

INFLUENCE OF BIO AND CHEMICAL NITROGEN FERTILIZERS ON THE GROWTH, YIELD AND ACTIVE CONSTITUENTS OF *Ammi visnaga* L. Plant

S.A. El-Shafie; S.A. El-Kholy; M.M. Mazrou and M.M.M. Afify
Horticulture Dept. Faculty of Agric. Minufiya University

(Received: Dec., 6 , 2009)

ABSTRACT: *This investigation was carried out at the Experimental Farm of the Faculty of Agriculture Minufiya University during two successive seasons of 2003/2004 and 2004/2005 for studying the effect of inoculation Ammi visnaga L. seeds with different N.F.B. strains such as Azospirillum lipoferum, Azotobacter chroococcum and the mixture between (Azospirillum + Azotobacter) separately or in combinations with urea as chemical fertilization at a rates of (0, 40, 80 and 120 kg/fed). The obtained results appeared that, the inoculation treatments with different N.F.B strains resulted in significantly taller plants, more branches number, heavier fresh herb of whole plant as well as wheavier both fresh and dry herb without umbels, in comparison with the uninoculated seeds during the two experimental seasons. The treatment of Azotobacter gave the best results in this trait. Also, both fresh and dry weights of umbels/plant were at the highest value when Ammi visnaga L. seeds treated with Azotobacter. Otherwise, both fruit yield per plant and per feddan reached to the maximum value when the seeds were inoculated with Azotobacter. Also, both khellin and visnagin yield were increased by the application of different N.F.B strains. The best results in this respect was obtained by inoculation the seeds with Azotobacter. In addition, the measured vegetative growth, umbels/yield as well as fruit yield per plant and per feddan was increased by fertilizing the plants with urea at the lowest dose (40 kg/fed) during the first and second seasons. Meanwhile, both khellin and visnagin yield followed the same abovementioned trend during the two experimental seasons. Otherwise, the best growth parameters, umbels/yield. Fruit yield as well as both total khellin and visnagin yield/plant and fed. were produced by the combination treatments between Azotobacter chroococcum + lower and or moderate urea doses (40 and or 80 kg/fed) in compared with the other combinations and the untreated plants (control) during the two experimental seasons.*

Key words: *Ammi visnaga L. bacterial strains, urea fertilization, Khellin, visnagin .*

INTRODUCTION

Ammi visnaga L. is a member of the Apiaceae or Umbelliferae, family which grow very well in Egypt. It represents one of the major plant producing

chromones, especially khellin and visnagin. Also, this plant is a bitter medical plant that is native to the Mediterranean area of North Africa and the Middle East. *Ammi visnaga* L. is one of the oldest herbs cultivated by the ancient Egyptians. Therefore, the medicinal usage of visnaga dates back to ancient Egypt. Over the ages visnaga's fruit have been used in case of kidney stones and even to this day. Egyptian herbal medicine practitioners use the visnaga to alleviate pains caused by kidney stones. Visnaga helps to reduce the pain caused by the stones trapped in kidney by loosening up the use tear muscles. In this way, it helps the locked stone in the kidney to ease down into the urinary bladder.

Researches have established that visnaga possesses anti-spasmodic features that's why it is widely used to heal asthma and is also considered to be a safe medication even for children. Visnaga is also an efficient medication for different respiratory ailments and is helpful in curing bronchitis, emphysema, and also whooping cough. Owing to the great importance uses of *Ammi visnaga* L. plants several investigations for optimizing fruit production and improving its contents from chromone (Khellin and visnagin) were carried by several investigates. In this trait, the fruit production and its contents from khellin and visnagin depended to a large extent on the application of proper agricultural practices such as supplying the plants with adequate nutrients, particularly nitrogen nutrition. Otherwise, nowadays there has been an increasing awareness of undesirable impact of using high doses from chemical fertilization, which have a dangerous effects on the environment and human health.

Therefore, many attempts were carried out by several investigators to minimize the nonstop addition of high doses from chemical fertilizers through the application of biofertilization by inoculating the seeds with N₂-fixing bacteria such as *Azospirillum lipoferum*, *Azotobacter chroococcum* and or the mixture between them, which are known to have a beneficial effects on the growth and chemical constituents of several medicinal and aromatic plants (Fayez *et al.*, 1985). In this respect El-Sawy *et al.*, (1998) and Nofal *et al.*, (2001) on *Ammi visnaga* reported that, the application of *Azotobacter* and *Azospirillum* separately or when interacted with suitable doses from chemical fertilization enhanced plant growth and improved its chemical constituents.

Furthermore, Afify (2002) on fennel plant, Goma (2002) on *Coriandrum sativum* and *Majorana hortensis*, Abdou and El-Sayed (2002) on *Carum carvi*, Shaalan (2005) on *Nigella sativa* and Dewidar (2007) on *Rosmarinus officinalis* L, *Mentha viridis* and *Origanum majorana* L plant mentioned that, the inoculation with different N₂-fixing bacterial strains in combinations with suitable chemical fertilization levels realized the best growth measurements and chemical component of the investigated plants. Therefore, the present investigation was conducted for studying the effect of inoculating the seeds

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of *Ammi visnaga* L with different N.F.B strains such as *Azospirillum lipoferum* , *Azotobacter chroococcum* and or the mixture between *Azotobacter* + *Azospirillum* separately or in combinations with different urea rates on the vegetative growth, fruit yield and active constituents of *Ammi visnaga* L plant grown under the environmental condition of El-Minufiya government.

MATERIALS AND METHODS

Two field experiments were carried out at the Experimental Farm of the Faculty of Agriculture, Minufiya University during two successive seasons of 2003/2004 and 2004/2005 for studying the effect of biofertilization with different N₂-fixing bacterial strains as well as the influence of different urea (46%, N) doses individually or in combinations with different N₂-fixing bacterial strains on the growth, yield and active constituents of *Ammi visnaga* L. plants.

