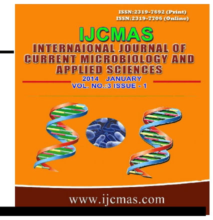

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Original Research Article

Bio-production of *Nigella sativa* L. seeds and oil in Taif area

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A B S T R A C T

Keywords

Biofertilizers; Cattle manure; Chlorophyll; Nitrate; Nitrite; Oil.

The response of black cumin plant to different treatments of cattle manure, biofetilizers and their combinations was investigated. The growth characters and seed yield components were increased as a result of applying different fertilization treatments compared to untreated control. Moreover, the volatile and fixed oil contents and their main components were also enhanced by using the previous treatments. All applied treatments increased total chlorophyll contents carbohydrate percentages in both herb and seeds and NPK percentages. Interestingly, the nitrate and nitrite contents in seeds were significantly reduced by applying different fertilization treatments. In order to reduce the production cost by minimizing or utterly eliminating the use of chemical fertilizers, decreasing environmental hazards, improve soil structure, promote leveraging agriculture and obtain high quality black cumin seeds as well as fixed and volatile oils, the treatment of cattle manure at 30 m^3/fed. combined with a mixture of NFB + PSB biofertilizer was recommended.

Introduction

Because the need of increasing the medicinal plant production all over the world, its production became an ultimate goal to meet the great increase of population to avoid chemical therapy side effects on human health through utilization of the medical herbs. However, the use of the most suitable and recommended agricultural practices in growing such crops could provide the producers with higher income, in comparison with many other traditional crops (Hassan *et al.* 2012).

Black cumin, *Nigella sativa*, L. plant belongs to *Ranunculaceae* family, common known as black cumin (is cultivated for seed yield and oil production. The whole seeds contain 30-35 % of oil which has several uses for pharmaceutical and food industries (Ustun *et al.* 1990). The black cumin seed cake is a by-product obtained from the black cumin seeds with cold pressing and it is used in the production of bio-oil (Sen and Kar 2012). Although the importance of chemical fertilizers, many constraints have been raised such as their adverse impacts on the public health, environment, increasing the production cost and deterioration of soil fertility (Boraste *et al.* 2009). It is very important to find alternative methods for supplying nutrients to the growing plant to confront the previous problems. Nowadays, various researchers consider the utilization of organic and bio-fertilizers as promising alternative nutrition especially for developing countries.

Organic fertilization provides the means for stabilizing soil fertility (especially in newly reclaimed soils). Converting nitrogen into a less soluble form is the main advantage of organic fertilizers compared to chemical fertilizers. The favorable effect of cattle manure on the vegetative growth of some medicinal plants had been reported by numerous investigators. The vegetative growth, fruit as well as oil yields were enhanced by using organic manure (Abdou and Mohamed 2003; Hassan and Ali 2013). Moreover, the photosynthetic pigments and carbohydrates percentage and N, P and K contents (Fernandes et. al. 2002; Abdou and Mohamed 2003) were also enhanced. On the other hand cattle manure application has been found to reduce nitrate and nitrite contents in seeds (Singh 2004; Zhou and Luo 2004; Hassan and Ali 2013). The oil content and its main components were also affected by cattle manure in different crops (Jhaa *et al.* 2011; Hassan and Ali 2013).

Recent awareness has been offered to reduce pollution practices in sustainable agriculture. One of the ways to minimize soil pollution is using bio-stimulants compounds without causing any harmful effects on aerial and soil environment and to retard nitrification for sufficiently longer time and increase the soil fertility (Yasin *et al.* 2012). The effective utilization of bio-fertilizers for crops not only provides economic benefits to the producers, but also improves, maintains the soil fertility and sustainability in natural soil ecosystem. The beneficial effects of plant growth promoting rhizobacteria on growth are not only through nitrogen fixed in the rhizosphere, but also related to the ability of these bacteria to synthesize antibiotics and growth-promoting substances including phytohormones and sometimes the ability to solubilize phosphates; the use of phosphate-solubilizing bacteria becomes necessary to minimize the dose of chemical P fertilizer that cause environmental pollution (Gendy *et al.* 2012; Hassan *et al.* 2012).
The mixed treatment of nitrogen fixing bacteria and phosphate solublizing bacteria resulted in the maximum increase in most of the growth and yield parameters of several medicinal and aromatic plants (Chen 2006; Helal *et al.* 2011; Akhani *et al.* 2012; Sokhangoy *et al.* 2012; Hassan *et al.* 2012; Hassan and Ali 2013).The essential oil content and its main components of various Apiaceae fruits were increased by using biofertilizers. In addition, carbohydrate percentage as well as nitrogen, phosphorus and potassium content in leaves were also promoted (Hassan *et al.* 2012; Dadkhah 2012; Hassan and Ali 2013).

