+AZ), chlorophyll a, b and total and carotenoids of onion leaves were significantly increased reaching the highest value by the treatment of 250 Kg fed.$^{-1}$ combined with the mixture of (AT+AZ) in the two seasons. Also, the results indicate that, the inoculation of onion plants with (AT+AZ) at a given level of mineral-N fertilizer, had a significant increase in the leaf pigments concentration compared to mineral-N treatments.

Data in Tables (5 & 6) indicate that, the treated plants with the mixture of (AT+AZ) singly recorded a significant increase in the concentration of N, P and K in leaves and bulbs and an insignificant increase with TSS% as compared to the control treatment in both seasons.

Concerning the effect of the combination between bio-and mineral-N fertilization treatments on N, P, K concentrations and TSS%, the data in Tables (5&6) indicate that N, P and K concentrations in both leaves and bulbs were significantly increased with inoculation of onion transplants, and by increasing the rates of mineral-N level reaching their maximum increase by the treatment of 250 Kg fed.$^{-1}$ in both seasons as compared to the corresponding mineral-N alone. With regard to TSS%, an insignificant increase was recorded by those treatments. However, the differences between the treatments of 250 Kg fed.$^{-1}$ and 125 Kg fed.$^{-1}$ combined with (AT+AZ) were insignificant. This insignificance could be referred to higher efficiency of biofertilizer (AT+AZ) under lower nitrogen level (Mehrotra and Lehri, 1997). Hanafy *et al.* (1997) suggested that the addition of biofertilizers increases the ability to convert N_2 to NH_4 and thus make it available to plant. Also, many investigators showed that inoculation of biofertilizers increased N concentration in potato (Abd El-Ati *et al.*, 1996), (Ouda, 2000) in tomato and (Gadallah *et al.*, 2004) in spinach. Also, the data show that the leaves of inoculated onion plants by biofertilizer (AT+AZ) and received either 125 Kg $fed.^{-1}$ or 250 Kg $fed.^{-1}$, contained higher P concentration in both seasons than plants fertilized with the mineral-N fertilizer only. The enhancing effect of biofertilizer on increasing P concentration in the leaves could be attributed to the beneficial effects of AT+AZ bacteria on reducing soil pH by secreting organic acids (e.g. acetic, propionic, fumaric and succinic), which brought about the dissolution of bounds forms of P and render them available for growing plants (Ibrahim and Abd- El-Aziz, 1977).

Table (4): Influence of bio- and mineral-N fertilization on leaf pigments of onion plants in both seasons of 2003/2004 and 2004/2005.

Treatment		Chlorophyll ($mg\ g^{-1}$ fresh weight)			Carotenoids ($mg\ g^{-1}$ fresh wt.)
Bio-F	Mineral-N (Kg $fed.^{-1}$)	A	B	Total	
		2003/2004			
Control		0.252	0.109	0.439	0.051
-	62.5	0.348	0.193	0.566	0.070
-	125	0.421	0.259	0.866	0.103
-	250	0.448	0.301	0.948	0.123
AT+AZ	-	0.350	0.244	0.606	0.103
AT+AZ	62.5	0.419	0.299	0.750	0.105
AT+AZ	125	0.497	0.366	0.975	0.127
AT+AZ	250	0.519	0.420	1.062	0.143
$LSD_{(0.05)}$		0.067	0.055	0.096	0.018
		2004/2005			
Control		0.253	0.115	0.383	0.069
-	62.5	0.350	0.201	0.561	0.093
-	125	0.412	0.270	0.770	0.115
-	250	0.437	0.313	0.840	0.122
AT+AZ	-	0.342	0.251	0.613	0.103
AT+AZ	62.5	0.402	0.302	0.737	0.109
AT+AZ	125	0.458	0.382	0.912	0.137
AT+AZ	250	0.475	0.412	1.010	0.148
$LSD_{(0.05)}$		0.041	0.047	0.124	0.015

AT = *Azotobacter chroococcum* **AZ** = *Azospirillium brasilense*
Bio-F = biofertilizer **Control** = Untreated plants

Moreover, Hanafy *et al.* (1997) mentioned that using *Azotobacters* increased root surface, root hairs and root elongation. Thus, factors caused by application of biofertilizers could improve P uptake and its concentration in the leaves of onion plants. With regard to K concentration, a significant increase of K concentration was obtained from combination between biofertilizer with the treatments of mineral-N fertilizer as compared to the treatment which only received mineral-N fertilizer in both seasons. These results were in agreement with those reported by Ouda (2000) on tomato and Gadallah *et al.* (2004) on spinach.

