Bull. Fac. Agric., Cairo Univ.,53 (2002): 93-114.

INFLUENCE OF COMBINED INOCULATION WITH DIAZOTROPHS AND PHOSPHATE SOLUBILIZERS ON GROWTH, YIELD AND VOLATILE OIL CONTENT OF CORIANDER PLANTS (*Coriandrum sativum* L.)

(Received: 8.5.2001)

By
H .E . Abou-Aly and A .O. Gomaa

*Agriculture Botany Department and * Horticulture Department, Faculty of Agriculture ,Moshtohor, Zagazig University (Benha Branch), Egypt.*

ABSTRACT

Two field trials were conducted during two successive seasons (1997/98 and 1998/99) to evaluate the effect of inoculation with diazotrophs (*Azotobacter chroococcum* or *Azospirillum brasilense*) combined with either *Bacillus megatherium var. phosphaticum* or (VAM) *Glomus mosseae* in the presence of half dose of inorganic N-fertilizer on growth, chemical constituents, yield and volatile oil content of coriander plants.

The results revealed that dual inoculation of coriander seeds with non-symbiotic N_2-fixers and phosphate solubilizers increased the available phosphorus content of the soil. This increase was accompanied by the highest values of phosphate dissolving bacteria densities and mycorrhizal root infection percentage. Combined inoculation of *Azotobacter chroococcum* and *Glomus mosseae* increased the values of nitrogenase activity and ammoniacal and nitrate nitrogen contents of coriander plant rhizosphere.

Inoculation with *Azotobacter* or *Azospirillum* combined with *Glomus mosseae* gave significant increases in vegetative growth, total carbohydrates, photosynthetic characteristics, essential oils, seed parameters, nitrogen, phosphorus and potassium contents of coriander plants.

Volatile oil constituents were determined with GLC analysis. It was found that linalool was the major constituent in the oil of coriander seeds. Maximum values of linalool were obtained in treatments inoculated with *Azotobacter* or *Azospirillum* in the presence of *Glomus mosseae*.

Key words: *azospirillum, azotobacter, Coriandrum sativum, glomus, vegetative growth, volatile oils.*

1. INTRODUCTION

Coriander (*Coriandrum sativum* L.) is a common condiment grown in many countries for its fresh herb and dry seeds. Coriander seeds and its oil are employed widely in some specific fresh drinks, essences and /or flavoring some kinds of food products. It is used also in pharmaceutical and other preparations to cover disagreeable odor and taste and as stimulant and carminative in medicine.

Biofertilization for many crops attracted the attention of researchers and it has become in the last few decades a positive alternative to chemical fertilizer (Suslow *et al*., 1979; Nieto and Frankenberger, 1990 and Wange, 1996). Several investigators reported that inoculation of plants by *Azotobacter* and /or *Azospirillum* combined with half of the recommended dose of nitrogen fertilizer can be used as substitutes for full dose of N-fertilizers to decrease the pollution of environment (Saber and Gomaa, 1993; El-Demerdash, 1994 and El-Khyat and Zaghloul, 1999).

Plant inoculation with the vesicular arbuscular mycorrhizae (VAM) in combination with N_2-fixing bacteria improved plant growth to a highly significant extent (Ishac *et al*., 1986 ; Yassen, 1993 and Fares, 1997).

Several investigators extensively studied phosphate dissolving bacteria and claimed their importance in supplying growing plants with available phosphorus when inoculated in soils (Khalil *et al*., 1991 and Ashoub and Abd El-Ghany, 1994).

The present work aimed to evaluate the effect of dual inoculation with *Azotobacter chroococcum* or *Azospirillum brasilense* combined with phosphate solubilizing microorganisms in the presence of half of the recommended dose of nitrogen fertilizer on productivity and volatile oil content of coriander plants.

2. MATERIALS AND METHODS

This experiment was carried out at the Experimental Farm, Fac. Agric., Moshtohor, Zagazig Univ., during 1997/98 and 1998/99 seasons. Mechanical and chemical analyses of the experimental soil are presented in Table (1-a) and (1-b). Mechanical analysis was estimated according to the methods of Jackson(1973), whereas, chemical analysis was estimated according to the methods of Black *et al.*, (1982).

Table (1-a): Mechanical analysis of the experimental soil.

Parameters	Unit	Seasons	
		1997/98	1998/99
Coarse sand	%	3.72	2.93
Fine sand	%	18.42	17.52
Silt	%	24.97	22.68
Clay	%	52.89	56.87
Textural class	------	Clay	clay

Table (1-b): Chemical analysis of the experimental soil.

Parameters	Unit	Seasons	
		1997/98	1998/99
$CaCO_3$	%	1.21	1.49
Organic matter	%	1.95	1.77
Total nitrogen	%	0.53	0.39
Total phosphorus	%	0.23	0.19
Total potassium	%	0.13	0.09
E.C	ds . m^{-1}	0.83	0.76
PH	---------	7.79	7.93

2. 1. N_2-fixing inoculants

Non-symbiotic N_2-fixers (*Azotobacter chroococcum* and *Azospirillum brasilense*) were used as seed inoculants. These strains were kindly provided by the Unit of Microbiology, Desert Research

Center, Mataria, Cairo , Egypt. The strains were grown on modified Ashby's medium (Abdel-Malek and Ishac, 1968) and semi-solid malate medium (Dobereiner, 1978), respectively, and incubated at 30°C for 7 days until the number of bacteria reached about $1x10^7$ cfu ml^{-1}.