The combination of organic manure and biofertilizers proved to be the superior treatment, compared to the individual application, which reflected in the greatest influence on growth, yield, oil content and chemical constituents of different medicinal and aromatic plants (Al-Fraihat *et al.* 2011; Shaalan 2005; Gendy *et al.,* 2012; Valadabadi and Farahani 2013).

Although cattle manure and biofertilizers are known to play an important role in the plant production, little is known about the interactive effects of both of them on growth and secondary metabolites accumulation in medicinal plants. To the best of our knowledge, few studies have been conducted so far to study the effect of organic manure and interactive effect of biofertilizers on black cumin plants. Moreover, improving not only the quantity but also the quality of black cumin yield was and still the main goal of several investigators. So, the aim of this study was to investigate the effect of different levels of cattle manure, some biofertilizers and their interactions on the growth, yield and oil content as well as its composition of black cumin plant to reveal the suitable treatment which maximizing seed and oil yields and obtaining the highest quality product.

Materials and Methods

Plant materials

This study was conducted at Biology Dep., Faculty of Science, Taif University during 2011/ 2012 and 2012/2013 seasons. Seeds of black cumin were sown in experimental units, each was one square meter including two rows with 50 cm apart and each row contained five hills at 20 cm distance in each side. After six weeks, the thinning was occurred leaving two seedlings/hill. The physical properties of soil used were (sand, 82.40%, silt 7.10% and clay 10.50%) and chemical properties were (pH, 8.37, EC, 2.33 dsm^{-1}, OM, 0.11%, Total $CaCO_3$, 0.98%, Total N, P, K were 0.17, 0.036 and 0.043 %, respectively). All other agriculture practices needed during black cumin growth were done when required.

Four levels of cattle manure were applied in this experiment i.e. 0, 10, 20 and 30 m^3/fed. during preparation of the experimental soil. The biofertilizers used were *Azotobacter chroococcum* (2.1×10^9 cell/cm^3) + *Azospirillum brasilense* (1.8×10^9 cell/cm^3) as nitrogen fixing bacteria, *Bacillus megatherium* var. phosphaticum (4.1×10^9 cell/cm^3) + *Bacillus polymyxa* (3.8×10^9 cell/cm^3) as phosphate solubilizing bacteria and the mixture between them as a combination treatment. Control plants were not treated with any biofertilizers. Nitrogen fixing bacteria, phosphate solubilizing bacteria are the efficient strains in suspension inoculate black cumin seeds by soaking them in the inoculum suspension for 15 minutes before planting according to El-Zeiny *et al.* (2001). After 6, 9, and 12 weeks from planting, the soil was inoculated with bacteria at side root zones of plants and irrigated immediately according to Gori and Favilli (1995). The treatments were arranged as a split plot in a complete randomized block design with four replicates. The cattle manure treatments were randomly distributed in the main plot while, biofertilizer treatments were in the sub-plots.

Data recorded

At harvesting stage, samples of twenty plants were randomly chosen from each experimental unit for determining the following characters i.e. plant height (cm), number of main branches/plant, fresh and dry weight of herb (g/plant), capsule number/plant, seed yield/plant (g) and weight of 1000 seeds (g).

Determination of oil

Volatile oil of black cumin seeds was determined using hydro distillation method according to British Pharmacopoeia (1963). Samples of 100 g were crushed (just before distillation) and were put directly in extraction units. The volatile oil percentage was measured and the oil yield /plant was calculated. The obtained volatile oil was dehydrated over anhydrous sodium sulphate and stored in refrigerator until Gas Chromatography (GC) analysis. Volatile oil samples were performed using a Varian GC CP-3800 and MS Saturn 2200 equipped with a Factor Four capillary column (VF-5 ms 30 X 0.25 mm ID and film thickness 0.25 µm). An electron ionization system with ionization energy of 70 eV was used for GC-MS detection. Helium was used as the carrier gas with a flow rate of 3 mL/min. Initial temperature 40 ^{0}C was held for 2 min, then programmed to rise from 40 to 190 ^{0}C at rate of 4^{0}C/min. The interface temperature was 190 ^{o}C, injector temperature was 190 ^{o}C, the final temperature was 175 ^{o}C for 3 min and the run time was 35.60 min. Det-gain 1.50 KV. The volatile oil components were identified by comparing their retention times and mass spectrum with those of standards, NIST library of the GC-MS system and literature data.