Table (5): Influence of bio-and mineral-N fertilization on N, P and K (mg g^{-1} dry matter of leaves) and NO_2 and NO_3 (mg kg^{-1} dry matter of leaves) in both seasons of 2003/2004 and 2004/2005.

Treatment		N	P	K	NO_2	NO_3
Bio-F	Mineral-N (Kg $fed.^{-1}$)	(mg kg^{-1} dry matter of leaves)			(mg g^{-1} dry matter of leaves)	
		2003/2004				
Control		1.11	1.60	14.40	118.42	1594.26
-	62.5	1.22	2.20	15.70	139.10	1819.46
-	125	1.35	2.90	17.10	183.88	2056.23
-	250	1.48	3.30	18.02	215.71	2297.66
AT+AZ	-	1.28	2.70	16.40	56.28	637.21
AT+AZ	62.5	1.35	2.80	17.60	64.12	863.29
AT+AZ	125	1.57	3.45	18.80	77.43	1088.16
AT+AZ	250	1.61	3.82	19.11	79.37	1276.38
$LSD_{(0.05)}$		0.08	0.51	1.01	10.45	217.00
		2004/2005				
Control		1.19	1.90	14.71	122.42	1583.62
-	62.5	1.27	2.70	15.97	142.46	1779.84
-	125	1.38	3.32	17.60	184.96	2077.33
-	250	1.45	3.71	18.45	218.53	2298.58
AT+AZ	-	1.37	3.02	17.53	58.04	749.71
AT+AZ	62.5	1.34	3.21	18.22	62.08	853.21
AT+AZ	125	1.53	3.87	19.40	74.19	1131.07
AT+AZ	250	1.58	4.31	20.10	82.62	1253.46
$LSD_{(0.05)}$		0.06	0.48	0.87	9.97	131.03

Table (6): Influence of bio- and mineral-N fertilization on N, P and K (mg g^{-1} dry matter of bulbs) and NO_2 and NO_3 (mg kg^{-1} dry matter of bulbs) and total soluble solids (TSS%) in both seasons of 2003/2004and 2004/2005.

Treatment		N	P	K	NO_2	NO_3	TSS%
Bio-F	Mineral-N (Kg $fed.^{-1}$)	(mg g^{-1} dry matter of bulbs)			(mg kg^{-1}dry matter of bulbs)		
			2003/2004				
Control		1.24	2.05	4.80	39.75	506.18	11.83
-	62.5	1.32	2.45	5.60	51.09	671.48	12.17
-	125	1.49	2.90	6.50	60.65	787.13	12.33
-	250	1.58	3.15	7.10	66.99	860.86	12.50
AT+AZ	-	1.33	3.00	6.50	37.16	494.44	11.15
AT+AZ	62.5	1.38	3.45	7.00	43.10	571.48	12.67
AT+AZ	125	1.69	3.81	8.20	47.93	607.21	12.83
AT+AZ	250	1.73	4.22	8.47	49.13	640.22	13.11
$LSD_{(0.05)}$		0.06	0.39	0.04	3.68	50.11	N.S
			2004/2005				
Control		1.28	2.08	4.50	37.80	510.44	11.50
-	62.5	1.35	2.43	5.20	52.99	667.30	12.30
-	125	1.46	3.10	6.85	63.83	785.88	12.50
-	250	1.57	3.38	7.70	70.51	853.83	12.81
AT+AZ	-	1.41	2.82	6.15	37.55	489.03	11.25
AT+AZ	62.5	1.46	3.27	6.90	41.03	558.98	13.17
AT+AZ	125	1.62	3.86	7.95	49.15	616.69	13.63
AT+AZ	250	1.70	4.11	8.80	51.86	633.71	13.72
$LSD_{(0.05)}$		0.09	0.33	1.02	6.29	96.86	N.S

