INFLUENCE OF BIOFERTILIZERS AND CHICKEN MANURE ON GROWTH, YIELD AND SEEDS QUALITY OF (*NIGELLA SATIVA,* L.) PLANTS

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(Manuscript received 7 July 2004)

Abstract

The experiment was conducted in Baramoon Experimental Station, Dakahlya Governorate, Egypt, during two successive seasons of 2001/2002 and 2002/2003. The aim of this study is to Investigate the response of black cumin plants to nitrogen fixing bacteria plus phosphate dissolving bacterial fertilizers in multi application combinations and chicken manure at rates of 12, 18 and 24 m^3/fed on growth, seeds yield and seeds quality. Moreover, investigating the effect of these biofertilizers combinations as a biological technique for reducing the dose of mineral fertilizer.

Seeds before sowing were treated with different strains of nitrogen fixing bacteria *"Azotobacter sp., Azospirilum sp.* and *Pseudomonas sp."* under the commercial name Biogene, Nitrobien and Microbien and phosphate dissolving bacteria *"Bacillus megaterium"* under the commercial name of Phosphorein.

Generally, results showed that biofertilization improved plant growth characters expressed as plant height, number of fruits, number of branches, seed yield/plant, seed yield/plot, fixed and volatile oil yield in the two seasons. The best results were obtained by inoculation with the mixture of Biogene, Nitrobien, Microbien plus Phosphorein combined with chicken manure at rate 24 m^3/fed. Data also revealed, that biofertilizer is a biological technique for reducing the dose of mineral fertilizer.

Treatment of chicken manure at 24 m^3/fed interacted with Phosphorein + Biogene + Nitrobein and Microbein resulted the most increment of all components percentage of fixed oil recorded by GLC chromatograms especially Linoleic acid, oleic acid and Palmetic acid, respectively. On same manner, this treatment proved same results on components of volatile oil especially Thymoquenone acid, β-Cymene acid and Geraniol acid, respectively.

INTRODUCTION

Black cumin (*Nigella sativa,* L.) is an annual herbaceous plant. Seeds contain volatile oil, fixed oil and saponin to which a toxic effect. The seeds are used medicinally to treat cough and bronchitis or as diuretic and carminative (Schouenberg

and Paris, 1977). Also, the seeds are used as flavoring agent for bakeries or as a spice instead of the black pepper, (Kybal, 1980).

Nevertheless, plant responses are sometimes evident, and thus explainations must be sought elsewhere than in N_2 metabolism. Among the more plausible alternative hypotheses are that species of Azotobacter produce compounds determental to pathogens or that act as plant growth regulators, and indeed azotobacters do synthesize stimulatory compounds such as gibberellins, cytokinins and indole acetic acid (Zumft and Mortenson, 1975).

Nowadays, soil alkalinity and pollution are considered as the most important problems in Egypt. Moreover, using of mineral fertilizers in agricultural production have resulted in serious problems in the soil and contaminate the underground water. It also accumulated in food chain causing hazardous effects. Many solutions were done to reduce the previously mentioned problems, out of them using biofertilization.

Numerous investigators stated out that biofertilization using different strains of bacteria induce stimulative effect on plant growth and productions by fixing atmospheric nitrogen (Saber, 1996). Certain bacteria can stimulate root growth considerably due to improving the mineral and water uptake (El Mandoh and Abdel-Magid, 1996). Many of these bacteria are diazotrophic bacteria e.g., *Azotobacter, Azospirillum,* or *Pseudomonas sp.* These beneficial bacteria are often classified as plant growth-stimulating bacteria, which induce their effect by various means (Jagnow *et al.,* 1991). One of their major effects is enhancing root growth by producing phytohormones like IAA by *Azospirillum brasilense* (Martin *et al.,* 1989), cytokinin by *Azotobacter* (Nieto and Frankenberger, 1990).

Awad (1998) reported that biofertilizer either with *Azospirillum sp.* or *Pseudomonas sp.* strains induced significant increases on both fresh and dry weight of tomato plants. On the other hand, Hewedy (1999) found that inoculation of tomato plant with either of *Azospirillum, Azotobacter, Pseudomonas* and *Bacillus megaterium* on single or multi application did not affect vitamin C contents in the fruit.

Phosporus is found in soil, plants and microorganisms in a number of organic and inorganic compounds. Thus, phosphorus occupies a critical position both in plant growth and in the biology of soil. The major microbiological means by which insoluble phosphorus compounds are mobilized is by the production of organic acids. The organic or inorganic acids convert $Ca_3(PO_4)_2$ to di and monobasic phosphates with the net result of an enhanced availability of element to plants, Cosgrove (1967).