The experimental soil was clay one and its physical and chemical properties were determined according to the method described by Jackson (1967) and recorded in Table (1a and 1b).

Table (1): The physical and chemical properties of the experimental soil according to the methods described by Jackson (1967).

a. Physical properties

Water field capacity	Organic matter %	Coarse sand %	Fine sand %	Silt %	Clay %	Texture grade
38.80	2.80	3.84	27.40	44.23	23.20	Clay loamy

b. Chemical properties

PH	E.C. min/hos/ cm at 25°	C.E.C. mg/ 100 g	Total CaCO ₃ %	Total N %	Total P ₂ O ₅ %	Soluble ions mg/100 g			
						Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺
7.9	1.73	25.60	2.32	0.12	0.26	0.42	0.68	0.60	0.12

The experiments were arranged in a factorial complete randomized block design with three replicates of 1.5 x 1.8 m for each treatment. The replicate included three rows 60 cms apart. During soil preparation, the recommended doses from calcium super phosphate (15.50% P₂O₅ 120 kg/fed) and potassium sulphate (48% K₂O 80 kg/fed) were added in the each growing season. Seeds of *Ammi visnaga* L. were soaked in different bacterial culture

namely Azospirillum, Azotobacter and their Mixture solely for 12 hrs, in addition to control seeds which were soaked in distilled water.

The aforementioned N₂-fixing bacterial strains were prepared for application according to the method described by Mahfouz (2003). The prepared culture from each bacterial type contained 10⁷ cell/ml.

The seeds were sown on the 10th of October during the two seasons at a distance of 30 cms apart and directly irrigated after sowing.

Urea (46% N) was experimented at the levels of (0, 40, 80 and 120 kg/fed.). The previous amount of fertilizers were added, in two equal side dressings, the first addition was after two and half months from planting and the second one at the beginning of flowering stage.

The treatments of bio and urea fertilization levels and their combinations could be arranged as follows:

- 1) Uninoculated bacteria and unfertilized with urea (control).
- 2) Uninoculated bacteria and fertilized with urea at 40 kg/fed.
- 3) Uninoculated bacteria and fertilized with urea at 80 kg/fed.
- 4) Uninoculated bacteria and fertilized with urea at 120 kg/fed.
- 5) Inoculation with Azospirillum and unfertilized with urea.
- 6) Inoculation with Azospirillum and fertilized with urea at 40 kg/fed.
- 7) Inoculation with Azospirillum and fertilized with urea at 80 kg/fed.
- 8) Inoculation with Azospirillum and fertilized with urea at 120 kg/fed.
- 9) Inoculation with Azotobacter and unfertilized with urea.
- 10) Inoculation with Azotobacter and fertilized with urea at 40 kg/fed.
- 11) Inoculation with Azotobacter and fertilized with urea at 80 kg/fed.
- 12) Inoculation with Azotobacter and fertilized with urea at 120 kg/fed.
- 13) Inoculation with Mixture (Azospirillum + Azotobacter) and unfertilized with urea.
- 14) Inoculation with Mixture (Azospirillum + Azotobacter) and fertilized with urea at 40 kg/fed.
- 15) Inoculation with Mixture (Azospirillum + Azotobacter) and fertilized with urea at 80 kg/fed.
- 16) Inoculation with Mixture (Azospirillum + Azotobacter) and fertilized with urea at 120 kg/fed.

The following data were recorded

1. Plant height.
2. Number of branches/plant.
3. Fresh weight of whole plant as well as fresh and dry weights of herb without umbels.
4. Fresh and dry weights of umbels/plant.

5. Fruit yield gm/plant and kg/per fed. (kg).

The statistical analysis was carried out according to Snedecor and Cochran (1980) during the two experimental seasons.

Both total khellin and visnagin percentages were determined according to the methods described by Martelli *et al.*, (1984).

RESULTS AND DISCUSSION

1. Plant height .

It is clear from the data in Table (2) that, the plant height of *Ammi visnaga* L. plant significantly increased by inoculation the seeds with different nitrogen fixing bacteria (N.F.B) strains in both growing seasons when compared with the uninoculated control. The tallest plants were produced by the treatment of inoculation with *Azotobacter chroococcum* during the two experimental seasons. Both *Azospirillum lipoferum* and the mixture between (Azospirillum + Azotobacter) resulted in taller plants than the uninoculated seeds (control) which produced the shortest plants in this trait during the seasons of 2003/2004 and 2004/2005. The mean plant height in the first season was 144.68, 169.88, 176.09 and 162.23 cms, while it was 136.56, 159.29, 166.91 and 153.50 cms. in the second one for the treatments of control, Azospirillum, Azotobacter and the mixture between Azotobacter + Azospirillum respectively.

The increment in the plant height as a result of using different N₂-fixing bacterial strains could be explained through the role of N₂-fixing microorganisms, in providing visnaga plants with the required nitrogen doses needed for protein and cytokinins synthesis and consequently enhancing cell division (Wagner and Michael, 1971). These results were similar to those obtained by El-Sawy *et al.*, (1998), Nofal *et al.*, (2001) on *Ammi visnaga* L. Afify (2002) on fennel plants and Mazrou (2003) on *Mentha piperita* plants.

The data in Table (2) generally show that, different urea doses caused an increase in plant height of *Ammi visnaga* L. plant in comparison to the control plants. The treatments of urea at 40 and or 80 kg/fed. caused an increase in plant height in comparison to the highest urea dose and the unfertilized plants during the first and second seasons.

The increment in the plant height as a result of nitrogen application at its suitable dose may be due to its effective role on synthesis of protein and formation of new tissues, consequently increasing plant length. Similar results were obtained by El-Sawy *et al.*, (1998), Nofal *et al.* (2001) on *Ammi visnaga* L. Afify (2002) on fennel plants and Dewider (2007) on *Rosmarinus officinalis*, *Mentha viridis* and *Origanum majorana*.

