RESPONSE OF *NIGELLA SATIVA*, L. PLANTS TO PLANT DENSITY AND MINERAL /BIOFERTILIZATION TREATMENTS

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Abstract

The present trial was conducted to explore the influence of 4 plant densities and 5 NPK/biogen treatments on growth, seed yield, volatile oil and chemical composition of *Nigella sativa*,L. plants.

Obtained results indicated that increasing plant density (up to 3 plants/hill at 20 or 30 cm distance on rows 50 cm apart) caused a reduction in herb dry weight, weight of capsules and seeds/plant and weight of seeds/ capsule, but augmented the seed yield / fed. In addition, such high density treatments reduced total chlorophylls, volatile oil yield / plant and herb uptake of N,P and K, while the yield of volatile oil/fed was increased.

In regard to fertilization treatments, mineral NPK treatment, followed by 1/2 NPK+biogen gave the best results of vegetative growth, yield and yield components, volatile oil and chemical composition. In response to the interaction between plant density and fertilization treatments, the highest seed yield and volatile oil yield / fed. were given due to planting *Nigella sativa* as 3 plants / hill at 20 cm distance and supplying them with NPK or 1/2 NPK+biogen.

INTODUCTION

Nigella sativa, L. (black cumin) is a winter annual herbaceous plant belonging to Fam. Ranunculaceae. It is a drug condiment crop widely grown in middle Egypt region especially Minia Governorate. The seeds are used as antiasthematic drug to treat cough and bronchitis, as diuretic and carminative and to strengthen the immunity system. In addition to their use as flavouring agent in bakeries and as spices.

Among the important agricultural practices are plant density, as well as, the replacement of mineral NPK by biofertilizers. Many authors declared that decreasing plant density augmented vegetative growth, seed yield, volatile oil per plant and chemical composition but reduced them per unit area such as Nofal *et al.*, (2001) on *Ammi visnaga*, Kandeel *et al.*, (2001) and Badran *et al.*, (2007) on fennel, Badran and Hafez (2002) on *Nigella sativa* and Badran *et al.*, (2003) on anise. Different investigators revealed the role of NPK fertilization in improving the above mentioned traits of medicinal and aromatic plants such as *Ammi visnaga* (Nofal *et al*, 2001), anise (Badran *et al.*, 2003) and fennel (Salah-Eldeen, 2005 and Badran *et al.*, 2007).

Meanwhile, the effective role of N-fixing bacteria was emphasized by Nofal *et al.,* (2001) on *Ammi visnaga*, Kandeel *et al.*, (2001), Badran and Safwat (2004) Salah-Eldeen(2005) and Badran *et al.,* (2007) on fennel. Chaudhary *et al.,* (2002) on coriander, Safwat and Badran (2002) and Badran *et al.,* (2007) on cumin and Badran *et al.,* (2003) on anise.

This experiment aimed to reach the ideal plant density and the partial replacement of NPK fertilizers by N- fixing biofertilzier (biogen).

MATERIALS AND METHODS

The present study was conducted during 2002/03 and 2003/04 seasons at the Experimental Farm,Fac. of Agric., Minia Univ. to explore the optimum plant density and the ideal NPK/biogen fertilization treatment for *Nigella sativa* plants. The seeds were sown on Oct 18th for both seasons, in 2x1.5m. plots containing 3 rows, 50cm apart. Farm yard manure was applied at the rate of 20 m3/fed. during soil preparation. Physical and chemical analysis of the soil are shown in Table (a).

Table a. Physical and chemical analysis of the soil .

Character	Value	Character	Value	Character		Value
Soil type	Clay loam	pH (1: 2.5)	7.76	Exch. Na mg/ 100 g		2.38
Sand %	26.2	EC(mmhos/cm)	1.02	Fe		8.54
Silt %	31.5	Total N %	0.08	DTPA ppm	Cu	2.06
Clay %	42.3	Avail. P. %	15.14	Zn		2.75
Org. Matt %	1.62	Exch. K mg/ 100 g	2.16	Mn		8.26
$CaCO_3$ %	2.12	Exc. Ca mg/ 100 g	31.66			

