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USING SOME ISOLATES AND TRANSFORMANTS OF AZOTOBACTER TO REDUCE CHEMICAL NITROGEN FERTILIZER RATES IN GARLIC PRODUCTION

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ABSTRACT

The present investigation was conducted at the laboratories of Genetics and Horticulture departments, Fac. of Agric., Minia University and farm of Mallawy Horticulture Research Station. This study was carried out during the two successive winter seasons of 2000/2001 and 2001/2002 to study the effect of application different genotypes of *Azotobacter* (Transformation procedure) and different rates of chemical N fertilizers in garlic production (cv. Chinese). Wild type, four transformants of two *Azotobacter* species (two from each species) and three nitrogen rates i.e. 25%, 50% and 100% of the recommended dose (120 kg N/Fed.) were used. The effects of *Azotobacter* transformants and chemical nitrogen fertilizers and its interactions on growth characters fresh and cured yield, yield components and nitrate and nitrite content in cloves were studied.

The obtained results demonstrated that: (1)- DNA extract from two *Azotobacter* species (*A. vinelandii* and *A. chroococcum*) grown under 200 mg/ml chloramphenical was used to transform resistant to antibiotics isolates of the same species sensitive to antibiotics. The highest frequency of transformants ($100x10^{-5}$) was obtained when *A. chroococcum* was a donor and *A. chroococcum* was a recipient. Therefore, the highest frequencies were obtained from the intraspecific transformation and the lowest frequencies were obtained from the interspecific transformation. (2)- Inoculation garlic plants cv "Chinese" with either transformants or wild type strain of *Azotobacter* improved most growth characters and yield addition to reducing nitrate content in cloves. (3)- Fertilization with 60 and 120 kg. N/Fed. gave significantly higher values compared with 30 kg. N/Fed. in respect of bulb weight and clove weight, as well as total fresh and curd yields. (4)- The differences between 60 and 120 kg. N/Fed. were insignificant in most growth and yield measurements. (5) The *Azotobacter* transformants differed significantly each from other in their effects on garlic growth and yields. The transformants (T_3) and (T_4) showed the highest values in both seasons. (6) The nitrate contents in garlic cloves samples were increased with increasing the applied N-chemical fertilizer to the combined biofertilizer treatment. An increase (21.8%) in nitrate level was detected in garlic samples produced from plants treated with chemical fertilizers compared with the *Azotobacter* wild type treatment. Generally the plants fertilized with chemical fertilizer contained highest value of nitrate (217 mg/kg) than those biofertilized with *Azotobacter*.

In conclusion, inoculation garlic plants with *Azotobacter* bacteria particularly transformants could be reduced using the chemical nitrogen fertilizer by about 25% from the recommended dose to obtain the same values of growth and yield. Moreover, nitrate content in cloves was reduced after inoculation garlic plants with *Azotobacter* transformants.

INTRODUCTION

Garlic (*Allium sativum* L.) is one of the oldest cultivated crops in the world. Egypt is considered to be a major producer for garlic in the world. In Egypt, particularly El-Minia governorate, garlic is grown widely for exportation and/or local consumption. The cultivated area of the garlic was about 9600 feddan in 2001 at El-Minia governorate. Thus, El-Minia governorate is considered the main production area of garlic in Egypt.

Many investigations have been done to study the effect of fertilizing garlic plant with different rates of nitrogen on the yield, its components and quality. Recently, nitrogen fertilizers have been increased in garlic cultivation. Whereas, El-Behaidi (1983), Maksoud *et al.* (1983) and Abdel-Hameid *et al.* (1991) reported that growth characters of garlic plants i.e. plant height, bulbing ratio, number of leaves / plant were improved with increasing the N level. Moreover both yields (first and second season) and yield components were increased with increasing N level up to 120 kg N/fed. as reported by Cardemas *et al* (1986), Hilman and Noordiyati (1988) and Osman *et al.* (1990). Zhang *et al.*, (1996) reported that nitrogen fertilizers applications are expected to be double or even triple within the next 30 years, making the problem of nitrogen fertilizer related to the pollution even more serious.

The major problem facing the farmers is that they can not afford the cost of these chemical N fertilizers. Moreover, in countries where fertilizers production relies on important raw material, the costs are even higher for farmer and the country. Besides chemical fertilizers production and utilization are considered as air, soil and water polluting operations. Moreover, after chemical nitrogen fertilization both nitrate and nitrite are present in water and vegetables such as garlic and tomato vary enormously, ranging from 1 to 1000 mg/kg of fresh weight (Maff, 1987). Therefore ingested food and water are the main sources of nitrate and nitrite exposure in an individual (Stuehr and Marletta, 1987). The possible bad effect of nitrate some products of the segregated pesticides to form nitrosamines. Nitrosamines compounds have been proved to cause cancer disease and mutations for animal and human cell. Nitrate levels in plant tissue should not exceed certain level - High nitrate content (1-2% in dry weight) in forage can be toxic to raminants (Prins, 1983).

Application of biofertilizers is important economically to reduce the cost of fertilizers and ecologically to reduce pollution of the environment (Rao and Tarafalx, 1990; Verma 1990; Shinde *et al.*, 1991; Manga, 1994). *Azotobacter* is non-symbiotic nitrogen fixing aerobic bacteria *Azotobacter* is highly motile and lives in close association with the roots of several grasses and crop plants (Okon, 1982 and El-Haddad *et al.,* 1993).

