EFFECT OF ROCK PHOSPHATE RATES, INOCULATION WITH *Bacillus megatherium* AND V A MYCORRHIZAS ON GROWTH, YIELD AND CHEMICAL CONSTITUENTS OF BLACK CUMIN (*Nigella sativa* L.) PLANTS

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ABSTRACT

A field experiment was conducted during two successive seasons 2006 / 2007 and 2007 / 2008 to determine the influence of phosphate fertilizer namely: calcium superphosphate at the recommended dose (200 kg / fed.), rock phosphate at the rates of (128, 256 and 384 kg / fed.) and bio-fertilizers. The bio-fertilizer used were *Bacillus megatherium* var. phosphaticum (BM) (used as seed inoculants) and vesicular arbuscular mycorrhizal fungi (VAM) (used as soil inoculants) and their interactions. Effect of the previous treatments on vegetative growth, yield, volatile and fixed oils percentage and yield, carbohydrates %, protein %, phosphorus % and P uptake of black cumin plants were studied in this investigation. The obtained data showed that plant height, branches number, herb dry weight, capsules number, seed yield / plant and / feddan, volatile and fixed oils % and yield / plant and / feddan, P % and uptake, protein % and carbohydrates % were led to the augmentation of due to phosphate fertilization treatments. The highest values of these previous parameters were observed when receiving the plants calcium superphosphate and / or rock phosphate at the high rate. In regard to bio-fertilizer treatments, all of them led to increase the growth, seed yield, volatile and fixed oils % and yield, P % and uptake, protein % and carbohydrates %. The best results in terms of these characters were obtained when inoculating the plants with BM + VAM. In most cases, the combined effect between phosphate fertilizer and bio-fertilizer treatments on black cumin parameters was statistically significant. In most cases, inoculating the plants in combination with calcium superphosphate and / or the high rate of rock phosphate was the most effective treatments concerning growth, yield as well as chemical constituents.

Key words: rock phosphate, *Bacillus megatherium* var. phosphaticum, vesicular arbuscular mycorrhizal and *Nigella sativa.*

INTRODUCTION

Black cumin plant (*Nigella sativa* L.) is an annual plant. It is an aromatic plant belongs to Family *Ranunculaceae*, it's seeds are reputed and used by common people for many purposes such drug, antiasthmatic, diuretic, cough, bronchitis and carminative (Schouenberg and Paris,1977) and flavoring agent for bakeries or as a spice (Kybal,1980) as well as used in fever, influenza, and hypertension. This plant has been extensively studied pharmacologically since the extracts of seeds have anti-inflammatory. CNS depressant and analgesic, antitumer, immunostimulant, antihistaminic, antidiabetic and antimicrobial activity. Some of these activities have been predominantly attributed to the volatile and fixed oils (Houghton *et al.*, 1995; Burites and Bucar, 2000; EL-Dakhakny *et al.,* 2000; Zaoui *et al.*, 2002; Nickvar *et al.*, 2003 and Ali and Blunden, 2003).

The promoting effect of phosphorus treatments on growth and yield was studied by Mallangounda *et al.,* (1995) and Ahmed (1997) on *Nigella sativa,* Sharma and Israel (1991), Abdel-Gawad (2001) and Ali *et al.,* (2003) on coriander. Tiwori and Banafar (1995), Ahmed (1995), Abdel-Gawad (2001) and Ali *et al.,* (2003) showed that P fertilization led to increase the oil in coriander. Ughreja and Chundawat (1992), Mallangounda *et al.*, (1995) and Abdel-Gawad (2001) found that P % was improved when fertilizing coriander plants with phosphorus. In addition, Ahmed (1997) on *Nigella sativa*, Abdel-Gawad (2001) and Ali *et al.,* (2003) on coriander, revealed that P fertilization led to an increase in P % and its content.

Rock phosphate was natural sources containing P, which has been used in some countries as a source of P fertilization. Awasthl *et al.*, (1977) showed that utilizing rock phosphate directly as a source of P_2O_5 for plants has been in vague since long in several countries and added that rock phosphate selected for direct application should have carbonate in crystallatic, contain high of CO_2/P_2O_5 ratio and less fluorine. The same investigators revealed that Mussoorie rock phosphate was economical to the farmer as a source of P_2O_5 which was 30 / 50 % less costly and are efficient as other water soluble phosphate sources for groundnut crops. Graham and Timmer (1985) mentioned that loss of P from superphosphate amended soil was exponential; whereas P release from rock phosphate amended soil was linear. They also added that plant growth responses were less via application of superphosphate than rock phosphate. Furthermore, rock phosphate is a long term of P availability in comparison with superphosphate that leached within weeks. Many authors studied the effect of rock phosphate on growth, yield and chemical constituents, Soliman (1997) on *Nigella sativa* plants, found that Abo-Tartor rock phosphate led to the augmentation of plant height, branches number, herb dry weight, oil % and oil yield, P % and P uptake. Badran *et al.*, (1988) concluded that Safaga rock phosphate was almost equal to calcium superphosphate in increasing plant height, herb, oil % and oil yield, P % and P uptake of yarrow plants. Omar (1996) revealed that plant height, branches number, shoot dry weight, P % and P uptake of guar were led to the augmentation of due to fertilizing the plants with Safaga or Sabaiaa rock phosphate. Ali (2001) on *Calendula officinalis*, emphasized that Safaga rock phosphate led to the augmentation of plant height, branches number, herb dry weight, P % and P content. Ali (2004) stated that the high rate of Safaga or Sabaiaa rock phosphate gave the best results concerning plant height, leaves dry yield, herb dry weight, oil % and oil yield in leaves and flowers, P % as well as P uptake of *Tagetes minuta*.

The use of phosphate solubilizing bacteria becomes necessary to minimize the use of large doses of chemical P fertilizers that cause environmental pollution (Fallik and Okon, 1988). In this respect, Koreish *et al.,* (1998) found that phosphate–solubilizing bacteria inoculation is recommended to decrease the mineral P fertilizer dose to 20 units / fed. Radwan and Farahat (2002) proved that bio-fertilizer (phosphate dissolving bacteria) significantly led to increase fruit yield of coriander plant. Inoculation of *Nigella sativa* seeds by *Bacillus megatherium* and some other strains, each of them individually or in combination, revealed that these treatments led to increase growth, yield characters and volatile, fixed oils % (Shaalan, 2005) and on *Coriandrum sativum*, L. (Abou-Aly and Gomaa, 2002; and Abd EL-Kader and Ghaly, 2003). Ibrahim (2000) conducted that phosphorein application stimulated the vegetative growth measurements such as plant height, number of branches, fresh and dry weights of leaves and stems of fennel (*Foeniculum vulgare,* L.) plants. Khater (2001) reported that Phosphorein inoculation treatment produced taller plants and heavier fresh and dry weights of *Coriandrum sativum,* L. herb than uninoculated control. Abd El-Latif (2002) cleared that the inoculated caraway seeds with the mixture of 1 kg/fed. nitrobein + 1 kg/fed. phosphorein led to increase plant height, number of branches, and fresh and dry weights of shoots.