Phosphate dissolving bacteria play a fundamental role in correcting the solubility problem in soil by transforming the insoluble phosphate to soluble forms by secreting organic acids such as formic, acetic, lactic, propionic, fumaric and succinic acids. Those acids decreased the pH and bring the dissolution of bond forms of phosphate and render them available for growing plants (Ashour, 1998). *Bacillus megaterium* was the important group in the solubilization process of insoluble phosphorus in soils (EI-Katkat, 1992).

The prepared biofertilizers or multiplication of various bacterial fertilizers have become recently new method which having a define role in plant growth and transformation nutrients (N, P and K) on plants. Gornaa (1989) found that the combination of biofertilizers led to increases in plant growth, N, P contents in the leaves, fruit yield and total dry weight of tomato plants than non-biofertilized control. He also added that inoculation tomato plants with mixture of nitrogen fixing bacteria and phosphate dissolving bacteria increased N and P contents in the leaves over inoculation with the nitrogen-fixing bacteria alone. Moreover, several investigators indicated that multi application of bacteria such as *Azospirillum, Pseudomonas, Azotobacter* and phosphate dissolving bacteria applications showed a significant efficiency in stimulating plant growth and uptake as well as tomato fruit yield with best quality than in case of single application (Terry *et al.,* 1995 and Hewedy, 1999).

Gomma (1989) found that combinations of biofertilizer contain *Azospirillum sp., Azotobacter sp.* and phosphate dissolving bacteria increased plant growth, N and P contents in the leaves and fruit yield than uninoculated and received 100% N, P, K recommendation only. Hewedy (1999) found that inoculation tomato plants with either *Bacillus megatrium, Azotobacter, Azospirillum* or *Pseudomonas* or their mixture in the presence of 75 % from NPK mineral fertilizer recommendation, significantly increased plant growth, N and P contents of tomato leaves and produced higher fruit yield with higher quality comparing with the uninoculated plants in the presence of 100 % NPK fertilizer recommendation.

The application of organic manures increased the amount of the total nitrogen in the soil and observed that the applied chicken manure significantly decreased the pH. Also, low pH value was noticed when chicken manure compost was added to the soil and the soil chemical properties were also improved (Abo El-Fadl *et al.*, 1990) on roselle plants.

The aim of this study is to investigate the response of black cumin plants to sources of organic manure, nitrogen fixing bacterial plus phosphate dissolving bacterial fertilizers in multi application combinations on growth, seed yield and volatile oil production. Moreover, investigating the effect of these biofertilizers combinations as a biological technique for reducing the dose of mineral fertilizer, as well as to improve soil characteristics, in order to reduce the production cost, reduce the environmental pollution and to improve soil fertility.

MATERIALS AND METHODS

This study was conducted during two successive seasons of 2001/2002 and 2002/2003 at the Experimental Farm of the Baramoon Experimental station, Egypt. The seeds of *Nigella sativa*, L. were obtained from Horticultural Research Station in Sids. The seeds were sown in October 15th in the two successive seasons in holes at space of 25 cm between two holes in plots of 3 x 2 m^2 (containing 5 rows). Thinning to 1 plant per hole was done 30 days after sowing. Seeds were inoculated before sowing with Biogene, Nitrobein, Mycrobein and Phosphorein. Chicken manure of levels 12, 18 and 24 m^3/fed were added when soil preparation before sowing. All the plants received normal agricultural practices.

Physical and chemical analysis of the experiment soil using standard method described by Jackson, (1967) are shown in Table 1.

Table 1. The physical and chemical analysis of the experiment soil (2001 season).

Sand %	Silt %	Clay %	$CaCO_3$ %	pH	Available nutrients (ppm)		
					N	P	K
24.8	31.1	43.4	3.4	7.5	14.3	11.6	32.8

Table 2 showed the physical and chemical analysis of chicken manure before soil treated in two seasons.

Table 2. The chemical analysis of the chicken manure (2001 and 2002 seasons).

Content	Chicken manure	
	First season 2001	Second season 2002
pH	6.9	6.72
EC, dS/m	1.38	1.24
Organic matter %	34.56	33.37
Available N %	1.68	1.78
Available P ppm	15.36	13.19
Exchangeable K^+	92.9	91.89

Treatments

Treatments were as follows:

1- Control (untreated plants).

2- Inoculation with *Azotobacter sp.* "nitrogen fixing bacteria" under the commercial name of "Biogene".

3- Inoculation with *Azospirillum sp.* "nitrogen fixing bacteria" under the commercial name of "Nitrobein".

4- Inoculation with *Pseudmonas sp.* "nitrogen fixing bacteria" under the commercial name of "Microbein".