It is evident from the data in Table (2) that, the plant height of *Ammi visnaga* L. reached to the maximum values (180.43 and 172.70 cms) when the plants were soaked in Azotobacter, then fertilized with urea at the lowest dose (40 kg/fed) during the first and second seasons. On the other hand, the shortest plants (136.67 and 126.33 cms) was obtained by the untreated plants during the first and second seasons.

These results could be supported by the findings of Menesi (1995) on *Ammi majus* and *Ammi visnaga*. Also, Helmy (2003) on roselle plants stated the same trend.

Table (2) : Effect of biofertilizers, urea and their combination on plant height (cms) of *Ammi visnaga* L. plant during the seasons of 2003/2004 and 2004/2005.

Seasons Urea levels(kg/fed) Bacterial strains	First season 2003/ 2004					Second season 2004/ 2005				
	Control 0	40	80	120	Mean	Control 0	40	80	120	Mean
Control	136.67	144.23	154.50	143.33	144.68	126.33	136.27	143.60	140.03	136.56
Azospirillum	164.97	169.00	173.60	171.93	169.88	153.70	160.20	162.97	160.27	159.29
Azotobacter	177.77	180.43	175.88	170.27	176.09	167.50	172.70	164.75	162.70	166.91
Mixture Azospirillum + Azotobacter	164.20	166.70	162.30	155.73	162.23	155.10	156.50	152.70	150.00	153.50
Mean	160.90	165.09	166.57	160.32		150.66	156.42	156.01	153.25	

	U	B	UB	U	B	UB
L.S.D. at 5%	3.19	3.19	6.38	2.99	2.99	5.69
L.S.D. at 1%	4.30	4.30	8.60	4.02	4.02	8.05
U : Urea		B : Biofertilizer		UB : Urea + Biofertilizer		

2. Number of branches :

From the data in Table (3) it is clear that, the inoculation of *Ammi visnaga* seeds with different N.F.B. strains significantly produced more number of branches per plant than the uninoculated seeds. The highest branches number/plant was produced by seeds inoculation with *Azotobacter chroococcum* followed by *Azospirillum lipoferum* then the mixture between (*Azotobacter* + *Azospirillum*). Meanwhile, the lowest branches number/ plant was obtained by the untreated seed during the first and second seasons. These results are in agreement with those obtained by Ibrahem (2000) on *Ammi visnaga* and *Foeniculum vulgare*, Afify (2002) on fennel plants and Mazrou (2008) on *Mentha piperita* L. plant.

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Table (3): Effect of biofertilizers, urea and their combination on branches number of *Ammi visnaga* L. plant during the seasons of 2003/2004 and 2004/2005.

Seasons Urea levels (kg/fed) Bacterial strains	First season 2003/ 2004					Second season 2004/ 2005				
	Control 0	40	80	120	Mean	Control 0	40	80	120	Mean
Control	6.40	7.30	8.10	7.50	7.33	6.17	6.77	7.47	7.10	6.88
Azospirillum	8.60	9.00	9.15	8.00	8.69	8.00	8.20	8.50	7.40	8.03
Azotobacter	9.45	9.05	8.70	8.35	8.89	8.35	8.95	8.15	7.90	8.34
Mixture Azospirillum + Azotobacter	8.25	8.45	8.20	7.00	7.98	7.75	8.15	7.70	7.18	7.69
Mean	8.18	8.45	8.54	7.71		7.57	8.02	7.96	7.39	
	U	B	UB			U	B	UB		
L.S.D. at 5%	0.39	0.39	0.77			0.48	0.48	0.97		
L.S.D. at 1%	0.52	0.52	1.04			0.65	0.65	1.30		
U : Urea		B : Biofertilizer				UB : Urea + Biofertilizer				

The data in Table (3) show clearly that, both low & moderate urea doses (40 and 80 kg/fed.) produced the highest number of branches/ plant than those of the unfertilized plants and the highest urea dose (120 kg/fed) which produced the lowest number of branches/plant during the first and second seasons. The results could be explained by the important role played by nitrogen in building protein molecules and enhancing cell division and cell elongation through its effect in raising the levels of GA₃ and cytokinins content in plant tissues (Wagner and Michael, 1971), which was reflected in promoting the growth of lateral buds and producing more branches.

These results are in harmony with those obtained by Badawi (2000) on roselle, Abd El-Kader (1992), Hassan (1997) and Afify (2002) on fennel plants. Also, Mazrou (2008) on *Mentha piperita* L. plants stated the same trend.

Furthermore, the data presented in Table (3) indicated that, the highest branches number was obtained by inoculation *Ammi visnaga* seeds with Azotobacter separately and or when interacted with urea at the lowest dose (40 kg/fed). Meanwhile, the lowest branches number was obtained by the plants didn't receive any of bio and urea fertilization levels during the two experimental seasons. These results are in accordance with those obtained by El-Sawy *et al.*, (1998) and Nofal *et al.*, (2001) on *Ammi visnaga*, Afify (2002) on fennel plants and Mazrou (2008) on *Mentha piperita* L. plant.

3. Fresh weight of whole plant as well as both fresh and dry weights of herb without umbels:

It is clear from the data in Tables (4, 5 and 6) that, the fresh weight of whole plant as well as both fresh and dry weights of herb without umbel significantly increased by soaking the seeds of *Ammi visnaga* with different N.F.B. strains Azospirillum, Azotobacter and the mixture between (Azospirillum + Azotobacter) when compared with the uninoculated seeds (control) during the first and second seasons. The best results in this respect was obtained by inoculation the seeds of *Ammi visnaga* L. with Azotobacter chroococcum. Also, inoculation the seeds with Azospirillum produced heavier fresh herb of whole plant as well as both fresh and dry herb without umbel than the treatment of the mixture between (Azospirillum + Azotobacter) and the control plants which resulted in the lowest measurements in this trait.

