ISSN 1110-0419

Annals Of Agric. Sc., Moshtohor,
Vol. 39(4): 2171-2181, (2001).

CO-INOCULATION EFFECT WITH *Rhizobium* AND *Azospirillum* ON GROWTH, NODULATION AND YIELD OF GUAR PLANT (*Cyamopsis tetragonoloba* L.).

BY

Abou-Aly, H.E.
Agric. Botany Dept., Fac. Agric., Moshtohor, Zagazig Univ., Egypt.

ABSTRACT

In two successive seasons, two field experiments were conducted at Experimental Farm, Fac. of Agric., Moshtohor, to study the influence of inoculation with mixed cultures of rhizobial strains and *Azospirillum lipoferum* on growth, nodulation, yield and nutrients content of guar (*Cyamopsis tetragonoloba L.*). Two rhizobial strains (ARC802 and local strain) were used singly and in combination with *A. lipoferum.*

The results showed that, all inoculated treatments had a significant effect in increasing the vegetative growth, nodulation, yield and NPK content of guar plants more than control treatments. Inoculation of guar with *Rhizobium* individually (especially ARC802) increased nitrogenase activity than *Azospirillum* individually or in combination with *Rhizobium.*

Addition of *Azospirillum* in combination with *Rhizobium* (especially ARC802) under the low rate of nitrogen fertilization was superior in increasing CO_2 evolution, nodulation, plant growth, yield and yield components of guar plants. It seemed that, a positive response was found between *Azospirillum* and *Rhizobium* depending on the *Rhizobium* strain.

INTRODUCTION

Guar (*Cyamopsis tetragonoloba L.*) is a leguminous crop grown for seed. Guar gum is extracted from the seeds (11 to 30%) which is being utilized in various industries such as pharmaceuticals, textile, paper manufacture and as a stabilizer of stiffener in foods and various other products (Stafford, 1975). The plant is used as a green manure in newly cultivated areas that could be used for poultry and animal nutrition. Guar meal, the by-product of guar industries is used as a concentrate food for animals which contains 42% crude protein (Ghonem, 1990).

Compatibility of *Rhizobium* with their host is an important factor to successful nodulation and nitrogen fixation by legumes. Fertilization trials with chemical and biofertilizers were found to improve nodulation, nitrogen fixation and seed quality of legumes (El-Sheikh and El-Zidany, 1997). Inoculation of plants by N_2-fixers combined with the half of recommended dose of nitrogen fertilizer had a beneficial effect on the productivity of the plants (Abou-Aly and Gomaa, 2002).

The growth enhancing effects of *Azospirillum* have been documented. Excretion of auxins, cytokinins and gibberellins into the growth medium is responsible at least in part of this direct effect on the plant (Bottini *et al.*, 1989).

The effect of combination with *Rhizobium* and *Azospirillum* was found to be important for improving and maximizing the plant growth and N_2-fixation (Itzigsohn *et al.*, 1993 and Okon *et al.* 1998). However, it was reported that *Rhizobium- Azospirillum* mixed cultures can have a negative effect on the *Rhizobium* –legume interaction (Jacek and Rolfe, 1985).

Few trials have been conducted to observe the response of guar to co-inoculation, so the present study was conducted to investigate the effect of *Azospirillum lipoferum* in combination with two strains of *Rhizobium* on nodulation, nitrogenase activity, growth, yield and mineral composition of guar under half dose of nitrogen fertilization.

MATERIALS AND METHODS

A field plot experiment was conducted in two successive summer seasons of 1999 and 2000 at the Agriculture Research and Experimentation Center, of Fac. Agric., Moshtohor. The soil of the experiment was analyzed to its physical and chemical properties (Table, 1)

Physical properties was estimated according to Jackson (1973) whereas, chemical properties was estimated according to Black *et al.* (1982).

Bacterial strains and inocula preparation

Rhizobium(ARC802) specific for guar (from Biofertilization Unit, Soil, Water and Environment Research Institute) and an active *Rhizobium* strain isolated from guar nodules (from Microbiology Unit, Desert Research Center, Mataria, Cairo, Egypt) were grown on Yeast Extract Mannitol Broth (YEMB) as a growth medium (Vincent, 1970) and incubated at 32°C for 7days. The initial numbers of cells used for seed inoculation were about 10^8 cells/ml.