5- Inoculation with *Bacillus megaterium* "phosphate dissolving bacteria" under the commercial name of "Phosphorein".

6- Inoculation with mixture of phosphorein + biogene.

7- Inoculation with mixture of phosphorein + nitrobein.

8- Inoculation with mixture of phosphorein + microbein.

9- Inoculation with mixture of phosphorein + biogene + nitrobein + microbein.

10- Plants received chicken manure at a rate of 12 m^3/fed.

11- Plants received chicken manure at a rate of 18 m^3/fed.

12- Plants received chicken manure at a rate of 24 m^3/fed.

13- Plants received combinations of phosphorein, biogene, nitrobein, microbein and chicken manure at a rate of 24 m^3/fed.

Each of biofertilizers was supplied at 7 kg/fed mixed with wet soil which contain sowing seeds (1: 10 ratio) into the root absorption zone of the plant.

The experiment was designed as complete randomize block design with three replicates, each replicate contain 13 treatments and were randomly distributed.

At harvesting (15th April), plant height (cm), branches No. per plant, seed yield per plant (gm) and per plot were recorded. Also, volatile oil, fixed oil percentage and yield per plant and plot were determined in the seeds.

All data obtained were statistically analyzed according to the methods of Snedecor and Cochran (1980).

Volatile oil obtained from GLC chromatograms and calculated as relative percentage in the second season 2002/2003. Volatile oil percentage was determined in seeds according to Guenther (1962). Fixed oil determination was obtained by soxhelet apparatus using n-hexane as a solvent according to A. O. A. C. (1970).

Chemical analysis:

Samples of both volatile and fixed oil obtained from treated plants were subjected to analysis to determine the physiochemical properties of fixed oil according to British Pharmacopoeia (1963). Both fixed oil and volatile oils were analyzed by GLC technique and was carried out in the central laboratory of Fac. Agric., Mansoura Univ. Protein, nitrogen percentages were calculated by multiplying the nitrogen by the factor 6.25 according to Tripathi *et al.* (1971).

Total carbohydrates in dry seeds were determined according to Dubais *et al.* (1956). The chemical analysis of soil and chicken manure were done at Water & Soil Laboratory of the Agricultural Rsearch Station in Mansoura (ARC), obtained analysis is shown in Tables 1 and 2.

RESULTS AND DISCUSSION

I. Effect of biofertilizers and chicken manures on vegetative growth of *Nigella sativa*, L. plants

I.1. Plant height (cm), fruits No./plant and branches No./plant:

Data in Table 3 showed that the use of biofertilizers in the form of nitrogen fixing bacteria, phosphate dissolving bacteria and different rates of chicken manure played a significant influence on the plant height (cm), number of fruits per plant and number of branches per plant. Although, biofertilizer treatments and chicken manure treated plants markedly increased these characters as compared with the untreated plants. Moreover, the highest values was obtained by using Phosphorein + Biogene + Nitrobein + Microbein + Chicken manure at rate of 24 m^3/fed and followed by Phosphorein + Biogene + Nitrobein + Microbein when compared with the other treatments.

From the same data, it was clear that a mixed biofertilizer, i.e. Phosphorein + a source of nitrogen fixing bacteria, had more stimulative effect than using phosphorein solely. The values recorded in Table 3 under the condition of single biofertilized application varied from treatment to another, but Nitrobein was the best single application treatment during the two seasons. On the other hand, plants which received chicken manure at different rates revealed also variation increments from treatment to another, but the treatment which treated by the rate of 24 m^3/fed was the best single application treatment comparing with other doses of chicken manure and the differences were significant.

Regarding measurements, it is evident from the data in Table 3 that average plant height (cm), number of fruits per plant and number of branches per plant were significantly increased with inoculating plants with biofertilization materials and chicken manure; either single or combined application compared with untreated plants.

Inoculation with a mixture of biofertilizers and chicken manure were the superior. These results hold true in the two growing seasons. The enhancing of these biofertilizing materials may be attributed to the ability of N-fixing bacteria to supply the plants with nitrogen and to release plants promoting substances which could stimulate absorption of nutrients and efficiency of nutrient metabolism. Also, such results of many investigators may explain the role of phosphate dissolving bacteria in availability of soil immobilized phosphorus. These results are in agreement with those obtained by Saber (1996) and Awad (1998) for fixing nitrogen bacteria and Gomaa (1989) on phosphate dissolving bacteria.

Also, results concerning chicken manure were in agreement with the results of Abo-El-Fadl *et al.* (1990) on roselle plants.

Table 3. Effect of biofertilizer, chicken manure and their combinations on growth of *Nigella sativa*, L. plants during the two seasons of 2001/2002 and 2002/2003.