2. 2. P- solubilizing inoculants

Bacillus megatherium var. phosphaticum (provided from the Microbiology Dept., Soil, Water & Environment Res. Inst., Agric., Res. Center, Giza, Egypt) was grown on Bunt and Rovira (1955) medium modified by Abdel-Hafez (1966), then incubated at 30°C for 7 days .

Mycorrhizal spores of *Glomus mosseae* were extracted from onion rhizosphere by using the wet sieving and decanting technique (Gerdmann and Nicolson, 1963).

2. 3. Inoculation

Coriander seeds were washed, air-dried and soaked in cell suspension of *Azotobacter* or *Azospirillum* (10^7 cell ml^{-1}) for 30 min . Arabic gum (16%) was used as an adhesive agent. Then the inoculated seeds were divided into three groups, the first group was left without further inoculation.The second group was inoculated with *Bacillus megatherium var. phosphaticum* (10^8 cell ml^{-1}) in the same manner mentioned above. The third group was prepared for cultivation of mycorrhizal treatments. Seeds of uninoculated treatments were treated with N-decifient medium without bacterial culture. Before sowing, mycorrhizal treatments were provided with a mycorrhizal spore suspension(100-200 spores ml^{-1} and was applied at a rate of 10 ml^{-1} m^{-1}) and infected onion root segments. Inoculated seeds were air dried at 25-28°C for one hour. After sowing, soil was immediately irrigated to provide a suitable moisture for the inocula.

2. 4. Experimental design

A randomized complete block design with four replicates was applied in the experiment. Seeds of coriander plants were planted in the first week of October in plots, the area of each plot was one square meter and included two rows with an inter-row distance of 50 cm. Seeds were planted in each row in hills with a distance of 20 cm. between hills. The seedlings were thinned one month later to two plants per hill.

Except for control treatments, all plots were fertilized with a half dose (30 kg-N) of ammonium sulphate (150 kg/feddan), 100 kg. superphosphate (15.5% P_2O_5) and 50 kg. potassium sulphate (48% K_2O) per feddan, added in two equal doses at 30 and 60 days after sowing.

2. 5. The experiment included the following treatments

1-Untreated plants (Control).
2- 60 kg. inorganic N-fertilizer.
3- *Azotobacter chroococcum* + 30 kg. N.
4- *Azotobacter chroococcum* + 30 kg. N.+P.S.B.
5- *Azotobacter chroococcum* + 30 kg. N.+ Mycorrhizae.
6- *Azospirillum brasilense* + 30 kg. N.
7- *Azospirillum brasilense* + 30 kg. N.+P.S.B.
8- *Azospirillum brasilense* + 30 kg. N.+ Mycorrhizae.

2. 6 . Sampling and Determinations

At the vegetative stage (60 days) and flowering stage (110 days), rhizosphere soil samples of the plants were taken to microbiological and chemical determinations. Also, coriander plants at maturity stage were obtained to determine the growth parameters and chemical analysis.

2. 7. Micorobiological analysis

- Rate of mycorrhizal infection was evaluated according to Mosse and Giovanetti (1980).
- Phosphate dissolving bacteria densities were determined by the standard plate method (Bunt and Rovira medium, 1955 modified by Abdel-Hafez, 1966) .
- Nitrogenase activity was estimated according to (Hardy *et al.*, 1973).

2. 8. Chemical analysis

-Available phosphorus and ammoniacal and nitrate nitrogen were determined according to Olsen *et al.*, 1954 and Bremner and Keeny, 1965 respectively.
-Nitrogen, phosphorus, potassium, chlorophyll and carotenoids were determined according to A.O.A.C. (1980).
-Total carbohydrates content of leaves was determined by Smith *et al.*,(1956).
-Essential oil content was determined by water distillation following the method described by Egyptian Pharmacopoeia (1984) .

2. 9. Growth parameters

Plant height (cm), fresh and dry weights of plant (g), no. of leaves /plant, no. of branches /plant, root length/plant (cm) and fresh and dry weights of roots / plant (g) were determined at maturity stage.

2. 10. Yield and its components

At harvest, no. of umbels/plant, no. of seeds (fruits)/umbel, seeds (fruits) yield (g/ plant), seeds (fruits) yield (kg/fed) and weight of 100 seeds (g) were recorded.

2. 11. Statistical analysis

Growth parameters, yield and chemical constituents of the plants were statistically analyzed using revised L.S.D. test according to the procedures outlined by Snedecor and Cochran (1989).

3. RESULTS AND DISCUSSION

3. 1. Phosphate dissolving bacteria, mycorrhizal root infection and available phosphorus

Results presented in Table (2) show the changes in the population of phosphate solubilizing bacteria in coriander plants rhizosphere. Densities of phosphate solubilizers were higher at the flowering stage than the vegetative stage. The beneficial effect of root secretions during the flowering stage may explain this increase. Inoculation of seeds with non-symbiotic N_2 fixers and receiving half dose of inorganic N-fertilizer gave higher densities of P-solubilizers than uninoculated plants supplemented with full dose of inorganic N-fertilizer.

