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**BIOFERTILIZATION AND ORGANIC MANURING EFFICIENCY ON
 GROWTH AND YIELD OF CARAWAY PLANTS (*Carum carvi* L.)
 BY**

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ABSTRACT

Two field experiments were carried out during 1996/1997 and 1997/1998 seasons to study the effect of inoculation with asymbiotic N₂-fixers i.e *A. chroococcum* or *A. lipoferum*, biogas manure application and inorganic N-fertilizer on the growth and yield of caraway plants.

The results of this study showed that, rhizosphere of caraway plants inoculated with asymbiotic N₂-fixers and provided with the half dose of inorganic N-fertilizer or fertilized with biogas manure recorded higher densities of *Azotobacters*, *Azospirilla* and inorganic phosphate dissolvers than those fertilized with the full dose of inorganic N-fertilizer.

Caraway seed inoculation with either *A. chroococcum* or *A. lipoferum* in the presence of the half dose of inorganic N-fertilizer treatments gave higher records of N₂-ase activity in rhizosphere soil than those fertilized with the full dose from either inorganic N-fertilizer or biogas manure. The rhizosphere soil of caraway plants inoculated with *A. chroococcum* showed the highest records of ammoniacal and nitrate nitrogen whereas, available phosphorus concentration was the highest in the treatment of biogas manure application.

The highest records of growth parameters and seed yield of *Carum carvi* were obtained with *Azotobacter chroococcum* or *Azospirillum lipoferum* inoculation treatments. Planting at narrow distance produced higher seed yield /fed. than the planting at wider distance. Caraway seed inoculation with *Azotobacter chroococcum* and sowing at 25 × 50 cm gave the highest seed yield (kg /fed.). Also, inoculation of caraway seeds with asymbiotic N₂-fixers gave higher records of N, P, K and total carbohydrates than those fertilized with either biogas manure or inorganic N-fertilizer. Whereas, biogas manure application treatment attained the highest records of oil percentage and this was observed in the two seasons.

INTRODUCTION

Many species of the family Apiaceae (umbelliferae) usually used in dried state or as fresh herb, as a household remedies or as medicinal ingredients. Among those Apiaceae plants, aromatic plants containing volatile oils represent an important source of the national income of Egypt for local consumption and export. One of the most important plants containing volatile oil is caraway (*Carum carvi* L.), fruits and its oil are used medicinally as carminatives, mild stomachic, antispasmodic, as a tonic in the treatment of digestive disorders. Dried fruits are widely employed for flavouring bread, cake confectionery cheese and kinds of food products. The odour and flavour of medicinal seed plants are due to its essential oil.

Biofertilization with nitrogen fixers gave an appreciable improvement of the growth and yield of different plants Faid, (1994). El-Sawy *et al.*, (1986) found that the growth of *Hyoscyamus muticus* and its content of hyoscyamine considerably increased by dual inoculation with *Azotobacter* and *Azospirillum* as well as organic amendment. Also, Saleh *et al.*, (1998) showed that growth of *Datura stramonium* and its content of alkaloids were greatly improved by biofertilization. Many investigators reported that half of the recommended dose of nitrogen fertilizer can be saved through inoculation of seeds or grains with asymbiotic nitrogen fixers (Ishac *et al.*, 1986; El-Haddad *et al.*, 1986 and El-Demerdash, 1994).

Respecting the effect of biogas manure on plant growth and microbial activities, Mahmoud *et al.*, (1984) and Zaghloul *et al.*, (1996) revealed that the application of biogas manure increased microbial densities and microbial activities in the soil, as well as there was a significant increase in macro and micro-nutrients and yield.

The present study aimed to investigate the effect of inoculation with asymbiotic N₂-fixers i.e. *A. chroococcum* or *A. lipoferum* and biogas manuring on growth and yield of caraway plants (*Carum carvi* L.).

MATERIAL AND METHODS

Two field experiments were carried out at the experimental farm, Fac. Agric., Moshtohor Zagazig Univ., during 1996 /1997 and 1997 /1998 seasons to study the response of caraway plants (*Carum carvi* L.) to inoculation with either *Azotobacter chroococcum* AC or *Azospirillum lipoferum* MN and fertilization with either biogas manure or ammonium nitrate. Chemical and mechanical analyses of the experimental soil as well as biogas manure analysis are presented in Tables (1) and (2).

Chemical analysis was estimated according to Black *et al.*, (1982), whereas, mechanical analysis was estimated according to Jackson (1973).

Table (1): Chemical and mechanical analyses of the experimental soil.

a- Chemical analysis .

Organic matter%	P ^B	T.N %	T.P %	E.C m mhos/cm	CaCO ₃ %
1.80	7.92	0.42	0.24	0.81	1.56
b- Mechanical analysis					
Coarse sand %	Fine sand %	Silt %	Clay %	Textural class	
3.34	18.86	22.12	55.68	clay	

T. N, Total nitrogen .

T. P, Total phosphorus .

E.C, Electric conductivity.

Table (2): Analysis of biogas manure.

Parameters	Unit	Value
Organic matter	%	56.2
Organic carbon	%	32.59
Total nitrogen	%	1.74
Total phosphorus	%	0.86
Total potassium	%	1.21
C: N Ratio		18.90
Iron	ppm	24
Zinc	ppm	11
Manganese	ppm	8
Copper	ppm	5
Density	Kg/m ³	275

Biogas manure was added before sowing at a rate of 90 Kg N/fed. While, the inorganic nitrogen fertilizer (NH₄ NO₃) was added at a rate of 90 Kg N/fed. in three equal doses i.e at sowing, after 30 and 60 days of sowing. All plots were supplemented with calcium superphosphate (15.5% P₂ O₅) and potassium sulphate (48% K₂O) at rates of 30 and 60 Kg P₂ O₅ and K₂O /fed., respectively. They were added in two equal doses at 30 and 60 days after sowing.

