THE ROLE OF SOME AZOSPIRILLUM ISOLATES AND THEIR PRODUCTIVITY OF (IAA) ON CUCUMBER PRODUCTION UNDER PLASTIC HOUSE CONDITIONS

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Abstract

Two experiments were carried out to estimate the productivity of two cucumber hybrids (i.e. Sivo and Asna) inoculated with five *Azospirillum* isolates (as biofertilizers) under the plastic house conditions through the two autumn seasons (2007 and 2008). The production of phytohormones by plant growth promoting rhizobacteria (*Azospirillum lipoferum*) is considered to be an important mechanism by which these bacteria promote plant growth. The mutagenesis of *Azospirillum lipoferum* with nitrous acid is studied. The results indicated that survivals of wild type decreased rapidly with the increase of the mutagen concentration. Out of 3299 colonies tested, 2.27% proved to be auxotrophic. One mutant (his^-1) altered in indole acetic acid biosynthesis (112.56%) when compared with wild type strain. In addition, three conjugants ($meth^-$ x $trpt^-$, $meth^-$ x his^- and his^- x lys^-) gave over production (104.95, 120.81 and 103.76%) respectively.

Data of yield of both cucumber hybrids were obtained using 50% and 100 % of recommended dose of mineral nitrogen with and without inoculations of *Azospirillum* treatments. The activity of the given isolates of *Azospirillum* bacteria to produce IAA and increase nitrogen fixing ability was also studied.

The obtained results showed that the productivity of Sivo hybrid was significantly higher than that of Asna hybrid. On the other hand, there were significant differences of IAA production between the used *Azospirillum* isolates (S1, S2, S3, S4 and S5). The highest early yield obtained when S4 and S5 isolates were applied while the highest total yield was from S3 isolate. It could be concluded that the mix usage of 50 % mineral nitrogen with biofertilizer (Azospirillum, wild type, mutants and their conjugants) was sufficient to give rise the same production of either early or total yield components.

Keywords: *Azospirillum*, nitrous acid, cucumber, productivity, mutagenesis, IAA

INTRODUCTION

Azospirillum spp. are commonly isolated bacteria from the rhizosphere of various grasses and cereals and well characterized as plant growth promoting rhizobacteria (PGPR). Many reports indicated to the use of *Azospirillum* spp. for inoculation of Cereals (Lucy *et al.*, 2004, Elmerich and Newton 2007). Plant growth promotion by

Azospirillum is not fully understood. Initially, it was known only as a nitrogen fixer, but the current opinion is that the primary mechanism is related to production of growth-promoting substances such as cytokinins, gibberellins, and auxins (Somers and Vanderleyden, 2004).

It is well known that bacteria of the genus *Azospirillum* synthesize auxins, especially indole -3-acetic acid (IAA) (Crozier *et al.*, 1988), and a variety of other auxins like indole -3-. pyruvic acid and indole -3- butric acid, (Crozier *et al.*, 1988, Costacurta *et al.*, 1994 and Martinez-Morales *et al.*, 2003). These bacterial compounds contribute to the plant auxin "pool" in such away that the effect of *Azospirillum* inoculation can be mimicked by exogenous auxin application (Glick *et al.*, 1999). Production of auxins by Azospirilla was related to the rapid establishment of a bigger root system that stimulates the general growth of the host plant.

Crop yields are enhanced in several regions of the world by inoculation of the plants with the plant growth promoting bacterium *Azospirillum* ssp. that also reduces the need for chemical fertilizers (Bashan, 1993).

In Egypt, the plastic house and the low tunnels have become one of the most important techniques for early vegetable production. Cucumber plant is one of the most important vegetable crops which grows successfully under protected cultivation yielding early and high production. Production of the clean product and reduction of chemical fertilizers treatments should be considered as the main goals in Egyptian agriculture for local market and exportation. Supplementing or substituting the inorganic N with bio or organic sources, to obtain uncontaminated product and overcome the problems of chemical fertilizers. The applications of chemical fertilizers caused increasing the total coast of production as well as the risk of the environmental pollution.