Nishi and Anil (1995) verified that protein levels and sugar of *Cyamopsis tetragonoloba* was led to the augmentation of due to mycorrhizal plants in comparison with non-micorrhizal ones. Tarafdar and Rao (1997) found that phosphate activity was significantly enhanced due to VAM inoculation of cluster bean (*Cyamopsis tetragonoloba)*, mung bean (*Vigna radiate*) and moth bean (*Vigna aconitifolia*).They added that dry matter production and green yield were improved by inoculation and the concentration of N, P, Cu and Zn in shoot were higher in the inoculated plants. Barakah *et al.*,(1998) stated that inoculation with micorrhizas and *Rhizobium meliloti* significantly led to increase plant growth, P and trace element uptake and N content of alfalfa (*Medicago sativa*). They proved that application of rock phosphate rather than superphosphate led to the augmentation of micorrhizal colonization and superphosphate fertilizer led to increase the dry weight compared to rock phosphate in plants grown without mycorrhizal inoculation, but after VAM inoculation their was less weight difference due to application of the two sources. Jain *et al.* (1998) studied the interactive effects between the VAM fungus *Glomus fasciculatum* and phosphorus levels on three forage legumes (*Trifolium alexandrinum, Medicago sativa* and *Vigna unguiculata*) and they found that growth and uptake of the plants were improved when inoculated with mycorrhizas. Rajagopal and Ramarethinam (1997) found that P uptake of tea roots was led to the augmentation of due to micorrhizae colonization and the effect was greater by superphosphate than rock phosphate. He added that P uptake was led to the augmentation of by increasing the use of P applied, except shoots given rock phosphate of non-inoculated plants. Hassan (2005) revealed that VAM with other treatments enhanced the growth, yield characters, P % and active ingredient materials in guar and fenugreek plants.

MATERIALS AND METHODS

This experiment was carried out in the Laboratories and Experimental Farm of Faculty of Agriculture, Al-Azhar University, Assiut, Egypt during the two successive seasons of 2006 / 2007 and 2007 / 2008 to study the effect of phosphate represented as rock phosphate rates and recommended doses of calcium superphosphate, and bio-fertilizers such as *Bacillus megatherium* var. phosphaticum (used as seed inoculants) and Vesicular arbuscular mycorrhizal fungi which contained three effective strains representing *Glomus etunicatum, Glomus intraradices* and *Glomus fasciculatum* (used for soil inoculation) on growth, yield, fixed oil, volatile oil, carbohydrates, protein and phosphorus uptake of black cumin (*Nigella sativa*, L) plants.

A split plot design with three replications, rock phosphate rates {**RP_1**=128, **RP_2**=256, and **RP_3**=384 kg/fed.} and recommended doses of calcium superphosphate (**SP**=200 kg/fed.) were added while soil preparation considered the main plots, bio-fertilizers treatment were assigned as sub plots. Black cumin seeds were sown on October 20^{th} of the two seasons. The experimental plot was 3.6 × 3.3 m and contained 5 rows, 60 cm apart. The distances between the hills were 30 cm. and the plants were thinned 45 days later to one plant / hill. Physical and chemical properties of the used soil are shown in Table (1). All agricultural practices were performed as usual. At the end of the experiment, the following data were recorded: plant height, number of branches / plant, herb dry weight (g) / plant, number of capsules / plant, seed yield (g) /plant and the seed yield (kg) /fed. was calculated.

Calcium superphosphate has 16 % of P_2O_5, and then 200 kg have 32 unit of P_2O_5. The rock phosphate was determined to have 25 % of P_2O_5, and then 128 kg have 32 unit of P_2O_5.

Chemical analysis

Fixed and volatile oil, carbohydrates, crude protein percentages in black cumin seeds, as well as, phosphorus percentage of the herb were determined according to A.O.A.C. (1990). Fixed and volatile oil yield per plant and per feddan, also phosphorus uptake were calculated. GC was carried out on the samples that represent the highest percentage of fixed oil of the second season. Fixed oil was methylated according to the method of Kates (1972) to obtain fatty acid methyl esters. Fatty acid methyl esters were analyzed with Hewlett Packard (HP) 6890 series GC system with capillary column (BP-70), length =60 m, diameter = 320 μm and film thickness = 0.25 μm equipped with (FID) detector and the detector temperature was 300°C. Temperature program was 70°C with initial time 2 min. Separation rate was (40 and 4 min.) with final temperature at (120 and 220°C) and final time at (2 and 10 min.), respectively. The inlet temperature was 250°C (splitless), with flow rate 2 ml/ min. Carrier gases were N_2 with 30 ml/min., H_2 at 30 ml/min. and air at 300 ml/min. Fatty acids percentage were expressed as percentage of total fatty acid methyl esters. The authentic samples of fatty acids methyl esters were also injected under the same condition for the identification of fatty acids.

Table (1): Physico-chemical properties of the used soil.

Texture	pH	E.C. (ds /m)	$CaCO_3$%	Available nutrients (ppm)			Water soluble Ions (meq /L) in the soil paste				
				N	P	K	Ca	Mg	CO_3+ HCO_3	CL	SO_4
Loamy	7.9	1.2	2.7	62.4	9.2	356	3.4	1.9	2.9	2.2	6.6

Statistical analysis was carried out according to the method of Gomez and Gomez (1984).

RESULTS AND DISCUSSION

1- Results:

Vegetative growth

Plant height:

Data listed in Table (2) reveal that the main effect of phosphate fertilizer treatments on plant height of *Nigella sativa* plants was statistically significant in the two experimental seasons. From the obtained data, it is obvious that fertilizing the plants with calcium superphosphate at recommended doses and all rates of rock phosphate treatments led to a significant increase in plant height compared to the check control treatment in both seasons.

Moreover, it was found that supplying black cumin plants with rock phosphate at the high rate or the recommended dose of superphosphate gave the tallest plants in the first season in comparison with other fertilizer treatments. Similar observations were observed in the second season which the tallest plants were noticed when treating the plants with the high rate of rock phosphate followed by the second rate of it. Numerically, these previous treatments led to the augmentation of plant height by 20.2, 19.1, 17.9 and 16.1 % than the control in the first and the second seasons, respectively.

Concerning the influence of bio-fertilizer treatments, data in Table (2) show that all of them significantly led to increase plant height comparing to uninoculated plants in the two seasons. It is clear that the tallest plants of black cumin plants were obtained when inoculating the plants with BM+VAM as reached 20.2 and 14.6 % over the check treatment in the two growing seasons, respectively.

With respect to the combined effect between phosphate fertilizer and bio-fertilizer treatments on plant height, it was significant in the first season only. The most effective treatments concerning plant height were the recommended dose of calcium superphosphate in combination with BM+VAM followed by the high rate of rock phosphate in combination with BM+VAM as compared to other combination treatments, as clearly illustrated in Table (2).