Treatments*	2001/2002 season			2002/2003 season		
	Plant height (cm)	Fruits no./plant	Branches no./plant	Plant height (cm)	Fruits no./plant	Branches no./plant
Control	66.3	55.3	9.5	67.8	57.8	8.9
Biogene	74.2	62.3	10.9	75.6	63.1	10.3
Nitrobein	81.6	78.7	15.6	82.7	76.1	14.7
Microbein	77.3	69.2	12.2	78.6	69.7	11.9
Phosphorein	69.8	59.4	10.1	70.9	60.7	9.9
Phos.+ Bio.	84.9	85.7	16.5	85.6	85.3	17.0
Phos.+ Nit.	87.8	87.2	17.8	87.9	87.1	17.7
Phos.+ Mic.	85.1	86.6	17.6	85.1	86.9	17.8
Phos.+ Bio.+ Nit.+ Mic.	88.9	89.2	19.2	89.8	89.7	18.9
Chicken manure 12 m^3/fed	75.3	67.7	11.7	74.6	68.6	11.2
Chicken manure 18 m^3/fed	79.6	75.8	12.8	79.7	72.9	12.2
Chicken manure 24 m^3/fed	83.2	83.6	16.4	83.4	82.8	16.2
Ch.24 m^3/fed + Phos. + Bio.+ Nit. + Mi	91.9	90.9	20.1	92.3	92.7	21.2
LSD at 5%	1.1	1.01	1.2	1.5	1.0	1.2

*Bio. = biogene, Nit.= nitrobein, Mic.= microbein, Phos.= phosphorein and Ch. = chicken manure

I.2. Seed yield per plant and per plot:

Data in Table 4 indicated that biofertilizers and chicken manure applications had a significant effect on the seed yield per plant and per plot. These parameters were increased with increasing chicken manure levels, so that 24 m^3/fed addition established the highest weight of seeds per plant and per plot during the two seasons. On the other hand, biofertilizers interactions revealed more values than using the biofertilizer solely and Phosphorein + Biogene + Nitrobein + Microbein application proved this true fact during two seasons. Phosphorein + Biogene + Nitrobein + Microbein + Chicken manure at a rate of 24 m^3/fed was the most superior at over all comparing with other all treatments. These results may be attributed to the more active combined role of both biofertilizers and chicken manure applications, than its utilization solely. All differences were significant comparing with control plants during the two seasons.

Table 4. Effect of biofertilizer, chicken manure and their combinations on seed yield per plant and plot (g) of *Nigella sativa*, L. plants during the two seasons of 2001/2002 and 2002/2003.

Treatments*	2001/2002 season		2002/2003 season	
	Seed yield (g/plant)	Seed yield (g/plot)	Seed yield (g/plant)	Seed yield (g/plot)
Control	9.43	565.8	8.97	538.2
Biogene	10.91	654.6	10.89	653.4
Nitrobein	12.61	756.6	12.97	778.2
Microbein	11.23	673.8	11.76	705.6
Phosphorein	10.02	601.2	10.71	642.6
Phos. + Bio.	13.87	832.2	14.12	847.2
Phos. + Nit.	15.81	948.6	14.92	895.2
Phos. + Mic.	14.78	886.8	14.81	888.6
Phos.+ Bio.+ Nit.+ Mic.	16.01	960.6	15.82	949.2
Chicken manure 12 m^3/fed	11.23	673.8	11.76	705.6
Chicken manure 18 m^3/fed	11.89	713.4	11.92	715.2
Chicken manure 24 m^3/fed	12.99	779.4	13.70	822.0
Ch. 24 m^3/fed + Phos. + Bio.+ Nit.+ Mic.	17.40	1044.0	16.98	1018.0
LSD at 5%	0.91	18.92	0.90	17.78

*Bio. = biogene, Nit.= nitrobein, Mic.= microbein, Phos.= phosphorein and Ch. = chicken manure

II- Effect of biofertilizers and chicken manure on the oils content of *Nigella sativa,* L. plants:

II.1. Volatile oil:

Data in Table 5 emphasized that biofertilizers and chicken manure rates applications significantly increased volatile oil yield per plant and per plot during two growing seasons. The treatment of chicken manure at rate of 24 m^3/fed with other all biofertilizers established the highest value of volatile oil ml/plant and ml/plot comparing with other rates of chicken manure and control plants during the two seasons. The combination of biofertilizers realized the best values of volatile oil per plant and per plot comparing with using the biofertilizer solely and control plants during the two seasons.