AT = *Azotobacter chroococcum* **AZ** = *Azospirillium brasilense*

Bio-F = biofertilizer **Control** = Untreated plants

Data in Tables (5&6) indicate that the treatment of the mixture of (AT+AZ) singly, showed a pronounced decrease in NO_2 and NO_3 concentration in leaves and bulbs of onion comparing to all other treatments. This decrease was;52.47% and 60.03% (in leaves) and 6.52% and 2.32% (in bulbs), respectively in the first season and 52.59% and 52.66% (in leaves) and 0.67% and 4.19% (in bulbs), respectively in the second one. As compared to the mineral-N levels, the decrease in the concentration of NO_2 and NO_3 (in the first season) was; 52.47%, 59.54% and 73.91% for NO_2 and 64.98, 69.01% and 72.27% for NO_3 (in leaves) and the decrease reached: 27.27%, 38.75% and 44.53% for NO_2 and 26.37%, 37.18% and 42.56% for NO_3 in bulbs as compared to the levels of 62.5, 125 and 250 Kg fed.$^{-1}$, respectively. In the second season, the decrease was: 59.26%, 68.36% and 73.44% for NO_2 and 57.88%, 63.91% and 67.38% for NO_3 (in leaves) and in bulbs the decrease reached; 29.14%, 41.17% and 46.75% for NO_2 and 26.72%, 37.77% and 42.73% for NO_3 as compared to the rates of 62.5, 125 and 250 Kg fed.$^{-1}$, respectively. Moreover, lower values of NO_2 and NO_3 concentration in leaves and bulbs were obtained from the treatments of the combination between biofertilizer (AT+AZ) and mineral-N fertilizer as compared to the treatments of mineral-N fertilizer only in both seasons. In this respect, the treatment of 62.5 Kg fed.$^{-1}$+(AT+AZ), caused a significant decrease in NO_2 and NO_3 concentration in the leaves and bulbs of onion in both seasons. In the first season, this decrease reached; 53.90% and 52.55% (in leaves) and 15.64% and 14.89% (in bulbs) for NO_2 and NO_3, respectively. The treatment of 125 Kg fed.$^{-1}$+(AT+AZ) showed a significant decrease in the concentration of NO_2 and NO_3 by 57.89% and 44.08% (in leaves) and 20.97% and 22.86% (in bulbs), respectively in the first season. In the second one, the decrease was; 59.89% and 45.55% (in leaves) and 23% and 21.53% (in bulbs) for NO_2 and NO_3, respectively as compared to the treatment of 125 Kg fed.$^{-1}$ only. The significant decrease in the concentration of NO_2 and NO_3 which was obtained by the treatment of 250 Kg fed.$^{-1}$ combined with (AT+AZ) as compared to the treatment of 250 Kg fed.$^{-1}$ singly in both seasons were; 63.21% and 44.45% (in the first season) and 62.19% and 45.47% (in the second season) for NO_2 and NO_3 (in leaves), respectively. In bulbs, the decrease reached; 26.66% and 25.63% (in the first season) and 26.45% and 25.78% (in the second season) for NO_2 and NO_3, respectively. In addition, the treatments of 62.5 and 125 Kg fed.$^{-1}$ in combination with the mixture of biofertilizer recorded a significant decrease for NO_2 and NO_3 which reached;70.27 % and 62.43%, respectively in the first season and by 71.59% and 62.88%, respectively in second one, as compared to the treatment of 250 Kg fed.$^{-1}$ alone. Thus, the combination between the treatments of mineral-N and biofertilizer had a greatly positive effect on total bulb yield and its components as compared to the treatments of mineral-N alone. It is worthy to note that, the application of biofertilizer alone or in combination with mineral-N fertilizer (at any level) produced lower values of NO_2 and NO_3 concentration in onion leaves and bulbs as compared to the plants which only received mineral-N fertilizer (regardless the level of mineral-N fertilizer). This might be due to the positive relationship between NO_2 and NO_3 concentration in leaves and nitrogen fertilization level. This result confirmed the suggestion that several plant species accumulate NO_3 as a result of excess of N uptake (Hanafy *et al.*, 1997 and 2000). The lower leaf NO_3 concentrations in biofertilizer treatments were attributed to the relative decrease in NO_3 uptake and increase in ammonium uptake due to the delayed mineralization and nitrification of the soil (Matsumoto and Yamagata, 1999). Hanafy *et al.*, (1997) indicated that using biofertilizers combined with 50% mineral-N supply, decrease NO_3 accumulation in Jew's mallow and radish leaves and this decrease may be due to the reduction in mineral-N application level. Also, it might be suggested that, under the effect of biofertilizers, some growth-promoting substances, e.g. auxins, gibberellins and cytokinins could be formed or released (Hartmann *et al.*, 1983). These phytohormones, especially cytokinins could be related to nitrate reductase content in plants which led to the reduction in NO_3 concentration. Knypl (1979) reported that, cytokinins enhance the activity of nitrate reductase and markedly enhanced the efficiency of nitrate reductase induction in many plants. Abdin *et al.* (1993) mentioned that, plant hormones like benzyladinine (as a cytokinin) increase the level of reductase gene expression.