Table (2): Effect of phosphate fertilizer and bio-fertilizer treatments on vegetative growth of *Nigella sativa* plant during 2006/2007 and 2007/2008 seasons

Phosphate fertilizer treatments (A)	Bio-fertilizer treatments (B)									
	Plant height cm/plant									
	First season					Second season				
	Cont	B.M	VAM	BM+VAM	Mean (A)	Cont	B.M	VAM	BM+VAM	Mean (A)
Control	55.33	57.00	56.67	63.33	58.08	55.00	59.33	62.67	65.33	60.58
S. P	59.67	65.67	72.00	76.67	68.50	66.67	67.67	72.00	74.33	70.17
R. P(1)	59.67	64.00	68.00	71.33	65.75	62.00	63.67	69.00	71.33	66.50
R. P (2)	60.67	65.67	69.33	72.33	67.00	65.00	69.33	72.67	74.33	70.33
R. P (3)	63.33	68.67	72.00	75.33	69.83	67.00	71.00	74.33	76.33	72.17
Mean (B)	59.73	64.20	67.60	71.80		63.13	66.20	70.13	72.33	
L.S.D.$_{0.05}$	A : 1.36 B : 1.23 AB : 2.74					A : 1.27 B : 1.17 AB : N.S				
	Number of branches / plant									
Control	10.67	11.67	12.67	13.00	12.00	12.67	13.67	14.67	15.67	14.17
S. P	12.33	13.33	15.00	17.00	14.42	15.67	16.67	18.33	20.00	17.67
R. P(1)	12.67	13.33	15.00	17.33	14.58	12.67	14.00	15.00	17.00	14.67
R. P (2)	12.67	13.67	14.67	15.67	14.17	13.00	15.67	17.00	18.00	15.92
R. P (3)	12.00	13.67	14.33	16.00	14.00	14.00	17.00	18.00	19.00	17.00
Mean (B)	12.07	13.13	14.33	15.80		13.60	15.40	16.60	17.93	
L.S.D.$_{0.05}$	A : 0.57 B : 0.25 AB : 0.56					A : 0.52 B : 0.18 AB : 0.41				
	Herb dry weight (g / plaut)									
Control	29.00	29.47	30.82	32.07	30.34	30.33	31.83	32.87	34.17	32.30
S. P	37.17	38.10	39.77	40.83	38.97	38.20	40.14	41.88	42.90	40.78
R. P(1)	29.17	30.70	31.87	33.00	31.18	31.17	32.40	34.03	34.85	33.11
R. P (2)	35.73	37.07	38.77	40.03	37.90	37.70	39.10	40.90	42.10	39.95
R. P (3)	37.93	39.15	40.90	41.90	39.97	39.87	41.00	42.81	43.67	41.84
Mean (B)	33.80	34.90	36.43	37.57		35.45	36.89	38.50	39.54	
L.S.D.$_{0.05}$	A : 0.46 B : 0.29 AB : N.S					A : 0.40 B : 0.37 AB :N.S				

{$\mathbf{RP_1}$=128, $\mathbf{RP_2}$=256, and $\mathbf{RP_3}$=384 kg/fed.}(SP=200 kg/fed.)

Number of branches:

Table (2) declares that the main effect of phosphate fertilizer treatments on branches number of black cumin plants was statistically significant in the two successive seasons. All phosphate fertilizer used significantly led to increase branch number compared to unfertilized control in the two seasons except, treating the plants with the low rate of rock phosphate in the second season. From the obtained data, it could be noticed that the differences between the phosphate fertilizer treatments were not significant in the first season. It seems that receiving the plants rock phosphate at the low rate or calcium superphosphate gave the maximum number of branches/plant which ranged 21.5 and 20.2 % over the control plants in the first season, respectively. Fertilizing black cumin plants with calcium superphosphate at the recommended dose followed by the high rate of rock phosphate recorded better results of branches number in comparison with other phosphate treatments in the second season. Numerically, these aforementioned treatments led to increase number of branches by 24.7 and 20.0 % over unfertilized ones, respectively. The role of P fertilization in increasing branches number was observed on *Nigella sativa* by Soliman (1997) and Ahmed (1997) and on guar by Omar (1996).

As for bio-fertilizer treatments, all of them significantly led to the augmentation of branches number / plant in comparison with untreated plants for the two consecutive seasons. From the obtained data, it is clear that the maximum number of branches were detected when inoculating black cumin plants with BM + VAM as reached 30.9 and 31.8 % over the check control in the two experimental seasons, respectively as indicated in Table (2).

The interaction between phosphate fertilizer and bio-fertilizer treatments on number of branches / plant had significant effect in the two successive seasons. It is obvious that treating the plants with rock phosphate at the low rate with BM+VAM or calcium superphosphate with BM+VAM gave the most effective treatments in the first season, calcium superphosphate with BM+VAM followed by rock phosphate at the high rate with BM+VAM recorded the most effective treatments in the second one, as clearly shown in Table (2)

Herb dry weight:

Data presented in Table (2) pointed out that the main effect of phosphate fertilizer treatments on herb dry weight / plant was statistically significant in the two seasons. It is noted from the obtained results that all phosphate fertilizer treatments significantly led to increase the herb dry weight compared to untreated control in both seasons.

Moreover, it was found that the heaviest weight of herb dry were observed due to receiving black cumin plants rock phosphate at the high rate followed by calcium superphosphate which led to increase it by 31.7, 29.5, 28.4 and 26.2 % over unfertilized control in the two growing seasons, respectively.

Herb dry weight of black cumin plants was significantly affected by inoculation treatments with BM, VAM and BM+VAM in the two experimental seasons. From the obtained data, it is noticed that all of them led to a significant increase in herb dry weight in comparison with the check treatment in the first and the second seasons. Inoculating the plants with BM+VAM gave better herb dry weight which led to increase it by 11.1 and 11.5 % over the control plants in both seasons, respectively, as clearly shown in Table (2).

The combination between phosphate fertilizer and bio-fertilizer treatments on herb dry weight had insignificant effect for the two seasons, as clearly tabulated in Table (2).

The yield

Number of capsules

Date recorded in Table (3) reveal that number of capsules / plant was significantly affected by phosphate fertilizer treatments in the two growing seasons. It is clear that all of them significantly led to the augmentation of capsules number compared to unfertilized plants in the two experimental seasons.

Moreover, the highest numbers of capsules were obtained when supplying *Nigella sativa* plants with rock phosphate at the high rate followed by the medium rate which led to the augmentation of it over the control plants by 27.4, 25.5, 19.3 and 17.9 % in the first and the second seasons, respectively.

Concerning bio-fertilizer treatments, data in Table (3) show that the influence of them on number of capsules/ plant was significant in both seasons. From the obtained data, it is noticed that the highest number of capsules were detected due to inoculating black cumin plants with BM+VAM reached 12.1 and 11.3 % over the check control for the two seasons, respectively.

The interacting effect between phosphate fertilizer and bio-fertilizer treatments on capsules number / plant was significant in the second season only. The most effective treatments were obtained from treating the plants with rock phosphate at the high rate in combination with BM+VAM followed by rock phosphate at the high rate plus VAM, as clearly shown in Table (3).

Seed yield per plant and per feddan:

Data presented in Table (3) reveal that the main effect of phosphate fertilizer treatments on seed yield of *Nigella sativa* / plant and / feddan was statistically significant in the two experimental seasons. It is concluded that all phosphate fertilizer treatments significantly led to increase seed yield/ plant and / feddan in comparison with no received ones in both seasons.