Genetic studies in *Azotobacter* and *Azosperillum* are essential for identifying the genes involved in nitrogen fixation, plant growth hormone production and other important phenotypes e.g. the complicated association of the bacterium with plant roots. A better understanding of these processes might help to improve the *Azotobacter* plant association in terms of crop yield. Although no indigenous genetic system e.g. conjugation, transformation or transudation, has been described for Azobacter, genetic material carried on

broad host range plasmids can be transferred and expressed in this bacterium (Singh, 1982).

Azotobacter chroococcum which in addition to its ability for nitrogen fixation is able to secrete same growth promoting substances, weak antibiotics and antifungal compounds (Pandey and Kumer, 1989); on potato plant. Also, *Azotobacter* resulted in a high increase in total microbial counts in rhizospere of soybean plants, addition to total N-content of plants and seeds which considerably increases by application of *Azotobacter* (Amara and Nas, 1995 and Wange, 1995). Improving yield and quality of carrot, potato and tomato has been obtained by inoculation with *Azotobacter* transformants have been reported by Dakhly and, Abdel-Mageed 1997. There fore the main objective of this study is to tray to reduced of the amounts of chemical N. fertilizer which have been applied to garlic plants by inoculation with different genotypes of *Azotobacter*.

MATERIALS AND METHODS

Laboratory experiments:-

1- Materials:-

a- Strains:-

Two wild types of *Azotobacter* species were used in this study. These wild types which were originally isolated in Genetics laboratory by (Abdel-Rahem *et al.*, 1995 and Dakhly and Abdel-Mageed, 1997).

b- Media:-

Complete medium (Strandberg and Wilson, 1968) for growing *Azotobacter*.

2- Methods:-

Transformation experiments:-

Isolation of chloramphenical resistance:-

Chloramphenical resistance was isolated from the wild types of *Azotobacter vinelandii* and *Azotobacter chroococum* to be used as a selective marker in transformational study. Eight concentrations of chloramphenical (25.0, 50.0, 75.0, 100.0, 125.0, 150.0, 175 and 200.0) were applied. One loop from each isolate was added to 5 ml sterilized distilled water in a test tube. 0.1 ml sample from each isolate of suitable dilution were plated on complete medium (five plates for each). The plates were incubated at 30°C for 3 days. Single colonies were tested on CM and CM supplemented with different concentration of chloramphenical at 30°C for 5 days and then the sensitive and resistant colonies were selected.

Transformation procedure:-

Total DNA of resistant colonies from two wild types of *Azotobacter* was extracted from 200 ml of late log-phase culture according to the procedure of Marmur (1961). The *Azotobacter* transformants were obtained using Page and von Tigerstrom (1979) procedure.

Field experiments:

Two experiments were conducted during the two successive winter seasons of 2000/2001 and 2001/2002 in a clay loam soil (The chemical and physical properties of this soil are presented in Table 1 at Mallawy Horticulture Research station, El-Minia, Egypt.

Table (1): The chemical and physical properties of studies soil.

Soil constituents	Physical properties				M.O.	Availble N%	E.C
	Sand	Silt	Clay	Texture			
Season 2000/2001	13.75	46.13	40.12	Slit clay	1.35	0.10	0.62
Season 2001/2002	13.74	46.12	40.14	Slit clay	1.37	0.12	0.76

Three nitrogen rates i.e. 30,60 and 120 kg. N/Fed. (the recommended dose 120 kg N/Fed.) in the form of ammonium sulphate 20.6% were used. Nitrogen fertilizer was splited into three equal doses and applied at 30, 60 and 90 days after planting. Four transformants of *Azotobacter chroococcum* and *Azotobacter vinelandii* addition to mixture from two wild types strain were used. The suspensions of each of these transformants and isolate, were mixed with white clones before inoculation cloves bulb planting and also were added to the plants after 20 days from sowing.

The experiment was arranged in split-plot design with four replicates. The chemical nitrogen rates were randomly in the main plots, while the bio-inoculation treatments were randomly arranged in the sub-plots. The plot area was 12 m^2 and consisted of 5 rows, 60 cm wide and 4 m long. Garlic cloves were planted on 15th October in the first and second season on both sides of each ridge at 7 cm apart.Calcium superphosphate (15.5 % P_2O_5) were added at rate of 300 kg/fed. during soil preparation potassium sulphate (48% K_2O) at rate of 150 kg/fed were added as 100 kg after 60 days and 50 kg after 95 days from planting. Other culture practices were carried out as recommended. The following data were recorded:-

(1) After 120 days from planting; ten plants from each experimental plot were taken randomly to determine the following parameters:-

a- Plant height (cm).
b- No. of leaves plant.
c - Leaves fresh weight/plant, gm.
d- Leaves dry weight/plant, gm.

(2) Bulbing ratio:

This character was measured during growth period whereas 5 plants were taken randomly of each experimental plot after 100, 130 and 160 days planting. It was measured as neck diameter/bulb diameter (cm).

(3) Fresh and cured yield:-

Garlic plants were harvested on the 24th and 25th of the April in the first and second season respectively . Garlic plants in the plot were weighted and total fresh yield was calculated. Garlic plants were left in the field to be cured for 3 weeks and after curing the cured yield kg/plot was calculated.