The experiment was arranged as complete randomized blocks in split plot design with three replicates. The main plots were designated to 4 plant densities, namely, 3 plants/hill at 20 or 30 cm distance and 2 plants/hill at 20 or 30 cm distance. These four treatments could be converted to 24,18,16 and 12 plants/m^2, respectively. The sub-plots included 5 fertilization treatments, namely, control, NPK, biogen, 1/2NPK+biogen and 1/4NPK+biogen. Mineral NPK treatment was applied at the rate of 300Kg/fed. ammonium nitrate 33.5% N,200 Kg/fed. calcium superphosphate 15.5% P_2O_5 and 100 Kg/fed. potassium sulphate 48.5% K_2O. Such fertilizer amounts were divided into 3 equal batches and applied after 6 weeks from planting date with 2 weeks intervals thereafter. While, biogen was mixed with the seeds before planting.

The plants were twice thinned to 2 or 3 plants / hill according to each treatment after 4 and 6 weeks from sowing date. All other regular agricultural practices were performed as usual.

Data were recorded, on the third week of April, for plant height (cm), herb dry weight (g), weight of capsules/plant(g), weight of seeds/capsule (mg) and weight of seed/plant(g) and per fed.(Kg). In addition, total chlorophylls (a+b) contents (mg/g F.W.) was estimated on mid March, for both seasons, according to Fadl and Seri-Eldeen (1978), and volatile oil% in the seeds was determined (Gad *et al,* 1963), then volatile oil yield per plant and per fed. were calculated. Meanwhile, N, P and K% were determined according to Page *et al.,* (1982) and herb uptake of the three nutrients were calculated. All obtained data were statistically analyzed following L.S.D. method (Little and Hills, 1978).

RESULTATS AND DISCUSSION

Vegetative Growth

The highest plant density (24 plants/m^2) caused significant increase in plant height and significant reduction in herb dry weight /plant in comparison with the lowest density (12 plants/m^2) in the two seasons as shown in Table (1). In agreement with these results were the findings of Kandeel *et al.*, (2001) on fennel, Badran and Hafez (2002) on *Nigella sativa* and Badran *et al.,* (2003) on anise. Concerning fertilization treatments, NPK, followed by 1/2 NPK+ biogen gave significantly the tallest plants and the heaviest herb dry weight, in both seasons, over those of the control as indicated in Table (1). In accordance with these results concerning NPK were the findings of Nofal *et al.*, (2001) on *Ammi visnaga* and Badran *et al.*, (2007) on fennel. Meanwhile, our results regarding N-fixing bacteria were on the line with those of Badran *et al.,* (2003) on anise, Badran and Safwat (2004) and Salah-Eldeen (2005) on fennel and Badran *et al.,* (2007) on cumin. The interaction between plant density and fertilization treatments was significant in the first season only. The tallest plants were due to the higher densities (24 or 18 plants/m^2) and NPK, while the heaviest dry weight was due to the lower densities (16 or 12 plants /m^2) and NPK.

Yield and Yield Components

Tables (1and 2) show clearly that each of weight of capsules/plant, weight of seeds/capsule and weight of seeds /plant were augmented parallel to the gradual reduction in plant density as the highest values were obtained, in the two seasons, due to the lowest density (12 plants/m^2). On the other hand, the highest seed yield/fed. was given due to the use of the highest density (24 plants/m^2). This

treatment increased the yield by 4.7,7.1 and 24.2% in the first season and 15.9,13.7 and 36.6% in the second one, respectively, in comparison with 18,16 and 12 plants/m^2) as shown in Table (2) .In agreement with these results were those of Nofal *et al.,* (2001) on *Ammi visnaga*, Badran and Hafez (2002) on *Nigella savita* and Badran *et al.,* (2007) on fennel. Data in Tables (1 and 2) show that most fertilization treatments gave significantly better results than control treatment. The highest values for the four tested treatments were given by NPK, and 1/2 NPK+biogen treatments in the two seasons. Mineral NPK treatment, overgrew the 1/2 NPK+biogen treatment, for the yield/fed. by only 14.9% in the first season and 14.8% in the second one, (Table 2). The role of NPK in promoting the yield and yield components was revealed by Salah-Eldeen (2005) and Badran *et al.,* (2007) on fennel, while that of N-fixing bacteria was reported by Nofal *et al.,* (2001) on *Ammi visnaga*, Chaudhary *et al.,* (2002) on coriander and Badran *et al.,* (2007) on fennel. The interaction between plant density and fertilization treatments was significant for weight of capsules/plant and weight of seeds/plant and fed. in both seasons The best combined treatments were 12 plant/m^2 x NPK or 1/2 NPK + biogen for the yield/plant, while it was 24 plant/ m^2 x NPK or 1/2 NPK+ biogen for the yield/fed. as clearly shown in Tables (1 and 2).