Inoculants:

Azotobacter chroococcum AC and *Azospirillum lipoferum* MN strains were provided from the unit of Biofertilizers, Fac. of Agric., Ain Shams Univ., Cairo, Egypt.

For preparation of *A. chroococcum* and *A. lipoferum* inocula, modified Ashby's medium (Abdel- Malek and Ishac, 1968) and semi- solid malate medium (Dobereiner, 1978) were inoculated with *A. chroococcum* and *A. lipoferum*, respectively then incubated at 30°C and 32°C for 7 days, respectively. Seeds of *Carum carvi* were successfully washed with water and air-dried. Thereafter, seeds were soaked in cell suspension of *A. chroococcum* or *A. lipoferum* (1ml contains 10⁸ viable cell) for 30 min. Gum arabic (16%) was

added as an adhesive agent, prior to soaking. Seeds of control treatment were treated with the same manner but using N-deficient medium instead of bacterial cultures. The inoculated seeds were air dried at room temperature for one hour before sowing.

Experimental design:

A split plot design with four replicates was used. The main plots were devoted for sowing distance, whereas the sub-plots were designated for inoculation and fertilization treatments.

This experiment included the following treatments:

- 1- Control (without any treatments).
- 2- A full dose (90 Kg N/fed) of inorganic N-fertilizer.
- 3- Biogas manure (90 Kg N/fed.).
- 4- *Azotobacter chroococcum* inoculum + a half dose of inorganic N-fertilizer (45Kg N/fed.).
- 5- *Azospirillum lipoferum* inoculum+ a half dose of inorganic N-fertilizer (45Kg N/fed.).

Cultivation process:

Cultivation process was performed at 10th November by sowing inoculated or uninoculated seeds in hills at rows with a distance of 25, 35 and 45cm between hills and 50 cm between rows.

After sowing, soil was directly irrigated to provide a suitable moisture for inocula. The normal culture practices for growing caraway plants were followed as recommended in the region, after germination the plants were thinned into two plants per hill.

Sampling and determinations:

After 35 and 70 days from sowing representative rhizosphere soil samples of the developed plants were taken. These periods were considered and referred to in the results discussion as the vegetative and flowering stages. The samples were microbiologically analyzed for Nitrogenase activities as an indication for N₂ fixing activity, densities of *Azotobacters* and *Azospirilla* as well as inorganic phosphate dissolvers. Also, rhizosphere soil samples were chemically analyzed for NH₄-N, NO₃-N and available phosphorus as follows :

1. Microbiological analyses:

- 1.1. Nitrogenase activity in the rhizosphere was determined according to Hardy *et al.*, (1973).
- 1.2. Densities of *Azotobacters* and *Azospirilla* were determined on modified Ashby's medium (Abdel-malek and Ishac, 1968) and semi-solid malate medium (Dobereiner, 1978), respectively using the most probable densities technique (Cochran, 1950). Whereas, the densities of inorganic phosphate dissolvers was determined on (Bunt and Rovira medium 1955, modified by Abdel-Hafez, 1966) using the plate count method.

2. Chemical analyses:

- 2.1. Ammoniacal and nitrate nitrogen were determined according to Bremner and Keeny (1965).
- 2.2. Available phosphorus extracted from soil according to Olsen *et al.*, (1954) and colourimetrically determined according to American Public Health Association (APHA, 1989) .

3. Growth parameters and yield:

Ten plants were randomly taken from each treatment for growth measurements and yield at harvest time i.e. plant height (cm), fresh and dry weights of herb (gm/ plant), seed yield (gm/plant) and (kg/fed.).

4. Chemical analysis of plant:

Total carbohydrates and N, P and K were determined in the dried leaves. The total carbohydrates were determined by Herbert *et al.*, (1971). Total nitrogen was determined by A.O. A.C (1980), total phosphorus was determined according to (APHA, 1989) and total potassium was determined by Dewis and Freitas (1970). Seed oil content was determined at harvest by water distillation following the method described by Egyptian pharmacopoeia (1984).

Statistical analysis:

Analysis of variance (ANOVA) of data obtained from growth parameters, yield and chemical analysis of the plants were carried out and significant differences among the means of various treatments were distinguished by L.S.D. (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Effect of different soil applications on microbial densities in rhizosphere of *Carum carvi*:

1-Changes in *Azotobacter spp* densities:

Data in Table (3) show that the densities of *Azotobacter spp* in rhizosphere soil were increased in all soil applications treatments compared to control one. The highest densities of *Azotobacter spp* were observed in the treatment of caraway seed inoculation with *Azotobacter chroococcum* and receiving half dose of inorganic N-fertilizer .

Irrespective of control treatment, the lowest densities of *Azotobacter spp* were resulted in the treatment of full dose of inorganic N-fertilizer application. Generally, it is worthy to notice that inoculation of caraway seeds with asymbiotic nitrogen fixers i.e. *Azotobacter chroococcum* or *Azospirillum lipoferum* gave higher densities of *Azotobacter spp* than biogas manure application. Similar trends of results were observed in the two growing seasons. Similar results were recorded by Mostafa (1997) who found that *Azotobacter* densities in rhizosphere soil were higher as a result of rape seed inoculation with *A. chroococcum* than organic manures amendment .