Bulb quality:

Five plants from each experimental plot were taken and the following parameters were measured:-

a- bulb fresh weight (gm). c- cloves number/bulb.

b- bulb dry weight (gm) after curing.. d- clove weight (gm).

5- Determination of nitrite (NO_2^-) and nitrate (NO_3^-):

Samples of cured cloves from e ach experimental plot were taken at random to determine nitrite (NO_2^-) and nitrate (NO_3^-) according to method described by Saad, (1991).

All data were statistically analyzed a nd treatments mean were compared using L.S.D. (at 0.05 level) meth od according Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Effect of antibiotic on *Azotobacter* species

Data in Tables (2 and 3) showed the effect of different levels of antibiotic (chloramphenicol) on number of antibiotic sensitive and resistant colonies on agar medium for *Azotobacter* species, which used as a recipient or donor in transformation. The results in these tables showed that the increase in dose rate of antibiotic chloramphenicol decreased the number of antibiotic resistant colonies of *Azotobacter vienlandii* and *Azotobacter chroococumi*. The complete medium (CM) not amended with antibiotic was used as a control. The less number of colonies (35.50 and 17.5 as means) were isolated at the concentration of 200 ? m/ml chloramphenicol with *Azotobacter vienlandii* and *Azotobacter chroococumi*.

Table (2): Effect of different levels of chloramphenicol on number of resistant antibiotic colonies of *Azotobacter vienlandii*

Conc. (µg/ml)	Replicates				Mean
	I	II	III	IV	
0.0	175	250	150	325	225.0
25.0	171	237	137	314	214.75
50.0	159	225	117	301	200.50
75.0	144	193	88	271	174.00
100.0	120	161	66	235	145.50
125.0	114	134	43	202	123.25
150.0	98	100	25	165	97.00
175.0	47	57	11	130	61.25
200.0	25	31	4	82	35.50

Data in Table (3) indicate also that frequencies of transformants, resulted from intraspecific crosses, are higher than frequencies resulted from interspecific crosses in all cases. Transformants were obtained with higher frequencies when donor DNA and recipient cells were incubated 2 hours that which incubated 1 hour. The higher frequency of transformants was obtained

from crossing of *Azotobacter chroococcum* donor X *Azotobacter vinelandii* recipient which have equal frequencies ($75x10^{-6}$ and 100×10^{-6}) for both 1 and 2 hours incubation respectively. The same trend was observed with using *A. vinelandii* as a donor and *A. vinelandii* as recipient ($52 X 10^{-6}$ and $68 X 10^{-6}$) for 1 and 2 hours respectively). The lower frequency in resulted from the cross *Azotobacter vinelandii* donor DNA x *Azotobacter chroococcum* cells (22×10^{-6} and $38 X 10^{-6}$)for both 1 and 2 hours incubation respectively.

Table (3): Effect of different levels of chloramphenicol on number of resistant antibiotics colonies of *Azotobacter chroococum*.

Conc. (µg/ml)	Replicates				Mean
	I	II	III	IV	
0.0	150	125	100	200	143.75
25.0	145	124	98	193	140.00
50.0	138	114	92	178	130.50
75.0	129	93	75	152	112.25
100.0	117	91	61	129	99.50
125.0	108	78	43	98	81.75
150.0	72	46	27	72	54.25
175.0	57	24	12	41	33.50
200.0	31	10	7	22	17.50

These results indicate that the highest frequencies transformants were obtained from mixing DNA from one of two wild types under study with *Azotobacter chroococcum* recipient cells for 1 and 2 hours incubation.

Table (4): Frequencies of transformants resulted from intraspecific and interspecific crosses between two wild types of sensitive or resistance *Azotobacter* .

Crosses	Recipient (sensitive)			
	A. chrococcum		*A. vilandii*	
	donors (resistance to 200.0 ? g chl. ml			
Treatment	*A.chrococcum*	*A. vienlandii*	*A. vienlandii*	*A. chrococcum*
Control				
No.	12000.000	18260.000	19180.000	19000.000
frequ. %	100	100	100	100
Transformented				
1 (hr)	9	4	10	8
freq. %	$75x10^{-6}$	$22x10^{-6}$	$52x10^{-6}$	$42x10^{-6}$
2 (hrs)	12	7	13	10
freq. %	100×10^{-6}	$38x10^{-6}$	$68x10^{-6}$	$52x10^{-6}$

These results may be attributed to the competence (ability of a cell to be transformed) of the recipient cells. In pneumococcus, Iyer and Ravin (1962) concluded that for any given transforming DNA, differences in the frequency of transformation of a recipient population may therefore be attributed to differences in the competence of the population. Beattie and

Selow (1970) found that heterospecific transformation between different species of Haemophilus was lower 1-6 times that homospecific transformation. Similar results were obtained in Rhizobia species by several authors (Raina and Modi, 1971; Rifaat *et al.*, 1974; Heumann and Springer, 1977 and Ali *et al.*, 1980).

Field experiments:

Plant height

Data presented in Table (5) showed that the inoculation garlic plants with either wild type strain or transformants of two species of *Azotobacter* increased plant height compared with uninoculated plants, but the differences were significant in the second season only.