Moreover, the maximum seed yield / plant and / feddan were observed when receiving the plants calcium superphosphate and / or rock phosphate at the high rate compared to other ones in the two seasons. Numerically, these previous treatments led to increase seed yield by 20.8 , 20.3 , 19.4 and 18.4 % than untreated control in the first and the second seasons, respectively and amounted 401.1, 418.6, 396.5 and 411.8 Kg / feddan seed compared to the check treatment (332.1 and 347.8) Kg / feddan seed in both seasons, respectively.

Seed yield of black cumin per plant and per feddan was significantly affected by inoculated the plants with BM, VAM and BM+VAM treatments in the two experimental seasons. The data show that all of them significantly led to the augmentation of seed yield / plant and / feddan in comparison with uninoculated ones in both seasons.

From the obtained results, it could be noticed that the highest seed yield / plant and / feddan were observed when treating *Nigella sativa* plants with BM+VAM which led to increase it by 11.9 and 11.9 % over the control in the two seasons, respectively. These aforementioned treatments recorded 396.1 and 414.9 Kg / feddan seed in comparison with untreated ones (353.9 and 370.5) Kg / feddan seed in the first and the second seasons, respectively, as clearly illustrated in Table (3).

The interaction between phosphate fertilizer and bio-fertilizer treatments on seed yield / plant and / feddan had significant effect for the two successive seasons. From the obtained data it is clear that inoculating the plants with BM+VAM and receiving calcium superphosphate and /or by rock phosphate at the high rate and produced the maximum seed yield in the first and the second seasons, respectively. These above treatments yielded 429.9, 446.1, 422.0 and

439.4 kg / feddan seed in the two seasons, respectively, while the check treatment amounted (315.9 and 322.4) kg / feddan seed in both seasons, respectively.

Table (3): Effect of phosphate fertilizer and bio-fertilizer treatments on yield of *Nigella sativa* plant during 2006 / 2007 and 2007 / 2008 seasons

Phosphate fertilizer treatments (A)	Bio-fertilizer treatments (B)									
	Number of capsules / plant									
	First season					Second season				
	Cont	B.M	VAM	BM+VAM	Mean (A)	Cont	B.M	VAM	BM+VAM	Mean (A)
Control	30.67	32.33	33.67	34.67	32.83	32.67	34.67	35.67	36.67	34.92
S. P	36.33	37.33	38.33	40.67	38.17	38.33	39.33	40.00	42.67	40.08
R. P(1)	34.33	35.33	36.33	38.67	36.17	36.33	37.33	38.33	40.67	38.17
R. P (2)	37.00	38.33	39.33	42.00	39.17	39.33	40.00	41.33	44.00	41.17
R. P (3)	40.00	41.00	42.33	44.00	41.83	42.00	43.00	44.33	46.00	43.83
Mean (B)	35.67	36.87	38.00	40.00		37.73	38.87	39.93	42.00	
L.S.D.$_{0.05}$	A : 0.48 B : 0.29 AB : N.S					A : 0.65 B : 0.32 AB : 0.71				
	Seed yield (g) / plant									
Control	18.77	19.08	19.82	21.23	19.73	19.15	19.68	21.35	22.45	20.66
S. P	22.38	23.18	24.20	25.53	23.83	23.50	24.62	24.83	26.50	24.86
R. P(1)	20.83	21.50	22.03	22.27	21.66	21.87	22.55	23.33	23.77	22.88
R. P (2)	21.07	22.07	22.83	23.53	22.38	22.53	23.18	23.80	24.40	23.48
R. P (3)	22.07	23.07	24.00	25.07	23.55	23.00	24.00	24.73	26.10	24.46
Mean (B)	21.02	21.76	22.58	23.53		22.01	22.81	23.61	24.64	
L.S.D.$_{0.05}$	A : 0.36 B : 0.20 AB : 0.44					A : 0.37 B : 0.22 AB : 0.49				
	Seed yield (kg) / feddan									
Control	315.9	321.3	333.6	357.5	332.1	322.4	331.4	359.4	378.0	347.8
S. P	376.8	390.3	407.4	429.9	401.1	395.6	414.4	418.1	446.1	418.6
R. P(1)	350.7	362.0	370.9	374.9	364.6	368.1	379.6	392.8	400.1	385.2
R. P (2)	354.7	371.5	384.4	396.2	376.7	379.4	390.3	400.7	410.8	395.3
R. P (3)	371.5	388.3	404.0	422.0	396.5	387.2	404.0	416.4	439.4	411.8
Mean (B)	353.9	366.7	380.1	396.1		370.5	384.0	397.5	414.9	
L.S.D.$_{0.05}$	A : 6.1 B : 3.3 AB : 7.4					A : 6.3 B : 3.7 AB : 8.2				

{**RP_1**=128, **RP_2**=256, and **RP_3**=384 kg/fed.}(SP=200 kg/fed.)

Chemical constituents

Volatile oil percentage

Data recorded in Table (4) show that the main effect of phosphate fertilizer treatments on volatile oil percentage of black cumin seeds was statistically significant in both seasons. From the obtained results, it was found that all of them significantly led to increase volatile oil percentage in comparison with unfertilized control in the two seasons.

Moreover, the maximum values of volatile oil percentage were detected due to fertilizing the plants with calcium superphosphate or rock phosphate at the high rate which led to increase it by 36.8, 36.1, 31.6 and 35.5 % than the check treatment in the first and the second seasons, respectively.

According to bio-fertilizer treatments, data in Table (4) reveal that all of these materials significantly led to the augmentation of volatile oil percentage compared to uninoculated control in the two seasons. It could be concluded that the best results were obtained when inoculating *Nigella sativa* plants with BM+VAM which was recorded the highest volatile oil percentage reached 25.0 and 25.1 % over untreated ones in the two experimental seasons, respectively. Positive effect of BM or VAM on increasing volatile oil % was reported by Shaalan (2005) on *Nigella sativa,* Abou-Aly and Gomaa (2002) and Abdel-Kader and Ghaly (2003) on coriander.

Table (4) pointed out that combined effect between phosphate fertilizer and bio-fertilizer treatments on volatile oil percentage was significant in the two growing seasons. It is clear that treating black cumin plants with calcium superphosphate in combination with BM+VAM or VAM resulted the most effective treatments concerning volatile oil percentage of black cumin seeds comparing to other combination treatments in the two experimental seasons.

Volatile oil yield / plant and / feddan

Data presented in Table (4) indicate that phosphate fertilizer treatments caused a significant effect on volatile oil yield of black cumin per plant and per feddan in the two successive seasons. From the obtained results, it seems that all phosphate fertilization led to a significant increase in volatile oil yield / plant and / feddan compared to untreated control in the two seasons.

Moreover, the data show that receiving the plants calcium superphosphate at the recommended dose or rock phosphate at the high rate produced the highest volatile oil yield ranged 66.2, 65.4, 57.4 and 61% over control in the two seasons and yielded 4.147, 4.421, 3.928 and 4.311 liter/ feddan oil in comparison with the check treatment (2.487 and 2.678) liter / feddan oil in the first and the second seasons, respectively.