Table 1. Effect of planting density and NPK / bioifertilization treatments on *Nigella sativa*, L. plants during 2002/ 2003 and 2003/ 2004 seasons.

	Planting density treatments (plant / hill x distance (cm)									
	3x20	3x30	2x20	2x30	Mean B	3x20	3x30	2x20	2x30	Mean B
	First season					Second season				
	Plant height cm									
Control	71.8	71.4	68.6	66.1	69.5	76.1	74.8	73.1	70.1	73.5
NPK	90.6	89.6	78.8	77.2	84.1	92.5	90.7	86.8	85.3	88.8
Biogen	78.4	78.6	71.9	70.5	74.9	79.0	77.9	76.3	73.4	76.7
1/2 NPK + biog.	83.4	83.6	75.6	74.3	79.2	84.7	84.3	84.2	82.1	83.8
1/4 NPK + biog.	80.7	80.9	73.1	73.4	77.0	81.7	81.5	79.6	75.7	79.6
Mean A	81.0	80.8	73.6	72.3		82.8	81.8	80.0	77.2	
L.S.D. 5 %	A:3.3	B:2.8	AB	5.6		A:4.0	B:3.0	AB	N.S.	
	Herb dry weight g									
Control	12.3	12.7	13.2	13.6	13.0	13.5	14.2	14.8	15.4	14.5
NPK	17.3	18.2	19.0	20.2	18.7	17.4	18.5	19.5	21.1	19.1
Biogen	12.8	13.6	14.0	14.6	13.8	14.1	14.6	15.2	16.0	15.0
1/2 NPK + biog.	15.7	16.2	16.9	18.0	16.7	16.8	17.7	19.2	19.8	18.4
1/4 NPK + biog.	15.0	15.5	15.9	16.7	15.7	15.9	16.9	18.4	19.4	17.7
Mean A	14.6	15.0	15.8	16.6		15.5	16.4	17.4	18.3	
L.S.D. 5 %	A:0.8	B:0.9	AB	1.8		A:1.2	B:0.8	AB	N.S.	
	Weight of capsules / plant									
Control	10.6	10.3	14.3	15.0	12.6	15.1	15.7	16.4	17.1	16.1
NPK	15.8	16.1	24.0	26.9	20.7	25.9	26.6	28.3	29.9	27.7
Biogen	11.3	11.2	16.4	19.9	14.7	18.1	18.7	19.9	21.1	19.5
1/2 NPK + biog.	13.2	13.1	22.1	24.2	18.2	21.3	22.2	24.5	28.1	24.0
1/4 NPK + biog.	12.4	12.7	19.8	22.5	16.9	19.9	20.3	22.3	25.4	22.0
Mean A	12.7	12.7	19.3	21.6		20.1	20.7	22.3	24.3	
L.S.D. 5 %	A:1.3	B:1.2	AB	2.4		A:1.3	B:1.3	AB	2.6	
	Weight of seeds/ capsule mg									
Control	162	180	179	206	182	140	156	172	190	165
NPK	230	244	256	276	252	233	252	271	284	260
Biogen	182	191	196	218	197	175	202	216	236	207
1/2 NPK + biog.	201	226	247	257	233	218	228	257	272	244
1/4 NPK + biog.	195	203	224	228	213	182	221	233	256	223
Mean A	194	209	220	237		190	214	230	248	
L.S.D. 5 %	A:17	B:19	AB	N.S.		A:14	B:11	AB	N.S.	