However, fixed oil was estimated by Soxhlet apparatus using petroleum ether (BP 40-60°C) as solvent according to the Association of Official Agricultural Chemists **(**A.O.A.C. 1980). Regarding the fatty acid composition of the fixed oil, the methyl ester of fatty acids were prepared using benzene:methanol: concentrated sulfuric acid (10: 8: 4) and methylation was carried out for one hour at 80-90°C. The methyl esters prepared from oil samples were analyzed by GC-MS equipped with dual flam ionization detector. The separation of fatty acid methyl esters was conducted with column: SP-2310, 55 % cyanopropyl phenyl silicon (1.5 x 4 mm). Column was used with a temperature program of 70-190° at 8°C /min. The injector and detector temperatures were maintained at 250 and 300°, respectively. The fixed oil components were identified by comparing their retention times and mass spectrum with those of standards, NIST library of the GC-MS system and literature data.

Chemical constituents

Chlorophyll content was determined in samples of fresh leaves according to Sadasivam and Manickam (1992). Extraction in acetone was repeated until all pigments were extracted. The absorbance of extracts was determined by a spectrophotometer (type Pharmacia, LKB-Novaspec II). The chlorophyll content was calculated as mg g^{-1} fresh weight. Total carbohydrate percentages were determined in both leaves and seeds. Samples were dried in an electric oven at 70 °C for 24 hours according to A.O.A.C. (1980). Then, the fine powder was used to determine total carbohydrate percentages according to Herbert *et al.* (1971). Nitrogen, phosphorus and potassium were determined in dried leaf samples, digested using sulphuric and perchloric acids method (Piper 1967; Black *et al.* 1965; Jackson 1978). Nitrate (NO_3) and nitrite (NO_2) concentrations in seeds were colorimetrically determined at 420 nm and 720 nm according to Jackson (1978) and Follett and Ratcliff (1963), respectively. The obtained values were assayed on fresh weight basis.

Statistical analysis

The results of two seasons were pooled and the analysis of variance (ANOVA) was performed using MSTAT program, USA. Means were separated using LSDs at a significance level of 0.05

Results and Discussion

Vegetative growth characters

Data presented in Table (1) indicated that plant height, branch number/plant as well as both fresh and dry weights of black cumin were significantly increased as a result of applying different cattle manure treatments compared to the control. The previous characters were gradually increased with increasing the cattle manure levels and the highest values in this respect were obtained by applying 30 m^3/fed treatment. All biofertilizers used in this experiment also enhanced the vegetative growth characters and this increase was significant in comparison with the control. The maximum values of growth parameters measured in this experiment were obtained by using a mixture of nitrogen fixing bacteria (NFB) and phosphate solublising bacteria (PSB) treatment. The interaction treatments between cattle manure and biofertilizers led to a significant increase in the vegetative growth characteristics compared to the control or the individual application by each of them.

Seed yield components

Application of cattle manure and biofertilizer treatments significantly increased the capsule number per plant, 1000-seeds weight and seed yield per plant compared to the control whether these treatments were separately applied or in combination (Table 1). The previous parameters were gradually increased with increasing the cattle manure level. In addition, all biofrtilizer used also increased the yield components under any level of cattle manure. The highest capsule number (27.89), the heaviest 1000-seeds weight (1.67 g) and the highest seed yield per plant (3.99 g) were recorded by the interaction treatment of cattle manure at 30 m^3/fed. and the mixture of NFB + PSB biofertilizer.

Volatile oil content

Data concerning the volatile oil percentage, as well as yield per plant and fed were presented in Table (2). The cattle manure treatments gradually increased the volatile oil percentage compared to control. In addition, biofertilizer treatments enhanced volatile oil percentage and the highest values were obtained by using a mixture of NFB + PSB treatment. The statistical analysis of results showed that the interaction between cattle manure and biofertilizer treatments were positively and significantly affected the volatile oil percentages in black cumin seeds. The same trend was recorded concerning volatile oil yield whether per plant or fed. the highest volatile oil yield obtained in our results (0.016 ml/ plant and 2.62 L/fed) were found by using the combined treatment of cattle manure at 30 m^3/fed. and the mixture of NFB + PSB biofertilizer.