Results of the present study clarify that the increase of chemical constituents by the increase of mineral-N fertilizer may be attributed to nitrogen fertilization which rise the capacity of plants to adsorb nutrients by the increase of root surface $unit^{-1}$ of soil volume as well as the high capacity of plants supplied with nitrogen in building metabolites as a result of nutrient uptake (Mandour *et al.*, 1986). Moreover, Midan (1995) mentioned that mineral-N fertilizer might promote metabolic processes within the plant, which could reflect a positive effect on chemical composition of pepper fruits but this is dependent on variety, soil fertility and cultivation date. In addition, many investigators explained the importance of biofertilizers in terms of reducing soil pH by secreting organic acids which bring about the dissolution of some bound nutrients and make them available for plants (Ibrahim and Abd-Aziz, 1977). The concentration of NO_3 in plant tissues is always in a dynamic state since it represents the differences between rate of N-absorption and rates of translocation and assimilation within the plant. These results confirmed the suggestion that several plants species accumulate NO_3 as a result of excess of N uptake over its reduction (Hanafy *et al.*, 1997 and 2000). Moreover, Rufty *et al.* (1982) reported that NO_3 is believed to accumulate in a storage pool; presumably in the vacuoles, from which it is not readily available. In the two seasons, bio-N showed lower values of NO_2 and NO_3 concentration in onion bulbs when compared with the plants which only received mineral-N fertilizer (regardless the different levels of mineral-N fertilizer). In addition, less values of NO_2 and NO_3 concentration were obtained by the treatments of bio-N in combination with mineral fertilizer in both seasons. In this respect, it is important to mention that, Hanafy *et al.*, (1997 and 2000) suggested that the increments in total soluble sugars concentrations in many plants play a role as an osmoticum and this might be implicated indirectly in decreasing NO_3 accumulation in plants.

The beneficial effect of biofertilizer (AT+AZ) on the detected chemical constituents of onion leaves and bulbs may be due to the releasing nitrogen and organic exudates and their role in facilitating the absorption of all nutrients by plants. Meanwhile, the increase in N, P and K uptake by plants could be, in general, due to the roots system size (Amara and Dahdoh, 1997). These results cope with the findings of El-Sawi *et al.* (2001); Abou-Ali and Gomaa (2002); Mohamed and El-Ganaini (2003) and Gadallah *et al.* (2004).

Volatile oil compounds:

The gas chromatography (GC) of onion volatile compounds as affected by mineral-N and/or bio-fertilization treatments is shown in Table (7) and Fig.1 (A,B,C,D,E,F,G and H) . In order to give a clear comparison for the effect of different treatments on the volatile compounds of onion oil, the total peak area% of the detected chemical classes of the volatile compounds were calculated and revealed in Table (7). The chromatograms (Fig. 1) show the presence of many compounds as eight of them were identified (Table, 7), while the other compounds remained unidentified due to the lack of authentic samples.

Table (7): Votalile compounds (%) in onion oil as affected by the application of mineral-N and/or biofertilization in the second season.