Variable responsiveness to the N supplying form is usually occurred among cucumber varieties and hybrids under greenhouse conditions. For example, the hydroponically grown cucumber cultivars with different No_3: NH_4 ratios exhibited differential responsiveness according to the source of N. It was found that cucumber plants grown in 60: 40 (No_3: NH_4 by 50 %) solution grew greater than those with No3 as N source (Zornoza *et al.*, 1992). A significant increase of the female flowers and total yield was found when Shou *et al.*, (1995) used NH_4^+ with 25% or 50% of the total N. In the same manner, the model position of the first female flower decreased and the production of female flower increased under these conditions.

The purpose of this study is mainly concerned with studying the efficiency of Nitrous acid for inducing mutations in *Azospirillum lipoferum*. Likewise, *Azospirillum*

mutants and their conjugants effectiveness on cucumber hybrids plants was also studied in terms of yield and yield components

MATERIALS AND METHODS

Lab. studies were carried out in Genetics and Horticultural Depts., Fac., Agric., Minia Univ., while the field experiment was done in newly reclaimed sandy soil at Shousha research station, Minia Univ., during two seasons (2007 and 2008). These studies have proved new isolates of *Azospirillum* and their effects on yield and yield components of two cucumber hybrids (Sivo and Asna) under plastic house conditions.

a- Laboratory studies

a- 1. Bacterial Strains

Azospirillum lipoferum was kindly provided by Microbiology Department, Faculty of Agriculture, Minia University.

a- 2. Media

Complete medium (CM) (Page and Tigerstrom, 1979) and minimal medium (MM) (Albrecht and Okon, 1980) were used to isolate the auxotrophic mutants of *Azospirillum lipoferum.*

a- 3. Induction, isolation and identification of auxotrophic mutants by Nitrous acid (NA)

Samples of 0.1 ml, *Azospirillum lipoferum* wild type aliquots, treated with different concentrations, (0.0, 20.0, 40.0, 60.0, 80.0 and 100.0 mM) of Sodium nitrite $NaNO_2$, (which ionized in water to produce nitrous acid and sodium hydroxide), with suitable dilutions were plated on complete medium (Abdel-Wahab, 1977). The plates were incubated at 30°C for three days and the surviving colonies were tested on MM and CM media after 5 days of incubation at 30°C. The survival percentage was scored by counting the growing cells:

$$\text{Survival percentage} = \frac{E \times 100}{C}$$

Where, E = total number of grown cells in each treatment, C = total number of grown cells in the control.

The plates were scored and biochemical mutants (which grow on CM and unable to grow on MM), were selected. Identification of *Azospirillum lipoferum* auxotrophic mutants were carried out according to Holiday (1956). In addition, the mutation frequency (MF) was calculated according to Green and Muriel (1976).

a- 4. Conjugation Procedure

Conjugation experiment was applied according to Abdel-Wahab (1977).

a- 5. Quantification of excreted IAA

The colorimetric reaction (Hartmann *et al.*, 1983) was used to quantify the amount of IAA excreted from *A. lipoferum* and the conjugants. Three samples from both wild type cultures and the isolates, each containing the same cell density, were tested. One mL aliquots of each were taken at 48 hrs intervals for IAA determination and growth measurements over a period of 10 days. A quantitative analysis of IAA production was conducted photometrically at 530 nm in a Spectronic Genesys 5 spectrophotometer using an IAA standard.

b- Field studies

Field experiments were done in reclaimed sandy soil at the Experimental Research Center Minia Univ., Shosha region, El-Minia Governorate during the two autumn seasons of 2007 and 2008 under plastic house conditions. Soil properties of these plastic houses are presented in Table (1) which were determined according to the standard method given by Page *et al.* (1982).

Table 1. The chemical and physical properties of the soil in plastic house.