The highest population of P-solubilizers were observed in the treatments inoculated with asymbiotic diazotrophs and phosphate solubilizing bacteria. Moreover, dual inoculation with diazotrophs and mycorrhizae led to an increase in densities of P-solubilizers compared to single inoculation with diazotrophs. These results confirmed those obtained by Saad and Hammad (1998) and Zaghloul (1999).

Data presented in Table (2), indicate that the combined inoculation with *Azotobacter* or *Azospirillum* and vesicular arbuscular mycorrhizae (VAM)*Glomus mosseae* in the presence of half dose of inorganic N-fertilizer gave the highest rate of coriander root infection with endomycorrhizae. These data are in agreement with those found by

Table (2): Effect of inoculation with diazotrophs and phosphate solubilizers on phosphate solubilizing bacterial densities (x 10^5/g dry soil), mycorrhizal root infection and available phosphorus content in rhizosphere of *Coriandrum sativum* L. plants during 1997/98and 1998/99 seasons.

Parameters / Treatments	Vegetative stage (60 days)						Flowering stage (110 days)					
	Phosphate solubilizing bacteria densities		Mycorrhizal root infection(%)		Available-P (ppm)		Phosphate solubilizing bacteria densities		Mycorrhizal root infection(%)		Available-P (ppm)	
	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99
Control (Untreated plants)	2.4	4.7	7.3	6.8	15.8	19.6	11.6	14.3	8.1	11.6	25.6	31.8
60 kg. inorganic nitrogen	13.5	18.9	10.2	12.5	31.6	38.2	20.4	25.8	13.7	15.1	42.5	56.4
Azotobacter + 30 kg. N.	38.2	42.6	11.8	14.2	51.2	59.8	64.5	88.7	17.2	20.9	120.9	124.1
Azotobacter + 30 kg. N.+P.S.B.	69.7	81.5	15.7	19.4	83.1	88.5	123.6	158.3	21.2	26.8	167.8	191.2
Azotobacter + 30 kg. N+Mycorrhizae	41.6	53.1	48.6	56.3	78.7	85.4	86.5	103.6	67.5	74.7	148.2	166.7
Azospirillum + 30 kg. N.	26.7	37.5	13.4	18.7	48.3	57.9	61.4	90.7	19.6	21.4	106.4	117.8
Azospirillum + 30 kg. N.+P.S.B.	62.8	77.9	17.7	16.5	79.4	84.2	118.7	136.9	20.1	23.3	150.5	161.3
Azospirillum+30kg. N.+Mycorrhizae	45.3	49.8	45.2	52.9	70.8	78.4	92.2	98.4	61.8	67.5	141.3	156.8

Fares (1997) and El-Sawy *et al.*,(1998). Low percentage of mycorrhizal root infection in the uninoculated coriander plants indicated that the native VAM fungi are present in the soil but in low density.

Data also show that the available phosphorus of coriander plants rhizosphere was remarkably increased in inoculation treatments in the presence of half dose of inorganic N-fertilizer compared to uninoculated treatment and supplemented with full dose of inorganic N-fertilizer. The increase was more prominent in the combined biofertilized treatments with non-symbiotic N_2-fixers and phosphate solubilizers than in single biofertilized treatments. The highest values of available phosphorus were recorded with *Azotobacter chroococcum* combined with phosphate solubilizing bacteria. This increase may be due to that the P-solubilizing bacteria utilize organic compounds as carbon and energy source and produce organic acids thereby solubilize insoluble phosphates (Gaur *et al.* 1979). These results were confirmed with those obtained by Barakah *et al.*, (1998) and El-Sayed (1998).

3.2. Nitrogenase activity and nitrogen forms in coriander rhizosphere

It is obvious from the data in Table (3) that nitrogenase activity increased in coriander plants rhizosphere which received asymbiotic diazotrophs and supplemented with half dose of inorganic N-fertilizer. This may be due to the low doses of N-fertilizer which encourage N_2-fixation (Saleh *et al.*, 1998). Nitrogenase activity increased to considerable extents in the presence of phosphate solubilizers. The highest rates of nitrogenase activity were obtained with combination of non-symbiotic N_2-fixers and VAM or phosphate solubilizing bacteria at flowering stage in the two seasons. Similar results were recorded by Barakah *et al.*, (1998) and Mikhaeel *et al.*, (2000) who found that nitrogenase activity increased by dual inoculation of the plants with diazotrophs and vesicular arbuscular mycorrhizae.

With respect to ammoniacal and nitrate nitrogen, data in Table (3) show that ammoniacal and nitrate nitrogen concentrations were apparently higher in the flowering stage than the vegetative one in the two seasons. Inoculation of coriander seeds with non-symbiotic N_2-fixers and phosphate solubilizers gave the highest values of ammoniacal and nitrate nitrogen concentrations. These increases may be attributed to the high values of phosphate solubilizing bacteria densities and

Table (3): Nitrogenase activity and nitrogen forms in the rhizosphere of *Coriandrum sativum* L. plants as affected by inoculation with diazotrophs and phosphate solubilizers .