Volatile oil composition

The results of GC-MS analysis of volatile oil showed that the main components found were α -pinene, γ -terpinene, limonene, caryophylene, borneol, thymoquinone, carvone, thymol and eugenol (Table 3). Thymoquinone recorded the highest percentage among the pervious components and carvone came in the second rank. The identified volatile oil components were positively affected by both cattle manure and biofertilizer treatments. An increase in the previous components was recorded as a result of applying cattle manure and biofertilizers separately or in combination. The combined treatment of cattle manure at 30 m^3/fed and the mixture of NFB + PSB biofertilizer recorded the highest quality of volatile oil.

Fixed oil content

Applying different levels of cattle manure significantly increased fixed oil percentage and its yield per plant and fed. in comparison with the control (Table 2). The best results in this concern were obtained by the highest level (30 m^3/fed). In the same line all biofertilizer used also significantly increased the fixed oil content of black cumin and the mixture of NPB + PSB treatment was superior to the individual ones. All possible interactions between cattle manure and biofertilizer treatments increased the previous parameters as well. Among all treatments applied, the interacted treatment of cattle manure at 30 m^3/fed. and the mixture of NFB + PSB biofertilizer resulted in the maximum fixed oil yield.

Fixed oil composition

Results of GC-MS analysis of fixed oil revealed that the main fatty acids presented were capric acid, lauric acid, myristic acid, palmatic acid, oleic acid, linoleic acid and linolenic acid (Table 4). The highest percentage among the pervious components was linoleic acid followed by oleic acid. The treatments of cattle manure and biofertilizers increased the previous fatty acids in the fixed oil compared to untreated control especially the highest level of cattle manure or using the mixture of NPB + PSB treatments. The highest quality of fixed oil was obtained by applying the combined treatment of cattle manure at 30 m^3/fed. and the mixture of NFB + PSB biofertilizer recorded the highest quality of volatile oil.

Chlorophyll and carbohydrates

The response of total chlorophyll content and carbohydrate percentage to various fertilizers applied in this experiment was tabulated in Table (2). The increase of cattle manure level, the increase of chlorophyll and carbohydrate was observed. Applying biofertilizer treatments enhanced the accumulation of these parameters too in comparison with control and the highest values were obtained by the mixture of NPB + PSB treatment. The total carbohydrates in seeds showed the similar trend of its in leaves however the values are higher. The interaction treatment of cattle manure at 30 m^3/fed. and the mixture of NFB + PSB biofertilizer gave the highest total chlorophyll content (1.42 mgg^{-1}) as well as total carbohydrate percentages (12.39 and 13.17 %) for leaves and seeds, respectively.

Nutrient content

An increment was occurred in nitrogen, phosphorus and potassium percentages in black cumin leaves when treated with cattle manure, biofertilizers or any combination treatment between them. The mixture treatment of NFB + PSB biofertilizer under the highest level of cattle manure resulted in the highest values in this concern. On the other hand, the nitrate and nitrite contents of seeds were significantly decreased as a result of applying different fertilizers compared to the untreated control. A gradual reduction in nitrate and nitrite contents was occurred by increasing the cattle manure level.

Table.1 Vegetative growth characters and yield components of black cumin treated by cattle manure, biofertilizers and their combination treatments