As for bio-fertilizer treatments, all of them significantly led to the augmentation of volatile oil yield/ plant and/ feddan in comparison with uninoculated ones in the two experimental seasons. It is noticed that application of BM+VAM gave the maximum volatile oil yield / plant and / feddan which increased by 40.7 and 40.3 % than uninoculated plants in both seasons which amounted to 4.078 and 4.401 liter / feddan oil while, the control gave 2.894 and 3.124 liter/ feddan oil for the two consecutive seasons, respectively, as shown in Table (4).

The interaction between phosphate fertilizer and bio-fertilizer treatments on volatile oil yield / plant and / feddan had significant effect in the two seasons. The data in Table (4) reveal that the most effective treatments of volatile oil were obtained from treating black cumin plants with calcium superphosphate and BM+ VAM followed by the high rate of rock phosphate in combination with BM+ VAM as they yielded 5.045 and 4.764 liter / feddan oil, respectively, in comparison with the check control (2.250) liter/ feddan oil in the first season.

Inoculating the plants with BM + VAM and received them calcium superphosphate or rock phosphate at the high rate produced the maximum volatile oil yield and recorded 5.354 and 5.146 liter / feddan oil, respectively compared to the control (2.357) liter / feddan oil in the second season.

Table (4): Effect of phosphate fertilizer and bio-fertilizer treatments on volatile oil content of *Nigella sativa* plant during 2006/2007 and 2007/2008 seasons

Phosphate fertilizer treatments (A)	Bio-fertilizer treatments (B)									
	Volatile oil percentage									
	First season					Second season				
	Cont	B.M	VAM	BM+VAM	Mean (A)	Cont	B.M	VAM	BM+VAM	Mean (A)
Control	0.713	0.740	0.763	0.780	0.749	0.733	0.760	0.787	0.803	0.771
S. P	0.817	0.950	1.163	1.170	1.025	0.837	0.990	1.173	1.197	1.049
R. P(1)	0.820	0.890	0.957	0.987	0.913	0.840	0.910	0.977	1.007	0.933
R. P (2)	0.847	0.903	0.983	1.030	0.941	0.867	0.923	1.003	1.080	0.968
R. P (3)	0.877	0.943	0.997	1.127	0.986	0.923	0.983	1.103	1.170	1.045
Mean (B)	0.815	0.885	0.973	1.019		0.840	0.913	1.009	1.051	
$L.S.D._{0.05}$	A : 0.007	B : 0.025	AB : 0.056			A : 0.066	B : 0.023	AB : 0.051		
	Volatile oil yield (ml / plant)									
Control	0.134	0.141	0.151	0.165	0.148	0.140	0.149	0.167	0.180	0.159
S. P	0.183	0.220	0.282	0.300	0.246	0.197	0.244	0.292	0.318	0.263
R. P (1)	0.171	0.191	0.211	0.220	0.198	0.183	0.215	0.228	0.239	0.216
R. P (2)	0.178	0.199	0.225	0.243	0.211	0.195	0.214	0.239	0.264	0.228
R. P (3)	0.193	0.218	0.239	0.283	0.233	0.212	0.233	0.273	0.306	0.256
Mean (B)	0.172	0.194	0.222	0.242		0.186	0.211	0.240	0.261	
$L.S.D._{0.05}$	A : 0.021	B : 0.007	AB : 0.016			A : 0.021	B : 0.007	AB : 0.017		
	Volatile oil yield (L) / feddan									
Control	2.250	2.374	2.542	2.783	2.487	2.357	2.508	2.817	3.030	2.678
S. P	3.081	3.709	4.753	5.045	4.147	3.311	4.102	4.916	5.354	4.421
R. P(1)	2.879	3.216	3.546	3.698	3.335	3.087	3.620	3.838	4.029	3.644
R. P (2)	3.002	3.356	3.782	4.091	3.558	3.289	3.608	4.023	4.444	3.841
R. P (3)	3.255	3.664	4.029	4.764	3.928	3.575	3.923	4.602	5.146	4.311
Mean (B)	2.894	3.264	3.730	4.078		3.124	3.552	4.039	4.401	
$L.S.D._{0.05}$	A : 0.346	B : 0.123	AB : 0.275			A : 0.355	B : 0.126	AB : 0.281		

{**RP_1**=128, **RP_2**=256, and **RP_3**=384 kg/fed.}(**SP**=200 kg/fed.)

Fixed oil percentage

Data in Table (5) indicate that fixed oil percentage of *Nigella sativa* seeds was significantly influenced by fertilizing the plants with phosphate fertilization in the two experimental seasons. From the obtained results, it is clear that all of them significantly led to increase fixed oil % compared to unfertilized ones in the two seasons.

Furthermore, the maximum values of fixed oil percentage were detected due to receiving the plants rock phosphate at the high rate followed by calcium superphosphate which led to increase by 28.09, 23.39, 26.39 and 22.02 % over the check control in the two experimental seasons, respectively.

With respect to bio-fertilizer treatments, it was found that these strains used significantly led to the augmentation of fixed oil percentage of black cumin seeds as compared to uninoculated ones in both seasons. The best result was observed when inoculating the plants with BM+VAM which led to increase it over the control treatment by 15.22 and 14.89 % for the two seasons respectively, as shown in Table (5).

Concerning the combined effect between phosphate fertilizer and bio-fertilizer treatments on fixed percentage, the data in Table (5) reveal that it was significant for the two experimental seasons. Moreover, it could be concluded that the most effective treatments were BM+VAM and fertilization with rock phosphate at the high rate followed by calcium superphosphate in comparison with other combination ones in the two growing seasons.

Fixed oil yield / plant and / feddan

Obtained data in Table (5) show that the main effect of phosphate fertilization on fixed oil yield / plant and / feddan was statistically significant in both seasons. It is obvious that all of them significantly led to increase fixed oil yield/ plant and / feddan in comparison with the check treatment in the two seasons. Receiving black cumin plants rock phosphate at the high rate or calcium superphosphate produced the highest fixed oil amounted 49.85, 45.57, 49.6 and 46.74 % over untreated ones in the two successive seasons, respectively which reached 110.75 , 138.05 , 108.24 and 132.32 liter/ feddan oil compared to control (72.31 and 79.85) liter / feddan oil for the two seasons, respectively.

In regard to bio-fertilizer treatments, data recorded show that all of them significantly led to the augmentation of fixed oil yield/ plant and/ feddan in comparison with uninoculated control for the two consecutive seasons. It was found that inoculating black cumin plants with BM+VAM gave the highest fixed oil yield ranged between 29.0

and 28.7 % than control plants in both seasons, respectively. This above treatment yielded 106.51 and 116.53 liter/ feddan oil while, untreated plants yielded 82.55 and 90.53 liter/ feddan oil in the first and the second seasons, respectively, as clearly revealed in Table (5).