The increment of fresh weight of total herb as well as both fresh and dry weights of herb without umbels might be due to its effective role of these N.F.B. strains on enhancement formation of available nitrogen which might play a direct or indirect role in plant metabolism through activating the photosynthetic process as well as accumulation of their products in plant organs resulting in more plant growth consequently more weight (Badawi 2000). These results are similar to those obtained by Afify (2002) on fennal plants, and El-Sawy *et al.*, (1998) on *Ammi visnaga* L. plant.

Table (4) : Effect of biofertilizers, urea and their combination on fresh weight of whole *Ammi visnaga* L. plants (g) during the seasons of 2003/2004 and 2004/2005.

Seasons Urea levels (kg/fed)	First season 2003/ 2004					Second season 2004/ 2005				
	Control 0	40	80	120	Mean	Control 0	40	80	120	Mean
Bacterial strains										
Control	368.80	444.13	458.20	426.61	424.44	339.15	370.53	439.70	405.40	388.70
Azospirillum	595.82	603.14	624.67	615.43	609.77	579.40	590.11	605.22	594.22	592.24
Azotobacter	667.31	716.90	654.25	613.80	663.07	649.85	683.25	630.92	579.55	635.89
Mixture Azospirillum + Azotobacter	533.24	516.55	495.20	471.10	504.02	499.22	475.05	456.37	437.90	467.14
Mean	541.29	570.18	558.08	531.74		516.91	529.74	533.05	504.27	

U B UB
 L.S.D. at 5% 17.74 17.74 35.49
 L.S.D. at 1% 23.90 23.90 47.79

U B UB
 16.70 16.70 33.39
 22.48 22.48 44.97

U : Urea

B : Biofertilizer

UB : Urea + Biofertilizer

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Table (5) : Effect of biofertilizers, urea and their combination on fresh weight of herb without umbels (gm) per *Ammi visnaga* L. plants during the seasons of 2003/2004 and 2004/2005.

Seasons Urea levels (kg/fed)	First season 2003/ 2004					Second season 2004/ 2005				
	Control 0	40	80	120	Mean	Control 0	40	80	120	Mean
Bacterial strains										
Control	254.40	302.13	311.70	290.21	289.61	233.91	255.53	303.20	272.80	266.36
Azospirillum	405.32	407.70	423.37	415.83	413.06	394.15	400.61	411.52	402.52	402.20
Azotobacter	467.11	494.40	454.00	423.30	459.70	452.50	470.75	441.80	401.15	441.55
Mixture Azospirillum + Azotobacter	367.74	356.25	341.50	324.90	347.60	340.72	325.45	315.15	301.80	320.78
Mean	373.64	390.12	382.64	363.56		355.32	363.09	367.92	344.57	

	U	B	UB	U	B	UB
L.S.D. at 5%	9.51	9.51	19.03	7.66	7.66	15.33
L.S.D. at 1%	12.81	12.81	25.62	10.32	10.32	20.64
U : Urea		B : Biofertilizer		UB : Urea + Biofertilizer		

Table (6) : Effect of biofertilizers, urea and their combination on dry weight of herb without umbels (gm) per *Ammi visnaga* L. plant during the seasons of 2003/2004 and 2004/2005.

Seasons Urea levels (kg/fed)	First season 2003/ 2004					Second season 2004/ 2005				
	Control 0	40	80	120	Mean	Control 0	40	80	120	Mean
Bacterial strains										
Control	124.43	147.32	154.49	146.47	143.18	115.40	124.67	148.53	136.37	131.24
Azospirillum	197.40	199.13	212.93	204.43	203.47	191.53	195.27	199.60	196.13	195.63
Azotobacter	228.20	240.50	224.40	216.40	227.38	221.77	234.73	220.80	197.17	218.62
Mixture Azospirillum + Azotobacter	179.93	174.33	167.43	157.23	169.73	167.30	161.97	150.63	148.07	156.99
Mean	182.49	190.32	189.81	181.13		174.00	179.16	179.89	169.74	

	U	B	UB	U	B	UB
L.S.D. at 5%	4.56	4.56	9.13	2.47	2.47	4.94
L.S.D. at 1%	6.15	6.15	12.29	3.33	3.33	6.65
U : Urea		B : Biofertilizer		UB : Urea + Biofertilizer		

The presented data in Tables (4, 5 and 6) showed that, the fresh weight of whole plant as well as both fresh and dry weights of herb without umbels increased as a result of fertilizing *Ammi visnaga* L. plant with urea (46% N) at the lowest and moderate doses (40 and or 80 kg/fed.) during the first and second seasons when compared with the unfertilized plants (control) and the highest urea level (120 kg/fed.) which resulted in the lightest weight during the first and second seasons. Such results were obtained by Afify (2002) on fennel plant, El-Sawy *et al.*, (1998) on *Ammi visnaga* L. plants and Ibrahem (2000) on *Ammi visnaga* and fennel plant.

From the data in Tables (4, 5 and 6) it could be mentioned that, the fresh weight of whole plant as well as both fresh and dry weights of herb without umbels of *Ammi visnaga* L. plants increased as a result of a combination treatment between biofertilization and the different urea doses when compared to the untreated plants (control) and those which fertilized with urea alone during the first and second seasons. Meanwhile, the combination treatments of Azotobacter and the different urea levels produced heavier parameters during the two experimental seasons followed by the combination between Azospirillum and different urea levels then the combination between the mixture between (Azotobacter + Azospirillum) and different urea levels. On the other hand, the lightest herb weight was obtained from the plants didn't receive any of bio and urea fertilizers. Similar findings on the effect of the interaction between bio and chemical nitrogen fertilizers were obtained by El-Sawy *et al.*, (1998) on *Ammi visnaga*. Also, Afify (2002) on fennel plants, Harridy and Amara (1998) on roselle plants and Mazrou (2008) on *Mentha piperita* L. plants stated the same previously trend.