Compound*	Control	Mineral-N (Kg fed.$^{-1}$)			Bio-F	Bio-F and Mineral-N		
		62.5	125	250		62.5	125	250
Propyl allyl sulfide	4.06	1.68	1.96	4.99	2.21	7.32	8.69	15.55
Methyl propyl disulfide	0.29	1.39	1.54	0.30	2.47	1.20	2.16	2.82
Allyl propyl disulfide	0.73	0.07	1.20	0.91	0.11	1.80	1.25	1.78
Methyl propyl trisulfide	0.44	1.15	2.25	1.16	2.13	2.88	1.76	4.92
Methyl allyl trisulfide	0.46	1.73	0.41	1.79	2.12	0.28	0.94	10.54
Ethyl-cis-propyl trisulfide	0.65	0.56	0.44	0.46	0.22	1.54	1.79	2.29
Dipropyl trisulfide	3.61	4.33	2.47	4.60	1.76	4.11	5.86	7.05
Propenol-cis-trisulfide	0.87	0.87	4.73	1.86	1.71	1.06	2.04	1.48
Peak area%	**7.86**	**11.75**	**15.00**	**16.07**	**12.73**	**20.19**	**24.49**	**46.43**

***Values** expressed as % volatile compounds from onion oil.
Control = Untreated plants **Bio-F** = biofertilizer

The identified compounds presented in Table (7) comprised sulfur containing compounds such as mono, di, and trisulfides. These compounds were previously found in onion oil by Galleto and Bednarezyk (1975) and Hegazy *et al.* (1994). It is clear from data in Table (7) that volatile oil compounds of onion were considerably influenced by the previous treatments.

The GC analysis indicated that bulb sulfur compounds (expressed as a peak area%) mostly increased in response to increase the rate of mineral-N fertilizer in combination with bio-N fertilizer. The least values (peak area%) were generally noticed with the uninoculated control plants (7.86%). The highest values (46.43%) of volatile oil compounds were produced with plants inoculated with the mixture of bio-N fertilizer in the presence full dose of mineral-N fertilizer (100%N) followed by the treatment of half dose of mineral-N fertilizer (50%N) combined with bio-N fertilizer (24.49%).