	Sand	Silt	Clay	Texture	$CaCo_3$ *	Total N%	O.M**	PH	E.C***
First Season	73.61	13.74	12.65	Sandy	8.81	0.015	0.11	8.4	1.85
Second Season	76.18	14.15	19.67	Sandy	8.11	0.018	0.14	8.3	1.76

*Calcium carbonate, ** Organic matter, *** Electric conductivity

Seeds of two cucumber hybrids namely "Asna" and "Sivo" were divided before sowing into 7 parts. Five parts of them were inoculated with 5 isolates of *Azospirillum* individually, (wild type (S1), histidineless1 (S2), histidineless3 (S3), methioneless x tryptophaneless (S4) and methioneneless x histidineless (S5)) the other two parts were without inoculation. The seeds of two cucumber hybrids were sowing directly in the soil of each plastic house. Three plastic houses (40 m long × 8.5 m width) were used as three replications. The seeds were sowed on August, 26th and September, 2nd in the first and second season respectively. Five rows (40 m long, 80 cm width, and 40 cm between the plants within row) were used for each hybrid. The experimental design was split plot with three replicates. The two cucumber hybrids were distributed randomly in the main plots. Seven treatments of biofertilizers in the subplots as follows:

A. plants fertilized with 100% recommended of chemical nitrogen rate without inoculation.

B. Plants fertilized with 50% of recommended nitrogen rates and inoculated with isolate 1,2,3,4 and 5 of *Azospirillum* in addition to treatment of uninoculated plants (control). These seven treatments were distributed in the sub plots (20 m long and 80 cm width).

During soil preparation 125, 150 and 200 g/m^2 of ammonium sulphate (20.6%), calcium supper phosphate (15.5%) and potassium sulphate, respectively were added to the soil.

In the first two weeks of sowing, plants were irrigated without fertilization while from 3^{th} week to 14^{th}, the quantity of nitrogen fertilizers (ammonium nitrate) were added as follows:

- from 3^{th} to 6^{th} week, 800 g of ammonium nitrate / m^3 of irrigation water.
- from 7^{th} to 9^{th} week, 650 g of ammonium nitrate / m^3.
- from 9^{th} to 12^{th} week, 750 g of ammonium nitrate / m^3.
- from 12^{th} to 14^{th} week, 500 g of ammonium nitrate / m^3.

These quantities were added to those plots which received 100% of chemical nitrogen fertilizers, while half of these quantities were added to those plants which received 50% of chemical nitrogen fertilizer with or without inoculation with biofertilizers. Other cultural practices for plastic house Cucumber production were followed. Data were recorded on the following charts:

1. Number of fruits / plant
2. Fruit diameter (cm).
3. Fruit length.
4. Early yield kg/plant.
5. Total yield kg/plant.

Data were subjected to the analysis of variance procedures and treatment means were compared using the L. S. D. values according to Gomez and Gomez 1984.

RESULTS AND DISCUSSION

Effect of nitrous acid (NA)

Table (2) shows the mean number and percentages of *Azospirillum lipoferum* survivals after treatments with different concentrations of Nitrous acid (NA).

The results indicated that survivals of wild type decreased rapidly with the increase of the mutagen (NA) concentration. Table (2) gives the number of colonies tested and the percentages of obtained mutants. It can be observed that the treatments, with the concentration 80.0 mM cell suspension for two hours, gave the highest frequency of mutants (10.61%) while the treatment with 20.0 mM cell suspension for one and two hours gave the lowest frequency of mutants (0.56% and 0.45% respectively). These mutants were found to be, nine argnineless, thirteen methionineless, eight tryptophaneless, eleven lysineless, fourteen histideneless, nine threonineless, eleven phenylalanineless, four revertante mutants and two were uncharacterized.