Table 5. Effect of biofertilizer, chicken manure and their combinations on volatile oil percentage, yield per plant and per plot of *Nigella sativa*, L. plants during the two seasons of 2001/2002 and 2002/2003.

Treatments*	2001/2002 season			2002/2003 season		
	Volatile oil %	Volatile oil yield (ml/plant)	Volatile oil yield (ml/plot)	Volatile oil %	Volatile oil yield (ml/plant)	Volatile oil yield (ml/plot)
Control	0.611	0.058	3.48	0.795	0.071	4.26
Biogene	0.981	0.100	6.00	0.990	0.108	6.48
Nitrobein	1.286	0.162	9.72	1.336	0.173	10.38
Microbein	1.100	0.123	7.38	1.190	0.140	8.40
Phosphorein	0.773	0.087	5.22	0.891	0.095	0.57
Phos. + Bio.	1.426	0.198	11.88	1.522	0.215	12.90
Phos. + Nit.	1.546	0.246	14.64	1.662	0.248	14.88
Phos. + Mic.	1.532	0.226	13.56	1.557	0.231	13.86
Phos. + Bio. + Nit. + Mic.	1.683	0.269	16.14	1.753	0.277	16.62
Chicken manure 12 m^3/fed	1.000	0.112	6.72	1.120	0.132	7.92
Chicken manure 18 m^3/fed	1.117	0.133	7.98	1.220	0.145	8.70
Chicken manure 24 m^3/fed	1.325	0.172	10.32	1.443	0.198	11.88
Ch. 24 m^3/fed + Phos. + Bio. + Nit. + Mic.	1.791	0.312	18.72	1.829	0.310	18.60
LSD at 5%	---	0.053	1.45	---	0.055	1.97

*Bio. = biogene, Nit.= nitrobein, Mic.= microbein, Phos.= phosphorein and Ch. = chicken manure

The highest and the superior values of the yield of volatile oil per plant and per plot were attended in the plants treated with Phosphorein + Biogene + Nitrobein + Microbein + Chicken manure at rate of 24 m^3/fed in both seasons.

The organic fertilizers and biofertilizers improve the growth and quality of product and these increments in results may be due to the effective role for the organic fertilizers which increased the amount of total nitrogen in the soil and the applied chicken manure significantly decreased the pH and the soil chemical properties were also improved (Abo El-Fadl *et al.*, 1990). On the other hand, the increments caused by nitrogen fixing bacteria and interactions produced of phytohormones and some organic acids could stimulated absorption of nutrients which promoted the production and growth generally. These results hold true in the two seasons and were found to be in accordance with Gomma (1989).

Gas liquid chromatography determination of volatile oil obtained from the seeds of *Nigella sativa*, L., was shown in Table 6. Results indicated that chemical composition of back cumin oil was as follows: α-Pinene, β-Pinene, β-Cymene, 1-8 Cineol, α-Terpineol, Linalool, Camphor, Borneol, Thymoquenone, Carvone, Citrol, Geraniol, Thymol, Egenol and Geranyl acetate.

In Thymoquenone the highest percentage obtained (49.31 %) from the treatment of Phosphorein + Biogene + Nitrobein + Microbein + Chicken manure at a rate of 24 m^3/fed followed by the treatment of Phosphorein + Biogene + Nitrobein + Microbein which achieved 47.33 %. In β-Cymene, the same previous treatments behaved the same manner. The highest percentage of Geraniol was obtained also in the treatment of Phosphorein + Biogene + Nitrobein + Microbein + Chicken manure at rate of 24 m^3/fed and it was 14.01 %.

Table 6. Effect of biofertilizer, chicken manure and their combinations on the identified constituents of volatile oil obtained from GLC at 2002/2003 season.