Data presented in Table (3) also show that densities of *Azotobacter* spp in rhizosphere of caraway plants tended to increase progressively in all treatments. At flowering stage, densities of *Azotobacter* spp were higher than vegetative stage. Such differences may be due to the changes in multiplication rate of *Azotobacters* as a result of qualitative changes in nature of the root exudates of the plant during the different growth stage (Abdel-Ati *et al.*, 1996).

2- Changes in *Azospirillum* spp densities:

Data in Table (3) clearly indicate that the densities of *Azospirilla* in rhizosphere soil of caraway plants increased in all soil treatments compared to control one. Inoculation of caraway seeds with *Azospirillum lipoferum* gave the highest densities of *Azospirilla*. While, the lowest densities of *Azospirilla* were recorded in the treatment of full dose inorganic N-fertilizer application and this was true in both vegetative and flowering stages. Biogas manure application gave higher densities of *Azospirilla* than seed inoculation with *Azotobacter chroococcum* and provided with half dose of inorganic N-fertilizer. Similar trends of results were observed in the two growing seasons. Similar results were obtained by Zaghoul *et al.*, (1996) who reported that the addition of organic manures increased the microbial densities and microbial activities in rhizosphere of wheat plants.

Data in Table (3) also emphasize that the densities of *Azospirilla* were higher at flowering than vegetative stage and this result was recorded in all treatments. This increase is likely to be due to the beneficial effect of root exudates and debris during flowering stage. This result was confirmed by that obtained by De-Freitas and Germida (1990).

3- Changes in phosphate dissolvers densities:

Data in Table (3) reveal that the densities of phosphate dissolvers in rhizosphere soil increased in all investigated treatments compared to control one. Application of biogas manure at a rate of 90 kg N/fed. gave the highest densities of inorganic phosphate dissolvers and this was observed in both vegetative and flowering growth stages of caraway plants.

Moreover, caraway seed inoculation with either *A. chroococcum* or *A. lipoferum* and received half dose of inorganic N-fertilizer gave higher densities of inorganic phosphate dissolvers than full dose of inorganic N-fertilizer application. Similar trends of results were observed in the two growing seasons.

Data in Table (3) also emphasize that the densities of inorganic phosphate dissolvers were higher at flowering than vegetative stage. This increase could be attributed to the high beneficial effect of roots secretions during flowering stage in most cultivated plants.

Table 3. *Azotobacters*, *Azospirilla* and inorganic phosphate dissolvers densities ($\times 10^4$ /g dry weight of soil) in rhizosphere of caraway plants in the two growing seasons .

Growth stage Treatments	Vegetative (35 days)						Flowering (70 days)					
	<i>Azotobacters</i>		<i>Azospirilla</i>		P.D.B		<i>Azotobacters</i>		<i>Azospirilla</i>		P.D.B	
	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
Control	61.8	65.5	48.4	56.2	60.4	72.3	72.0	74.4	56.8	60.4	82.3	91.6
Full dose of N-fertilizer	100.4	120.5	82.6	94.3	180.6	200.2	121.8	135.0	110.3	116.6	210.5	225.0
Biogas manure	140.2	156.4	120.6	128.4	420.8	450.5	163.0	168.1	148.1	156.8	486.3	493.5
<i>A. chroococcum</i> inoculum + half dose of N-fertilizer	210.6	225.8	96.4	102.2	250.3	270.6	240.7	256.6	115.3	123.0	271.6	286.3
<i>A. lipoferum</i> inoculum + half dose of N-fertilizer	180.2	195.4	172.5	180.3	210.1	240.2	260.3	272.2	186.4	200.6	246.8	278.4

P.D.B. : phosphate dissolving bacteria

N. : Nitrogen

Effect of different soil applications on nitrogenase activity in rhizosphere of *Carum carvi*:

It is obvious from data in Table (4) that N_2 -ase activity as an indication for N_2 -fixing activity in rhizosphere of *Carum carvi* plants was considerably influenced by biofertilization with asymbiotic N_2 -fixers and biogas manure amendment. The highest records of N_2 -ase activity were recorded in the rhizosphere of inoculated plants with *A. chroococcum* and supplemented with the half dose of inorganic N-fertilizer. Also, caraway seed inoculation with *A. lipoferum* and received half dose of inorganic N-fertilizer gave higher records of N_2 -ase activity than full dose of nitrogen application from either ammonium nitrate or biogas manure.

Table (4): Nitrogenase activity (μ moles C_2H_4 /hr./g dry weight of soil) in rhizosphere of caraway plants in the two growing seasons.

Treatments	Growth stage		Flowering (70 days)	
	Vegetative (35 days)		1997	1998
Control	24.8	28.5	36.6	40.2
Full dose of N-fertilizer	36.3	38.3	45.5	50.6
Biogas manure	64.3	68.7	73.3	81.5
<i>Azot. chroococcum</i> Inoculum + half dose of N fertilizer	98.6	103.6	136.3	148.4
<i>Azos. lipoferum</i> Inoculum + half dose of N fertilizer	92.6	94.4	120.2	126.0

Abbreviations: as those stated for Table (3) .

The same trend of results previously mentioned was recorded in the two growing seasons as well as at vegetative and flowering stages of caraway plants. These results are in harmony with El-Sawy *et al.*, (1986) and Saleh *et al.*, (1998). They reported that inoculation of medicinal plants i. e. *Hyoscyamus muticus* and *Datura stramonium* with asymbiotic N_2 -fixers increased N_2 -ase activity compared to uninoculated ones.

Results in Table (4) also show that, increasing the dose of inorganic N-fertilizer resulted in decreasing N_2 -ase activity. It could be noticed that inorganic N-Supplementation exhibited a negative effect on biological N_2 -fixation. Low doses of N-fertilizer promoted the response of plants to inoculation with asymbiotic N_2 -fixers (Ishac *et al.*, 1986 and El-Demerdash, 1994). They reported that half of the recommended field rate of added inorganic N-fertilizer can be saved by seed inoculation with asymbiotic N_2 -fixers. Such trends support the obtained results in the current study.