From the above mentioned results it could be concluded that, the best measurements of the vegetative growth were obtained by inoculated *Ammi visnaga* seeds with Azotobacter individually or when combined with the lower urea dose in most cases during the two experimental seasons.

4. Fresh and dry weights of umbels:

Data dealing with the effect of different bacterial strains of (N.F.B) on fresh and dry weights of umbels per visnaga plant are presented in Table (7 and 8). Results showed that, the inoculation of visnaga seeds with different (N.F.B) strains significantly produced heavier fresh and dry umbels/plant than the untreated seeds during the first and second seasons. Inoculation *Ammi visnaga* L. seeds with Azotobacter produced the heaviest fresh and dry umbels/plant (205.86 and 194.34 gm) for fresh umbels and (134.90 and 133.12 gm) for dry umbels gm/plant during the first and second seasons respectively. Also, inoculation the seeds with Azospirillum significantly resulted higher fresh and dry umbels than those of the mixture (Azotobacter + Azospirillum). The lightest fresh and dry umbels were obtained by the uninoculation seeds during the two experimental seasons.

The increase in both fresh and dry weight of umbels/plant as a result of using different (N.F.B.) strains may be due to its production of growth regulators such as gibberellins cytokinins and indole acetic acid (Shalan *et al.*, 2001).

These results were similar to those obtained by Nofal *et al.*, (2001) and El-Sawy *et al.*, (1998) on *Ammi visnaga* . Also, Afify (2002) on fennel plants stated the same trend.

It is evident from the data in Tables (7 and 8) that, the mean fresh and dry weights of umbels/plant increased as a result of using urea fertilizer doses of (40 and 80 kg/fed). In most cases the highest urea dose (120 kg/fed) produced the lightest fresh and dry umbels/plant. Such results were obtained by Abd El-Wahab (1997) on *Nigella sativa.*, Gomaa (2002) on coriander plants and Afify (2002) on fennel plants.

It is clear from the data in Tables (7 and 8) that, both fresh and dry umbels weight/plant gradually increased by increasing urea dose up to the moderate level (80 kg/fed), then decreased with the highest dose (120 kg/fed) during the first and second seasons. Meanwhile, Azotobacter when interacted with urea at the lowest dose (40 kg/fed.) produced the heaviest fresh and dry umbels during the two seasons. On the hand, the untreated plants gave the lightest fresh and dry umbels/plant during the first and second seasons. These results were similar to those obtained by Afify (2002) on fennel plants, and Harridy and Amara (1998) on roselle plant.

5. Fruit Yield per plant (gm) and per fed. (kg):

From the results in Tables (9 and 10) it is clear that, both fruit yield per plant and per feddan significantly raised by inoculation *Ammi visnaga* L. seeds with different nitrogen fixing bacteria (N.F.B) strains i.e. Azospirillum, Azotobacter and the mixture (Azospirillum + Azotobacter) than the uninoculated seeds (control) during the first and second seasons. Inoculation visnaga seeds with Azotobacter resulted in significantly heaviest fruit yield per plant (49.90 and 47.44 gm/plant) and per feddan (1108.88 and 1053.87 kg/fed) during the two experimental seasons. Also, the treatment of Azospirillum produced higher fruit yield per plant and per feddan than those of the treatment of the mixture (Azotobacter + Azospirillum) and the uninoculated seeds (control) which resulted in the lightest yield per plant and per feddan during the first and second seasons. These results are in agreement with those obtained by Amin (1997) and Afify (2002) on fennel plants. Also, Shalan *et al.*, (2001) on rosella plants found the same previously mentioned trend.

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Table (9) : Effect of biofertilizers, urea and their combination on fruit yield (g) per *Ammi visnaga* L. plant during the seasons of 2003/2004 and 2004 and 2005.

Seasons Urea levels (kg/fed)	First season 2003/ 2004					Second season 2004/ 2005				
	Control 0	40	80	120	Mean	Control 0	40	80	120	Mean
Bacterial strains										
Control	28.14	32.35	36.84	29.37	31.68	23.20	29.75	34.41	30.15	29.38
Azospirillum	44.83	48.50	43.15	38.50	43.75	40.15	43.50	39.85	36.46	39.99
Azotobacter	47.06	52.94	54.50	45.10	49.90	43.70	49.75	51.50	44.75	47.44
Mixture Azospirillum + Azotobacter	41.50	39.80	34.36	31.70	36.84	38.10	36.50	31.90	28.08	33.65
Mean	40.38	43.40	42.21	36.17		36.29	39.88	39.42	34.86	

U B UB U B UB
L.S.D. at 5% 9.97 9.97 19.95 3.43 3.43 6.86
L.S.D. at 1% 13.43 13.43 26.86 4.62 4.62 9.24
U : Urea B : Biofertilizer UB : Urea + Biofertilizer

Table (10): Effect of biofertilizers, urea and their combination on fruit yield (kg/fed.) per *Ammi visnaga* L. plant during the seasons of 2003/2004 and 2004 and 2005.