Parameters / Treatments	Vegetative stage (60 days)						Flowering stage (110 days)					
	N_2 ase activity (n moles C_2H_4/hr/g dry soil)		NH_4^+- N (ppm)		NO_3^- -N (ppm)		N_2 ase activity (n moles C_2H_4/hr/g dry soil)		NH_4^+- N (ppm)		NO_3^-- N (ppm)	
	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99
Control (Untreated plants)	9.6	11.4	14.6	22.5	18.3	24.1	12.8	13.2	23.7	27.2	29.5	32.4
60 kg. inorganic nitrogen	18.7	23.5	29.8	33.7	27.5	30.6	24.1	30.5	31.5	43.6	34.3	41.5
Azotobacter + 30 kg. N.	41.6	55.3	32.7	35.2	35.6	41.4	62.4	78.3	44.7	48.6	49.3	56.9
Azotobacter + 30 kg. N.+P.S.B.	58.5	70.4	38.1	44.3	41.7	53.9	76.2	106.4	49.4	56.3	53.1	66.8
Azotobacter + 30 kg. N.+Mycorrhizae	64.7	79.2	40.5	48.6	54.4	58.2	89.1	113.5	62.4	68.2	61.7	73.9
Azospirillum + 30 kg. N.	38.1	47.9	30.4	36.2	38.3	42.8	60.7	70.6	50.7	54.5	42.6	51.3
Azospirillum + 30 kg. N.+P.S.B.	48.7	58.6	35.1	42.3	40.5	48.7	68.1	81.9	53.6	55.2	48.5	58.4
Azospirillum + 30 kg. N.+Mycorrhizae	52.9	64.8	41.2	45.8	45.6	52.3	74.2	88.6	56.9	60.1	59.4	63.2

Table (4): Effect of inoculation with diazotrophs and phosphate solubilizers on growth parameters of *Coriandrum sativum* L. plants during 1997/98 and 1998/99 seasons.

Parameters / Treatments	Plant height (cm)		Fresh weight of plant (g)		Dry weight of plant (g)		Number of leaves/ plant		Number of branches/ plant		Root Length / plant (cm)		Fresh weight of Root (g)		Dry weight of Root (g)	
	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99
Control(Untreated plants)	64.25	72.75	94.00	94.50	13.00	13.01	76.50	77.50	4.00	4.25	22.50	22.75	12.75	13.00	2.12	2.17
60 kg. inorganic nitrogen	81.00	86.75	111.00	119.25	15.21	16.37	81.25	82.25	4.75	5.25	24.50	24.75	15.50	15.50	2.56	2.58
Azotobacter + 30 kg. N.	86.00	90.00	120.00	123.75	17.15	17.09	86.00	86.00	5.75	6.25	29.00	29.75	19.25	19.75	3.20	3.31
Azotobacter + 30 kg. N.+P.S.B.	93.75	96.00	124.50	129.00	17.20	17.91	89.75	90.00	6.50	6.75	30.75	32.25	22.50	22.75	3.74	3.82
Azotobacter + 30 kg. N.+ Mycorrhizae	105..50	109..0	130.50	136.50	18.13	19.06	96.50	97.25	7.50	7.50	35.75	36.75	24.50	24.75	4.11	4.16
Azospirillum + 30 kg. N.	85.00	87.50	118.00	121.75	16.22	16.80	84.00	85.50	5.50	6.00	27.25	28.50	19.00	19.00	3.14	3.18
Azospirillum + 30 kg. N.+P.S.B.	91.00	94.50	122.00	126.50	16.81	17.52	87.00	87.75	6.25	6.50	29.75	31.25	22.00	22.25	3.65	3.74
Azospirillum + 30 kg. N.+ Mycorrhizae	97.25	103.0	127.75	131.75	17.98	18.25	95.50	95.75	7.00	7.00	34.50	35.50	23.25	24.00	3.78	4.02
L. S. D. at 5%	3.28	2.19	3.68	1.54	0.47	0.33	3.12	1.19	0.84	0.97	1.55	1.16	0.96	0.76	0.17	0.13
L. S. D. at 1%	4.47	2.99	4.89	2.09	0.65	0.45	4.25	1.61	1.15	1.32	2.11	1.44	1.30	1.04	0.23	0.18

mycorrhizal root infection percentage with the same treatments as was observed in this study (Table 2) which enhanced the availability of nutrients in soil (Barakah *et al.*, 1998).

3 . 3. Growth characters

Data in Table (4) clearly show that all growth characters of coriander plants significantly improved by biofertilization as compared with uninoculated plants. Dual inoculation with diazotrophs and phosphate solubilizers significantly increased growth characters i.e. plant height, plant fresh and dry weights, number of leaves and branches per plant, root length and fresh and dry weights of root per plant compared to single inoculation with asymbiotic N_2-fixers. It was found that maximum growth characters were observed in treatments inoculated with *Azotobacter chroococcum* and VAM or phosphate solubilizing bacteria. These results are in agreement with those obtained by Fares, (1997) and El-Sayed, (1998) who stated that combined inoculation with diazotrophs and VAM had a positive effect on plant growth. The beneficial effects of N_2-fixers on plant growth can be attributed not only to the N_2-fixation process but also to the production of growth promoting substances such as auxins, gibberellins and cytokinins (Yahya and Al-Azawi, 1989) which might improve plant growth and stimulate the microbial development. Whereas, the combined application of asymbiotic N_2-fixers and phosphate solubilizers improved growth parameters, the stimulatory effect of phosphate solubilizers can be attributed to the absorption of nutrients by plants especially phosphorus from soil as previously observed in Table (2). Cooper and Tinker (1978) stated that the mechanism of increasing plant phosphorus content by VAM is based on the high activity of VA mycorrhizal hyphae in absorbing soil phosphorus and then translocate onto the host roots through a specific efficient active translocation.