Among different transformants the T_2 followed by the T_1 in the first season and T_4 followed by T_2 in the second season showed the highest values of plant height. These effects for inoculation with *Azotobacter* strain on plant height character particularly with transformants were reported by many investigators i.e. Dakhly and Abdel-Mageed (1997).

Regarding to the chemical fertilization effects on plant height of garlic plants, results in Table (5) showed that the second and third rates (60 and 120 kg. N/Fed.) had significant effects to increase this trait compared to the first rate (30 kg. N/Fed.). The differences between the second and third rate were not significant as data showed. This mean that using the second rate was enough to obtain the heights values of this character.

Number of leaves/plants:

Data presented in Table (5) showed that number of leaves of garlic plants was unaffected by inoculation with *Azotobacter* strains either wild type or transforamants in both seasons.

On the other hand, the chemical fertilizer rates showed significant effect on this trait. The second and third rates showed significant increased in number of leaves/plant compared to the first rate in both seasons. This mean that this character need to high levels of nitrogen to aberrance. For this reason, the using of chemical nitrogen fertilizers was very important to obtain the large number of leaves.

Leaves fresh and dry weight/plant:

Inoculated garlic plants with either wild type strain or some transformants of *Azotobacter* increased significantly both leaves fresh and dry weights/plants as shown in Table (5). The highest values of leaves fresh weight/plant was obtained after inoculated garlic plants with wild type strain (33.242 g/plant) and T_4 (42.442 gm/plant) in the first and second season respectively. While the lowest values of this trait was obtained with those plants which uninoculated (control plants) in both seasons. These results showed that the inoculation garlic plants with *Azotobacter* isolates were important to improve this trait.

The highest values of fresh and dry weight of leaves/plant were obtained when 60 kg N/Fed was applied in both seasons except with the dry weight of leaves in the second season which obtained after using 120 kg

Table (5): The effects of inoculation with *Azotobacter* transformants and rates of nitrogen fertilizers on plant height (cm); number of leaves and bulbing ratio in two seasons 1999/2000 and 2000/2001

Isolates (A)	Chemical Fertilization (B)	Season 1999/2000				Season 2000/2001			
		Plant height (cm)	No. of leaves	Leaves fresh weight/plant	Leaves dry weight/plant	Plant height (cm)	No. of leaves	Leaves fresh weight/plant	Leaves dry weight/plant
I	1	58.325	8.100	28.550	5.200	61.750	8.325	31.425	6.125
W.T.	2	65.125	8.400	36.575	7.725	68.225	8.575	46.875	8.025
	3	64.650	8.350	34.600	7.487	66.575	8.150	45.650	8.725
Mean A		62.700	8.283	33.242	6.804	65.517	8.350	41.317	7.625
II	1	60.225	7.875	21.300	5.200	62.200	8.375	28.750	5.625
T1	2	65.600	9.175	35.325	7.625	67.325	8.550	45.375	7.900
	3	65.300	8.125	43.200	7.600	66.650	8.525	44.575	8.525
Mean A		63.708	8.392	30.275	6.808	65.392	8.483	39.567	7.350
III	1	60.000	8.100	24.125	5.575	63.575	8.325	32.625	6.350
T2	2	66.620	9.100	37.175	8.200	67.850	8.550	47.225	8.325
	3	66.250	8.350	35.700	7.725	65.725	8.300	46.125	9.300
Mean A		64.492	8.517	32.333	7.167	65.717	8.392	41.992	7.992
IV	1	59.195	8.075	22.225	4.900	61.175	8.375	30.575	6.100
T3	2	64.650	9.150	36.150	8.200	66.600	8.600	45.600	8.825
	3	64.150	8.250	35.475	7.550	66.175	8.050	45.100	8.750
Mean A		62.658	8.492	31.283	6.883	64.650	8.342	40.425	7.892
V	1	61.225	8.225	24.525	5.625	64.600	8.425	33.150	6.550
T4	2	63.325	9.350	36.625	8.250	69.900	8.825	47.600	8.300
	3	64.225	8.100	35.675	7.600	67.575	8.575	46.575	7.925
Mean A		62.925	8.558	32.275	7.158	67.358	8.608	42.442	7.592
VI	1	58.575	7.725	21.600	3.625	56.600	8.400	24.300	5.00
Control	2	60.200	8.300	32.150	6.575	63.700	8.650	42.900	8.250
	3	65.100	9.200	36.600	8.225	66.825	8.950	46.250	7.925
Mean A		61.292	8.408	30.117	6.142	62.375	8.667	37.483	7.058
Mean B	B1	59.688	8.017	23.721	5.021	61.650	8.371	30.137	5.958
	B2	64.254	8.912	35.667	7.762	67.267	8.625	45.762	8.271
	B3	64.946	8.396	35.375	7.698	66.587	8.425	45.712	8.525
LSD	A	N.S	N.S	4.281	0.482	1.724	N.S	0.301	2.001
	B	2.341	0.236	2.324	0.261	2.134	0.238	0.263	2.617
	AB	2.497	0.245	4.641	0.863	2.781	0.451	0.718	3.411

N/fed with insignificant differences. On the other hand, the lowest values of this trait were obtained with the lower rate (30 kg/fed.) of fertilizers on both seasons. These increasing in leaves weights may cause increased, the photosynthesis products in the vegetative growth. These improvements in photosynthesis products help to improve yield and yield components of garlic plants.