Table (5): Effect of phosphate fertilizer and bio-fertilizer treatments on fixed oil content of *Nigella sativa* plant during 2006/2007 and 2007/2008 seasons

Phosphate fertilizer treatments (A)	Bio-fertilizer treatments (B)									
	Fixed oil percentage									
	First season					Second season				
	Cont	B.M	VAM	BM+VAM	Mean (A)	Cont	B.M	VAM	BM+VAM	Mean (A)
Control	20.10	21.10	22.07	23.57	21.71	21.32	22.30	23.27	24.63	22.88
S. P	25.00	26.33	27.33	28.51	26.79	26.10	27.50	28.45	29.62	27.92
R. P(1)	22.13	23.33	24.00	25.04	23.63	23.17	24.70	25.61	26.44	24.98
R. P (2)	23.30	24.44	25.70	26.60	25.01	24.44	25.47	26.58	27.54	26.01
R. P (3)	25.40	27.62	28.34	29.87	27.81	26.47	28.44	29.40	31.37	28.92
Mean (B)	23.19	24.56	25.49	26.72		24.30	25.68	26.66	27.92	
L.S.D.$_{0.05}$	A : 0.59 B : 0.18 AB : 0.41					A : 0.55 B : 0.24 AB : 0.53				
	Fixed oil yield (ml / plant)									
Control	3.773	4.027	4.377	5.003	4.295	4.083	4.393	4.970	5.527	4.743
S. P	5.603	6.213	6.617	7.283	6.429	6.133	6.777	7.070	7.860	6.960
R. P (1)	4.613	5.017	5.293	5.577	5.125	5.070	5.573	5.983	6.293	5.730
R. P (2)	4.913	5.397	5.870	6.273	5.613	5.510	5.907	6.333	6.730	6.120
R. P (3)	5.613	6.397	6.807	7.497	6.578	6.090	6.830	7.277	8.200	7.099
Mean (B)	4.903	5.410	5.793	6.327		5.377	5.896	6.327	6.922	
L.S.D.$_{0.05}$	A : 0.290 B : 0.085 AB : 0.191					A : 0.262 B : 0.111 AB : 0.248				
	Fixed oil yield (L / feddan)									
Control	63.53	67.79	73.68	84.23	72.31	68.74	73.96	83.67	93.04	79.85
S. P	94.33	104.60	111.39	122.62	108.24	103.26	114.08	119.02	132.32	117.17
R. P(1)	77.66	84.45	89.11	93.88	86.28	85.36	93.83	100.73	105.95	96.47
R. P (2)	82.72	90.85	98.82	105.61	94.50	92.76	99.44	106.62	113.30	103.03
R. P (3)	94.50	107.69	114.59	126.21	110.75	102.52	114.98	122.50	138.05	119.51
Mean (B)	82.55	91.08	97.52	106.51		90.53	99.26	106.51	116.53	
L.S.D.$_{0.05}$	A : 4.88 B : 1.44 AB : 3.21					A : 4.41 B : 1.87 AB : 4.17				

{**RP_1**=128, **RP_2**=256, and **RP_3**=384 kg/fed.}(**SP**=200 kg/fed.)

According to the interaction between phosphate fertilizer and bio-fertilizer treatments, data in Table (5) declare that it was significant effect on fixed oil yield/ plant and / feddan in the two seasons. It could be noticed that inoculating black cumin with BM+VAM in combination with the high rate of rock phosphate followed by calcium superphosphate produced the maximum values of fixed oil yield as recorded 126.21, 138.05, 122.62 and 132.32 liter/ feddan oil in the two seasons, respectively. Meanwhile, the check treatment yielded 63.53 and 68.74 liter / feddan oil in both seasons, respectively.

Phosphorus percentage

Table (6) shows that phosphorus percentage in the herb of *Nigella sativa* plants was significantly affected by fertilizing the plants with phosphate fertilizer in the two experimental seasons. From the obtained data, it could be noticed that all of these materials used significantly led to the augmentation of phosphorus percentage comparing to control in both seasons.

Moreover, the highest percentages of phosphorus were obtained from receiving the plants calcium superphosphate or rock phosphate at the high rate as they led to increase it by 32.56, 30.00, 24.93 and 23.92 % over that of the check control in the two seasons, respectively.

Concerning bio-fertilizer treatments, data in Table (6) reveal that utilizing these strains led to a significant increase in phosphorus % compared to uninoculated ones in the successive seasons. It is clear that inoculating black cumin plants with BM + VAM recorded the maximum value of P % reached 15.09 and 13.34 % than control plants in the two seasons, respectively.

As for the combined effect between phosphate fertilizer and bio-fertilizer treatments, it was significant on phosphorus percentage in the two consecutive seasons. The most effective treatments were observed from inoculation the plants with BM+VAM and fertilization with the high rate of rock phosphate and/or seasons, as shown in Table (6).

Table (6): Effect of phosphate fertilizer and bio-fertilizer treatments on phosphorus percentage and content of *Nigella sativa* plant during 2006/2007 and 2007/2008 seasons

Phosphate fertilizer treatments (A)	Bio-fertilizer treatments (B)									
	phosphorus percentage									
	First season					Second season				
	Cont	BM	VAM	BM+VAM	Mean (A)	Cont	B.M	VAM	BM+VAM	Mean (A)
Control	0.360	0.370	0.380	0.390	0.375	0.380	0.393	0.403	0.410	0.397
S. P	0.453	0.463	0.487	0.507	0.478	0.473	0.480	0.503	0.527	0.496
R. P(1)	0.380	0.403	0.427	0.443	0.413	0.413	0.423	0.443	0.467	0.437
R. P (2)	0.397	0.433	0.450	0.467	0.437	0.420	0.453	0.470	0.487	0.458
R. P (3)	0.430	0.450	0.497	0.517	0.473	0.450	0.470	0.517	0.530	0.492
Mean (B)	0.404	0.424	0.448	0.465		0.427	0.444	0.467	0.484	
L.S.D.$_{0.05}$	A : 0.008 B : 0.004 AB : 0.009					A : 0.010 B : 0.004 AB : 0.009				
Phosphorus uptake (g / plant)										
Control	0.104	0.109	0.117	0.124	0.113	0.110	0.119	0.126	0.135	0.123
S. P	0.168	0.176	0.193	0.207	0.186	0.176	0.188	0.204	0.219	0.197
R. P (1)	0.111	0.124	0.136	0.146	0.129	0.115	0.127	0.140	0.151	0.133
R. P (2)	0.141	0.161	0.174	0.187	0.166	0.146	0.166	0.180	0.194	0.171
R. P (3)	0.163	0.176	0.203	0.216	0.189	0.168	0.181	0.208	0.221	0.194
Mean (B)	0.137	0.149	0.164	0.176		0.143	0.156	0.172	0.184	
L.S.D.$_{0.05}$	A : 0.007 B : 0.002 AB : 0.004					A : 0.051 B : N.S AB : 0.096				

{**RP_1**=128, **RP_2**=256, and **RP_3**=384 kg/fed.}(**SP**=200 kg/fed.)