Seasons Urea levels (kg/fed)	First season 2003/ 2004					Second season 2004/ 2005				
	Control 0	40	80	120	Mean	Control 0	40	80	120	Mean
Bacterial strains										
Control	625.33	718.88	818.66	652.66	706.88	515.55	661.10	764.66	669.99	652.83
Azospirillum	996.21	1077.77	958.88	855.55	972.10	892.21	966.66	885.55	810.21	888.66
Azotobacter	1045.77	1176.43	1211.10	1002.21	1108.88	971.10	1105.54	1144.43	994.43	1053.87
Mixture Azospirillum + Azotobacter	922.21	884.43	763.55	704.44	818.66	846.66	811.10	708.88	623.99	747.66
Mean	897.38	964.38	938.05	803.72		806.38	886.10	875.88	774.66	

U B UB U B UB
L.S.D. at 5% 17.73 17.73 35.46 14.00 14.00 28.01
L.S.D. at 1% 23.88 23.88 47.76 18.86 18.86 37.72
U : Urea B : Biofertilizer UB : Urea + Biofertilizer

Data recorded in Table (9 and 10) showed that, the fruit yield of *Ammi visnaga* per plant (gm) and per feddan (kg) was at the highest value when the

plants were fertilized with the lowest urea dose (40 kg/fed), then decreased by the moderate and the highest urea levels (80 and 120 kg/fed). The unfertilized plants (control) produced heavier fruit yield per plant and feddan than the plants which fertilized with the highest urea dose (120 kg/fed) during the two experimental seasons. These results are in accordance with the findings of Afify (2002) on fennel plants and Meawad and El-Deep (1991) on anise plants.

It is clear from the data in Tables (9 and 10) that, the fruit yield per plant and per feddan increased by using different N.F.B strains separately or when combined with urea application especially at the lowest dose (40 kg/fed) during the first and second seasons. Also, soaking *Ammi visnaga* L. seeds with *Azotobacter* then interacted with urea at the moderate dose (80 kg/fed) produced the heaviest fruit yield per plant (54.50 and 51.50 gm) and per feddan (1211.10 and 1144.43 kg) during the first and second seasons. On the other hand, the lowest fruit yield per plant (28.14 and 23.20 gm/plant) and per feddan (625.33 and 515.55 kg) were produced by the plants didn't receive any of bio and urea fertilizers during the two experimental seasons. These results are in accordance with those confirmed by Mahgoub (2002) on fennel and coriander, Afify (2002) on fennel and Amin (1997) on fennel coriander and caraway plants

Active constituents.

1. Total khellin percentages in the dried fruits:

Data in Table (11) clearly indicate that, the total khellin percentages in the dried fruits of *Ammi visnaga* L. plants increased by using different N.F.B strains in compared with the uninoculated seeds during the two experimental seasons. Inoculation *visnaga* seeds with *Azotobacter* resulted in the highest value in this respect, then followed by the Mixture (*Azospirillum*, *Azotobacter*), then *Azospirillum* and the control during the first and second seasons.

The obtained results of total khellin percentages in dried fruits of *Ammi visnaga* L. plants emphasized the beneficial role of the inoculation *visnaga* seeds with different N.F.B strains on the metabolic process which is responsible for the quantity of total khellin content in *Ammi visnaga* L. plants. The same observation was obtained by El-Sawy *et al.*, (1998) and Ibrahim *et al.*, (1984) on *Ammi visnaga* L.

Data in Table (11) show that, the total khellin percentages in the dried fruit of *visnaga* plants increased gradually by increasing urea levels up to the moderate dose (80 kg/fed) then slightly decreased by the highest urea dose (120 kg/fed). The lowest khellin percentages was obtained by the untreated plants during the two experimental seasons. Similar results were obtained by El-Sawy *et al.*, (1998) and Ibrahim (1984) on *Ammi visnaga* L.

Table (11): Effect of biofertilizers, urea and their combination on total khellin percentages and total khellin yield per plant and per fed. in the dried fruits of *Ammi visnaga* L. plants during the seasons of 2003/2004 and 2004/2005.

Bacterial strains	Control			40			80			120			Mean			
	Urea levels(kg)	Khellin %	Khellin Yield/g plant	Khellin k. g plant	Khellin %	Khellin Yield/g plant	Khellin k. g plant	Khellin %	Khellin Yield/g plant	Khellin k. g plant	Khellin %	Khellin Yield/g plant	Khellin k. g plant	Khellin %	Khellin Yield/g plant	Khellin k. g plant
First season 2003/ 2004																
Control	1.02	287.03	6.38	0.99	320.27	7.12	1.06	390.50	8.68	1.03	303.06	6.72	1.03	352.22	7.28	
Azospirillum	1.14	511.06	11.36	1.17	567.45	12.61	1.15	496.22	11.03	1.15	442.75	9.84	1.15	504.37	11.21	
Azotobacter Mixture	1.20	564.72	12.55	1.25	661.75	14.70	1.30	708.50	15.74	1.31	583.14	13.13	1.27	629.53	14.03	
(Azospirillum +Azotobacter)	1.24	477.25	11.44	1.21	461.68	10.70	1.22	405.45	9.32	1.19	370.89	8.38	1.22	428.82	10.82	
Mean	1.15	460.02	10.43	1.16	502.79	11.28	1.18	500.17	11.19	1.17	424.96	9.52				
L.S.D. at 5%		U	U		B	B		U.B	U.B							
L.S.D. at 1%		47.78	0.23		47.78	0.23		95.55	0.45							
		64.34	0.30		64.34	0.30		128.68	0.61							
Second season 2004/ 2005																
Control	0.89	206.48	4.59	0.91	270.73	6.02	1.09	375.07	8.33	1.05	316.58	7.03	0.98	292.22	6.49	
Azospirillum	1.16	465.74	10.35	1.18	513.30	11.41	1.10	438.35	9.74	1.11	404.71	8.99	1.14	455.53	10.12	
Azotobacter Mixture	1.23	537.51	11.94	1.22	606.77	13.49	1.28	659.20	14.65	1.24	554.90	12.33	1.24	589.60	13.10	
(Azospirillum +Azotobacter)	1.20	457.70	10.16	1.19	434.35	9.65	1.19	379.61	8.43	1.16	325.73	7.36	1.18	399.22	8.90	
Mean	1.12	416.73	9.26	1.13	456.29	10.14	1.17	463.06	10.29	1.14	400.48	8.93				
L.S.D. at 5%		U	U		B	B		U.B	U.B							
L.S.D. at 1%		39.64	0.17		39.64	0.17		79.29	0.34							
		53.39	0.23		53.39	0.23		106.78	0.46							