Treatments		Plant height (cm)	Branch number /plant	Fresh weight (g/plant)	Dry weight (g/plant)	Capsule number/ plant	1000-seeds Weight (g)	Seed yield (g/plant)
Cattle manure (A)	Biofertilizers (B)							
Control	Control	38.54	6.72	38.65	12.48	13.35	1.23	1.89
	NFB*	44.61	7.88	40.52	12.67	15.24	1.24	1.92
	PSB*	43.27	8.11	40.87	12.88	16.47	1.26	1.94
	NFB + PSB	46.38	8.96	42.15	13.27	18.23	1.27	2.14
10 m^3/fed	Control	40.23	7.32	39.67	12.51	15.25	1.24	2.23
	NFB	46.12	8.24	41.87	12.72	17.74	1.27	2.29
	PSB	45.17	9.45	42.16	13.36	18.84	1.28	2.37
	NFB + PSB	48.47	10.16	44.55	13.89	22.16	1.33	2.49
20 m^3/fed	Control	43.51	8.23	42.17	13.12	17.24	1.27	2.45
	NFB	48.62	8.86	45.21	14.22	19.36	1.29	2.78
	PSB	47.50	8.87	45.89	14.74	20.28	1.31	3.24
	NFB + PSB	51.18	10.92	49.26	15.36	24.81	1.36	3.67
30 m^3/fed	Control	45.29	9.14	44.67	13.89	20.28	1.29	2.88
	NFB	51.22	9.87	47.87	14.61	23.17	1.34	3.14
	PSB	50.43	10.16	48.41	15.14	24.72	1.42	3.47
	NFB + PSB	54.72	11.47	53.56	15.89	27.89	1.67	3.99
LSD 0.05 A		2.46	0.72	2.75	1.98	2.23	0.08	0.39
B		2.13	0.63	2.21	1.13	1.82	0.06	0.32
AXB		3.74	0.88	3.83	2.25	3.37	0.13	0.51

***NFB means nitrogen fixing bacteria (mixture of *Azotobacter chroococcum* and *Azospirillum brasilense*), PSB means phosphate solubilizing bacteria (mixture of *Bacillus megatherium* var. phosphaticum and *Bacillus polymyxa*).**

Moreover, the mixture treatment of NFB + PSB biofertilizer was superior to the individual application. The interaction treatment of cattle manure at 30 m^3/fed. and the mixture of NFB + PSB biofertilizer recorded the lowest values of nitrate (260.25mgL^{-1}) and nitrite (29.41mmL^{-1}) contents of black cumin seeds (Table 5).

Our results clearly indicate that all fertilization sources treatments enhanced the growth characters and increased the yield components of black cumin plants compared to the control. The promotion effect of cattle manure on vegetative growth could be explained through its effects in improving soil properties by decreasing bulk density, increasing water holding capacity, preventing nutrient leaches and providing the plant with nutrient requirements (Gichangi *et al.* 2009). The previous positive effects of cattle manure may be reflected on vegetative growth promotion as our data indicated and hence the seed yield was increased (Table 1). It has been reported that organic manure is a product contains many elements which improve the soil fertility and increase the availability of nutrient elements by holding them on mineral surfaces and, consequently, affect plant growth and yield (El-Sharkawy and Abdel-Razzak, 2010). Our results support the others obtained by Abdou and Mohamed (2003), Jhaa *et al.* (2011), Raissi *et al.* (2012) and Hassan and Ali (2013).

Table.2 Oil content, total chlorophyll and carbohydrate of black cumin treated by cattle manure, biofertilizers and their combination treatments

Treatments		Volatile oil (%)	Volatile oil yield (ml/plant)	Fixed oil (%)	Fixed oil yield (ml/plant)	Total Chloro-phyll (mgg^{-1})	Total carbo-hydrates (%)
Cattle manure (A)	Biofertilizers (B)						
Control	Control	0.24	0.005	22.31	0.422	1.13	12.37
	NFB*	0.26	0.005	22.98	0.441	1.15	12.82
	PSB*	0.27	0.005	23.17	0.449	1.17	12.76
	NFB + PSB	0.28	0.006	23.89	0.511	1.22	13.45
10 m^3/fed	Control	0.25	0.006	23.11	0.515	1.19	13.57
	NFB	0.28	0.006	23.87	0.547	1.23	13.89
	PSB	0.29	0.007	23.98	0.568	1.25	14.12
	NFB + PSB	0.31	0.008	24.10	0.600	1.29	14.74
20 m^3/fed	Control	0.26	0.006	24.12	0.591	1.21	14.86
	NFB	0.29	0.008	24.78	0.689	1.27	15.64
	PSB	0.32	0.010	24.89	0.806	1.32	15.82
	NFB + PSB	0.34	0.012	25.12	0.922	1.35	16.76
30 m^3/fed	Control	0.30	0.009	24.88	0.717	1.28	15.32
	NFB	0.34	0.011	25.76	0.809	1.34	16.87
	PSB	0.37	0.013	26.25	0.911	1.36	17.21
	NFB + PSB	0.41	0.016	27.74	0.107	1.42	19.39
LSD 0.05	A	0.02	0.003	0.76	0.112	0.053	1.33
	B	0.01	0.002	0.61	0.083	0.037	1.11
	AXB	0.04	0.005	0.98	0.159	0.064	1.48

*NFB means nitrogen fixing bacteria (mixture of *Azotobacter chroococcum* and *Azospirillum brasilense)*, PSB means phosphate solubilizing bacteria (mixture of *Bacillus megatherium* var. phosphaticum and *Bacillus polymyxa*).