3 . 4 . Chemical constituents

The nitrogen, phosphorus and potassium contents as well as photosynthetic characteristics in coriander plants showed similar trends to those recorded in growth characters. Data in Table (5) revealed that biofertilization of coriander plants with non-symbiotic N_2-fixers increased mineral contents and photosynthetic characteristics more than the control. The highest N, P and K contents as well as photosynthetic

Table (5): Effect of inoculation with diazotrophs and phosphate solubilizers on some chemical characteristics of *Coriandrum sativum* L. plants during 1997/98 and 1998/99 seasons.

Parameters / Treatments	Total nitrogen %		Total phosphorus%		Total potassium %		Chlorophyll (a) mg/g		Chlorophyll (b) mg/g		Carotenoids mg/g		Total carbohydrates%	
	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99
Control (Untreated plants)	1.12	1.18	0.38	0.40	1.10	1.11	0.59	0.61	0.40	0.44	0.50	0.55	10.72	11.74
60 kg. inorganic nitrogen	1.48	1.47	0.41	0.42	1.22	1.19	0.78	0.78	0.50	0.51	0.61	0.65	12.27	12.49
Azotobacter + 30 kg. N.	1.65	1.65	0.43	0.45	1.28	1.29	0.81	0.83	0.55	0.53	0.63	0.70	12.99	13.15
Azotobacter + 30 kg. N.+P.S.B.	1.78	1.76	0.45	0.48	1.29	1.30	0.83	0.84	0.56	0.55	0.68	0.77	13.97	13.99
Azotobacter + 30 kg. N.+ Mycorrhizae	1.83	1.89	0.56	0.58	1.33	1.32	0.86	0.88	0.59	0.58	0.72	0.78	14.44	15.13
Azospirillum + 30 kg. N.	1.61	1.61	0.41	0.43	1.27	1.28	0.80	0.81	0.55	0.52	0.62	0.67	12.69	12.99
Azospirillum + 30 kg. N.+P.S.B.	1.69	1.71	0.43	0.47	1.28	1.29	0.81	0.84	0.57	0.53	0.66	0.73	13.48	13.62
Azospirillum + 30 kg. N.+Mycorrhizae	1.79	1.85	0.48	0.53	1.29	1.31	0.83	0.86	0.58	0.56	0.71	0.76	14.07	14.94
L. S. D. at 5%	0.03	0.05	0.01	0.02	0.01	005	0.01	0.01	0.01	0.01	0.01	0.02	0.39	0.35
L. S. D. at 1%	0.04	0.06	0.02	0.03	0.02	0.06	0.01	0.01	0.01	0.02	0.02	0.02	0.53	0.48

Table(6): Effect of inoculation with diazotrophs and phosphate solubilizers on seeds parameters and essential oil of *Coriandrum sativum* L. plants during 1997/98and 1998/99 seasons.

Parameters / Treatments	Number of umbels/ plant		Number of seeds / umbels		Seed yield / plant (g)		Seed yield / fed. (kg.)		Weight of 100 seeds (g)		Essential oil of seeds %	
	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99	97/98	98/99
(Control) Untreated plants	37.00	38.00	24.75	25.00	8.00	8.75	640.00	700.00	1.11	1.15	1.02	1.07
60 kg. inorganic nitrogen	50.75	53.25	30.25	32.25	12.25	13.00	980.00	1040.00	1.30	1.28	1.18	1.21
Azotobacter + 30 kg. N.	63.00	65.25	35.75	35.75	14.25	14.75	1140.00	1180.00	1.31	1.33	1.23	1.28
Azotobacter + 30 kg. N.+P.S.B.	67.25	68.25	37.25	38.25	16.75	17.50	1340.00	1400.00	1.36	1.36	1.25	1.29
Azotobacter + 30 kg. N.+ Mycorrhizae	69.75	70.25	39.75	40.00	19.50	19.75	1560.00	1580.00	1.38	1.38	1.30	1.39
Azospirillum + 30 kg. N.	57.25	65.00	34.50	35.25	13.50	14.00	1080.00	1120.00	1.30	1.29	1.21	1.25
Azospirillum + 30 kg. N.+P.S.B.	62.75	66.25	36.75	38.00	15.75	16.75	1240.00	1340.00	1.32	1.35	1.24	1.28
Azospirillum + 30 kg. N.+Mycorrhizae	65.75	68.75	38.25	38.50	18.25	17.75	1460.00	1420.00	1.35	1.36	1.28	1.35
L. S. D. at 5%	3.61	1.69	2.14	0.89	0.97	0.57	78.08	45.61	0.01	0.02	0.030	0.029
L. S. D. at 1%	4.92	2.30	2.92	1.22	1.32	0.77	106.30	62.09	0.02	0.03	0.42	0.039

characteristics were recorded with *Azotobacter* or *Azospirillum* combined with VAM or phosphate solubilizing bacteria. These results are in agreement with Barakat and Gaber (1998) and Mikhaeel *et al.*, (2000). They stated that single and mixed biofertilizers significantly increased both mineral contents and leaf chlorophyll concentration than the control. These results are good explanation to the obtained results regarding the favorable role of biofertilization on growth characters (Table 4). The availability of N and P for plant growth due to diazotrophs and phosphate solubilizers inoculaled to large increase in the rate of photosynthesis by the plants which is sufficient to support plant growth.