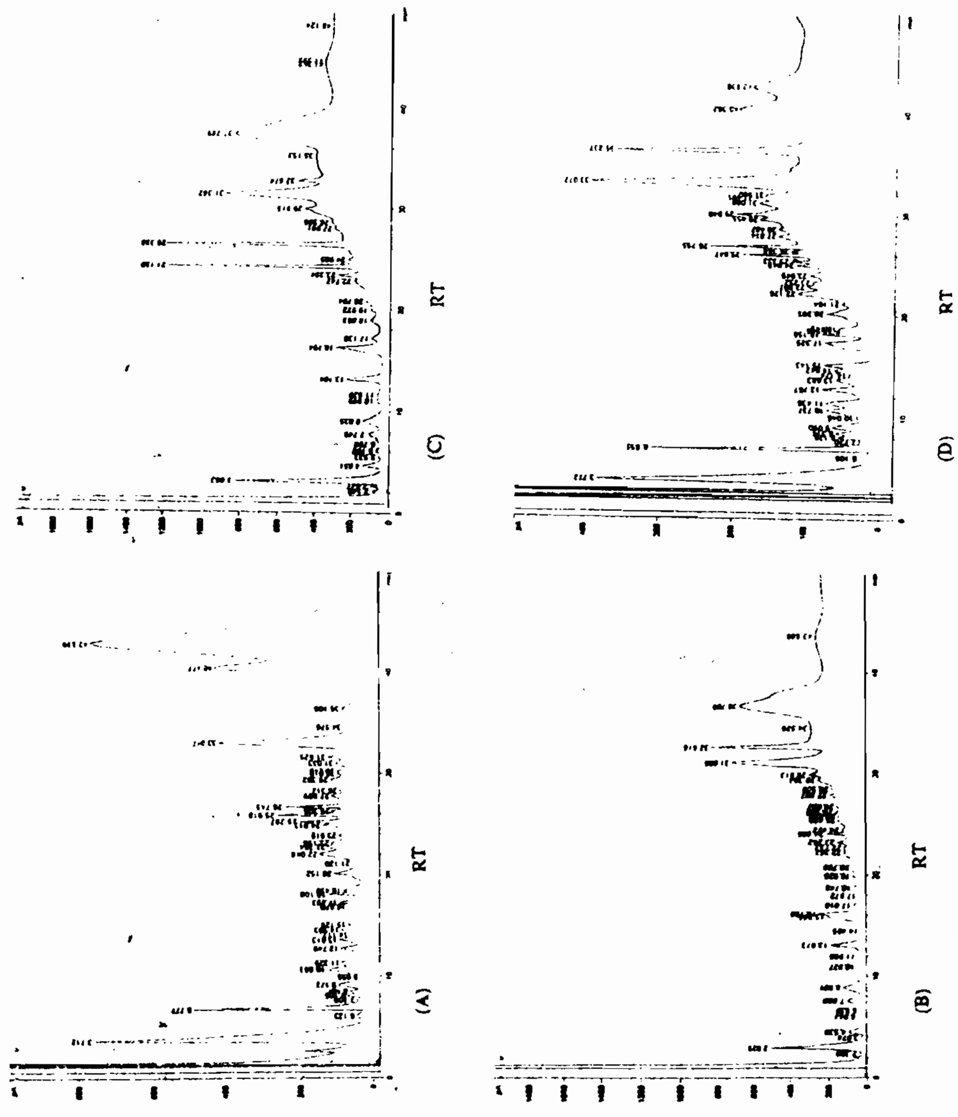

Fig. (1): The chromatograms of GC of Volatile compounds in onion oil as affected by the application of mineral-N and/or biofertilization.
A)- Control : Untreated plants. B)- 62.5 kg fed^{-1} mineral-N fertilizer.
C)-125 kg fed^{-1} mineral-N fertilizer. D)- 250 kg fed^{-1} mineral-N fertilizer