Table.3 The main components of black cumin volatile oil treated by cattle manure, biofertilizers and their combination treatments

Treatments		α -Pinene	γ -terpinene	Limonene	Caryophylene	Borneol	Thymoquinone	Carvone	Thymol	Eugenol
Cattle manure (A)	Biofertilizers (B)									
Control	Control	1.46	3.87	6.52	2.37	6.82	22.67	13.47	3.54	6.37
	NFB*	1.49	3.92	6.68	2.42	6.91	23.15	13.63	3.67	6.54
	PSB*	1.48	3.91	6.65	2.44	6.94	23.47	13.81	3.71	6.63
	NFB + PSB	1.51	4.22	6.96	2.56	6.98	24.19	14.15	3.79	6.72
10 m^3/fed	Control	1.37	3.74	6.82	2.36	6.81	23.71	13.49	3.71	6.42
	NFB	1.39	4.13	7.11	2.46	7.12	25.64	13.78	3.82	6.58
	PSB	1.36	4.21	7.34	2.44	7.31	26.17	14.11	3.91	6.62
	NFB + PSB	1.42	4.37	7.85	2.67	7.39	27.38	14.24	4.13	6.89
20 m^3/fed	Control	1.44	3.85	6.81	2.39	6.98	23.88	14.10	3.85	6.45
	NFB	1.46	4.67	7.13	2.67	7.24	26.94	14.47	3.96	6.76
	PSB	1.45	4.92	7.27	2.68	7.62	27.43	14.89	3.99	6.77
	NFB + PSB	1.52	5.11	8.56	2.92	7.89	28.82	15.12	4.24	6.95
30 m^3/fed	Control	1.44	3.98	7.92	2.51	7.17	24.17	14.23	4.11	6.52
	NFB	1.39	4.47	8.43	2.87	7.82	27.32	15.21	4.34	6.67
	PSB	1.45	4.97	8.56	2.85	8.24	29.14	15.34	4.32	6.72
	NFB + PSB	1.53	5.84	10.72	3.46	9.45	31.92	15.64	4.76	6.98

*NFB means nitrogen fixing bacteria (mixture of *Azotobacter chroococcum* and *Azospirillum brasilense)*, PSB means phosphate solubilizing bacteria (mixture of *Bacillus megatherium* var. phosphaticum and *Bacillus polymyxa*).

The results obtained here also declared that biofertilizers treatments enhanced the vegetative growth characters as well as seed yield components of black cumin plants. This promotion could be explained through the effect of non symbiotic N_2-Fixing bacteria and phosphate solubilizing in exerting a positive effect on plant growth through the synthesis of phytohormones, N_2 fixation, reduction of membrane potential of the root, synthesis of some enzymes (such as ACC deaminase) that modulate the level of plant hormones as well as the solubilization of inorganic phosphate and mineralization of organic phosphate, which make phosphorus available to the plants (Rodriguez and Fraga 1999). In addition, the promotion effects of biofertilizers on vegetative growth may be reflected on increasing the seed yield. Biofertilizer has a great tendency to produce substances like Indole acetic acid (IAA), Gibberellins, vitamin B complex and growth hormones having great potential increasing the growth and development and ultimately the yield of crops (Yasin *et al.* 2012). These products increase the surface area/unit root length and improved the root hair branching with an ultimate increase on the uptake of nutrients and adsorption of water from the soil that eventually yield larger and in many cases, more productive plants (Dobbelaere *et al.* 2001). Otherwise, phosphate solublizing bacteria have a great role in growth and yield promotion since during phosphorus solubilization, some organic acids are produced which decrease the pH and acid phosphatases convert the organic phosphorus into inorganic form (Khan *et al.* 2009).

Decrease in the pH and the production of organic acid have combine effect in the solublization of phospate (Fankem *et al.* 2006). Several authors recorded similar trends concerning the promotion effects of biofertilizers on medicinal and aromatic plants (Hellal *et al.* 2011; Dadkhah 2012; Sokhangoy *et al.* 2012; Akhani *et al.* 2012; Hassan *et al.* 2012; Hassan and Ali 2013).