Among the obtained auxotrophic mutants there was an excess of methionine and histidine auxotrophs. Similar results were previously reported with Tn5 mutagenesis in E. coli K 12 (Shaw and Berg 1979). The same effect was found with Tn5 mutagenesis in *Pseudomonus aeruginosa* for cysteine and methionine auxotrophs (O' Hoy and Krishnapillai 1985). This finding may be due to the existence of hot spots in the genome for Tn5 mutagenesis of the genes involved.

Table 2. Numbers and percentages of survivals and mutants after treatment with five concentrations of nitrous acid (NA).

Conc. of the NA	Time of treatment	Survival cells	Survival %	No. of colonies tested	No. of mutants	Mutants %	No. and requirements of single mutants								
							arg⁻	meth⁻	trypt.⁻	lys⁻	his⁻	thr⁻	phala⁻	revertant	Other mutants
0.00	1 hrs	100,000	100.00	600	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20.00		90,000	90.00	180	1.00	0.56	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40.00		60,000	60.00	320	2.00	0.63	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
60.00		30,000	30.00	414	4.00	0.97	1.00	0.00	1.00	1.00	0.00	1.00	0.00	0.00	0.00
80.00		10,000	10.00	280	22.00	7.86	2.00	3.00	4.00	3.00	5.00	2.00	3.00	0.00	0.00
100.00		0.0000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	2 hrs	100,000	100.00	350	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20.00		83,000	83.00	220	1.00	0.45	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40.00		51,000	51.00	310	3.00	0.97	1.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	0.00
60.00		18,000	18.00	295	7.00	2.37	2.00	3.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00
80.00		32,000	3.20	330	35.00	10.61	2.00	5.00	2.00	5.00	7.00	6.00	8.00	4.00	2.00
100.00		0.0000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Conjugation

In our efforts to obtain higher conjugation rates, different mating conditions were tested (e. g. different conjugation media and various temperatures). During these experiments, two sizes of colonies were noticed on complete medium. Colonies of average size appeared during three days of growth, but some small sized colonies could be found with low rate.

According to the present results it was observed that the high conjugation frequency occurred of Hfr his^- x arg^- and this means that conjugation rate was increased. Abd El-Salam and klingmuller (1987) tried to optimize the donor-recipient ratio to obtain a good yield of conjugants in. *Azospirillum lipoferum.* It was found that the optimum ratio was 1 donor: 10-20 recipients. More than 600 conjugants were obtained per plate when 1×10^9 cells of *A. lipoferum* were conjugated with 5×10^7 *E. coli* donor strain.

Indole acetic acid production (IAA)

The excretion of IAA by the conjugants was compared with that of the wild type and mutants (Table 3).

All mutants exhibited normal growth behavior when compared with that of the wild type, therefore, difference in IAA production does probably not due to growth abnormality.

The detected amounts of IAA excreted by 21 mutants and 12 hybrids (conjugants) are presented in Table (3). Among them 5 (2 mutants and 3 conjugants) were found to be overproducers of IAA relatively to wild type strain.

These differences in producing IAA among mutants, conjugants and wild type strains may be due to the three IAA synthetic pathways in *Azospirillum* depending on one or more copies of the gene involved in one pathway relating to the growing media contents (Bashan and Holguin, 1995). Kleckner *et al.*, (1977) concluded that at least two different pathways for IAA biosynthesis, or more than one copy of the genes involved in one pathway, are found in *A. lipoferum.*

These results are in agreement with those which reported by Pedrosa and Yatos, (1984) who obtained *nif*-mutants of *Azospirillum* detective in nitrogen fixation by using N-methyl-N-nitro-N-nitrosoguanadine mutagenesis. Also, IAA-overproducing mutants of *Azospirillum* have been isolated by Hartmann *et al* (1983). This mutagensis has been frequently used to obtain auxotrophic and IAA overproducing mutants of *Azospirillum* (Abd El-Salam and klingmtiller 1987).

Table 3. Production of IAA by *Azospirillum lipoferum* mutants and their Conjugants in relation to control.