Azospirillum lipoferum strain (from Microbiology Unit, Desert Research Center) was grown on modified N-deficient semi-solid malate medium (Hegazi *et al.*, 1979) and incubated at 30°C for 7 days. The numbers of viable cells at sowing time were about 10^8 cells/ml.

Cultivation process

Except the control treatments experiment soil plots, were supplied with half dose of nitrogen fertilizer (20 kg N/fed. as Ammonium nitrate), calcium superphosphate at a rate of 30 kg P_2O_5/fed. and potassium sulphate at a rate of 48 kg K_2O/fed. in two equal doses after 15 and 45 days of planting.

Table (1). Some physical and chemical properties of the experimental soil.

Soil properties	Unit	Seasons	
		1999	2000
1- Particle size distribution			
Coarse sand	%	9.10	12.08
Fine sand	%	17.21	17.26
Silt	%	20.50	17.60
Clay	%	53.19	53.06
Textural class		Clay	Clay
2-Chemical properties			
Organic matter	%	1.72	1.65
Total nitrogen	%	0.32	0.36
Total phosphorus	%	0.18	0.15
Total potassium	%	0.49	0.52
Iron	ppm	22.6	25.1
Zinc	ppm	2.34	2.78
Manganese	ppm	19.26	19.42
Copper	ppm	2.48	2.94
Cations			
Ca^{++}	meq/L	11.24	12.16
Mg^{++}	meq/L	7.80	6.86
Na^{+}	meq/L	5.72	5.97
K^{+}	meq/L	1.88	0.98
Anions			
$CO3^{--}$	meq/L	6.92	5.45
$HCO3^{-}$	meq/L	6.36	6.32
Cl^{-}	meq/L	8.47	7.45
$SO4^{--}$	meq/L	4.89	6.75
CaCO3	%	0.56	0.66
pH		7.81	8.07

Guar seeds were washed, air-dried and soaked in cell suspension of *Rhizobium* inoculum and/or *Azospirillum* inoculum for 30 min. and coated with gum arabic as an adhesive agent (Mor *et al.*, 1995). Uninoculated seeds were treated by using uninoculated media. The control treatments which seeds were not inoculated were divided into two treatments, the first was left without fertilization and the second was fertilized with the recommended dose of nitrogen (40 kg N/fed.), phosphorous and potassium (as mentioned above).

The experiment concluded seven treatments with three replicates arranged in a completely randomized block design. Inoculated or uninoculated seeds were sown in hills at rows with 24cm. distance between hills and 50 cm. between rows. The experiment included the following treatments:

1-Control (without fertilization or inoculation).
2-Fertilized control (without inoculation).
3-*Rhizobium* sp. (ARC802).
4-*Rhizobium* sp. (local strain).
5-*Azospirillum lipoferum*

6- *Rhizobium* sp. (ARC802) +*Azospirillum lipoferum.*
7- *Rhizobium* sp. (local strain) + *Azospirillum lipoferum.*

Sampling and determination

Samples of rhizosphere soil were taken at vegetative (35 days) and flowering (65 days) stages to be analyzed for CO_2 evolution according to Page *et al.* (1982) and N_2-ase activity in nodules according to Hardy *et al.* (1973).

At flowering stage, plant height, number of branches/plant, number of nodules, dry weight of nodules, dry weight of plant were estimated. At harvesting, number of pods, dry weight of pods and seed yield were estimated.

Total nitrogen, phosphorus and potassium were determined in guar plants according to A.O.A.C.(1980), A.P.H.A.(1992) and Dewis and Freitas (1970), respectively. Also, guar gum was estimated using the method described by Das *et al.* (1977). Fe, Zn, Mn and Cu were determined in the plants by using flame ionization atomic absorption spectrometer according to the method of Chapman and Pratt (1978).