It is clear from the data in Table (11) that, the different combinations between urea doses and N.F.B strains resulted in higher total khellin percentages in the dried fruits of *Ammi visnaga* L. plants during the two experimental seasons. Inoculation *visnaga* seeds with *Azotobacter* then combined with urea at moderate and the highest levels recorded the highest total khellin percentages followed by the mixture (*Azospirillum*, *Azotobacter*) when combined with different urea doses then *Azospirillum* when interacted with different urea levels during the two experimental seasons. These results were similar to those obtained by El-Sawy *et al.*, (1998) on *Ammi visnaga* L. and Shaalan (2005) *Nigella sativa*.

2. Khellin yield in the dried fruits (mg/plant and kg/fed):

Presented data in Table (11) showed that, inoculation *Ammi visnaga* L. seeds with *Azotobacter* resulted in the highest total khellin yield per plant (629.53 and 589.60 mg/plant) and (14.03 and 13.10 kg/fed) during the first and second seasons. On the other hand, the uninoculated seeds (control) resulted in the lowest total khellin yield per plant and per feddan (325.22 and 292.22 mg/plant) and (7.23 and 6.49 kg/fed) during the first and second seasons. These results were in agreement with those obtained by El-Sawy *et al.*, (1998) and Inrahim *et al.*, (1984) on *Ammi visnaga* L. plants.

Data recorded in Table (11) show that, the both lowest and moderate urea levels (40 and 80 kg/fed) gave more total khellin yield per plant and per feddan when compared with the highest urea dose and the unfertilized plants during the two experimental seasons. Meanwhile, the unfertilized plants (control) produced higher total khellin yield per plant and per feddan than those fertilized with the highest urea dose during the first and second seasons. These results were in agreement with those obtained by El-Sawy *et al.*, (1998) and Ibrahim *et al.*, (1984) on *Ammi visnaga* L. plants.

The different urea doses significantly produced higher total khellin yield per plant and per feddan than the unfertilized plants. Meanwhile, the different N.F.B. strains individually or when combined with different urea doses significantly produced higher total khellin yield than the plants didn't receive any of bio and chemical nitrogen fertilizer and those which fertilized with urea alone during the first and second seasons. Also, it could be noticed that, the seeds which inoculated with *Azotobacter* and combined with urea at moderate dose produced highest total khellin yield per plant and per feddan than the other combinations during the first and second seasons. These results were similar to those obtained by El-Sawy *et al.*, (1998) and Ibrahim *et al.*, (1984) on *Ammi visnaga* L. plants.

C : Total visnagin percentages in the dried fruits:

The obtained data in Table (12) illustrated that, the total visnagin percentages in the dried fruits of *visnaga* plants increased as a result of inoculation *Ammi visnaga* L. seeds with different N.F.B. strains when

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compared with the uninoculated seeds. In the same time, the highest total visnagin percentages (0.33 and 0.33%) was obtained by using the mixture between (*Azotobacter* + *Azospirillum*) during the two experimental seasons. Also, inoculated the seeds with *Azotobacter* resulted in more visnagin percentages than those which treated with *Azospirillum* during the first and second seasons. Similar results were obtained by Ibrahim *et al.*, (1984) on *Ammi visnaga* L.

It is clear from the data in Table (12) that, there were no clear differences was observed when *Ammi visnaga* L. plants were fertilized with different urea doses in compared with the unfertilized plants. Meanwhile, the visnagin content was at the highest value (0.30 and 0.28%) during the first and second seasons when visnaga plants were fertilized with urea at the lowest dose (40 kg/fed). These results were similar to those obtained by Ibrahim (1984) on *Ammi visnaga* L. plants.

From the data in Table (12) it could be mentioned that, the different combinations between (N.F.B) strains and different urea doses resulted in higher total visnagin content than the plants which fertilized with different urea doses alone and the unfertilized plants. The highest total visnagin content was obtained when the seeds were inoculated with the mixture between (*Azospirillum* + *Azotobacter*) then fertilized with urea at moderate and high doses during the first and second seasons.

D: Total visnagin yield per plant and per feddan:

It is clear from the data in Table (12) that, both total visnagin yield (mg/plant and kg/fed) significantly increased as a result of inoculation the seeds with different N.F.B. strains when compared to the control plant. Inoculated *Ammi visnaga* L. seeds with *Azotobacter* recorded the highest total visnagin yield (mg/plant and kg/fed) during the first and second seasons followed by the mixture (*Azotobacter* + *Azospirillum*) then *Azospirillum*, respectively.

The important role of N.F.B strains in increasing the percentages of visnagin content and consequently reflexed on increasing total visnagin yield per plant and per feddan on the enzymatic systems responsible for the biosynthesis of these compounds (Kandeel *et al.*, 2001). These results are in agreement with those obtained by Ibrahim *et al.*, (1984) on *Ammi visnaga* and Shaalan (2005) on *Nigella sativa*.

Table (12): Effect of biofertilizers, urea and their combination on total visnagin percentages and total visnagin yield per plant and per fed. in the dried fruits of *Ammi visnaga* L. plants during the seasons of 2003/2004 and 2004/2005.