Data in Table (4) also clearly indicate that the N_2 -ase activity was higher at flowering than vegetative stage and this was observed in all investigated treatments and in the two growing seasons. This increase in N_2 -ase

activity at flowering stage is likely to be due to the high densities of *Azotobacter spp* or *Azospirillum spp* which were recorded at flowering stage (Table, 3).

Effect of different soil applications on nitrogen forms and available phosphorus in rhizosphere of *Carum carvi*:

Data presented in Table (5) indicate that ammoniacal and nitrate nitrogen as well as available phosphorus concentrations were remarkably increased in rhizosphere soil in all investigated treatments compared to untreated one (control). The rhizosphere soil of caraway plants inoculated with *A. chroococcum* and supplemented with the half dose of inorganic N-fertilizer showed the highest records of ammoniacal and nitrate nitrogen. This increase of $\text{NH}_4^- \text{N}$ and $\text{NO}_3^- \text{N}$ in case of inoculation with *A. chroococcum* may be due to high N_2 fixation by *Azotobacter chroococcum*. Similar trend of results was observed in the two seasons as well as at vegetative and flowering stages

Data in Table (5) also show that, the rhizosphere soil of plants inoculated with *A. lipoferum* and supplemented with the half dose of inorganic N-fertilizer contained higher concentrations of ammoniacal and nitrate nitrogen than full dose of nitrogen application from either ammonium nitrate or biogas manure. Similar results were obtained by Zaghoul *et al.*, (1996). They found that ammoniacal and nitrate nitrogen contents were higher in case of biofertilization than organic manuring and inorganic N-fertilization in rhizosphere soil of wheat plants.

It is obvious from data recorded in Table (5) that available phosphorus concentration was the highest in the treatment of biogas manure application. This result was confirmed by that obtained by Mahmoud *et al.*, (1984) and Neweigy *et al.*, (1997). They reported that biogas manure application increased total and available phosphorus in rhizosphere soil.

Respecting the effect of inoculation with asymbiotic N_2 -fixers on available -P, data in Table (5) clearly show that inoculation of caraway seed with *A. lipoferum* and provided with the half dose of inorganic N-fertilizer gave higher records of available -P than those inoculated with *A. chroococcum*. This finding was observed in the two seasons as well as at vegetative and flowering stage.

Generally, ammoniacal and nitrate nitrogen as well as available phosphorus concentrations were higher at flowering than vegetative stage. This result could be attributed to the high densities of *Azotobacters*, *Azospirilla* and inorganic phosphate dissolvers which were recorded at flowering stage (Table, 3).

Table 5. Ammoniacal and nitrate nitrogen content and available phosphorus concentration in rhizosphere of caraway plants in the two growing seasons .

Growth stage Treatments	Vegetative (35 days)						Flowering (70 days)					
	Ammoniacal nitrogen (ppm)		Nitrate nitrogen (ppm)		Available phosphorus (ppm)		Ammoniacal nitrogen (ppm)		Nitrate nitrogen (ppm)		Available phosphorus (ppm)	
	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
Control	19.4	21.0	28.6	32.3	23.3	26.2	31.1	33.6	35.0	38.0	38.1	41.3
Full dose of N-fertilizer	28.6	32.3	40.2	45.5	50.8	54.0	52.0	56.3	60.5	64.3	71.6	68.3
Biogas manure	42.2	46.4	66.6	72.3	101.0	108.4	78.4	84.6	80.5	83.6	126.4	130.1
<i>A. chroococcum</i> inoculum + half dose of N-fertilizer	83.3	87.5	81.3	90.1	80.4	83.3	135.2	140.1	96.3	101.6	98.5	103.3
<i>A. lipoferum</i> inoculum + half dose of N-fertilizer	78.8	80.5	76.5	78.8	94.5	98.6	116.4	125.5	84.3	88.2	113.1	117.6

Abbreviations : as those stated for Table (3) .

Effect of different soil applications on growth parameters and yield of *Carum carvi*:

A-Growth parameters.

The growth parameters of caraway plants were affected by different sowing distances as shown in Table (6). Increasing of sowing distance decreased plant height. The maximum plant height was recorded with 25 cm between plants and this finding was observed in the two growing seasons. Narrow distance resulted in reduction of light intensity on caraway canopy which encouraged IAA synthesis. Increasing of IAA concentration in stem tissues caused cell enlargement and hence plant height.

At the wider sowing distance fresh and dry weights of caraway herb were higher than those of narrow sowing distance in both growing seasons. Fresh and dry weights per plant were significantly increased with the increasing of sowing distance between hills. This result might attributed to the low competition between plants for available radiant energy. These results are in agreement with Omer *et al.*, (1993).

It is obvious from data in Table (6) that, plant height, fresh and dry weights of caraway plants were significantly increased with different soil applications compared to control one. In the 1st season, the highest records of plant height were resulted in the treatment of caraway seed inoculation with *Azotobacter chroococcum* and provided with the half dose of inorganic N-fertilizer. Whereas, the highest values of plant height in the 2nd season were recorded in case of biogas manure application.

Irrespective of control treatment, the lowest records of plant height were resulted from the full dose of inorganic N-fertilizer application and this was observed in the two growing seasons.