Statistical analysis

Data were assessed by analysis of variance to determine the significant differences between treatments by L.S.D. according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

CO_2 evolution and nitrogenase activity

Data presented in Table (2) showed that guar seed inoculation with symbiotic and/or asymbiotic N_2-fixers significantly increased CO_2 evolution (as indication for soil microorganisms activity) and nitrogenase activity than uninoculated treatments.

Inoculation of guar seed by *Rhizobium* (ARC802) combined with *Azospirillum* gave higher values of CO_2 evolution than each of the two strains of rhizobia or *Azospirillum* alone.

As regard to nitrogenase activity, plants inoculated with *Azospirillum* strain alone showed little amount of acetylene reduction activity but still more than the control treatments. Nitrogenase activity slightly increased with the dual inoculation by *Rhizobium* (ARC802) and *Azospirillum* than *Rhizobium* (local strain) combined with *Azospirillum*. While the highest values of N_2-ase activity were observed with seed inoculation by specific *Rhizobium* (ARC802) alone. This may be due to nitrogen fixation by *Azospirillum* might be a secondary importance to growth substances production (Plazinski and Rolfe 1985). Similar trends were noticed in the two growing seasons. These results are in agreement with Swelim and Abdel-Wahab (2000).

Table (2): Effect of co-inoculation with *Rhizobium* and *Azospirillum* on CO_2 evolution and nitrogenase activity of Guar plant.

Treatments	CO_2 evolution (µg / g dry soil / hr)				N_2-ase activity (n moles C_2H_4 / hr / g dry nodules)			
	1999		2000		1999		2000	
	Pre-fl.	Fl.	Pre-fl.	Fl.	Pre-fl.	Fl.	Pre-fl.	Fl.
Control	65.10	106.9	81.6	146.2	18.9	32.6	25.3	41.0
Fertilized control	143.17	171.8	161.8	210.3	43.7	77.4	59.6	98.1
Rhizobium (ARC 802 strain)	150.30	195.2	168.5	234.7	132.5	172.2	141.9	185.4
Rhizobium (local strain)	153.71	186.0	159.0	240.3	126.4	154.0	138.1	176.8
Azospirillum	178.6	206.3	193.8	253.2	105.8	130.8	92.6	141.2
Rhizo. (ARC802 strain)+Azosp.	184.5	235.7	199.6	260.5	131.7	166.9	133.4	169.7
Rhizo (local strain) + Azosp.	171.2	217.1	195.3	255.1	116.9	153.0	121.3	160.9
L.S.D at 5%	1.463	1.785	19.2	10.20	3.959	10.88	7.277	13.30

Rhizo. : *Rhizobium*
Azosp. : *Azospirillum*

Pre-fl. : pre-flowering stage (35 days).
Fl. : flowering stage (65 days).

Nodulation status

Concerning the effect of co-inoculation in presence of half dose of inorganic nitrogen on nodulation, data presented in Table (3) showed that co-inoculation of guar plants gave higher records of nodulation compared to guar plants inoculated with *Azospirillum* individually.

With respect to inoculation with a single isolate, higher values of number and dry weight of nodules were obtained in the case of inoculation with *Rhizobium*(ARC802). The improvement of nodulation pattern was further strengthened in dual inoculation of *Rhizobium*(ARC802) and *Azospirillum*. This result is in accordance with those obtained by Swelim and Abdel-Wahab (2000) who reported that the highest value of nodulation was observed in case of co-inoculation of soybean seeds with *Bradyrhizobium* and *Azotobacter* or *Azospirillum*.

Plant growth

Data in Table (3) also indicated that uninoculated plants gave the lowest records of plant growth characters (plant height, number of branches per plant and dry weight of plant). All studied growth characters of guar were significantly improved by inoculation compared to the control. The highest response was recorded on the application of combined *Azospirillum* and specific *Rhizobium* (ARC802). Specific *Rhizobium* as active symbiotic bacteria encourage the plant growth by supplying plants with sufficient quantities of nitrogen. (Wynne, *et al.*, 1980). Combined treatment of *Azospirillum* with *Rhizobium* (local strain) came on the second rank among all treatments. This showed the effective role of *Azospirillum* on plant growth in general. The beneficial effect of *Azospirillum* on the plant development could be attributed to its production of broad spectrum of plant growth substances (Tren *et al.*, 1979) which improve the growth of entire root system, which result in enhanced mineral and water uptake.