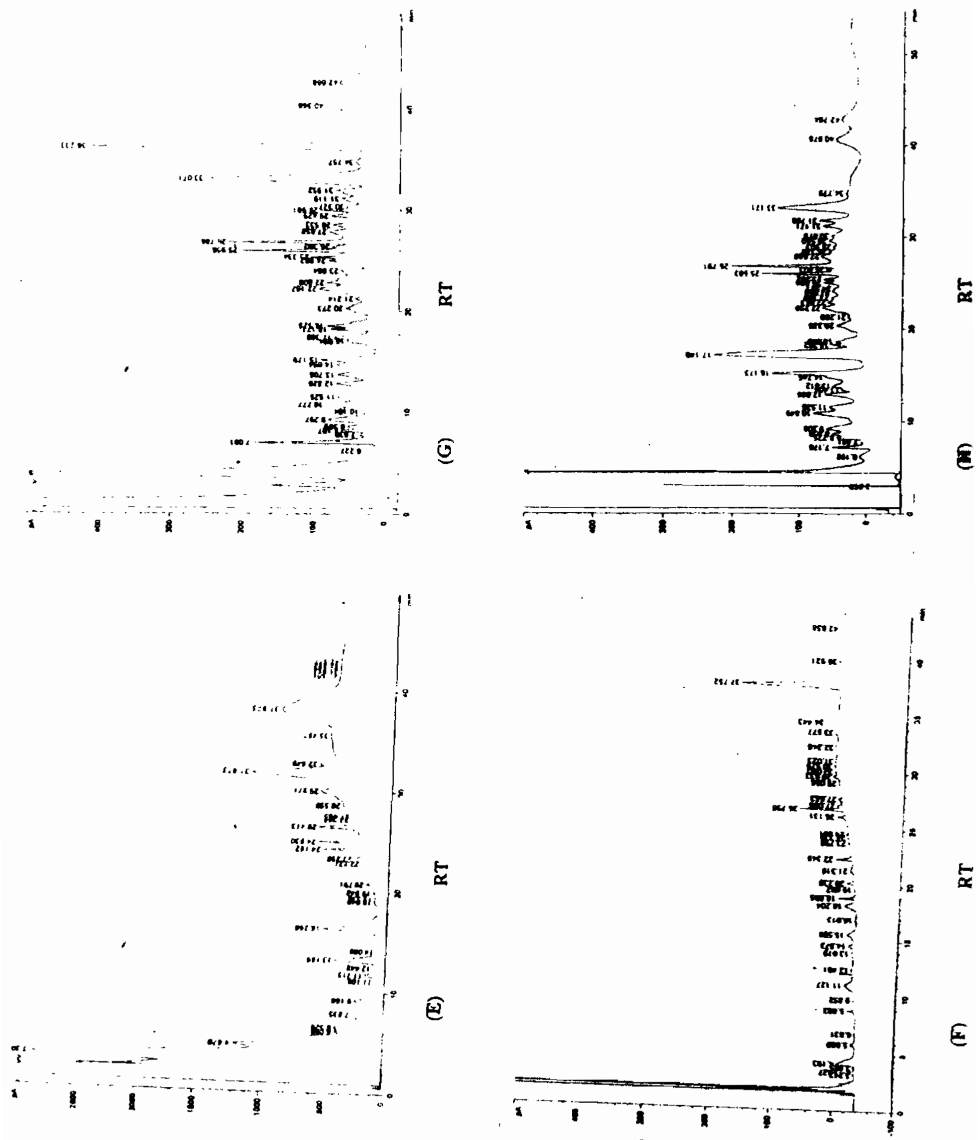

Fig. (1): The chromatograms of GC of Volatile compounds in onion oil as affected by the application of mineral-N and/or biofertilization.

E)- The mixture of *Azotobacter chroococcum* (AT) and *Azospirillum brasilense* (AZ) [1:1].

F)- 62.5 kg fed^{-1} mineral-N fertilizer+(AT+AZ).

G)- 125 fed^{-1} mineral-N fertilizer+(AT+AZ).

H))- 250 fed^{-1} mineral-N fertilizer+(AT+AZ).

The obtained data indicate that the major compounds in onion oil sample of control treatment were: propyl ally sulfide (4.06%) and dipropyl trisulfide (3.61%). while the other compounds were found in small quantities. Generally, it is clear that the concentration of propyl ally sulfide and dipropyl trisulfide was increased due to the inoculation with the mixture of bio-N fertilizer in the presence of mineral-N fertilizer reaching the maximum with 100%N combined with the mixture of bio-N treatment (15.55%) as compared to the control. Meantime, the concentration of methyl ally trisulfide was clearly increased due to the inoculation with the mixture of bio-N fertilizer in the presence of full dose mineral-N fertilizer (10.54%).

From the above mentioned results, it may be concluded that the change in mineral-N amount levels alone or inoculation with bio-N fertilizer strongly affect the biosynthetic formation of the volatile compounds in onion oil. In this respect, El-Sawy *et al.* (1986) stated that the content of hyoscyamine in *Hyoscyamus muticus* plants was considerably increased by dual inoculation with *Azotobacter* and *Azospirillium.* The concentration of alkaloids was greatly improved by inoculation with *Azotobacter* and *Azospirillium* (Salah *et al.*, 1998). Also, the inoculation with the mixture of *Azotobacter* and *Azospirillium* with full dose of inorganic-N fertilizer remarkedly increased the production of Khelin in *Ammi visnaga* plants (El-Sawy *et al.*, 1998) and increased methyl charvical concentration in *Ocimum basilicum* (Kandeel *et al.*, 2002).

Finally, it could by concluded that, the use of only bio-N fertilizers for the production of onion plants is insufficient, so, they must be used together with mineral-N fertilizer. Adding biofertilizer (namely, *Azotobacter chroococcum* and *Azospirillium brasilense*) to 50% of the mineral-N fertilizer (125 kg ammonium nitrate feddan^{-1}) induced an increase in the most studied plant growth parameters as well as the chemical constituents which in turn could be positively reflected on yield of bulbs. Such enhancement might be related to the improvement effects of biofertilizer on physical and biological properties of the soil which promotes the release of some nutrients and/or some growth substances in rooting zone in readily available forms which consequently might increase their uptake by plants. The application of biofertilizer (*Azotobacter* and *Azospirillium)* in combination with 125 kg feddan^{-1}; ammonium nitrate) is recommended for improving growth, yield (appr. 20.30% more over the recommended dose) and some chemical constituents including the major compounds of volatile oils which are responsible about the flavour and pungency of onion plants. In addition, it can be suggested that, the reduction of 50% from mineral-N fertilizer might help for reducing the production costs as well as diminishing the environmental pollution by minimizing the harmful effects of using chemical fertilizers on human health.