Respecting the effect of various treatments on fresh and dry weights of caraway plants, data in Table (6) clearly indicate that the highest records of fresh and dry weights were resulted in the treatment of caraway seed inoculation with *Azotobacter chroococcum* followed by biogas manure amendment. The same trend of results was obtained in the two growing seasons

Moreover, it is worthy to notice that inoculation of caraway seed with *Azospirillum lipoferum* and the half dose of inorganic N-fertilizer supplementation gave higher records of fresh and dry weights than the full dose of inorganic N-fertilizer application. These results are in harmony with El-Haddad *et al.*, (1986) and El-Demerdash (1994). They reported that half of the nitrogen fertilizer can be saved through inoculation of seed with asymbiotic N₂-fixers.

Table 6. Effect of different soil applications on some growth parameters and yield of caraway plants in the two growing seasons .

Treatments	The first season (1997)																			
	Plant height (cm)				Fresh weight (g/plant)				Dry weight (g/plant)				Seed yield (g/plant)				Seed yield (kg/ha.)			
	25cm	35cm	45cm	Mean	25cm	35cm	45cm	Mean	25cm	35cm	45cm	Mean	25cm	35cm	45cm	Mean	25cm	35cm	45cm	Mean
Control	116.7	103.7	75.8	98.6	143.6	217.0	148.2	169.6	18.7	19.1	28.0	21.9	16.60	17.80	19.57	17.99	1062.4	813.7	695.8	857.3
Full dose of N-fertilizer	126.3	116.1	91.5	111.3	171.2	267.0	267.3	235.2	20.2	28.0	27.5	25.2	17.40	18.18	20.47	18.68	1113.6	831.1	727.8	890.8
Biogas manure	134.4	125.4	96.6	118.8	182.4	317.0	326.2	275.2	21.5	35.4	35.0	30.6	17.0	18.62	20.87	19.05	1132.8	851.7	740.3	908.1
<i>A. chroococcum</i> inoculum	138.3	125.7	124.2	129.4	310.1	307.1	346.1	321.1	33.8	34.0	38.5	35.34	18.65	20.35	21.15	20.05	1193.6	930.3	752.0	958.6
+ half dose N-fertilizer																				
<i>A. lipoferum</i> inoculum + half dose N-fertilizer	139.2	131.4	96.3	122.3	267.9	196.7	275.9	246.8	28.8	29.6	26.0	28.1	18.05	18.65	20.87	19.19	1155.2	852.6	742.0	961.6
Mean	130.98	120.36	96.88		215.04	260.96	272.7		24.6	29.22	31.0		17.68	18.72	20.58		1131.52	855.78	731.58	
LSD at 5 %																				
S.D (a)	9.8				10.8				2.1				1.76				28.4			
S.A. (b)	10.6				15.4				3.2				1.82				16.7			
a X b	11.5				17.8				5.7				2.35				42.3			
The second season (1998)																				
Control	117.9	120.6	94.2	110.9	141.5	178.7	156.8	159.0	19.9	21.1	23.9	21.6	15.75	22.25	22.25	20.08	1008.0	1017.1	791.1	938.73
Full dose of N-fertilizer	133.7	130.3	102.9	122.3	180.8	247.5	285.8	238.0	23.2	33.7	32.3	29.7	18.00	23.10	23.17	21.42	1152.0	1056.0	823.8	1010.6
Biogas manure	146.1	146.4	109.8	134.1	244.4	332.7	384.0	320.36	28.7	34.5	42.8	35.3	19.50	23.12	25.20	22.61	1248.0	1056.9	896.0	1067.0
<i>A. chroococcum</i> inoculum	126.7	124.6	122.9	124.73	260.0	374.0	393.7	342.56	31.0	33.9	47.5	37.5	21.30	25.57	27.0	24.62	1363.2	1168.9	960.0	1164.0
+ half dose N-fertilizer																				
<i>A. lipoferum</i> inoculum + half dose N-fertilizer	149.1	130.4	112.8	130.7	225.0	285.0	236.0	248.7	27.5	30.8	36.0	31.4	19.75	25.0	26.62	23.79	1264.0	1142.9	946.5	1117.0
Mean	134.7	130.46	108.52		210.34	283.58	291.32		26.06	30.8	36.5		18.86	23.81	24.85		1207.04	1088.3	883.48	
LSD at 5 %																				
S.D (a)	8.6				13.7				3.4								34.6			
S.A. (b)	9.2				19.5				5.8								30.8			
a X b	10.3				23.9				6.3								39.5			

Abbreviation : as those stated for Table (3)

S. D, sowing distance

S. A, soil application

Data in Table (6) indicate that plant height and fresh and dry weights were significantly affected by sowing distance in combination with various soil applications. The combination of planting distance of 25 cm with *Azospirillum lipoferum* inoculation produced the highest records of plant height. Whereas, the interaction between planting distance of 45 cm and *Azotobacter chroococcum* inoculation gave the highest records of fresh and dry weights of caraway plants. These findings were observed in the two growing seasons.

B. Seed yield:

Data presented in Table (6) also indicate that seed yield either (g/plant) or (kg /fed.) was significantly affected by the sowing distance.

Despite of the wider sowing distance increased seed yield per plant, the opposite was obtained for the yield of unit area (kg /fed.). These results indicate that the increase of seed yield /plant in wider sowing distance was not enough great to counter balance the increase in plants densities per unit area in case of narrow sowing distance. In other words, plants in dense cultivation were efficient in utilizing light, water and available nutrients per unit area and consequently the seed yield per unit area was greater for narrow than wider sowing distance. These results are in accordance with those obtained by Ezz-Eldin (1989) and Omer *et al.*, (1993).