Phosphorus uptake:

Data presented in Table (6) reveal that the main effect of phosphate fertilizer treatments on phosphorus uptake of black cumin herb was statistically significant in the two experimental seasons. From the obtained data, it could be noticed that all phosphate fertilizer, except the low and medium rates of rock phosphate in the second season significantly led to the augmentation of phosphorus uptake in comparison with the check treatments in the two seasons.

Moreover, it was found that the highest phosphorus uptake of black cumin herb were recorded when treating the plants with rock phosphate at the high rate or calcium superphosphate (absorbed 0.189, 0.194, 0.186 and 0.197 g / plant) while untreated ones gave 0.113 and 0.123 g / plant in the two seasons, respectively.

Regarding bio-fertilizer treatments, the data in Table (6) show that it had no significant effect on phosphorus uptake of black cumin herb in the two experimental seasons.

The interaction between phosphate fertilizer and bio-fertilizer treatments on phosphorus uptake of black cumin herb had statistically significant in both seasons. The maximum values of phosphorus uptake were obtained due to inoculating black cumin with BM + VAM in combination with the high rate of rock phosphate followed by in combination with calcium superphosphate which gave 0.216, 0.221 and 0.219 g / plant meanwhile, untreated ones amounted to 0.104 and 0.110 g / plant in the first and the second seasons, respectively as illustrated in Table (6).

Protein percentage:

Data recorded in Table (7) reveal that the main effect of phosphate fertilizer on protein percentage of black cumin seeds was statistically significant in both seasons. It is clear that all phosphate fertilizers used led to a significant increase in protein percentage in comparison with control in the two experimental seasons.

Moreover, the maximum values of protein % were observed due to receiving the plants calcium superphosphate followed by rock phosphate at the high rate which they led to increase it over the check treatment by 47.55, 33.15, 44.42 and 29.87 % in the first and the second seasons, respectively.

In regard to bio-fertilizer treatments, data illustrated in Table (7) show that all of them significantly led to the augmentation of protein % compared to uninoculated ones in the two seasons. From the obtained results, inoculating black cumin plants with BM + VAM gave the highest protein percentage as reached 14.57 and 14.08 % in the two seasons, respectively compared to control plants. The positive effect of BM or VAM on increasing of protein % was reported by Shaalan (2005) on *Nigella sativa* and Hassan (2005) on guar and fenugreek plants

The combined effect between phosphate fertilizer and bio-fertilizer treatments on protein % of black cumin seeds had statistically significant in the two growing seasons. Receiving the plants calcium superphosphate and inoculating them with BM + VAM followed by VAM led to obtain the most effective treatments concerning protein % in comparison with other treatments in the consecutive seasons, as clearly declared in Table (7).

Carbohydrate percentage:

Table (7) pointed out that phosphate fertilizer treatments significantly affected on carbohydrate % of black cumin seeds in the two seasons. The data show that supplying the plants with phosphate fertilizer significantly led to increase carbohydrate percentage compared to unfertilized ones in both seasons. Moreover, it is obvious that the highest values of carbohydrate % were obtained when utilizing of calcium superphosphate followed by the high rate of rock phosphate which were 43.58, 26.81, 31.87 and 19.64 % over the control in the two experimental seasons, respectively.

Concerning bio-fertilizer treatments, data in Table (7) indicate that all of them significantly led to the augmentation of carbohydrate % in black cumin seeds in comparison with untreated control for the two growing seasons. From the obtained results, it seems that BM + VAM treatments gave maximum values of carbohydrate percentage which led to increase it by 13.45 and 13.01 % than those of uninoculated plants in the first and the second seasons, respectively.

As for the interaction between phosphate fertilizer and bio-fertilizer treatments on carbohydrate % of black cumin seeds, it was significant effect in both seasons. It is appears that the maximum values of carbohydrate % of black cumin seeds resulted from supplying the inoculation calcium superphosphate and BM + VAM followed by VAM in comparison with other treatments in the two successive seasons, as clearly shown in Table (7). The positive effect of BM on increasing of carbohydrate % was reported by Shaalan (2005) on *Nigella sativa*.

Table (7): Effect of phosphate fertilizer and bio-fertilizer treatments on protein and carbohydrate percentage of *Nigella sativa* plant during 2006/2007 and 2007/2008 seasons.

Phosphate fertilizer treatments (A)	Bio-fertilizer treatments (B)									
	protein percentage									
	First season					Second season				
	Cont	B.M	VAM	BM+VAM	Mean (A)	Cont	B.M	VAM	BM+VAM	Mean (A)
Control	16.11	16.40	17.07	17.63	16.80	17.28	17.67	18.22	18.60	17.94
S . P	23.43	24.38	25.14	26.20	24.79	24.38	25.49	26.24	27.54	25.91
R. P(1)	17.03	17.77	19.02	20.04	18.46	17.98	18.44	20.15	21.17	19.44
R. P (2)	18.27	19.15	20.34	21.14	19.72	19.20	20.17	21.22	22.39	20.74
R. P (3)	20.50	21.67	23.07	24.23	22.37	21.61	22.57	24.14	24.90	23.30
Mean (B)	19.07	19.87	20.92	21.85		20.09	20.86	21.99	22.92	
L.S.D.$_{0.05}$	A : 0.39 B : 0.17 AB : 0.37					A : 0.40 B : 0.21 AB : 0.47				
	Carbohydrate percentage									
Control	21.17	22.23	23.12	24.17	22.67	23.70	24.87	25.53	26.30	25.10
S. P	29.57	30.80	34.47	35.37	32.55	30.77	32.04	35.40	36.45	33.66
R. P (1)	24.40	25.57	26.45	27.04	25.86	24.77	26.50	27.39	27.93	26.65
R. P (2)	25.14	26.00	26.77	27.70	26.40	26.18	26.91	27.63	28.07	27.20
R. P (3)	27.17	28.25	29.24	30.33	28.75	28.24	29.13	30.47	32.30	30.03
Mean (B)	25.49	26.57	28.01	28.92		26.73	27.89	29.28	30.21	
L.S.D.$_{0.05}$	A : 0.64 B : 0.19 AB : 0.43					A : 0.54 B : 0.23 AB : 0.52				

{**RP**$_1$=128, **RP**$_2$=256, and **RP**$_3$=384 kg/fed.}(**SP**=200 kg/fed.)

Fatty acid content.

Table (8) indicated the fractionated fatty acid methyl esters of the selected five treatments which yielded the highly content of fixed oil percentage. There is an obvious difference between them in their fatty acids content. The plants received the high rate of rock phosphate and inoculated by BM +VAM gave the best oil quality. Linoleic acid represents the major content of the fractionated fatty acid methyl esters. All of the plants received rock phosphate and inoculated with BM + VAM are nearly had similar in their fatty acid quality. Lauric acid is not found in the control treatment but, is found in treated ones with traces quantity in the treated black cumin with recommended dose of superphosphate and inoculated with BM + VAM. Linoleic and Erucic acids led to increase gradually in the seeds of plants treated with increasing the rates of rock phosphate and inoculation with BM +

VAM in comparison with the control. Linolenic acid was not detected in the control one but, was found in the others in trace amounts. Also, erucic acid is in tolerance led to increase when the rate of rock phosphate is led to increase with the inoculation of BM + VAM in comparison with control and superphosphate treatments. Total unsaturated fatty acids are also led to increase due to rock phosphate rates increment in comparison with the control and superphosphate treatments. These obtained data are agreed with that obtained by Shaalan (2005) and Kokdil and Yilmaz (2005) on *Nigella sativa*, L.