Strains	IAA (µg/ml)					Mean	%
	2 days	4 days	6 days	8 days	10 days		
Wild type	15.9	27.6	36.2	37.2	40.7	31.52	100.0
Mutants							
Arg⁻ 1	1.9	3.7	4.1	4.0	3.9	3.52	11.17
Arg⁻ 2	2.2	2.8	2.7	2.8	2.7	2.64	8.38
Arg⁻ 3	1.8	3.1	3.7	2.9	3.2	2.94	9.33
Meth⁻ 1	6.7	9.2	12.7	12.8	15.2	11.32	35.91
Meth⁻ 2	7.2	14.0	15.2	12.9	12.7	12.32	39.09
Meth⁻ 3	2.9	4.0	5.1	5.7	5.8	4.78	15.16
Trypt⁻ 1	11.2	18.3	22.5	23.7	23.8	19.90	63.13
Trypt⁻ 2	8.2	13.7	17.5	19.9	20.5	15.96	50.63
Trypt⁻ 3	6.9	15.3	18.7	20.2	21.3	16.28	51.65
Lys⁻ 1	12.4	18.9	22.5	25.3	30.4	21.90	69.48
Lys⁻ 2	11.7	19.2	25.7	26.1	29.7	22.48	71.32
Lys⁻ 3	10.0	20.7	27.3	31.2	35.2	24.88	78.93
His⁻ 1	16.2	25.9	39.2	45.9	50.2	35.48	112.56
His⁻ 2	9.8	18.7	27.1	33.2	41.2	26.00	82.49
His⁻ 3	15.2	30.9	40.1	47.2	55.3	37.74	119.73
Thr⁻ 1	6.4	12.1	13.2	18.3	19.2	13.84	43.91
Thr⁻ 2	5.9	11.0	15.7	17.2	20.3	14.02	44.48
Thr⁻ 3	7.2	13.1	20.2	22.7	27.3	18.10	57.42
Phen.ala⁻ 1	2.9	4.7	9.2	11.8	15.0	8.72	27.66
Phen.ala⁻ 2	4.3	9.8	11.2	18.3	19.8	12.54	39.78
Phen.ala⁻ 3	5.1	10.2	18.8	25.3	28.9	17.66	56.03
Conjugants							
Hfr Meth⁻ ×arg⁻	15.3	19.2	29.7	37.3	40.2	28.34	89.91
Hfr Meth⁻ ×Trypt⁻	17.2	28.1	32.2	39.7	48.2	33.08	104.95
Hfr Meth⁻ ×Lys⁻	5.4	12.7	22.8	25.3	33.2	19.98	63.39
Hfr Meth⁻ ×His⁻	19.2	32.8	40.2	47.0	51.2	38.08	120.81
Hfr Meth⁻ ×Thr⁻	13.7	21.2	32.2	36.1	41.2	28.88	91.62
Hfr Meth⁻ ×Phen.ala⁻	12.2	23.2	29.7	37.2	42.3	29.92	91.75
Hfr His⁻ ×arg⁻	16.2	19.3	32.2	37.7	39.9	29.06	92.20
Hfr His⁻ ×Trypt⁻	19.7	22.2	35.7	40.2	41.0	31.76	100.76
Hfr His⁻ ×Lys⁻	17.3	25.2	37.8	39.7	43.2	32.64	103.76
Hfr His⁻ ×His⁻	19.2	27.2	33.2	37.8	40.0	31.48	99.87
Hfr His⁻ ×Thr⁻	12.3	17.7	22.2	29.3	30.8	22.46	71.26
Hfr His⁻ ×Phen.ala⁻	17.3	21.2	27.2	32.2	37.0	26.98	85.60

Number of fruits / plant

Data in Table (4) show that both two hybrids of cucumber are significantly different in their number of fruits per plant. "Sivo" hybrid showed higher number of fruits per plant (29.61 and 29.27 in the first and second season, respectively) when compared to "Asna" hybrid (28.34 and 27.70 fruits per plant in the first and second season, respectively).