Data in Table (5) also, indicate that total carbohydrates showed the same general trend concerning the beneficial effect of dual inoculation of non-symbiotic N_2-fixers and phosphate solubilizers which gave the highest values of total carbohydrates. The enhancing effect of the biofertilizers on growth and photosynthetic characters with the same treatments (Table 4 and 5) may explain the increase in total carbohydrates.

3 . 5 . Yield and its components

Data presented in Table (6) show that there was significant increases in the yield values in both seasons when the plants received biofertilizers. Maximum yields of coriander plants expressed as number of umbels per plant, number of seeds (fruits) per umbel, seed yield (fruits yield) per plant and feddan, weight of 100 seeds and essential oil of seeds were obtained by using dual inoculation with *Azotobacter* or *Azospirillum* combined with VAM or P-solubilizing bacteria. These results are in harmony with those obtained by Fares (1997) and Zaghloul (1999) who mentioned that inoculation of the plants with diazotrophs and P-solubilizers significantly improved the yield of the plants. These increases may be attributed to the beneficial effects of N_2-fixers and P-solubilizers on plant growth, NPK contents and photosynthetic characteristics observed in this work (Tables 4 and 5).

3 . 6 . Essential oil constituents

Concerning the effect of biofertilization on essential oil constituents of coriander plants, data in Table (7) indicate that GLC analysis of the volatile oil of seeds revealed the presence of α -pinene, linalool, camphor, myrcene, borneol, nerol, cis-geraniol and geranyl acetate . All treatments

Table (7): G.L.C. analysis of the freshly distilled oil of *Coriandrum sativum* L. as affected by inoculation with diazotrophs and phosphate solubilizers.

Treatments		Area Percentage							
	Ret. time	**1.843**	**6.256**	**8.250**	**10.078**	**15.964**	**22.738**	**25.766**	**28.642**
	Name of compound	**α-Pinene**	**Linalool**	**Camphor**	**Myrcene**	**Borneol**	**Nerol**	**Cis-geraniol**	**Geranyl acetate**
	Peak number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Control (Untreated plants)		5.316	54.537	10.472	15.288	0.924	0.724	0.678	0.632
60 kg. inorganic nitrogen		6.061	62.456	6.672	13.782	0.970	0.507	1.105	2.471
Azotobacter + 30 kg. N.		6.105	64.127	4.831	12.739	1.898	1.984	1.898	0.989
Azotobacter + 30 kg. N.+P.S.B.		5.040	65.580	5.681	13.671	0.850	0.407	1.315	1.470
Azotobacter + 30 kg. N.+ Mycorrhizae		4.240	66.283	6.017	9.885	1.513	1.766	1.223	4.744
Azospirillum + 30 kg. N.		6.117	63.213	4.831	13.739	1.898	1.884	1.011	1.877
Azospirillum + 30 kg. N.+P.S.B.		4.859	65.266	5.929	14.022	1.018	1.282	1.397	1.479
Azospirillum + 30 kg. N.+ Mycorrhizae		7.228	66.269	3.720	13.728	0.896	0.863	0.878	0.988

showed that linalool is the main compound of coriander seeds volatile oil. These results are in accordance with those of Hussein (1995) who reported that the main component of coriander oil is linalool. Minimal levels of linalool were obtained in uninoculated treatments. It was found that linalool reached its maximal levels in plants inoculated with asymbiotic diazotrophs and VAM.

It can be concluded from the results of the present study that combined biofertilization of coriander plants with asymbiotic diazotrophs and phosphate solubilizing bacteria or VAM in the presence of half dose of inorganic N-fertilizer had a beneficial effect on the productivity of coriander plants to a significant extent. It can be save 30 kg N per feddan and decrease the possible pollution of environment and enhance the utilization of soil nutrients, particularly unavailable phosphorus by phosphate solubilizers.

4. REFERENCES

Abdel-Hafez A.M.(1966). Some studies on acid producing microorganisms in soil and rhizosphere with special reference to phosphate dissolvers. Ph. D. Thesis, Agric. Botany Dept., Fac. of Agric., Ain Shams Univ., Egypt, 31 .

Abdel-Malek Y. and Ishac Y.Z. (1968). Evaluation of methods used in counting azotobacters. J. appl. Bact., 31: 267-275.

A.O.A.C.(1980). Official Methods of Analysis,12Th Ed. Association of Official Analysis Chemists: Washington, D.C., U.S.A.

Ashoub A.H. and Abd El-Ghany B.F. (1994). Effect of phosphate-dissolving bacteria on chickpea production under calcareous soil. Egypt. J. Appl. Sci.; 9(4): 345-360 .