Treatments* Components	Control	Bio.	Nit.	Mic.	Phos.	Phos.+Bio	Phos.+ Nit.	Phos.+ Mic.	Phos.+Bio. +Nit.+Mic.	Ch. 12 m^3/fed	Ch. 18 m^3/fed	Ch. 24 m^3/fed	Ch. 24 m^3/fed+ Phos.+Bio.+Nit. +Mic.
α-Pinene	2.20	2.45	3.00	2.79	2.31	3.80	3.32	3.18	3.71	2.71	2.90	3.60	3.85
β-Pinene	1.10	1.48	1.75	1.70	1.33	1.97	2.20	2.00	2.25	1.65	1.71	1.87	2.40
β-Cymene	12.66	14.63	17.92	15.84	14.11	18.65	19.65	19.10	19.92	15.01	17.31	18.21	20.11
1-8 Cineol	1.00	1.30	1.72	1.62	1.20	1.89	2.15	1.98	2.20	1.41	1.68	1.82	2.32
α-Terpineol	1.28	1.72	2.30	2.70	1.53	2.59	2.70	2.65	2.75	2.60	2.10	2.40	2.91
Linalool	2.25	2.65	3.10	2.98	2.45	3.15	3.35	3.24	3.75	2.86	3.00	3.12	3.91
Camphor	1.38	1.54	2.33	2.10	1.66	2.71	3.00	2.95	3.20	2.00	2.15	2.41	3.25
Borneol	1.14	1.60	1.87	1.73	1.40	2.00	2.23	2.10	2.28	1.70	1.79	1.90	2.45
Thymoquenone	34.67	38.97	40.55	39.52	37.23	43.27	46.22	44.56	47.33	39.01	39.71	41.11	49.31
Carvone	2.83	3.22	4.89	4.20	2.99	5.63	6.27	5.91	6.73	3.65	4.61	5.11	6.90
Citrol	2.76	3.11	4.78	4.10	2.89	5.52	6.15	5.80	6.71	3.46	4.52	5.03	6.73
Geraniol	10.21	11.54	12.73	12.01	10.97	13.00	13.87	13.25	13.98	11.81	12.43	12.91	14.01
Thymol	1.15	1.68	1.98	1.92	1.48	2.10	2.25	2.14	2.30	1.90	1.96	2.00	2.51
Egenol	1.20	1.70	2.28	1.98	1.50	2.50	2.68	2.60	2.70	1.97	2.00	2.38	2.89
Geranyl acetate	2.65	3.10	4.63	4.09	2.73	5.41	5.98	5.73	6.62	3.31	4.41	5.00	6.71

*Bio. = biogene, Nit.= nitrobein, Mic.= microbein, Phos.= phosphorein and Ch. = chicken manure

II.2. Fixed oil:

Data presented in Table (7) revealed that fixed oil percentage significantly increased by the application of both biofertilizers and chicken manure at different treatments in comparison with control, in the same way, fixed oil yield per plant and per plot showed the same behavior of volatile oil i.e. fixed oil yield ml/plant and ml/plot were increased with increasing rates of chicken manure and the highest value was obtained by 24 m^3/fed and the most superior value was obtained at the combination of biofertilizers and chicken manure especially the treatment of Phosphorein + Biogene + Nitrobein + Microbein + Chicken manure at rate of 24 m^3/fed followed by Phosphorein + Biogene + Nitrobein + Microbein per plant and per plot during two seasons. These results are in harmony with those of Mehrotra and Lehri (1971).

Table 7. Effect of biofertilizer, chicken manure and their combinations on fixed oil percentage, yield per plant and per plot of *Nigella sativa*, L. plants during the two seasons of 2001/2002 and 2002/2003.

Treatments	2001/2002 season			2002/2003 season		
	Fixed oil %	Fixed oil yield (g/plant)	Fixed oil yield (g/plot)	Fixed oil %	Fixed oil yield (g/plant)	Fixed oil yield (g/plot)
Control	25.86	2.44	146.4	27.75	2.49	149.4
Biogene	33.22	3.62	217.2	34.34	3.74	224.4
Nitrobein	38.49	4.85	291.0	38.55	5.00	300.0
Microbein	36.21	4.07	244.2	35.72	4.20	252.0
Phosphorein	30.71	3.08	184.8	31.72	3.40	204.0
Phos.+Bio.	41.98	5.82	349.2	41.87	5.91	354.6
Phos.+ Nit.	42.37	6.99	419.4	42.31	6.58	394.8
Phos.+ Mic.	42.15	6.23	373.8	42.83	6.34	380.4
Phos.+ Bio. + Nit. + Mic.	42.83	7.13	427.8	42.87	6.94	416.4
Chicken manure 12 m^3/fed	35.42	3.98	238.8	34.51	3.89	233.4
Chicken manure 18 m^3/fed	36.71	4.36	261.6	35.82	4.27	256.2
Chicken manure 24 m^3/fed	40.21	5.22	313.2	39.87	5.46	327.6
Ch. 24 m^3/fed + Phos. + Bio. + Nit. + Mic.	43.19	7.94	476.4	43.98	7.68	460.8
LSD at 5%	---	0.81	11.31	---	0.72	10.61

Bio. = biogene, Nit.= nitrobein, Mic.= microbein, Phos.= phosphorein and Ch. = chicken manure

The gas liquid chromatography determination of fixed oil obtained from the dry seeds of black cumin was shown in Table 8. The results revealed the presence of the following fatty acids: caproic acid, carpylic acid, capric acid, lauric acid, myristic acid, pentadecylic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linoleoic acid, cis II Elcosenoic and erucic acid. The high percentage from the fatty acids obtained were linoleic acid, oleic acid and palmetic acid, respectively.