Regarding the effects of nitrogen rates on fresh and dry weights of leaves/plant, the obtained results in Table (5) showed significant effect in both seasons.

The interaction effects showed significant differences on this trait. The highest values of leaves fresh weight/plant were obtained with fertilized garlic plant with the highest rate of fertilizer (120 kg N/plant) and inoculation these plants with (T_1) and T_4 in the first and second season respectively. In the meantime the highest values of leaves dry weight/plant were obtained with those plants which inoculated with T_4 and fertilized with the second rate of fertilizer in the first season. While in the second season the highest values were obtained after inoculated plants with T_2 and fertilized these plants with the highest rate of fertilizers. These results are in partial agree with those obtained by Osman *et al* (1990) that increasing N levels had increased leave weights.

Bulbing ratio:

Data presented in Table (6) indicated insignificant differences in both seasons in bulbing ratio between inoculated plants and uninoculated. Also, the differences among different isolates were not significant on this trait in both seasons.

Regarding to the effects of the chemical fertilizers rates data in Table (6) showed that the bulbing ratio was decreased significantly with increasing the fertilizer rate. These results were in line with the feeding with Shalaby *et al.* (2002), who found that garlic plants received both organic and bio fertilizers formed earliest bulbing than those plants which received chemical fertilizers. The high rate of fertilizer may give high vegetative growth which reduces the bulbing ratio.

Bulb fresh and dry weights (g):

Data in Table (7) showed that both fresh and dry weights of garlic bulb were significantly increased in both seasons after inoculation of plants with either wild type strain or tansformants of *Azotobacer* in comparison to uninoculated plants. Observed increasing in bulb weights might be due to the increasing in cloves number and weights in accordance to inoculation with *Azotobacter*. Inoculated plants with T_4 showed the highest values of bulb fresh and dry weights in both seasons except for the bulb fresh weight in the second season where T_3 was the best. On the other hand control plants (theses plants which uninoculated) showed lowest values of these traits in both seasons.

Table (6): The effects of inoculation with *Azotobacter* transformants and rates of nitrogen fertilizers on Bulbing ratio, Yield weight kg/plot and cured yield (ton/fed) in two seasons 1999/2000 and 2000/2001

Isolates	Chemical	Season 1999/2000			Season 2000/2001		
(A)	fertilization (B)	Bulbing ratio	Yield weight kg/plot	cured yield (ton/fed)	Bulbing ratio	Yield weight kg/plot	cured yield (ton/fed)
I	1	0.452	15.300	2.957	0.413	14.578	3.404
W.T.	2	0.406	22.675	5.793	0.396	21.357	6.322
	3	0.435	21.600	5.644	0.367	20.302	6.380
Mean A		**0.431**	**19.858**	**4.798**	**0.392**	**18.746**	**5.369**
II	1	0.452	14.600	2.598	0.413	14.720	3.532
T1	2	0.431	22.225	5.567	0.373	21.100	6.523
	3	0.396	20.750	5.428	0.366	20.050	6.612
Mean A		**0.426**	**19.192**	**4.531**	**0.384**	**18.473**	**5.556**
III	1	0.441	16.325	3.260	0.412	15.345	3.418
T2	2	0.421	23.600	6.337	0.367	21.700	6.627
	3	0.399	20.675	6.179	0.366	21.283	6.530
Mean A		**0.420**	**20.200**	**5.259**	**0.382**	**19.443**	**5.525**
IV	1	0.451	15.400	3.277	0.414	14.267	3.493
T3	2	0.421	22.325	6.410	0.353	21.380	6.545
	3	0.401	21.375	6.268	0.352	20.275	6.810
Mean A		**0.424**	**19.700**	**5.318**	**0.373**	**18.641**	**5.616**
V	1	0.442	16.675	3.495	0.398	15.550	3.720
T4	2	0.420	24.175	6.548	0.341	22.277	6.740
	3	0.414	22.725	6.482	0.359	21.713	6.515
Mean A		**0.425**	**21.192**	**5.508**	**0.366**	**19.847**	**5.658**
Vi	1	0.459	14.350	2.481	0.422	18.145	2.712
Control	2	0.441	21.325	4.332	0.413	19.347	4.717
	3	0.422	25.950	6.563	0.362	21.375	6.620
Mean A		**0.441**	**20.542**	**4.455**	**0.399**	**19.622**	**4.683**
	B1	0.449	15.442	3.009	0.412	15.359	3.380
Mean B	B2	0.423	22.721	5.831	0.374	21.194	6.246
	B3	0.411	22.179	6.094	0.362	20.833	6.578
	A	n.s	1.902	0.110	n.s	n.s	0.078
LSD	B	0.98	1.405	0.718	0.193	2.550	0.110
	AB	0.56	2.535	0.182	0.272	3.092	0.704

Also, chemical fertilizer rates of nitrogen had significant effects on both bulb fresh and dry weights. Using the higher rates (second and third rats) of nitrogen fertilizer increased significantly bulb fresh and dry weights in both seasons, as results shown in Table (7). The differences between using the second and third rate of nitrogen fertilizer had significant effects in both seasons on weights of fresh and dry bulbs.

Regarding to the interaction effects data in Table (7) showed that there are significant effects among bio- and chemical fertilizers on fresh and dry weights of bulbs in both seasons.