Inoculation cucumber plants with *Azospirillum* isolates gave a significant high number of fruits in both seasons (Table 4). Plants which received 100 % of chemical nitrogen rate without inoculation exhibited highest number of fruits per plant (31.12 and 31.15 fruits / plant) in the first and second seasons, respectively. The lowest values of this character (26.61 and 27.13) in the first and second seasons respectively were observed with control plants.

The inoculation process improved these values in compared with the control plants. The highest values of this character (30.63 and 30.16) in the first and second seasons were obtained after inoculation with isolate (4). Otherwise, these values are not different from 100 % mineral nitrogen treatment in the first season only. These results confirm the advantages of using the biofertilizer instead of chemical fertilizer. Biofertilizer (*Azospirillum*) may excreted growth promoting substances (Somers and Vanderleyden, 2004) and also, indole acetic acid (Crozier, *et al.*, 1988). It is well known that IAA enhanced the production of female flowers which consequently increased the number of fruits numbers per plants.

Table 4. the effects of some isolates of *Azospirillum* on some growth parameters and yield component of two Cucumber hybrids.

Treatments hybrids	Isolates	No. of fruits/plant		Fruit length		Fruit diameter		Early yield kg/plant		Total yield kg/ m^2	
		First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Sivo	100% M. F.	32.74	33.15	16.66	15.80	3.20	3.20	0.751	0.698	10.17	9.98
	50% + S 1	29.45	30.11	16.10	15.77	3.29	2.97	0.632	0.601	10.01	9.10
	50% + S 2	28.16	27.12	16.17	16.10	3.20	3.27	0.513	0.597	9.13	9.36
	50% + S 3	30.50	26.11	16.66	15.90	3.23	3.17	0.664	0.543	9.75	9.86
	50% + S 4	31.16	32.11	16.97	15.70	3.13	3.13	0.661	0.628	9.04	9.01
	50% + S 5	28.14	28.15	15.87	15.97	3.10	3.13	0.647	0.673	9.45	9.95
	50% without	27.11	28.14	15.43	14.73	3.17	3.07	0.457	0.458	8.13	8.61
Mean of A		29.61	29.27	16.27	15.71	3.19	3.13	0.618	0.599	9.38	9.41
Asna	100% M. F.	29.50	29.14	15.87	15.17	3.10	3.13	0.663	0.692	9.28	9.72
	50% + S 1	27.12	28.12	15.33	13.87	3.10	3.10	0.601	0.563	8.10	8.42
	50% + S 2	28.15	27.11	14.63	14.40	2.93	3.66	0.572	0.550	8.12	8.31
	50% + S 3	28.33	27.15	16.13	14.60	3.37	3.30	0.541	0.581	8.24	8.66
	50% + S 4	30.1	28.20	15.00	15.57	3.10	3.13	0.488	0.561	8.13	7.61
	50% + S 5	29.1	28.10	16.37	14.70	2.93	3.00	0.601	0.567	8.15	8.51
	50% without	26.11	26.11	14.93	14.93	3.00	3.00	0.478	0.485	7.16	7.40
Mean of A		28.34	27.70	15.46	14.75	3.08	3.19	0.563	0.571	8.169	8.376
Mean of B											
	100% M. F.	31.12	31.15	16.27	15.49	3.15	3.17	0.692	0.695	10.01	9.85
	50% + S 1	28.28	29.12	15.72	14.82	3.14	3.04	0.617	0.582	9.06	8.77
	50% + S 2	28.15	27.12	15.40	15.25	3.07	3.97	0.543	0.574	8.03	8.84
	50% + S 3	29.42	26.63	16.34	15.00	3.30	3.24	0.593	0.537	8.99	9.26
	50% + S 4	30.63	30.16	15.99	15.64	3.12	3.13	0.575	0.595	8.59	8.31
	50% + S 5	28.58	28.13	16.12	15.36	3.02	3.07	0.651	0.590	8.80	9.23
	50% without	26.61	27.13	15.19	14.83	3.04	3.08	0.466	0.470	7.35	8.01
L.S.D.	A	0.491	0.615	0.963	0. 815	0.031	0.028	0.081	0.092	0.612	0.514
	B	1.011	0.418	0.011	0.014	0.070	0.061	0.193	0.185	0.824	0.911
	AB	1.737	1.939	1.131	1.181	0.912	0.815	0.264	0.272	1.601	1.04

Fruit length

The fruit of hybrid "Sivo" was longer than fruit of hybrid "Asna" as data showed in Table (4).