Table (3): Effect of co-inoculation with *Rhizobium* and *Azospirillum* on nodulation and growth characters of guar plant.

Treatments	Nodules No. /plant		Nodules dry weight mg/plant		Plant height (cm)		Branches No. / plant		Dry weight g / plant	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Control	93.4	8.2	0.91	0.78.0	138.0	146.0	8.0	9.0	17.3	18.1
Fertilized control	19.2	16.8	210.0	197.0	150.0	152.0	10.0	11.0	19.5	20.7
Rhizobium (ARC 802 strain)	51.2	54.6	543.0	518.0	162.0	165.0	12.0	13.0	22.1	23.7
Rhizobium (local strain)	45.3	51.8	538.0	436.0	156.0	155.0	12.0	11.0	22.4	22.5
Azospirillum	26.2	21.7	286.0	216.0	157.0	159.0	12.0	11.0	22.9	21.2
Rhizo.(ARC802 strain)+Azosp.	61.3	67.9	682.0	657.0	182.0	181.0	14.0	14.0	26.8	27.1
Rhizo (local strain) + Azosp.	53.7	58.4	594.0	622.0	174.0	172.0	13.0	14.0	23.4	25.5
L.S.D at 5%	6.956	1.616	1.902	2.690	2.0	1.6	1.22	0.67	0.05	0.04

Rhizo. : *Rhizobium*
Azosp. :*Azospirillum*

Also, inoculation with *Rhizobium* alone may have needed to achieve a specific threshold of nodules before a positive effect could be seemed on the plant. This period represents the crucial stage at which the plants would be under considerable stress owing to high nodulation that required conceivable amount of energy. The presence of *Azospirillum* may help the plants to overcome this initial stress (Itzigsohn *et al.*, 1993). Effect of *Azospirillum* on nodulation and specific activity of nodule N_2-fixation, leading to growth promotion, may be attributed to early nodulation, an increase in the total nodule number and a general improvement in mineral and water uptake by the roots (Volpin and Kapulnik, 1994). These results are in full agreement with Abd El-Fattah (2001).

Pods and yield

Pods parameters (number of pods and pods dry weight per plant) and seed yield per plant as recorded in Table (4) showed that pods parameters increased with all inoculated treatments. There is a sort of correlation between plant growth (Table, 3) and pods parameters of guar plants. The inoculation with *Rhizobium* ARC 802 and *Azospirillum* seemed again to be superior. The seed yield data showed a similar trend to that observed in pod parameters.

The yield increase, due to biofertilizers application, might be attributed to the increase in vegetative growth in general which increase pods parameters and leading to total yield increments. Similar results were obtained by Sorial and Abd El-Fattah (1998) and Tewfike (2000).

Table (4): Effect of co-inoculation with *Rhizobium* and *Azospirillum* on yield and yield component of guar plant.

Treatments	Pods No. / plant		Pods dry weight g / plant		Seed yield g / plant	
	1999	2000	1999	2000	1999	2000
Control	68.4	77.1	11.9	12.6	7.7	8.1
Fertilized control	79.2	84.4	13.7	15.4	10.5	11.3
***Rhizobium* (ARC 802)**	98.1	93.6	17.3	16.8	11.6	11.9
***Rhizobium* (local)**	92.6	87.7	16.3	15.3	11.3	11.2
Azospirillum	94.4	88.3	16.6	15.9	11.8	11.4
Rhizo. (ARC802) + Azosp.	103.6	110.1	20.9	21.2	12.7	12.4
Rhizo (local) + Azosp.	99.5	96.8	20.4	19.8	12.3	12.5
L.S.D at 5%	1.0	1.26	0.3	0.3	0.41	0.4

Plant constituents

NPK content of guar plant

Plant content of nutrients could be taken as an indication to nutrients availability in the soil. NPK content of guar plant as recorded in Table 5 showed that combination of *Rhizobium* and *Azospirillum* increased these contents. This was followed by the treatments that inoculated with *Rhizobium* alone. These results are in harmony with Barakat and Gaber (1998) who reported that single and mixed biofertilizers significantly increased minerals content. This could be attributed to the increase in the nitrogen fixing efficiency of inoculated plants. It is normally expected that the N content increases as a direct result for the increased N_2 fixation. As a result the P and K content coincidelly increased.