Table (7): The effects of inoculation with *Azotobacter* transformants and rates of nitrogen fertilizers on bulb fresh weight (g), bulb dry weight during harvest, cloves No./bulb and cured yield (ton/fed) in two seasons 1999/2000 and 2000/2001

Isolates	Chemical	Season 1999/2000				Season 2000/2001			
(A)	Fertilization (B)	bulb fresh weight	bulb dry weight (g)	Cloves No./bulb	Cloves weight (g)	Bulb fresh weight	bulb dry weight (g)	Cloves No./bulb	Cloves weight (g)
I	1	32.100	17.750	16.375	1.518	34.550	20.325	16.550	2.100
W.T.	H	43.325	25.300	17.500	2.095	45.300	27.550	17.675	2.400
	3	42.575	24.575	17.209	2.295	44.325	26.125	17.525	2.025
Mean A		39.333	22.542	17.025	1.969	41.392	24.667	17.250	2.175
II	1	31.575	17.125	16.450	1.425	34.225	19.625	16.525	2.100
T1	2	43.650	24.525	17.575	2.178	44.175	25.525	17.425	2.175
	3	42.700	24.175	17.300	2.340	42.550	25.550	17.550	2.175
Mean A		39.308	21.942	17.105	1.981	40.317	23.933	17.167	2.150
III	1	32.550	18.250	16.550	2.030	35.575	20.675	16.650	2.475
T2	2	44.200	25.600	17.700	2.155	44.725	26.125	17.700	2.425
	3	43.625	24.175	17.400	2.213	46.550	24.575	17.525	2.125
Mean A		40.125	22.675	17.217	2.133	42.283	23.792	17.292	2.342
IV	1	31.575	18.300	17.150	1.617	36.850	20.180	17.275	2.300
T3	2	43.160	24.550	17.525	2.110	45.175	25.675	17.525	2.400
	3	42.625	24.175	17.200	2.160	44.200	25.125	17.525	2.100
Mean A		39.120	22.342	17.252	1.989	42.075	23.660	17.442	2.267
V	1	31.650	18.600	17.425	1.815	34.175	21.200	17.350	2.675
T4	2	43.675	26.075	17.700	2.515	44.325	26.625	17.525	2.325
	3	43.600	25.875	17.350	2.298	43.100	26.200	17.550	2.025
Mean A		39.642	23.517	17.492	2.189	40.533	24.675	17.475	2.342
VI	1	31.200	15.275	16.925	1.408	33.250	18.200	17.175	2.100
Control	2	32.050	19.443	17.200	2.248	37.625	22.600	17.550	2.425
	3	43.575	23.650	17.575	2.830	45.650	25.600	17.575	2.300
Mean A		35.608	19.456	1.233	2.162	38.842	22.133	17.433	2.275
	B1	31.775	17.550	16.613	1.649	34.771	20.034	16.921	2.292
Mean B	B2	41.677	24.249	17.533	2.217	43.554	25.867	17.567	2.358
	B3	43.117	24.438	17.337	2.346	44.396	25.529	17.542	2.125
	A	2.774	2.214	n.s	1.103	1.530	1.206	n.s	n.s
LSD	B	2.642	2.336	0.298	2.618	1.359	2.090	0.214	0.631
	AB	4.318	3.388	0.485	4.286	2.143	3.376	0.370	0.556

Number of cloves/bulb:

Data presented in Table (7) showed that the inoculation process of garlic plants with *Azotobacter* strain had insignificant effects on number of cloves/bulb in both seasons. On the other hand the high rates of nitrogen fertilizer showed significant increased in number of cloves/bulb in both seasons compared to the lower rate as shown in Table (7). The differences between the second and third rate was not significant on this trait. These results are in the contrast with those which obtained by Cardemas (1986) who found that application of N fertilizer at several levels did not affect cloves per bulb.

Regarding to the interaction, effects, data show that significant differences. The highest number of cloves/bulb (17.70) was obtained after inoculation with T_4 and T_2 in the first and second season respectively, and fertilization with 60 kg. N / Fed. of chemical fertilizer.

Clove weight (g):

Inoculation garlic plants with those transformants in our investigation of *Azotobacter* caused significant increases in clove weight compared to both of uninoculated plants or those, which inoculated, with wild type strain of *Azotobacter* as shown in Table (7).

Also, the clove weight (gm) was increased with using high rates of nitrogen fertilizers in both seasons, but the differences were significant in the first season only. The highest significant values of cloves weight were obtained after inoculated garlic plants with T_4 and fertilized it with the second rate and first rate of chemical fertilizers in the first and second season respectively. These results are in partial agree with those obtained by Osman *et al* (1990) who found that increasing N levels had increased clove weight.

Fresh yield weight kg/plot:

Data of this character are presented in Table (6) the fresh yield weight kg/plot was increased after inoculated garlic plants with some *Azotobacter* transformants particularly with (T_4) in both season, but the differences were significantly in the second season only. These results agree with those obtained with Shalaby *et al.* (2002).

Clear significantly increasing was observed with using the second rate of chemical fertilizer (60 kg/Fed.) compared with the first rate as shown in Table (6). This increasing was about 6 and 7 kg/plot in the first and second season respectively. In meantime the differences between the second third rate of fertilizer were insignificant in both season. These results mean that the second rate of fertilizer was sufficient to obtained the highest fresh yield as kg/plot in both season. These results agree with those obtained by Cardemas *et al* (1986), El-Beheidi (1983) Osman *et al* (1990) who found that increasing N level increased the yield of garlic.