Regarding to the biofertilizers (*Azospirillum*) effect, data show that fruit length of cucumber plants was significantly affected in plants inoculated with *Azospirillum* isolates (S1, S2, S3, S4 and S5). The fruit length increased significantly with increasing the rate of mineral fertilizer to 100% of recommended dose and or with inoculation with *Azospirillum* isolates (S1, S2, S3, S4 and S5) as compared to those plants which received 50% of mineral nitrogen without inoculation process. Data show (Table 4) that inoculation cucumber plants with these isolates (isolates 3, 4 and 5) with 50 % mineral nitrogen rate was sufficient to obtain nearly the same values of cucumber fruit length without any significant differences (16.34, 15.99 and 16.12 cm, respectively) when compared to 16.27 cm (with 100 % mineral nitrogen rate) in the first season and in the second season (15.64 and 15.36 for isolate 4 & 5 respectively). This improvement in fruit length with inoculation by *Azospirillum* may due to its role in nitrogen fixing ability and improving nitrogen uptake by plants.

Fruit diameter

Data in Table (4) showed that Sivo hybrid in both seasons exhibited the higher values of fruit diameter.

The inoculation of cucumber plants by all isolates of *Azospirillum* (except S5) with 50% mineral nitrogen gave the similar values of fruit diameter in comparison with that of 100% mineral nitrogen.

In addition, data show that, no significant differences were obtained in fruit diameter between inoculated plants which received 50% mineral nitrogen and uninoculated plants which received 100% of mineral nitrogen in both seasons. These results are in agreement with many workers on different crops after inoculation with some biofertilizers i.e. increase root diameter of carrot (Dakhly and Abdel-Mageed, 1997), and bulb diameter of garlic (Shalaby *et al.*, 2000 and Foly *et al.*, 2002).

Early yield

Data presented in Table (4) indicate that early yield kg/plant was significantly affected to depend on hybrid in both seasons. The "Sivo" hybrid showed the higher value of early yield (0.618 and 0.599 kg/plant) as compared to 0.563 and 0.571 kg/plant for "Asna" hybrid in the first and second season respectively. This superiority for "Sivo" hybrid in early yield character may be due to the short period needed for female flower production as well as the short time needed from flowering to fruit picking, when compared to those of "Asna" hybrid.

Regarding the effect of inoculation process, data in Table (4) show that inoculation cucumber plants with *Azospirillum* isolates increased significantly early yield as compared to control plants in both seasons. On the other hand, fertilization with 100% mineral nitrogen dose showed high values of early yield in both seasons. The differences between using 100 % mineral fertilizer effect and 50 % mineral fertilizer addition to inoculation with *Azospirillum* (isolate 1,2,3,4 and 5) on this trait were not significant in both seasons. These results indicated to the possibility of using 50 % only of mineral fertilizer and inoculation with *Azospirillum* isolates to obtain the same quantity of early yield.

Total yield kg / m^2

The total yield of Sivo hybrid was significantly higher than that of Asna hybrid in both seasons (Table 4).

Regarding to the fertilizer treatments data in Table (4) show that the highest values of total yield of cucumber were obtained when 100 % mineral nitrogen dose was added to.

Inoculation cucumber plants with all *Azospirillum* isolates (1,2,3,4 and 5) increased significantly total yield character in comparison with that of control plants. The highest values of total yield were obtained with 100% mineral nitrogen treatment which followed by inoculation those plants with isolates 2 and 3.