3x20 = 24 plants /m^2 – 3x30 = 18 plants /m^2

2x20 = 16 plants /m^2 – 2x30 = 12plants /m^2

Table 2. Effect of planting density and NPK / bioifertilization treatments on Nigella sativa, L. plants during 2002/ 2003 and 2003/ 2004 seasons.

	Planting density treatments (plant / hill x distance (cm)									
	3x20	3x30	2x20	2x30	Mean B	3x20	3x30	2x20	2x30	Mean B
	First season					Second season				
	Weight of seeds / plant									
Control	5.5	7.6	7.9	9.3	7.6	5.9	6.9	8.1	9.2	7.5
NPK	10.7	12.4	14.2	16.6	13.5	12.7	15.1	15.6	17.3	15.2
Biogen	6.7	8.5	8.5	10.4	8.5	8.0	9.2	10.5	11.5	9.8
1/2 NPK + biog.	8.7	11.3	12.7	14.4	11.8	10.9	12.1	14.4	16.1	13.4
1/4 NPK + biog.	7.3	9.5	11.3	11.9	10.0	9.1	10.3	12.9	14.2	11.6
Mean A	7.8	9.9	10.9	12.5		9.3	10.7	12.3	13.7	
L.S.D. 5 %	A:1.1	B:1.3	AB	2.6		A:1.3	B:0.9	AB	1.8	
	Weight of seeds / fed.									
Control	394	412	379	335	380	427	317	319	331	380
NPK	768	668	680	596	678	912	817	749	624	777
Biogen	485	457	407	375	431	579	499	506	413	499
1/2 NPK + biog.	626	608	608	519	590	785	653	693	578	677
1/4 NPK + biog.	528	527	542	430	507	658	558	618	512	587
Mean A	560	535	523	451		672	580	591	492	
L.S.D. 5 %	A:23	B:27	AB	54		A:36	B:21	AB	42	
	Total chlorophylls a + b mg/ g F.W.									
Control	2.48	2.58	2.63	2.70	2.60	2.52	2.62	2.68	2.77	2.65
NPK	2.96	3.07	3.15	3.22	3.10	3.04	3.12	3.19	3.24	3.15
Biogen	2.53	2.60	2.70	2.77	2.65	2.74	2.83	2.91	2.87	2.84
1/2 NPK + biog.	2.75	2.84	2.94	3.02	2.89	2.88	2.99	3.07	3.16	3.03
1/4 NPK + biog.	2.64	2.73	2.84	2.88	2.77	2.79	2.91	2.99	3.07	2.94
Mean A	2.68	2.76	2.85	2.92		2.80	2.90	2.97	3.02	
L.S.D. 5 %	A:10	B:10	AB	N.S.		A:0.7	B:0.13	AB	N.S.	
	Essential oil yield / plant cm									
Control	.039	.058	.062	.075	.059	.041	.050	.060	.070	.055
NPK	.090	.108	.126	.151	.119	.103	.126	.133	.152	.129
Biogen	.051	.067	.069	.087	.069	.057	.070	.082	.090	.075
1/2 NPK + biog.	.072	.095	.108	.127	.101	.086	.097	.117	.135	.109
1/4 NPK + biog.	.058	.080	.094	.102	.084	.069	.081	.103	.116	.092
Mean A	.062	.082	.092	.108		.071	.085	.099	.113	
L.S.D. 5 %	A:.005	B:.005	AB	.010		A:.007	B:.005	AB	.010	