Table (5): Effect of co-inoculation with *Rhizobium* and *Azospirillum* on NPK of guar plant and gum content of guar seeds.

Treatments	N-content %		P-content %		K-content %		Seed gum %	
	1999	2000	1999	2000	1999	2000	1999	2000
Control	2.8	2.5	0.57	0.53	2.1	2.3	23.2	22.6
Fertilized control	2.9	3.2	0.68	0.61	2.6	2.4	24.6	22.8
Rhizobium (ARC 802 strain)	4.0	3.7	0.88	0.91	4.1	4.0	26.4	26.8
Rhizobium (local strain)	3.9	3.5	0.87	0.83	3.5	3.6	25.7	26.1
Azospirillum	3.3	3.4	0.80	0.80	3.1	3.2	26.5	25.8
Rhizo. (ARC802 strain)+Azosp.	4.5	4.6	0.96	0.94	4.4	4.1	28.2	27.6
Rhizo (local strain) + Azosp.	4.2	4.0	0.91	0.92	4.1	3.8	26.8	25.9
L.S.D at 5%	0.05	0.06	0.09	0.06	0.1	0.4	1.03	1.21

Guar gum in seed

Guar gum is extracted from guar seed. Data presented in Table (5) showed that all inoculated treatments gave percentage of guar gum (guaran %) more than control treatments. The inoculation by *Rhizobium* ARC802 combined with *Azospirillum* gave the highest values of seed gum percentage. The obtained results are in agreement with those obtained by Tewfike (2000).

Micronutrients of guar plants

It is evident from the data presented in Table (6) that in the two growing seasons changes in iron, zinc, manganese and copper of guar plants showed the same trend. The highest Fe and Cu content of plants accompanied the treatments of *Azospirillum* and *Rhizobium* (ARC802). Treatment of *Azospirillum* combined with *Rhizobium* (local) increased Fe and Cu content but to a less extent. It is clear from the data presented in Tables (3 and 4) that, the excellent effect of combined treatments of *Azospirillum* and *Rhizobium* on the plant growth and yield components may be an acceptable explanation to these increases in mineral content.

All treatments did not gave any positive effect on increasing zink content of plants. Manganese content of plant was increased by inoculation. Treatments of *Rhizobium* significantly increased Mn content.

Summarizing the results of this study, it could be concluded that nitrogen fixation could be maximized in guar through co-inoculation with specific *Rhizobium* of guar and plant growth promoting rhizobacteria including *Azospirillum* in combination with the half dose of N-fertilizer. This treatment experimentally increased all the yield parameters including green growth, seeds and gum.

Table (6): Effect of co-inoculation with *Rhizobium* and *Azospirillum* on mineral contents of guar plant.

Treatments	Fe (ppm)		Zn (ppm)		Mn (ppm)		Cu (ppm)	
	1999	2000	1999	2000	1999	2000	1999	2000
Control	90	107	89	90	70	81	7	9
Fertilized control	102	121	88	92	80	86	10	12
Rhizobium (ARC 802 strain)	154	148	85	94	132	119	12	13
Rhizobium (local strain)	143	129	89	87	151	131	11	14
Azospirillum	148	133	88	95	87	94	7	10
Rhizo. (ARC802 strain)+Azosp.	172	194	89	88	104	99	14	13
Rhizo (local strain) + Azosp.	163	155	90	97	117	104	13	14
L.S.D at 5%	6.8	6.2	N.S.	N.S.	3.66	8.29	2.07	2.24

REFERENCES

Abd El-Fattah, F.K. (2001): Stimulatory effect of *Azospirillum* and *Azotobacter* on symbiotic efficiency between *Bradyrhizobium* and soybean under different rates of nitrogen fertilization.J. Agric. Sci., Mansoura Univ., 26(6): 3961-3973.