Sivo hybrid showed the higher total yield value due to its superiority in number of fruits per plant and fruit diameter. These results are in agreement with Lucy *et al.*, 2004 (on cereals), Shan *et al.*, 1995 (on cucumber), Dakhly and Abdel-Mageed, 1997 (on potato, carrot and tomato), and Foly *et al.*, 2002 (on garlic).

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دور بعض عزلات الأزوسبيريلايم وإنتاجها من الأندول أسيتك أسيد وتأثير ذلك على الصفات المحصولية للخيار تحت ظروف الصوب البلاستيكية

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أجريت هذة الدراسة فى معمل الوراثة الميكروبية بقسم الوراثة ومعمل قسم البساتين والتجارب الحقلية خلال الموسمين الخريفين ٢٠٠٧،٢٠٠٨ فى مزرعة التجارب والبحوث الزراعية بجامعة المنيا تحت ظروف الزراعة المحمية (تحت الصوب البلاستيكية).

إنتاج الهرمونات النباتية بواسطة بكتيريا الريزوسفير (أزوسبيريلام ليبوفيرم) عامل مهم فى زيادة نمو النباتات بواسطة هذة البكتيريا. وقد تم استخدام حمض النيتروز فى استحداث الطفرات فى الأزوسبيريلام ليبوفيرم. النتائج المتحصل عليها تدل على أن أعداد المستعمرات الحية تتناقص بشدة مع زيادة تركيز المطفر. من ٣٢٩٩ مستعمرة تم اختيارها كانت هناك نسبة ٢,٢٧ % طافرة. طفرة واحدة هى التى أظهرت انتاج عالى من الأندول أسيتك أسيد وهى طفرة ذات العوز للهستدين١ والتى أعطت ١١٢,٥٦ % عند مقارنتها بالطراز البرى، بالاضافة الى ثلاثة مقترنات وهى مثيونين × تربتوفان، مثيونين × هستدين، هستدين × ليسين أعطت إنتاج عالى (١٠٤,٩٥، ١٢٠,٨١، ١٠٣,٧٦) على التوالى.

وقد استخدم فى الدراسة الحقلية هجينين من هجن الخيار تحت الزراعة المحمية وهما (سيفو ، أسنا). حيث تم تلقيحهم بأستعمال خمس عزلات من الأزوسبيريلام كمخصب حيوى. و تم تسجيل صفات المحصول لكلا الهجينين تحت مستويين من التسميد الأزوتى الكيماوى وهما ٥٠ % ، ١٠٠ % من الجرعة الموصى بها مع استخدام أو عدم استخدام المعاملة بالبكتيريا. ولقد اختبرت قدرة سلالات البكتيريا على انتاج الأندول أسيتك أسيد والنتائج المتحصل عليها أظهرت اختلافات معنوية فى كلا الهجينين فى صفات النمو والمحصول. هجين "سيفو" أظهر انتاج أعلى فى بعض مكونات المحصول مثل طول وقطرالثمرة علاوة على المحصول المبكر و المحصول الكلى / متر مربع عن الهجين "أسنا" وأيضا السلالات الخمسة المختبرة من الأزوسبيريلام اختلفت فيما بينها فى أنتاج الأندول أسيتك أسيد. وكذلك فى تأثيرها على صفات مكونات المحصول و المحصول المبكر والكلى من خيار الصوب. وكانت أفضل السلالات للحصول على محصول مبكر هى السلالاتين رقم ٤ ،٥ بينما كانت أفضل السلالات للحصول على أعلى محصول كلى هى السلالة رقم ٣.

ومن خلال هذة الدراسة يمكن التوصية باستخدام ٥٠ % فقط من السماد المعدنى النيتروجينى مع تلقيح النباتات ببكتيريا الأزوسبيرليم دون حدوث نقص معنوى فى صفات المحصول المبكر أو الكلى.