3x20 = 24 plants /m^2 – 3x30 = 18 plants /m^2

2x20 = 16 plants /m^2 – 2x30 = 12plants /m^2

Volatile Oil per plant and per Fed

Volatile oil yield /plant was gradually increased according to the gradual reduction in plant density, with the highest values being obtained, in the two seasons, from the lowest density (12 plants/m^2). However, volatile oil yield/fed. behaved differently, as the highest yield was obtained from the highest density (24 plants/m^2) in both seasons as indicated in Tables (2 and 3). Such results were similar to those reported by Kandeel *et al.,* (2001), Badran and Hafez (2002) and Badran *et al.,* (2003) on fennel, *Nigella sativa* and anise, respectively. All four tested fertilization treatments caused significant increases of volatile oil yield per plant and per fed. in both seasons over those of control treatment. The highest overall yield was due to NPK followed by 1/2 NPK + biogen. Such two treatments increased volatile oil yield / fed. by 104.1 and 72.3% in the first season and 137.0 and 98.6% in the second one in comparison with control treatment. The role of NPK fertilization in augmenting volatile oil was emphasized by Salah- Eldeen (2005) and Badran *et al.,* (2007) on fennel. Meanwhile, that of N-fixing bacteria was revealed by Kandeel *et al.,* (2001) and Badran and Safwat (2004) on fennel and Safwat and Badran (2002) and Badran *et al.,* (2007) on cumin. The interaction between plant density and NPK/ biogen treatments was significant in both seasons. The highest volatile oil yield / plant was due to the lowest density x NPK, while that per feddan was due to the highest density x NPK treatment, Tables (2 and 3).

Chemical Composition

The lowest two density treatments, 12 followed by 16 plants / m2, gave the highest total chlorophylls content, as well as, nitrogen, phosphorus and potassium herb uptake in the two seasons as shown in Table (3). These results are in agreement with those of Badran and Hafez (2002) on *Nigella sativa* and Badran *et al.,* (2007) on fennel concerning chlorophylls, and those of Nofal *et al.,* (2001) on *Ammi visnaga* and Badran *et al.,* (2003) on fennel in regard to N, P and K uptake. Concerning fertilization treatments, NPK followed by 1/2 NPK+ biogen gave the highest values, in both seasons of chlorophylls and N,P and K uptake . In accordance with these findings were those of Badran *et al.,* (2003) on anise and Salah-Eldeen (2005) and Badran *et al.,* (2007) on fennel for NPK fertilizers. While, Kandeel *et al.,* (2001) and Salah Eldeen (2005) on fennel, Chandhary *et al.,* (2002) on coriander and Badran *et al.,* (2003) on anise came to similar results concerning N-fixing bacteria. The interaction between the two studies factors was significant only for herb N and P uptake in both seasons, with the best results being obtained due to the lowest density (12 plant/ m2) in combination with NPK fertilization treatment as shown in Table (3).

Table 3. Effect of planting density and NPK / bioifertilization treatments on *Nigella sativa*, L. plants during 2002/ 2003 and 2003/ 2004 seasons.

	Planting density treatments (plant / hill x distance (cm)									
	3x20	3x30	2x20	2x30	Mean B	3x20	3x30	2x20	2x30	Mean B
	First season					Second season				
	Essential oil yield/ fed. liter									
Control	2.83	3.15	2.98	2.70	2.92	2.93	2.70	2.88	2.53	2.76
NPK	6.48	5.85	6.06	5.44	5.96	7.44	6.84	6.40	5.46	6.54
Biogen	3.67	3.62	3.33	3.12	3.44	4.13	3.80	3.32	3.23	3.62
1/2 NPK + biog.	5.18	5.15	5.20	4.58	5.03	6.17	5.26	5.63	4.87	5.48
1/4 NPK + biog.	4.18	4.34	4.53	3.66	4.16	4.94	4.37	4.93	4.19	4.61
Mean A	4.47	4.42	4.42	3.90		5.12	4.59	4.63	4.06	
L.S.D. 5 %	A:.19	B:.22	AB	.43		A:.31	B:.23	AB	.46	
	Herb nitrogen uptake mg									
Control	149	156	160	169	159	169	178	185	194	182
NPK	261	282	305	330	295	265	287	317	353	306
Biogen	161	172	179	193	176	177	188	198	214	194
1/2 NPK + biog.	204	231	252	280	242	230	258	290	312	273
1/4 NPK + biog.	193	215	227	250	221	208	237	267	295	252
Mean A	194	211	225	244		210	230	251	274	
L.S.D. 5 %	A:13	B:17	AB	34		A:17	B:15	AB	31	
	Herb phosphorus uptake mg									
Control	23.8	24.5	24.8	26.2	24.8	26.7	28.6	28.4	30.2	28.5
NPK	64.6	71.7	76.6	83.2	74.0	66.0	73.8	78.8	88.4	76.8
Biogen	25.1	28.0	29.9	31.7	28.7	28.2	30.9	33.2	36.9	32.8
1/2 NPK + biog.	49.6	52.1	56.5	64.9	55.8	53.2	58.8	64.7	73.1	62.5
1/4 NPK + biog.	44.0	47.8	49.2	60.7	50.4	44.6	53.5	58.8	68.3	56.3
Mean A	41.4	44.8	47.4	53.3		43.8	49.1	52.8	59.4	
L.S.D. 5 %	A:4.8	B:5.5	AB	11.2		A:9.0	B:7.6	AB	15.2	
	Herb potassium uptake mg									
Control	234	246	259	270	252	262	277	293	310	286
NPK	400	424	448	480	438	406	433	463	509	453
Biogen	245	264	279	299	272	274	287	307	333	300
1/2 NPK + biog.	330	348	370	401	362	356	386	421	444	402
1/4 NPK + biog.	312	330	343	367	338	336	362	401	431	383
Mean A	304	323	340	364		327	349	377	405	
L.S.D. 5 %	A:25	B:39	AB	N.S.		A:32	B:22	AB	N.S.	