Table 8. Effect of biofertilizer, chicken manure and their combinations on the identified constituents of fixed oil obtained from GLC chromatograms and calculated as relative percentage in the second season 2002/2003.

Treatments* Components	Control	Bio.	Nit.	Mic.	Phos.	Phos. + Bio	Phos.+ Nit.	Phos. + Mic.	Phos. + Bio. + Nit. + Mic	Ch. 12 m^3/fed	Ch. 18 m^3/fed	Ch. 24 m^3/fed	Ch. 24 m^3/fed + Phos.+ Bio. + Nit + Mic.
Caproic acid	---	---	---	---	---	0.10	0.10	0.10	0.10	0.10	---	---	0.20
Carprylic acid	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.20	0.20	0.20	0.20	0.30
Capric acid	---	0.20	0.35	---	---	0.41	0.51	0.20	0.62	---	---	---	0.81
Lauric acid	---	0.20	0.35	---	---	0.41	0.51	0.60	0.62	---	---	---	0.81
Myristic acid	0.10	0.45	0.59	0.50	0.38	0.69	0.85	0.80	0.87	0.48	0.56	0.61	0.90
Pentadecylic acid	---	---	---	---	---	---	---	---	0.10	---	---	---	0.20
Palmetic acid	10.22	10.92	12.31	11.92	10.59	13.37	13.63	13.54	13.72	11.23	12.25	12.43	14.59
Stearic acid	0.10	1.79	1.89	1.10	0.71	2.59	3.41	2.85	3.70	0.80	1.50	2.30	4.89
Oleic acid	13.10	14.22	15.31	15.21	13.91	16.21	16.98	16.23	17.86	15.61	15.28	16.11	20.61
Linoleic acid	46.36	42.22	45.32	44.91	40.31	46.97	47.32	47.15	48.41	42.81	45.30	46.72	49.98
Linolenic acid	---	---	---	---	---	---	---	---	2.9	---	---	---	0.90
Cis I I Eicosenoic	0.20	---	1.90	1.20	0.80	2.60	3.50	2.90	9.80	0.90	1.60	2.30	10.90
Erucic acid	---	---	---	---	---	0.10	---	0.60	0.71	---	---	---	0.91

*Bio. = biogene, Nit.= nitrobein, Mic.= microbein, Phos.= phosphorein and Ch. = chicken manure

Table 9. Effect of biofertilizer, chicken manure and their combinations on total carbohydrates and protein percentage in seeds of *Nigella sativa*, L. plants during the two seasons of 2001/2002 and 2002/2003.

Treatments* / Chemical properties	Control	Bio.	Nit.	Mic.	Phos.	Phos. + Bio.	Phos. + Nit.	Phos. + Mic.	Phos. + Bio.+ Nit. + Mic.	Ch. 12 m^3/fed	Ch. 18 m^3/fed	Ch. 24 m^3/fed	Ch. 24 m^3/fed + Phos. + Bio. + Nit. + Mic.
Total carbohydrates (%)	25.98	30.86	35.96	33.92	28.97	36.87	37.57	36.91	37.87	32.78	35.71	36.12	38.23
Protein (%)	20.21	22.56	23.98	23.75	21.78	24.91	25.76	24.98	25.83	22.86	23.86	24.90	25.97

*Bio. = biogene, Nit.= nitrobein, Mic.= microbein, Phos.= phosphorein and Ch. = chicken manure

Table 10. Effect of biofertilizer, chicken manure and their combinations on the physiochemical analysis of the fixed oil of *Nigella sativa*, L. in the second season 2002/2003.

Treatments* / Components	Control	Bio.	Nit.	Mic.	Phos.	Phos.+ Bio.	Phos. + Nit.	Phos. + Mic.	Phos. + Bio.+ Nit. + Mic.	Ch. 12 m^3/fed	Ch. 18 m^3/fed	Ch. 24 m^3/fed	Ch. 24 m^3.fed+ Phos. + Bio.+ Nit. + Mic.
Specific gravity	0.9210	0.9228	0.9247	0.9236	0.9220	0.9357	0.9367	0.9364	0.9491	0.9228	0.9240	0.9250	0.9670
Refractive index	1.4481	1.4494	1.4542	1.4520	1.4490	1.461	1.4690	1.4650	1.4711	1.4499	1.4591	1.4583	1.4780
Acid value	28.91	29.17	33.87	29.87	29.10	33.98	34.91	34.90	35.70	29.81	29.91	34.00	36.91
Ester number	190.2	194.6	197.5	196.7	193.4	198.8	207.9	203.6	213.1	194.9	196.9	197.9	220.9

*Bio. = biogene, Nit.= nitrobein, Mic.= microbein, Phos.= phosphorein and Ch. = chicken manure

The highest of lenoleic acid, oleic acid and palmitic acid respectively were obtained from the plants treated with combined Phosphorein + Biogene + Nitrobein + Microbein + Chicken manure at a rate of 24 m^3/fed; the values were 49.98 %, 20.61 % and 14.59 %, respectively, followed by thetreatment of Phosphorein + Biogene + Nitrobein + Microbein and values were 48.41 %, 17.86 % and 13.72 %, respectively.