Applying cattle manure or biofertilizer treatments also promoted the volatile and fixed oil percentages and yields as well as interestingly affected their main components as shown in Tables (2, 3 and 4). Increasing the uptake of nutrient by root of plant especially phosphorus element, as a result of applying bifertilizers or organic manure, which is a main constituent of phospholipids, phosphoproteins, nucleic acids and coenzymes. However, the most important compound in which phosphate group one linked by pyrophosphate bonds is adenosine triphosphate (ATP). The energy absorbed during photosynthesis or released during respiration is utilized in the synthesis of the pyrophosphate bounds in ATP. In this form, the energy can by conveyed to various undergoing processes such as activation uptake and the synthesis of various organic compounds such as volatile oil (El-Ghadban *et al.* 2003). Increasing both percentages of oil and seed yield is a reasonable factor in increasing oil yield as our data indicated. Increasing the oil content and changing its main components by using organic manure and biofertilizers has been previously reported (Shaalan 2005; Mahfouz and Sharaf-Eldin 2007; Jhaa *et al.* 2011; Hassan and Ali 2013; Valadabadi and Farahani 2013).

The chemical constituents of black cumin herb and seed also affected by different organic and biofertilizer treatments. Increasing chlorophyll content by organic and biofertilizer as presented in Table (2)

Table.4 The main components of black cumin fixed oil treated by cattle manure, biofertilizers and their combination treatments

Cattle manure (A)	Biofertilizers (B)	Capric acid	Lauric acid	Myristic acid	Palmatic acid	Oleic acid	Linoleic acid	Linolenic acid
Control	Control	0.67	0.51	0.44	12.67	25.32	35.47	1.47
	NFB*	0.71	0.53	0.49	12.72	25.47	35.81	1.48
	PSB*	0.72	0.54	0.50	12.74	26.13	36.24	1.52
	NFB + PSB	0.79	0.57	0.52	12.96	26.78	36.89	1.64
10 m^3/fed	Control	0.68	0.54	0.46	12.78	25.44	35.52	1.52
	NFB	0.73	0.57	0.51	13.11	26.11	36.17	1.59
	PSB	0.76	0.58	0.54	13.24	26.98	36.98	1.67
	NFB + PSB	0.81	0.61	0.57	13.87	27.23	37.23	1.72
20 m^3/fed	Control	0.69	0.56	0.48	13.22	25.88	35.70	1.52
	NFB	0.74	0.59	0.51	13.97	26.79	37.24	1.57
	PSB	0.76	0.64	0.55	14.21	28.19	38.89	1.59
	NFB + PSB	0.83	0.68	0.58	14.97	30.42	39.47	1.64
30 m^3/fed	Control	0.74	0.58	0.49	13.27	26.17	35.89	1.53
	NFB	0.82	0.63	0.54	14.24	28.87	38.26	1.62
	PSB	0.85	0.67	0.57	14.87	29.18	38.95	1.67
	NFB + PSB	0.96	0.71	0.63	15.34	31.57	40.21	1.74

*NFB means nitrogen fixing bacteria (mixture of *Azotobacter chroococcum* and *Azospirillum brasilense)*, PSB means phosphate solubilizing bacteria (mixture of *Bacillus megatherium* var. phosphaticum and *Bacillus polymyxa*).

Table.5 Nitrogen, phosphorus and potassium percentages as well as nitrate and nitrite (mgL^{-1}) of black cumin treated by cattle manure, biofertilizers and their combination treatments