Barakah F.N., Salem S.H. and Heggo A.M. (1998). Effect of inoculation with endomycorrhizal fungi (VAM) and *Rhizobium meliloti* on nodulation, nutrition and N_2-fixation of alfalfa grown on calcareous soil. Annals Agric. Sci., Ain Shams Univ.,Cairo, 43(1): 49-64.

Barakat M.A.S. and Gaber S.M. (1998). Effect of different biofertilizer types and nitrogen fertilizer levels on tomato plants. Alex. J. Agric. Res., 43(1): 149-160.

Black C.A., Evans D.O., Ensminger L. E. , White , Clark F. E . and Dinauer R. C. (1982) . Methods of Soil Analysis. Part 2.

Chemical and Microbiological Properties. 2nd Ed. Am. Soc. Agron., Inc. Pub., Madison, Wisconsin, U. S.A.

Bremner J.M. and Keeny D.R. (1965). Steam distillation method for determination of ammonium, nitrate and nitrite. Annals Chem. Acta, 32: 485-495 .

Bunt J.S. and Rovira A.D. (1955). Microbiological studies of some subantractic soils. J. Soil Sci., 6: 119-128 .

Cooper K.M. and Tinker P.B. (1978). Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizae. IV. Effect of environmental variables on movement of phosphorus. New Phytol.; 88, 327.

Dobereiner J. (1978). Influence of environmental factors on the occurrence of *S. lipoferum* in soil and roots. Ecol. Bull. (Stockholm), 26: 343-352.

Egyptian Pharmacopoeia (1984). General Organization for Governmental Printing Office, Cairo, Egypt.

El-Demerdash M.E. (1994). Growth and yield of maize plants as affected by bacterization with diazotrophs and supplementation with half the dose of N-fertilizer. Annals, of Agric. Sci., Moshtohor, 32(2): 889-898.

El-Khyat A.S. and Zaghloul R.A. (1999). Biofertilization and organic manuring efficiency on growth and yield of Caraway plants(*Carum carvi* L.). Annals of Agric. Sci., Moshtohor, 37(2): 1379-1397.

El-Sawy M., Saleh E.A., El-Borollosy M.A., Nokhal T.H. , Fendrik I. and Sharaf M.S.(1998). Effectiveness of dual inoculation with diazotrophs and vesicular arbuscular mycorrhizae on the growth and khellin content of *Ammi visnaga* . Arab Univ. J. Agric. Sci., 6 (2): 357-371.

El-Sayed S.A.M. (1998). Influence of biofertilization with *Rhizobium* and Phosphate solubilizing bacteria on nutrient uptake and yield of lentil. Egypt, J. Microbiol., 33, No. 1 : 61-71.

Fares C.N. (1997). Growth and yield of wheat plants as affected by biofertilization with associative symbiotic N_2-Fixers and endomycorrhizae in the presence of different P-fertilizers. Annals Agric. Sci., Ain Shams Univ., Cairo, 42 (1) : 51- 60 .

Gaur A.C., Arora D. and Prakash N. (1979).Electron microscopy of some rock-phosphate dissolving bacteria and fungi.Folia Micriobiol.,24, 314.

Gerdmann J. W. and Nicolson T. H. (1963). Spores of mycorrhizal endogene species extracted from soil by wet sieving and decanting. TRANS. Brit. Mycol. Soc., 46 :235-244 .

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

Hussein M.S. (1995). Response of growth, yield and essential oil of coriander and dill to different nitrogen resources. Egypt, J. Hort. 22 (1) : 1 .

Ishac Y.Z., El-haddad M.E., Daft M.J. , Ramadan E.M. and El-Demerdash M.E. (1986). Effect of seed inoculation, mycorrhizal infection and organic amendment on wheat growth. Plant and Soil, 90: 373-383.

Jackson M.L.(1973). Soil Chemical Analysis . Prentice-Hall of Indian private, New Delhi.

Khalil K.W., El-Sersawy M.M., Abd El-Ghany B.F. and Hashem F.A. (1991). Profitability of using some organic wastes with P-fertilization on wheat production under saline irrigation water and Wadi Sudr conditions. Egypt. J. Appl. Sci.; 6 (7): 267-284 .

Mikhaeel F., Shalaby A.M. and Hanna M.M. (2000). Dinitrogen fixation and nitrogen assimilation as influenced by dual (VA-mycorrhizal fungi-*Rhizobium*) inoculation in soybean plants. Annals Agric. Sci., Ain Shams Univ., Cairo, 45 (1) : 67-77.

Mosse B. and Giovanetti M. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New phytologist, 84: 489-500 .

Nieto K.F. and FranKenberger W.T.J. (1990). Influence of adenine, isopentyl alcohol and *Azotobacter chroococcum* on the growth of *Raphanus sativus* . Plant & Soil, 127 (2): 147-156.

Olsen S.R., Cole O.V. , Watanabe F.S. and Del A.L. (1954). Estimation of available phosphorus in soil by extraction with sodium bicarbonate . U. S. Dept., Agric. Circ., 939.

Saad O.A.O. and Hammad A.M.M. (1998). Fertilizing wheat plants with rockphosphate combined with phosphate dissolving bacteria and VA Mycorrhizae as alternative for superphosphate. Annals Agric. Sci., Ain Shams Univ., Cairo, 43 (2): -460.