It is noted rankly distinguishede to mention that the content of unsaturated fatty acids were over 70 % which reflects the medicenal value of the oil as a potential meventive agents in a number of health disorders as heart diseases and hyper chalsteraloenia. The obtained results agreed with Hewedy (1999).

The organic fertilizers and effects on black cumin plants were in accordance with those obtained by El-Nadi *et al.* (1995). They stated that chicken manure contained the principle elements needed for plant growth, it also had characteristics that makes its applications to soil has many advantages, it has a large content of nutrients and greater water holdings capacity and it is also simple and easy to handle.

Data presented in Table 9 showed that total carbohydrate contents of the *Nigella* seeds was ranged from 25.98 % to 38.23 % in the seeds. The results showed that the treatment of Phosphorein + Biogene + Nitrobein + Microbein + Chicken manure at a rate of 24 m^3/fed were the superior one comparing with control and other treatments.

Also, it was shown in Table 9 that protein percentage realized the most superior increasing with treatment Phosphorein + Biogene + Nitrobein + Microbein + Chicken manure at a rate of 24 m^3/fed. All treatments showed significant increments between different treatments and control.

3- Physiochemical analysis of fixed oil:

The results showed in Table 10 indicated the physiochemical properties of black cumin seeds of fixed oil and the treatments behaved in the same manner of protein and all treatments were agreed with those obtained by El-Nadi *et al.* (1995).

RECOMMENDATIONS

Biofertilizers and organic manure could be used to enhance the vegetative growth and superior black cumin seeds without chemical fertilization.

The application of Phosphorein + Biogene + Nitrobein + Microbein + Chicken manure at a rate of 24 m^3/fed was the most increasing at all characteristics.

Generally, the application of the two kinds of fertilization increased the main constituents in volatile and fixed oil obtained from black cumin seeds.

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تأثير الأسمدة الحيوية وسماد الدواجن على نمو وانتاج وجودة البذور فى نبات حبة البركة

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قسم بحوث النباتات الطبية والعطرية ــ معهد بحوث البساتين ــ مركز البحوث الزراعية ــ القاهرة

تمت هذه الدراسة فى مزرعة البرامون بمحافظة الدقهلية التابعة لإدارة بحوث البساتين خلال موسمى ٢٠٠١/٢٠٠٢ ، ٢٠٠٢/٢٠٠٣ واستخدم فيها أنواع مختلفة من الأسمدة العضوية وهى النتروبين والبيوجين والفوسفورين والميكروبين، كما استخدم سماد الدواجن بمعدلات إضافة ١٢ ، ١٨، ٢٤ م^{٣}/فدان وكانت النتائج التى تم الحصول عليها هى:

١ــ أدى استخدام الأسمد ة الحيوية وسماد الدواجن كلا على حده أو متفاعلة إلى زيادة معنوية فى طول نبات حبة البركة وعدد ثمار ووزن البذور للنبات وكذلك محصول الزيت الطيار والثابت للنبات والقطعة التجريبية وذلك مقارنة بالكنترول كانت أعلى قيمة عند استخدام المعاملة التى تحتوى على كل من المصادر الآتية مضافة مع بعضها البعض وهى: الفوسفورين + البيوجين + النتروبين + الميكروبين + سماد الدواجن عند مستوى ٢٤ م^{٣}/ف وكان ذلك فى موسمى التجربة.

٢ــ أعطى التحليل الكروماتجرافى للزيت الطيار لنبات حبة البركة أعلى قيمة من المركب الرئيسى (Thymoquenone) ، أما بالنسبة للزيت الثابت فكانت أعلى قيمة هى المركب الرئيسى حمض اللينوليك Lionoleic acid وقد تحققت تلك القيم الفائقة للمركبات الرئيسية عند استخدام المعاملة الفوسفورين + البيوجين + النتروبين + الميكروبين + سماد الدواجن عند مستوى ٢٤ م^{٣}/ف.

٣ــ قد تلاحظ أن المعاملات التى تحتوى على متفاعلات أعطت دائما نتائج أفضل من استعمالها منفردة.