Cured yield Ton /Fed.:

In Table (6) data showed that the inoculation garlic plants with either wild type stain or transformants of *Azotobacter* significantly increased cured yield (ton/Fed.) for both season compared with those uninoculated plants.

The highest values of cured yield (ton/Fed.) were obtained after inoculated plants with T_4 (5.508 and 5.658 ton/Fed.) in the first and second season respectively. In meantime the lowest values of this trait were obtained with those plants which uninoculated. This increasing in cured yield of garlic plants after inoculation with biofertilizer may be due to its effects on plant growth improvement i.e. number of leaves/plant and fresh and dry weight of plants. It is well known that *Azotobacter* bacteria have ability to fix nitrogen and supply the growing plants with it. Moreover, this bacteria had a role to inhance the phytohormones in plants that could stimulate the elements absorption and translocation which in turn improve the physiological process in plants such as photosynthesis process. These results are in agreement with those obtained by Dakhly and Abd El-Mageed (1997) on some vegetable crops, Shalaby *et al* (2002) on garlic plants. Mahendran and Kumar (1996) who found that the inoculated of garlic plants with biofertilizers (phosphobacterium) increased total bulb yield. Also, Dakhly and Abdel-Mageed (1997) reported that total yield of carrot, tomato and potato was markedly increased after inoculated these plants with *Azotobacter chroococcum* transforamnts.

Regarding to the chemical fertilizer rates effects on cured yield, data in Table (6) show that, both second and third rate of chemical fertilizer were significantly increased garlic curid yield compared with the first rate (25% of recommended dose). The increasing in cured yield after using the second rate was bout 2.822 and 2.866 tons/fed. in the first and second season respectively. These results are in similar with those obtained by Cardemas *et al* (1986) and Osman *et al* (1990) who found that cured yield of garlic plants was increased with increasing N level up to 120 kg N/Fed.

Nitrite and nitrate contents:

The effect of various treatments on the levels of nitrate in garlic plants is presented in Table (8). The nitrate content ranged from 169.7 to 217 mg/kg)and the lowest level of nitrate was recorded in the sample of wild type plants followed by those produced from plants subjected to biofertilizer treatments (treated with T_4). The nitrate contents in garlic samples were increased with increasing the applied N-chemical fertilizer to the combined biofertilizer treatment. An increase (21.8%) in nitrate level was detected in garlic samples produced from plants treated with chemical fertilizers compared to the wild type treatment. Generally the plants fertilized with chemical fertilizers contained highest value of nitrate (217 mg/kg) than those biofertilized with *Azotobacter*.

The levels of nitrite in garlic produced from plants subjected to various treatments are shown in Table (8). The results show similar trends to those observed for nitrate contents. Nitrite contents ranged from 2.5 mg/kg for in the sample of wild type plants to 8.8 mg/kg for fertilized plants with chemical fertilizers. Abdel-Naem *et al*., (1999) reported that the levels of nitrite in potato tuber produced from plants subjected to various bioferilization treatments ranged from 2.16 to 4.07 mg/kg in potato tubers.

Table (8): Nitrate and nitrite concentrations in garlic plants inoculated with *Azotobacter* transformants and treated by rates of nitrogen fertilizers in two seasons 1999/2000 and 2000/2001

Isolates (A)	Chemical fertilization (B)	Average of Nitrate NO_3^- mg kg^{-1}	Average of Nitrites NO_2- mg kg^{-1}
I	25%	149	2.128
W.T.	50%	171	2.585
	100%	189	2.785
Mean A	-	**169.7**	**2.499**
	25%	159	4.639
II	50%	182	6.889
T_1	100%	185	6.574
Mean A		**175.3**	**6.034**
	25%	148	2.985
III	50%	190	4.000
T_2	100%	194	4.388
Mean A	-	**177.3**	**3.791**
	25%	162	5.778
IV	50%	198	6.962
T_3	100%	210	7.892
Mean A	-	**190**	**6.877**
	25%	151	3.112
V	50%	178	3.255
T_4	100%	184	3.925
Mean A	-	**171**	**3.430**
	25%	209	8.590
VI	50%	217	8.887
Control	100%	225	8.976
Mean A	-	**217**	**8.817**

Using biofertilizers for vegetable plants as a substitute for the N-chemical fertilizer may be recommended to reduce nitrite contents and improve the yield quality (Hammad and Abdel-Ati, 1998; Abdel Naem *et al.*, 1999).

CONCLUSION

These improvements in garlic plant growth, fresh and cured yields after inoculation with either wild type strain or transformants of *Azotobacter* compared to uninoculated plants, may be due to; produce bacteria growth regulators such as indole acetic acid and gibberelines. The presence of these plant growth regulators particularly in the rhisosphere of plant uptake may be subjected to direct uptake by plant roots because of the intimate contact between microbial and plant cell improve plant growth and ions absorption and translocation.