Saber M.S.M. and Gomaa A.M.H. (1993). Associative action of multi-strain biofertilizer on tomato plants grown in a newly reclaimed soil. 6^{th} Inter. Symp. on Nitrogen Fixation with Non-Legumes, Ismailia. Egypt, 495-497.

Saleh E.A., Nokhal T.H. , El-Borollosy M.A. , Fendrik I., Sharaf M.S. and El-Sawy M. (1998). Effectiveness of dual inoculation with diazotrophs and vesicular arbuscular mycorrhizae on growth and medicinal compounds of *Datura stramonium*. Arab Univ. J. Agric. Sci. 6(2) : 343-355 .

Smith P.F, Giller M.A. and Gasses P.A. (1956). Colorimetric methods for determination of sugar and related substances. Annals. Chem., 28:350.

Snedecor G.W. and Cochra W.G. (1989). Statistical Methods. 8^{th} Ed. Iowa State Univ. Press, Iowa ,U.S.A.

Suslow T.V., Kloepper J.W., Schroth M.N. and Burr, T.J. (1979). Beneficial bacteria enhance plant growth. California Agriculture, 33 (11/12): 15-17.

Wange S.S. (1996). Effect of biofertilizer under graded nitrogen levels on carrot(*Daucus carota*,L.). Annals of Plant Physiology,10(1):96-98.

Yahya A.L. and Al-Azaw S.K. (1989). Occurrence of phosphate-solubilizing bacteria in some Iraqi soils. Plant & Soil., 117-135.

Yassen A.M. (1993). Studies on endomycorrhizae in Egypt. M. Sc. Thesis , Fac. Agric., Ain Shams Univ., Cairo, 32-40.

Zaghloul R.A. (1999). Effectiveness of dual inoculation with *Azospirillum* and phosphate solubilizing microorganisms on growth and yield of *Zea mays* L. Zagazig J.Agric. Res., 26(4): 1005-1025 .

تأثير التلقيح المزدوج بمثبتات الازوت الجوي والميكروبات المذيبة للفوسفات علي النمو والمحصول ومكونات الزيت الطيار لنبات الكسبرة

حامد السيد أبو علي - أنور عثمان جمعة *

قسم النبات الزراعي، * قسم البساتين
كلية الزراعة بمشتهر -جامعة الزقازيق - فرع بنها

ملخص

أجريت تجربتان حقليتان بمزرعة النباتات الطبية والعطرية بكلية زراعة مشتهر خلال موسمي الزراعة ٩٨/٩٧& ٩٩/٩٨ لدراسة تأثير التلقيح المزدوج بالبكتيريا المثبتة لأزوت الهواء الجوي لا تكافليا (*Azotobacter chroococcum or Azospirillum brasilense*) في وجود البكتيريا المذيبة للفوسفات (*Bacillus megatherium var. phosphaticum*) أو فطر الميكورهيزا *Glomus mosseae* مع استخدام نصف جرعه التسميد النيتروجيني المعدني علي النمو والتحليل الكيماوي والمحصول ومحتوي الزيت الطيار لنبات الكسبرة .

وقد أوضحت النتائج أن تلقيح بذور الكسبرة بالبكتيريا المثبتة لأزوت الهواء الجوي لا تكافليا مع وجود الكائنات المذيبة للفوسفور قد أدي إلى زيادة محتوي الفوسفور الميسر في التربة. وكانت تلك الزيادة مصحوبة بارتفاع في أعداد البكتيريا المذيبة للفوسفات وكذلك نسبة إصابة الجذور بالميكورهيزا. زادت أيضا قيم نشاط إنزيم النيتروجينيز ومحتوي التربة من النيتروجين الأمونيومي والنتراتي عند التلقيح بالآزوتوباكتر في وجود فطر الميكورهيزا.

وقد دلت النتائج أيضا علي أن تلقيح البذور بأي من الأزوتوباكتر أو الأزوسبيريللام مع وجود فطر الميكورهيزا أعطت زيادة معنوية في كل من صفات النمو (طول النبات ، الوزن الطازج والجاف للنبات ، عدد الأوراق ، عدد الأفرع ، طول المجموع الجذري ، الوزن الطازج والجاف للمجموع الجذري) ونسبة الكربوهيدرات والكلورفيل والكاروتينات وكذلك محتوي النبات من النيتروجين والفوسفور والبوتاسيوم وأيضا عدد النورات لكل نبات وعدد البذور لكل نورة ومحصول البذرة للنبات ثم للفدان ووزن مائة بذرة ونسبة الزيت الطيار في البذرة.

ولقد تم التعرف علي ثمانية مركبات عند تحليل الزيت الطيار لبذور الكسبرة باستخدام G L C وهي (ألفا بينين ، لينالول ، كامفور ، ميرسين ، بورنيول ، نيرول ، سيس جيرانيول ، جيرانيل أسيتات) ووجد أن المركب الرئيسي هو لينالول حيث سجل أعلي قيم له عند التلقيح بالآزوتوباكتر أو الآزوسبيريللام مع وجود مثبتات الفوسفور وخصة فطر الميكورهيزا .

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٥٣) العدد الاول
(يناير ٢٠٠٢): ٩٣-١١٤.