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تأثير التسميد الحيوى على نمو ومحصول البصل

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أجريت هذه الدراسة خلال موسمين متتاليين هما ٢٠٠٣/٢٠٠٤، ٢٠٠٤/٢٠٠٥ على نباتات البصل (صنف جيزة ٦) بإحدى المزارع الخاصة-الفيوم-مصر، وذلك لدراسة إمكانية إحلال التسميد الحيوى بدلا من التسميد المعدنى لنباتات البصل. استخدم فى هذه الدراسة نترات الأمونيوم (٣٣,٥% نتروجين) كمصدر للسماد النتروجيني المعدني، وذلك بمعدلات صفر، ٦٢,٥، ١٢٥و٢٥٠ كجم/فدان والتى تمثل الكنترول ،٢٥%، ٥٠% و ١٠٠% على التوالي من المعدلات الموصى بها لتسميد نباتات البصل.أضيف السماد المعدني الي المعاملات اما منفردا او مع التسميد الحيوى وهو عبارة عن خليط من الأزوتوباكتر والأزوسبيريلليم بنسبة ١:١ أوضحت النتائج المتحصل عليها مايلي:

- أدت معاملات التسميد النتروجيني المعدني سواء بصورة منفردة أو مع التسميد الحيوى (مخلوط من بكتريا الأزوتوباكتر و الأزوسبيريلليم) الى حدوث تأثير إيجابى فى كلا موسمي الدراسة وذلك لجميع صفات النمو التى تم دراستها (ارتفاع النبات - عدد الأوراق للنبات- الوزن الطازج والجاف لأوراق النبات - الوزن الطازج والجاف للبصلة للنبات الوزن الطازج والجاف الكلي للنبات).
- أظهرت معاملة التسميد النتروجيني المعدنى بمعدل ٢٥٠كجم/فدان بالإضافة إلي معاملة التسميد الحيوى أعلى زيادة فى صفات النمو سالفة الذكر وذلك مقارنة بجميع المعاملات الأخرى.
- أدت نفس المعاملات السابقة إلى حدوث زيادة معنوية فى المحصول الكلى للفدان مقارنة بباقى المعاملات.
- كان للتسميد النتروجيني المعدني بمعدل ٢٥٠كجم /فدان بالإضافة إلى التسميد الحيوى أثر واضح في زيادة تركيز الصبغات الورقية (الكلوروفيل و الكاروتينويدات) وكذلك محتوى الأوراق والأبصال من النتروجين-الفوسفور-البوتاسيوم فى كلا موسمى الدراسة).
- بالنسبة لتركيز النتريت والنترات فى كل من الأوراق والأبصال، فقد أدت معاملات التسميد الحيوى سواء بصورة منفردة أو مخلوطا مع السماد النتروجينى المعدنى إلى حدوث نقص معنوى فى تركيز النتريت والنترات فى كلا موسمي الدراسة.
- سجلت أعلى نسبة مئوية لمكونات الزيت الطيارة بواسطة المركبات: بروبايل اليل سلفيت- مثيل اليل تراى سلفيت- داى بروبايل تراى سلفيت، وذلك بتأثير المعاملة ٢٥٠كجم/فدان بالإضافة الى التسميد الحيوى.
- معاملة التسميد النتروجيني المعدني بمعدل ١٢٥كجم/فدان + التسميد الحيوى كان لها نفس التأثير للمعاملة ٢٥٠كجم/فدان + التسميد الحيوى حيث لم يكن هناك اى فرق معنوى بينهما في كل من موسمي الدراسة.
- وهكذا، فإن استخدام التسميد النتروجينى المعدني بمعدل ١٢٥كجم/فدان من نترات الأمونيوم فى وجود التسميد الحيوى (مخلوط من بكتريا الأزوتوباكتر و الأزوسبيريلليم) يؤدى إلى تحسين النمو والقدرة المحصولية (حدوث زيادة فى المحصول بمقدار ٢٠,٣٠% أعلى من معدل التسميد الموصى به) والمكونات الكيميائية بما فيها المكونات الرئيسية للزيت الطيار المسئوله عن الحرافة والنكهة فى البصل، علاوة على خفض تكلفة الإنتاج من خلال نقص كمية السماد النتروجيني المعدني المستخدم (بمقدار ٥٠%) مؤديا بذلك الى حدوث خفض فى معدل تلوث البيئية.