Respecting the effect of different soil applications on caraway seed yield, data in Table (6) emphasize that caraway seed inoculation with *Azotobacter chroococcum* and provided with the half dose of inorganic N-fertilizer gave the highest records of seed yield / plant as well as seed yield /fed. Also, caraway seed inoculation with *Azospirillum lipoferum* and provided with the half dose of inorganic N-fertilizer gave higher records of seed yield than the full dose of nitrogen application from either ammonium nitrate or biogas manure. The same trend of results was observed in the two growing seasons. These results were confirmed by those obtained by Faid (1994) and Saleh *et al.*, (1998). They reported that biofertilization with asymbiotic N₂-fixing bacteria gave an appreciable improvement of the growth and yield of different plants.

Data presented in Table (6) also show that seed yield was significantly affected by sowing distance in combination with various soil applications. The combination of sowing distance 25 cm with *Azotobacter chroococcum* inoculation gave the highest records of seed yield (kg /fed.) while, the lowest records of seed yield were resulted in the treatment of full dose of inorganic N-fertilizer and 45 cm sowing distance. Similar trends of results were recorded in the two growing seasons.

Effect of different soil applications on some chemical constituents of *Carum carvi*:

A. N, P and K and total carbohydrates percentage:

Data in Table (7) clearly show that, increasing the sowing distance increased N, P and K and total carbohydrates percentage in the two seasons. The wider distance attained highest records of these parameters compared with narrow and middle distances and this was observed in the two seasons. These results are in agreement with those obtained by Mohamed and Wahba (1993) on *Tagetes erecta*.

Data in Table (7) also show that all investigated treatments gave an appreciable improvement of N, P and K and total carbohydrates percentage compared to control plants in the two seasons.

Caraway seed inoculation with *Azotobacter chroococcum* and provided with the half dose of inorganic N-fertilizer gave the highest records of N, P and total carbohydrates compared with other treatments. But caraway seed inoculation with *Azospirillum lipoferum* and provided with the half dose of inorganic N-fertilizer gave higher records of K percentage than other treatments.

The various positive effects of biofertilizers (*Azotobacter chroococcum* and *Azospirillum lipoferum*) on growth, nutritional status of the plants and productivity could be due to :

- 1- They encourage the uptake of various elements .
- 2- They activate the photosynthesis process and both cell division and cell enlargement.
- 3- They control the incidence of pests and disease.

All of these merits greatly affected the yield and advance seed ripening.

In addition, biogas manure application gave higher records of N, P and K and total carbohydrates percentage than the full dose of inorganic N-fertilizer application and this was observed in the two seasons. Also, data recorded in Table (7) show that, the combination between wider distance (35 or 45 cm) and *Azotobacter chroococcum* inoculation attained higher records of N and P as well as total carbohydrates percentages compared with other treatments in the two seasons. While, the highest records of K in caraway herb were attained with wider distances combined with *Azospirillum lipoferum* inoculation.

B- Oil percentage:

Data recorded in Table (7) indicate that, increasing the planting distance increased the oil percentage in the seeds compared to the narrow distance (25 cm) which produced the lowest oil percentage in the seeds in the two seasons.

Table 7. Effect of different soil applications on some chemical constituents of caraway plants in the two growing seasons .

Treatments	The first season (1997)																			
	Total nitrogen (%)				Total Phosphorus (%)				Total Potassium (%)				Total carbohydrates (%)				Oil content (%)			
	25cm	35cm	45cm	Mean	25cm	35cm	45cm	Mean	25cm	35cm	45cm	Mean	25cm	35cm	45cm	Mean	25cm	35cm	45cm	Mean
Control	1.61	1.70	1.92	1.74	0.47	0.48	0.51	0.49	1.41	1.46	1.52	1.46	21.13	22.07	23.0	22.06	3.10	3.00	3.24	3.11
Full dose of N-fertilizer	1.70	1.78	2.23	1.90	0.48	0.50	0.52	0.50	1.46	1.55	1.65	1.55	21.88	24.13	26.38	24.13	3.44	3.00	3.60	3.35
Biogas manure	1.78	1.88	2.26	1.97	0.48	0.52	0.54	0.51	1.55	1.68	1.79	1.67	22.63	25.25	27.09	24.99	3.54	3.80	3.70	3.68
<i>A.chroococcum</i> inoculum + half dose N-fertilizer	2.10	2.41	2.53	2.35	0.53	0.54	0.55	0.54	1.56	1.72	1.88	1.72	23.57	92.57	28.21	27.11	3.30	3.50	3.64	3.53
<i>A. lipoferum</i> inoculum + half dose N-fertilize	1.80	2.02	2.28	2.03	0.48	0.53	0.54	0.52	1.64	1.80	1.94	1.79	23.57	26.27	25.44	25.11	3.44	3.84	3.80	3.65
Mean	1.80	1.96	2.24		0.49	0.51	0.53		1.52	1.64	1.76		22.55	25.46	26.02		3.36	3.93	3.60	
LSD at 5 %																				
S. D (a)	0.17				N.S				0.11				1.83				0.04			
S. A (b)	0.23				N.S				0.08				1.97				0.12			
a X b	0.25				0.02				0.13				2.12				0.15			
The second season (1998)																				
Control	1.74	1.81	1.93	1.83	0.50	0.52	0.54	0.52	1.57	1.62	1.67	1.62	22.06	22.99	23.75	22.93	3.04	3.45	3.52	3.34
Full dose of N-fertilizer	1.80	1.92	2.09	1.94	0.51	0.53	0.54	0.53	1.63	1.71	1.82	1.72	22.81	25.06	27.19	25.02	3.31	3.39	3.47	3.39
Biogas manure	1.88	2.00	2.19	2.02	0.53	0.54	0.55	0.54	1.72	1.86	1.97	1.85	23.37	25.62	27.91	25.63	3.47	3.87	3.76	3.70
<i>A.chroococcum</i> inoculum + half dose N-fertilizer	2.08	2.27	2.47	2.27	0.56	0.56	0.56	0.56	1.69	1.88	2.08	1.88	24.31	28.88	31.43	28.20	3.68	3.52	3.58	3.59
<i>A. lipoferum</i> inoculum + half dose N-fertilize	1.92	2.07	2.27	2.09	0.53	0.54	0.55	0.54	1.81	1.90	2.03	1.91	23.75	25.99	28.43	26.05	3.64	3.46	3.55	3.55
Mean	1.88	2.01	2.19		0.52	0.54	0.55		1.68	1.79	1.91		23.26	25.70	27.74		3.43	3.54	3.58	
LSD at 5 %																				
S. D (a)	0.09				N.S				0.04				1.46				N.S			
S. A (b)	0.11				N.S				0.09				2.18				0.09			
a X b	0.18				N.S				0.19				3.27				0.16			