Abou-Aly, H.E. and Gomaa, A.O. (2002): Influence of combined inoculation with diazotrophs and phospshate solubilizers on growth, yield and volatile oil content of coriander plants(*Coriandrum sativumL.*).Bull. Fac.Agric.CairoUniv.,53:93,114.

A.O.A.C. (1980): Official Methods of Analysis Association of Official Agriculture Chemists.10th Ed. Washington, D.C., U.S.A.. P:832.

A.P.H.A., American Public Health Association (1992): Standard methods for the examination of water and waste water. Washington, D.C., U.S.A..

Barakat, M. A. S. and Gaber, S. M. (1998): Effect of phosphate dissolving bacteria on Chickpea production under calcareous soil. Egypt. J. Appl. Sci., 9(4): 345-360.

Black, C.A.; Evans, D.O.; Ensminger, L.E.; White, J.L.; Clark, F.E. and Dinauer, R.C. (1982): Methods of Soil Analysis. Part 2. Chemical and microbiological properties. 2nd Ed. Soil Sci., Soc. of Am. Inch. Publ., Madison, Wisconsin,U.S.A.

Bottini, R.;M.; Fulchieri, D. Pearce and Pharis, R.P. (1989): Identification of gibberellins A1,A3 and iso-A3 in culture of *Azospirillum lipoferum*. Plant Physiol., 89:1-3.

Chapman, H.D. and Pratt, P.F. (1978): "Methods of Analysis for Soils, Plants and Waters", California, Univ. Div. Agric. Sci. Priced Pub. 4034.

Das, B.; Thereja, S.K. and Singh, K. (1977): Quality of guar seeds as influenced by different agronomic practices. Proceedings of the First Guar Research Workshop, Organized by the Indian Council of Agricultural Research, at Central Arid Zone Research Institute, Jodhpur, pp. 98:103.

Dewis, G. and Freitas, F. (1970): Physical and chemical methods of soil and water analysis. F.A.O., Bull., No (10).

El-Sheikh, E.A.E. and El-Zidany, A.A. (1997): Effects of *Rhizobium* inoculation, organic and chemical fertilizers on yield and physical properties of faba bean seeds. Plant Food Human Nutr. 51, 137-144.

Ghonem, S.A. (1990): Response of guar to nitrogen and phosphorus fertilization. Zagazig J. Agric. Res., 17(2):199-210.

Hardy, R.W.F.; Burns, R.C. and Holsten, R.D. (1973): Application of the acetylene ethylene assay for measurement of nitrogen fixation. Soil Biol.Biochem.,5:47-81.

Hegazi, N.A.; Amer, H.A. and Monib, M. (1979): Enumeration of N2-fixing Spirilla. Soil Bio. Biochem., 11:437-438.

Itzigsohn, R.; Kapulink, Y.; Okon, Y. and Dovart A. (1993): Physiological and morphological aspects of interaction between *Rhizobium meliloti* and alfalfa in association with *Azospirillum brasilense*. Can. J. Microbiol. 39:610-615.

Jacek, P. and Rolfe, B.G. (1985): Interaction of *Azospirillum* and *Rhizobium* strains leading to inhibition of nodulation. Appl. Environ. Microbiol., Apr, 1985, P. 990-993.

Jackson, M.L. (1973): Soil Chemical Analysis. Prentice-Hall of India, Private New Delhi.

Mor, S.; Dogra, R.C. and Kishan, S. (1995): Effect of adhesive on nitrogen fixation legumes and cereals. Legume Research, 18(4):205-210.

Okon, Y.; Bloemberg, G.V. and Lugtenberg, B.J.J. (1998): Biotechnology of Biofertilization and Phytostimulation. In: Agricultural Biotechnology (A. Altman, ed.), Marcel Dekker, Inc., New York, pp. 327-349.

Page, A.L.; Miller, R.H. and Keeny, D.R. (1982): Methods of Soil Analysis, Part 2,2nd Ed., Am. Soc. Agronomy, Inc. Mad. Wisconsin, U.S.A.

Plazinski, J. and Rolfe, B.G. (1985): Influence of *Azospirillum* strains on the nodulation of clover by *Rhizobium* strains. Environ. Microbiol., 49:984-989.