3x20 = 24 plants /m^2 – 3x30 = 18 plants /m^2

2x20 = 16 plants /m^2 – 2x30 = 12plants /m^2

Decreasing plant density supplies the plants with more room for root system, growth and adequate nutrients, water and light, thereby produces thicker stems with more branch number capable of holding more capsules with higher seed and oil yield /

plant. On unit area basis, however, seed and oil yield should be increased by increasing plant density and this trend was insured in the present study. Concerning N,P and K fertilizers, their fundamental involvement in very large number of enzymatic reactions were the cause for enhancing and augmenting vegetative growth, yield components and finally seed and oil yield production of *Nigella sativa* plants. In regard with the N-fixing bacteria product, biogen, it was found to have not only the ability to fix nitrogen but also to release certain phytohormones of gibberellins and indolic nature, which could stimulate plant growth, absorption of nutrients and photosynthesis process (Hegde *et al,* 1999). The substitute of considerable amounts of NPK mineral fertilizers by biogen product gave, in the present experiment reasonable results in growth and seed and oil production which could be an accepted solution, from the environmental and economical point of view, in growing *Nigella sativa* plants.

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استجابة نباتات حبة البركة لمعاملات الكثافة النباتية والتسميد المعدنى / الحيوى

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تم اجراء هذه التجربة لمعرفة تاثير أربعة معاملات كثافة نباتية وخمسة معاملات تسميد معدنى وحيوى (بيوجين) على النمو والمحصول والزيت الطيار والتركيب الكيماوى لنباتات حبة البركة . ولقد أظهرت النتائج أن زيادة الكثافة النباتية (٣ نباتات فى الجورة على مسافة ٢٠ أو ٣٠ سم) قد سببت نقصا فى الوزن الحاف للعشب ووزن الكبسولات والبذور للنبات ووزن البذور للكبسولة ، فى حين تسببت هذه المعاملة فى زيادة محصول البذور للفدان . كذلك فقد تسببت معاملات الكثافة النباتية العالية فى نقص محتوى الكلوروفيلات ومحصول الزيت الطيار للنبات ومحتوى العشب من عناصر النتروجين والفوسفور والبوتاسيوم فى حين زاد محصول الزيت الطيار للفدان. وفيما يخص معاملات التسميد فقد اعطت معاملة التسميد المعدنى الكامل وتليها معاملة ٢/١ التسميد المعدنى + البيوجين أفضل النتائج بالنسبة للصفات الخضرية ومحصول البذور ومكوناته والزيت الطيار والتركيب الكيماوى . أما فيما يتعلق بالتفاعل بين معاملات الكثافة النباتية ومعاملات التسميد المعدنى / الحيوى فقد تم الحصول على أعلى محصول من البذور والزيت الطيار للفدان نتيجة زراعة حبة البركة بمعدل ٣ نباتات فى الجولة على مسافة ٢٠ سم بين الجور مع امداد النباتات بالتسميد المعدنى الكامل أو ٢/١ التسميد المعدنى + السماد الحيوى (بيوجين).