Urea levels(kg)	Control			40			80			120			Mean		
	visnagin %	Yield/g plant	visnagin/g plant	visnagin %	Yield/g plant	visnagin/g plant	visnagin %	Yield/g plant	visnagin/g plant	visnagin %	Yield/g plant	visnagin/g plant	visnagin %	Yield/g plant	visnagin/g plant
First season 2003/ 2004															
Control	0.23	64.72	1.44	0.25	80.88	1.80	0.21	77.36	1.72	0.22	64.11	1.44	0.23	71.77	1.60
Azospirillum	0.28	125.52	2.79	0.27	130.95	2.91	0.25	107.88	2.40	0.25	96.25	2.14	0.26	115.15	2.56
Azotobacter	0.29	136.47	3.03	0.32	169.41	3.76	0.30	163.50	3.63	0.27	121.77	2.71	0.30	147.79	3.28
Mixture (Azospirillum +Azotobacter)	0.31	128.65	2.86	0.34	135.32	3.01	0.36	123.70	2.75	0.29	91.93	2.04	0.33	119.90	2.66
Mean	0.28	113.84	2.53	0.30	129.14	2.87	0.28	118.11	2.63	0.26	93.52	2.08			
L.S.D. at 5%		U	U		B	B		U.B	U.B						
L.S.D. at 1%		11.02	0.079		11.02	0.079		22.03	0.158						
		14.83	0.106		14.83	0.106		29.67	0.213						
Second season 2004/ 2005															
Control	0.20	46.40	1.03	0.21	62.48	1.39	0.20	68.82	1.53	0.19	57.29	1.27	0.20	58.75	1.31
Azospirillum	0.25	100.38	2.30	0.25	108.78	2.42	0.22	87.67	1.95	0.23	83.86	1.86	0.24	95.17	2.13
Azotobacter	0.30	131.10	2.91	0.34	169.15	3.76	0.32	164.80	3.66	0.32	143.20	3.18	0.32	152.06	3.38
Mixture (Azospirillum +Azotobacter)	0.29	110.49	2.46	0.32	116.80	2.60	0.35	111.65	2.48	0.36	101.24	2.25	0.33	110.05	2.45
Mean	0.26	97.09	2.18	0.28	114.30	2.54	0.27	108.24	2.41	0.27	96.40	2.14			
L.S.D. at 5%		U	U		B	B		U.B	U.B						
L.S.D. at 1%		9.52	0.045		9.52	0.045		14.82	0.091						
		19.04	0.061		19.04	0.061		25.64	0.122						

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increased by fertilizing visnaga plants with urea at low and moderate doses (40 and 80 kg/fed), Meanwhile, the lowest urea dose produced the highest visnagin yield. On the other hand, the lowest visnagin yield per plant and per feddan was obtained by fertilizing visnaga plants with the highest urea dose (120 kg/fed) during the two experimental seasons. These results were in agreement with those obtained by Ibrahim *et al.*, (1984) on *Ammi visnaga* L. plants.

From the data in Table (12) it could be noticed that, generally the untreated plants resulted in the lowest visnagin yield per plant and per feddan when compared with the other treatments. Meanwhile, the different combination between different bacterial strains and different urea doses produced higher total visnagin yield than the control and different urea doses separately. Otherwise, inoculated visnaga seeds with *Azotobacter* then combined with urea at the lowest dose (40 kg/fed) produced the highest visnagin yield per plant (169.41 and 169.15 mg/plant) and per feddan (3.76 and 3.76 kg/fed) during the first and second seasons.

Conclusion

From aforementioned results it could be concluded that, inoculation the seeds of *Ammi visnaga* L. with different N.F.B strains especially *Azotobacter chroococcum* and fertilizing the plants with urea at low and moderate doses (40 and or 80 kg/fed) led to the best parameters of growth, fruit yield and active constituents of *Ammi visnaga* L. plants.

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تأثير الأسمدة الحيوية والكيميائية على النمو والمحصول

والمكونات الفعالة في نبات الخلة البلدى

صلاح الدين عبد السلام الشافعى ، سمير عبد الفتاح الخولى

متولى مسعد مزروع ، محمد محمد موسى عفيفى

قسم البساتين - كلية الزراعة بشبين الكوم - جامعة المنوفية

الملخص العربى

تم إجراء هذا البحث بمزرعة كلية الزراعة - جامعة المنوفية خلال موسمين متتاليين ٢٠٠٣/٢٠٠٤ و ٢٠٠٤/٢٠٠٥ وذلك بهدف دراسة تأثير الأسمدة الحيوية والكيميائية على النمو والمحصول والمكونات الفعالة في نباتات الخلة البلدى . وفى هذه الدراسة تم تلقيح بذور نباتات الخلة بثلاث سلالات مختلفة من البكتيريا المثبتة للأزوت الجوى بتركيز (١٠^٧ خلية/لتر) وهى أزوسبيريليم ليبوفورم ، أزوتوباكتر كروكوكم والخليط بين الأثنين بالإضافة إلى ذلك فقد تم دراسة تأثير التسميد باليوريا (٤٦% نيتروجين) بمعدلات (صفر ، ٤٠ ، ٨٠ ، ١٢٠ كجم/فدان). وقد تم دراسة تأثير كلا النوعين من الأسمدة إما بصورة منفردة أو متداخلة مع بعضها .

وقد أتضح من نتائج الدراسة أن استعمال كل من نوعى الأسمدة إما بصورة فردية أو متداخلة مع بعضها قد أدت إلى زيادة معنوية في صفات النمو ، محصول النورات ، ومحصول الثمار وكذلك المحصول من كل من الخللين والفرناجين مقارنة بنباتات الكنترول الغير معاملة . وقد أتضح من الدراسة أيضا أن أفضل المعاملات في هذا الشأن هى معاملة التلقيح بالأزوتوباكتر مع التسميد بالمستوى المنخفض والمتوسط من اليوريا (٤٠ ، ٨٠ كجم/فدان) حيث أن هاتين المعاملتين انتجتا أعلى محصول من الثمار وكذلك من الخللين والفرناجين بالنسبة للنبات والفدان خلال موسمى التجربة .