Snedecor, G.W. and Cochran, W.G. (1989): Statistical Methods. 8th Ed., IowaState Univ., Press, Iowa, U.S.A.

Sorial, M. E. and Abd El-Fattah,M. A. (1998): The possibility of using the biofertilizers as a completely substitute of NPK-fertilizers in plant production. Annals of Agric. Sci., Moshtohor,36(3): 1683-1700.

Stafford, RE. (1975): Other uses of guar under exploited tropical plants with promising value, Washington, D.C. Nationed Academy of Science, pp. 30-34.

Swelim, D. and Abdel-Wahab, S.M. (2000): Co-inoculation effect with *Rhizobium* and *Azospirillum* on nodulation and nitrogenase activity of Leucaena leucocephala. The 10th Conf. of Microbiol. Cairo, Egypt. Nov., 12-14.

Tewfike, T.A. (2000): Effect of dual biofertilization, biogas manure and mineral fertilizer on guar plants (*Cyamopsis tetragonoloba L.*). J. Agric. Sci., Mansoura Univ., 25(10): 6481-6492.

Tren, T.M., Gaskins, M. H. and Hubbell, D. H. (1979): Plant growth substances produced by *Azospirillum brasilense* and their effect on growth of pearl millet *(Pennisetum americanum)*. Appl. Environ. Microbiol. 37: 1016-1024.

Vincent, J.M. (1970): "Manual for the Practical Study of Root-Nodule Bacteria".IBP Handbook No.15, Blackwell Sci. Pub., Oxford, pp.14-25.

Volpin, H. and Kapulnik, Y. (1994): Interaction of *Azospirillum* with beneficial soil microorganisms. *Azospirillum* /Plant Associations (Y.Okon, ed.), CRC Press, Boca Raton, FL, pp. 111-118.

Wynne, J.C.; Elkan, G.H.; Meisner, C.M.; Schneeweis, T.J. and Ligon, J.M. (1980): Greenhouse evaluation of strains of *Rhizobium* for peanuts. Agron. J.,72: 645-649.

تأثير التلقيح المشترك ببكتريا الريزوبيا والأزوسبيريللام على النمو وتكوين العقد والمحصول لنبات الجوار

حامد السيد أبوعلي

قسم النبات الزراعى (ميكروبيولوجيا زراعية)-كلية الزراعة بمشتهر-جامعة الزقازيق-مصر.

أجريت تجربتان حقليتان بمزرعة مركز البحوث والتجارب بكلية الزراعة بمشتهر لدراسة أثر التلقيح المشترك بمزارع من *Rhizobium* و*Azospirillum lipoferum* على النمو وتكوين العقد والمحصول وكذلك محتوى بعض العناصر لنباتات الجوار. في هذه الدراسة استخدمت سلالتين من الريزوبيا هى ARC802 المتخصصة لنبات الجوار وسلالة أخرى معزولة من عقد جذرية لنبات الجوار كل على حده أو مختلطة مع *A. lipoferum*. وقد أوضحت النتائج الآتى:

أعطت كل المعاملات الملقحة سواء الفردية أو المختلطة زيادة معنوية فى معدل انطلاق CO_2 فى التربة وكذلك النمو الخضرى وتكوين العقد والمحصول وأيضا محتوى النبات من NPK عن النباتات الغير ملقحة.

كما أظهرت النتائج أن التلقيح ببكتريا الريزوبيا بمفردها وخاصة ARC802 أعطت زيادة فى معدل نشاط إنزيم النيتروجينيز مقارنة بالتلقيح سواء ببكتريا الأزوسبيريلام بمفردها أو مختلطة مع الريزوبيوم.

ولقد أدت إضافة *A. lipoferum* بالاشتراك مع *Rhizobium* ARC802 وذلك فى وجود نصف جرعة السماد النيتروجينى المعدنى إلى الحصول على أعلى معدل نمو وعدد ووزن العقد الجذرية وكذلك المحصول لنبات الجوار.

ومن هذا يتضح أن هناك استجابة جيدة لنبات الجوار لإضافة الأزوسبيريللام مع الريزوبيوم ويعتمد ذلك على وجود السلالة المتخصصة من الريزوبيوم.