Abbreviation : as those stated for Table (3) .

S. D, sowing distance

S. A, soil application

N S, non significant

Essential oil percentage was significantly increased with the different soil applications compared with the untreated plants. The highest records of oil percentage in the two seasons were attained with biogas manure application compared with all other treatments.

It is worthy to notice that caraway seed inoculation with either *Azotobacter chroococcum* or *Azospirillum lipoferum* and provided with the half dose of inorganic N-fertilizer gave higher records of oil percentage than the full dose of inorganic N-fertilizer application. These results are in agreement with those obtained by El-Sawy *et al.*, (1986) and Saleh *et al.*, (1998). They reported that the medicinal compounds (hyoscyamine and alkaloids) were greatly improved by biofertilization with asymbiotic N₂-fixers.

The correlation between dry weight accumulation and essential oil formation was observed. The biochemical parameters that have been studied indicated that total nitrogen and total carbohydrates content were increased with different fertilizers.

Thus, the increase of total nitrogen and total carbohydrates content could be said to remain associated with increasing synthesis of essential oil. Franz and Wunsch (1972) reported a very interesting relationship between soluble nitrogen and essential oil content in *Mentha piperita*.

In other words the excessive fertilization which can be considered as a stress condition may alter the biosynthetic processes to the advantage of methyl chavicol from shikimic acid or mevalonic acid since green ruffles are dual biosynthetic pathways (Lawrence, 1988 and Omer *et al.*, 1998).

The combination between sowing distance of 35 cm with *Azospirillum lipoferum* inoculation gave more records of oil percentage in the first season, but in the second season the highest records of oil percentage were attained by planting distance 35 cm and biogas manure application.

REFERENCES

- Abdel-Ati, Y.Y.; Hammad, A.M.M. and Ali, M.Z. (1996): Nitrogen fixing and phosphate solubilizing bacteria as biofertilizers for potato plants under Minia conditions. 1st Egyptian Hungarian Horticultural Conf. Kafr El-Sheikh, Egypt Vol. (1): 25-34.
- 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. P.31.
- Abdel-Malek, Y. and Ishac, Y.Z. (1968): Evaluation of methods used in counting azotobacter. J. Appl. Bact., 31: 267-275.

- A.O.A.C., Association of Official Agricultural Chemists (1980): Official Methods of Analysis, 10th Ed. Washington, D.C., U.S.A. P: 832.
- APHA, American Public Health Association (1989): Standard methods for the examination of water and waste water. Washington, Dc, U.S.A.
- 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. Inc. publ., 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 subarctic soils. *J. Soil Sci.*, 6: 119-128.
- Cochran, W.G. (1950): Evaluation of bacterial densities by means of the "most probable densities" *Biometrics*, 6: 105-116.
- De-Freitas, J.R. and Germida, J.J. (1990): Plant growth promoting rhizobacteria for winter wheat. *Can. J. Microbiol.* 36:265-272.
- Dewis, J. and Freitas, F. (1970): Physical and chemical methods of soil and water analysis. F.A.O., Bull, No. 10.
- 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-Haddad, M.E.; Ishac, Y.Z.; Saleh, E.A.; El-Borollosy, M.A.; Reffat, A.A. and El-Demerdash, M.E. (1986): Comparison of different methods of inoculation with asymbiotic N₂-fixers on plant growth. *proc. 2nd AABNF, Cairo, Egypt, Dec. 15-19* .
- El-Sawy, M.; Saleh, E.A.; Abdel-Fattah, M.K.; El-Borollosy, M.A. and Sharaf, M.S. (1986): Hyoscyamine content of *Hyoscyamus muticus* L. as affected by inoculation with *Azotobacter* and /or *Azospirillum* strains. *Proc. 2nd Conf. AABNF, Cairo, Egypt, PP. 498-513*.
- Ezz-Eldin, A.A. (1989): Response of seeds and petal yield of safflower to nitrogen fertilization and plant density. M. Sc. Thesis, Fac. Agric., Ain-Shams University, Cairo Egypt, P 76.
- Faid, E.M.Y. (1994): Studies on Biofertilization in Desert Soils. M.Sc. Thesis, Fac. Agric., Ain Shams Univ., Cairo, Egypt, PP. 54-89.
- Franz, C.H. and Wunsch, A. (1972): Effect of K and N nutrients on the formation of essential oil of *Mentha piperita*. *Plant Med.* 22, 160.
- 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.
- Herbert, D.; Phlipps, P.J. and Strange, R.E. (1971): Determination of total carbohydrates. *Method in Microbiol*, 58: 209-344 .