Cattle manure (A)	Biofertilizers (B)	N (%)	P (%)	K (%)	Nitrate in fruits (mgL^{-1})	Nitrite in fruits (mgL^{-1})
Control	Control	1.42	0.234	1.76	274.68	33.28
	NFB*	1.54	0.253	1.82	272.47	33.12
	PSB*	1.47	0.262	1.86	2.71.54	32.99
	NFB + PSB	1.61	0.275	1.92	268.23	32.84
10 m^3/fed	Control	1.45	0.246	1.85	272.15	33.09
	NFB	1.63	0.259	1.93	270.26	32.74
	PSB	1.52	0.278	2.04	271.42	32.46
	NFB + PSB	1.69	0.294	2.15	268.47	32.01
20 m^3/fed	Control	1.49	0.264	1.98	270.59	32.86
	NFB	1.68	0.285	2.33	268.74	32.54
	PSB	1.56	0.312	2.37	267.18	32.47
	NFB + PSB	1.73	0.334	2.41	264.47	31.35
30 m^3/fed	Control	1.51	0.287	2.12	268.58	31.76
	NFB	1.82	0.298	2.37	265.66	30.82
	PSB	1.73	0.334	2.41	262.31	30.59
	NFB + PSB	1.94	0.357	2.52	260.25	29.41
LSD 0.05	A	0.14	0.005	0.06	15.45	2.79
	B	0.09	0.004	0.04	12.64	2.14
	A X B	0.21	0.020	0.09	21.15	3.65

*NFB means nitrogen fixing bacteria (mixture of *Azotobacter chroococcum* and *Azospirillum brasilense)*, PSB means phosphate solubilizing bacteria (mixture of *Bacillus megatherium* var. phosphaticum and *Bacillus polymyxa*).

may be due to the promotion of cytokinins, which secreted by N_2-fixers is known to delay senescence of plant tissues through its effect on reducing the loss of chlorophyll (Gaballah 1995). In addition, phosphate solublising bacteria stimulated chlorophyll synthesis through encourages pyridoxal enzymes formation that plays an important role in α-amino levulinic acid synthetase as a primary compound in chlorophyll synthesis. Different authors recorded similar trends (Mahfouz and Sharaf-Eldin 2007; Al-Fraihat *et al.* 2011; Hassan and Ali 2013). A positive relationship between leaf carbohydrate and leaf pigments has been reported (Hassan and Ali 2013). Therefore, the synthesis of photosynthetic pigments in leaves may be an induced factor for carbohydrate synthesis and hence the carbohydrate percentage was increased in black cumin herb (Table 2). These results support the previous findings in different medicinal and aromatic plants (Hassan *et al.* 201**2**; Abdel-Razzak and El-Sharkawy 2013).

Increasing the microorganisms in the soil as a result of applying biofertilizers had a positive effect in converting the unavailable forms of nutrient elements to available forms. The microorganisms also produce growth promoting substances resulting in more efficient absorption of nutrients and consequently N, P and K percentages were increased (Table 5). The non symbiotic N_2-fixing bacteria produced adequate amounts of IAA and cytokinins with increasing the surface area per unit root length and enhanced the root hair branching with an eventual increase on the uptake of nutrients from the soil (Rodriguez and Fraga 1999). Phosphate solubilizing bacteria release organic and inorganic acids which reduce soil pH leading to change of phosphorus and other nutrients to available forms ready for uptake by plants (Singh and Kapoor 1999). The improvement of nutrient availability as a result of the application of organic manure may be due to the reduction in soil pH or the improvement of physical and chemical properties of the treated soils (Sikora and Azad 1993).

It is very interesting here to report that the individual or combined application of organic or biofertilizers significantly reduced the nitrate and nitrite contents in black cumin seeds (Table 5). Therefore, organic or biofertilizers treatments increased the quality of seeds. Decreasing the nitrate and nitrite contents in seeds is a critical factor in exporting most seeds and fruits of medicinal and aromatic plant included black cumin. Moreover, reducing the nitrate and nitrite contents in seeds is an important factor for obtaining high quality oil. Similar results achieved by inorganic and organic fertilizers were reported (Mahfouz and Sharaf-Eldin 2007; Hassan and Ali 2013). It is very clear from our results that the combined treatment of organic and biofertilizers was superior to the individual application in all parameters studied. These results may be due to the combined promotion effects of each fertilizer source and consequently recorded better results compared to each of them alone. Similar trends have been previously reported (Shaalan 2005; Valadabadi and Farahani 2013; Al-Fraihat *et al.* 2011; Gendy *et al.* 2012).

This study illustrated that applying organic and biofertilizers surge nitrogen and phosphorous availability in black cumin plants and consequently promoted the vegetative growth and increased the seed yield. In order to reduce the production cost by minimizing or utterly eliminating the use of chemical fertilizers, decreasing environmental hazards, improve soil structure, promote leveraging agriculture and obtain high quality black cumin seeds as well as fixed and volatile oils, the treatment of cattle manure at 30m^3/fed. combined with a mixture of NFB + PSB biofertilizer was recommended.

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