Brown *et al.*, (1968) found that combination of IAA and GA applied to tomato seedlings produced effects on plant growth similar to those of pure culture of *Azotobacter* . Also, Azon *et al.*, (1978) reported that cell-free of *Azotobacter* and other microorganisms affected plant growth similarly as added the combined application of IAA and gibberellic acid. Biofertilizer does not replace mineral fertilizers but significantly reduce their rate of application. Biofertilization is the most significant tool for sustainable development of agriculture and improved environment within the frame of bio-organic farming systems (Saber, 1993).

The use of microbial isolates and transformants has become a hope for the Egyptian agriculture particularly in the field of production of plants, especially when the economical and environmental points of view are considered, sine they reduce the environmental pollution and production costs, in addition to improving the quality.

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استخدام بعض عزلات وتحولات الازوتوباكتر لخفض معدلات التسميد الآزوتى فـي إنتاج الثوم

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آجري هذا البحث في معامل قسم الوراثة وقسم البساتين بكلية الزراعة جامعة المنيـــا ومزرعـة محطة بحوث البساتين بملوي وذلك خلال الموسـمين الشـتويين (٢٠٠٠/٢٠٠١ ، ٢٠٠١/٢٠٠٢) لدراسـة استخدام طرز وراثية مختلفة من الازوتوباكتر (ناتجة بعملية التحول الوراثي) مـع معـدلات مـن التسـميد الكيماوي الآزوتى (٢٥%،٥٠%،١٠٠% من المعدل الموصى به وهو ١٢٠كجم نتروجين للفدان) على نمـو النتاج محصول الثوم (الصنف الصيني) حيث استخدام مخلوط مـن الطـراز الـبرى لكـلا النوعيـن مـن الأزوتوباكتر (ازتوباكتر فلنديای-وازوتوباكتر كروكوكم) بالإضافة إلى أربعة متحولات (أثنين من كل نـوع) مع معدلات التسميد الآزوتى الثلاثة وذلك لدراسة تأثير استخدام هذه البكتريا ومعـدلات التسـميد الآزوتـى المستخدمة والتفاعل بينهما على الصفات الخضرية والمحصولية (الوزن الطـازج- وزن المحصـول بعـد المعالجة مكونات المحصول – محتوى الفصوص من النترات والنتريت) ويمكن تلخيص النتـائج المتحصـل عليها كما يلي

[١] الحمض النووي الـــــــــ DNA المستخلص من نوعين الازوتوباكتر والنامية على بيئة تحتوى على المضاد الحيوي الكلورفينكول بتركيز ٢٠٠ ميكروجرام /مل لتحويل المقاومة للمضاد الحيوي فى بعـض طرز البكتريا الحساسة لهذا والتي لم يمكنها النمو على ٥٠ ميكروجرام / مل المضاد الحيـوي (وكـانت أعلى تحولات (١٠٠×١٠^{-٦}) عندما كان المعطى بكتريا ازوتوباكتر كروكوكم مقاوم والمستقبل ازوتوباكتر كروكوكم حساس وكانت اقلها (٥٢×١٠^{-٦}) عندما كان المعطى الازوتوباكتر كروكوكـم × ازوتوبـاكتر فلنديای كمستقبل كما لوحظ أن أعلى تكرارات نمت عندما كان التحول داخل الأنواع وأقل التكرارات نمت عندما كان التحول بين الأنواع .

[٢] تلقيح نباتات الثوم الصيني بالمتحولات والطرز البرى من الازوتوباكتر أحدث تحسينات فى معظم صفات النمو والمحصول وذلك بالإضافة لخفض محتوى الفصوص من النترات والنتريت

[٣] أعطى التسميد بالمعدل الثاني والثالث من التسميد الآزوتي (٥٠%،١٠٠%) من المعدل الموصى أعلـى قيم معنوية مقارنة بالمعدل الأول ٢٥%) وذلك لصفات وزن الـرأس -وزن الفصـوص -المحصـول الطازج – المحصول بعد المعالجة

[٤] كانت الاختلافات بين التسميد بالمعدل الثاني والثالث من التسميد الآزوتي غير معنوي لمعظم صفات النمو والمحصول

[٥] أظهرت النتائج اختلاف المتحولات لبكتريا الازوتوباكتر بقيم معنوية فيما بينها فى التـأثير علـى النمـو والمحصول للثوم . حيث كان للمتحولات T_5 ,T_4 أعلى قيم فى كلا الموسمين كما كانت قيم المتحـولات أعلى من الطراز البرى لمعظم الصفات

[٦] انخفضت نسبة النترات بحوالي (٢١ %) فى فصوص الثوم الملقحة بسلالات الطراز البرى وبحـوال (٢٠ %) فى فصوص الثوم الملقح بالمتحولات الازوتوباكتر

وبوجه عام فإنه أمكن خفض التسميد الآزوتي بحوالي ٢٥% من المعدل الموصى به للثوم الصيني باستخدام التلقيح ببكتريا الازوتوباكتر والمتحولات مع المحصول على نفس معدلات النمو والمحصول تقريباً بالإضافة إلى خفض نسبة النترات والنتريت فى الفصوص

ولذلك فان نتائج هذا البحث توصى باستخدام التسميد الحيوي مع محصول الثوم سواء للاستهلاك المحلـى أو للتصدير باستخدام أي من الطراز البرى و المتحولات من بكتيريا الازوتوباكتر لتقيـل اسـتدام التسميد الكيماوي وللحصول على منتج ذات محتوى منخفض من النتريت والنترات .