- 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*, 90:373-382.
- Jackson, M.L. (1973): *Soil Chemical Analysis*. Prentice -Hall of Indian Private, New Delhi.
- Lawrence, B.I.M. (1988): A future examination of the variation of *Ocimum basilicum* L. in flavours and fragrances. A world perspective Lawrence B. M; B. D Mookherjee and B. J. Willis, (Ed) Elsevier. New York .
- Mahmoud, M.H.; Alaa El-Din, M.N.; Imam, M.M. and Arroug, S.M. (1984): Evaluation of biogas manure as a fertilizer for wheat. *Agric. Res. Review, Soil & water (Abstracts)* 62 (4c): 548 .
- Mohamed, S.M. and Wahba, H.E. (1993): Effect of planting distance pinching and zinc application on growth, flowering and carotene content of *Tagetes erecta*. *Annls of Agric. Sc. Moshtohor*, Vol. 31(2).
- Mostafa, M.H. (1997): Effect of seed inoculation with *Azotobacter* on rape yield in Egypt. *Egypt. J.Agric. Res.*, 75 (2): 295-302.
- Neweigy, N.A.; Elhsan A. Hanafy; Zaghloul, R. A. and El-Sayeda, H. El-Badawy (1997): Response of sorghum to inoculation with *Azospirillum*, organic and inorganic fertilization in the presence of phosphate solubilizing microorganisms. *Annals of Agric. Sci., Moshtohor*, 35(3): 1383-1401.
- 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.
- Omer, E.A.; Refaat, A.M.; Ahmed, S.S.; Kamel, A. and Hammouda, F.M. (1993): Effect of spacing and fertilization on the yield and active constituents of milk thistle, *silybum marianum* L. *Herbs, spices and Medicinal Plants*, 1 (4): P. 17 .
- Omer, E.A.; Khattab, M.E. and Ibrahim, M.E. (1998): Production and volatile oils of new four cultivars of basil cultivated in Egypt. *Egypt. J. Hort.*, 25, No. 2 .
- Salah, 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., Ain Shams Univ., Cairo*, 6 (2): 343-355 .
- Snedecor, G.W. and Cochran, W.G. (1989): *Statistical Methods*. 8th Ed., Iowa State Univ., Press, Iowa. U. S. A.
- Zaghloul, R.A.; Amer, A.A. and Mostafa, M.H. (1996): Efficiency of some organic manures and biofertilization with *Azospirillum brasilense* for wheat manuring. *Annals of Agric. Sci., Moshtohor*, 34 (2): 627-640.

فعالية التسميد الحيوى والعضوى على نمو وإنتاجية نباتات الكراوية

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أقيمت تجربتان حقليتان بمزرعة مركز البحوث والتجارب الزراعية بكلية الزراعة بمشهر خلال موسي ١٩٩٦/١٩٩٧ . ١٩٩٧/١٩٩٧ لدراسة تأثير التلقيح بالبكتريا المثبتة لأزوت الهواء الجوى (الازوتوباكتر او الأزوسبيريلام) *A. chroococcum* or *A. lipoferum* . ومقارنة ذلك بالتسميد العضوى بسماد البيوجاز والتسميد المعدنى بسماد نترات الامونيوم على نمو وإنتاجية نباتات الكراوية وقد أظهرت هذه الدراسة أهم النتائج التالية:

عند التلقيح بالبكتريا المثبتة لأزوت الهواء الجوى (الازوتوباكتر أو الأزوسبيريلام) أو التسميد بسماد البيوجاز أعطى ذلك أعدادا أعلى من الازوتوباكتر والازوسبيريلام وكذلك البكتريا المذيبة للفوسفات فى منطقة الريزوسفير وذلك بالمقارنة بالتسميد بسماد نترات الأمونيوم .

عند التلقيح بالبكتريا المثبتة للأزوت (الازوتوباكتر أو الأزوسبيريلام) وإمداد النباتات بنصف الجرعة الموصى بها من السماد الأزوتى ، أعطى ذلك أعلى معدل من نشاط إنزيم النيتروجيناز فى منطقة الريزوسفير بالمقارنة بالتسميد بالجرعة الكاملة سواء من سماد البيوجاز أو نترات الامونيوم . أعطت المعاملة الملقحة بالازوتوباكتر أعلى تركيز من النيتروجين الامونيومى والنتراتى فى منطقة الريزوسفير بينما أعلى تركيز من الفوسفور الميسر لوحظ عند التسميد بسماد البيوجاز .

لوحظت فروق معنوية فى صفات النمو وكذلك فى المحصول حيث إزدادت هذه القياسات عند التلقيح بالازوتوباكتر أو الأزوسبيريلام مع إضافة نصف الجرعة من السماد الأزوتى وذلك بالمقارنة بالتسميد بالجرعة الكاملة من سماد البيوجاز أو نترات الامونيوم .

أدت الزراعة على مسافات ٢٥ سم بين الجور إلى الحصول على محصول أعلى من البذور/فدان وذلك بالمقارنة بالزراعة على مسافات واسعة بين الجور . عند التلقيح بالازوتوباكتر والزراعة على مسافة ٢٥ × ٥٠ سم أدى ذلك إلى الحصول على أعلى إنتاج من البذور/ فدان .

كذلك أدى التلقيح بالبكتريا المثبتة لأزوت الهواء الجوى (الازوتوباكتر أو الأزوسبيريلام) إلى زيادة معنوية فى محتوى نباتات الكراوية من النيتروجين والفوسفور والبوتاسيوم وكذلك الكربوهيدرات الكلية ، بينما أدى التسميد بسماد البيوجاز إلى الحصول على أعلى محتوى من الزيت فى البذور .