Table (8) relative amount of Nigella sativa fixed oil constituents from GC of control and treated plants (20067 / 2008).

Fatty acid		Control	SP (BM+VAM)	RP_1 (BM+VAM)	RP_2 (BM+VAM)	RP_3 (BM+VAM)
Lauric		--	0.07	0.96	0.89	0.75
Myristic		0.12	0.64	0.69	0.65	0.32
Palmitic		11.09	12.00	13.20	13.09	12.41
Stearic		3.12	2.47	3.04	3.12	2.05
Oleic		14.62	17.05	15.99	16.54	17.26
Linoleic		44.07	52.00	58.62	59.07	59.22
Linolenic		--	0.08	0.11	0.16	0.25
Eicosenoic		2.41	2.41	2.17	2.41	2.08
Erucic		0.15	1.86	2.48	2.59	2.64
Total	saturated	14.33	15.18	17.89	15.53	17.89
	unsaturated	61.25	73.40	79.37	81.45	80.37

{RP_1=128, RP_2=256, and RP_3=384 kg/fed.}(SP=200 kg/fed.)

The chemical properties of Nigella sativa fixed oil revealed that no significant differences between all tested of treated seeds oil. All of acid, ester, saponification, iodine and peroxide values were approximately around the trusted factors (Table 9)

Table (9): Chemical properties of black cumin fixed oil

Treatment	Acid Value	Ester Value	Sap. value	Iodine value	Peroxide value
Control	28.17	161.83	190.0	88	7.9
SP (BM+VAM)	29.10	162.5	191.2	90	8.0
RP_1 (BM+VAM)	29.68	163.42	193.1	89	8.2
RP_2 (BM+VAM)	29.82	163.28	193.1	89	8.1
RP_3 (BM+VAM)	29.92	164.98	194.9	89	8.2

{RP_1=128, RP_2=256, and RP_3=384 kg/fed.}(SP=200 kg/fed.)

2- Discussion

The obtained results could be discussed as follows: the positively effect of phosphorus on plant growth, seed yield, volatile and fixed oil % as well as other chemical constituents of black cumin plants obtained in this work was due to the role of phosphorus, that it has been long recognized as an essential constituent in all living cells. No single living cell can survive without phosphoric acid. Nucleoproteins constituting the essential substances of cell nuclei contain phosphoric acid. Phosphorus deficiency leads to stunting, delayed maturity and shriveled seeds. Phosphate components are necessity for photosynthesis, metabolism of amino acids and fat and inter-conversion of carbohydrates. (Bidwell, 1974; Devlin, 1979 and Yagodin, 1984).

The increment of growth, yield, essential and fixed oils % and yields as well as protein % and carbohydrates % of black cumin plants as a result of inoculated the plants with BM, VAM and BM + VAM separately each of it or in combinations may be due to the positive effect of these materials. Many investigators studied the influence of *Bacillus megetherium* and vesicular arbuscular mycorrhizal such as, El-Katkat, (1992) who concluded that *Bacillus megetherium* was the important group in the solubilization process of insoluble phosphorus in soils. Mycorrhizal fungi were found to produce compounds which stimulate branching of roots or increase the hormonal status of the plant host (Allen *et al.*, 1982). The role of VA mycorrhizas in increasing phosphate uptake by the plant is therefore of paramount importance in the tripartite association. However, Ross (1971) showed that mycorrhizal infection led to increase yield and nitrogen content compared to non- mycorrhizal controls. This finding was confirmed by the use of $^{15}N_2$ tracer technique (Kucey and Panl, 1982).

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).

The fixed oil of *Nigella sativa* has antioxidant effect (Houghton *et al.*, 1995; Ali and Blundon, 2003). It has also been shown that black cumin oil was effective on liver and pancreas in rata. The oil was found to reduce significantly serum total cholesterol, low-density lipoproteins, triglycerides and glucose levels in rats (El-Dakhakny *et al.,* 2000; Zaoui *et al.,* 2002). This study showed that the fixed oil of black cumin is rich in polyunsaturated fatty acids which play an important role in human health. One interpretation of this could be that at higher applications of P, when less carbon is allocated to the roots, there is less of a surplus of carbohydrates that can be used by the fungus for storage structures). At high applications of P, there is a delay in sporulation due to the carbon flux from the plant being reduced (Olson *et al.,* 1997). Abbott *et al.* (1984) observed that vesicle formation (fungal storage structures in the root) was more sensitive to P applications than the amount of intraradical hyphae was. The distribution of an arbuscular mycorrhizal (AM) fungus between soil and roots, and between mycelial and storage structures, was studied by use of the fatty acid signature 16:1ω5. Increasing the soil phosphorus level resulted in a decrease in the level of the fatty acid 16:1ω5 in the soil and roots. A similar decrease was detected by microscopic measurements of root colonization and of the length of AM fungal hyphae in the soil. The fatty acid 16:1ω5 was estimated from two types of lipids, phospholipids and neutral lipids, which mainly represent membrane lipids and storage lipids, respectively. The numbers of spores of the AM fungus formed in the soil correlated most closely with neutral lipid fatty acid 16:1ω5, whereas the hyphal length in the soil correlated most closely with phospholipid fatty acid 16:1ω5 (Olson *et al.,* 1997).

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تأثير إستخدام معدلات من الفوسفات الصخري، التلقيح ببكتيريا باسللس ميجاتيريم و فطريات الميكورهيزا على النمو، المحصول والمكونات الكيميائية لنباتات حبة البركة.

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أجريت هذه التجربة في مزرعة كلية الزراعة – جامعة الآزهر – أسيوط خلال موسمين متتاليين 2006 / 2007، 2007 / 2008 لمعرفة تأثير التسميد الفوسفاتي حيث أستخدم سماد سوبر فوسفات الكالسيوم بالمعدل الموصى به (200 كجم / فدان) وسماد الفوسفات الصخري بمعدلات 128، 256، 384 كجم / فدان وأيضا التسميد الحيوي بإستعمال البكتيريا الميسرة للفوسفات (باسللس ميجاتيريم) كتلقيح للبذور قبل زراعتها وكذا تلقيح التربة بفطريات الميكورهيزا والتداخل بينهما على النمو الخضري والمحصول والنسبة المئوية للزيت الطيار والثابت ومحصولهما والنسبة المئوية للكربوهيدرات والبروتين وأيضا النسبة المئوية لعنصر الفوسفور وإمتصاصه لنباتات حبة البركة.

أوضحت النتائج المتحصل عليها أن إرتفاع النبات وعدد الأفرع ووزن العشب الجاف وعدد الكابسولات ومحصول البذور للنبات وللفدان والنسبة المئوية للزيت الطيار والثابت ومحصولهما للنبات وللفدلن والنسبة المئوية لعنصر الفوسفور وإمتصاصه والنسبة المئوية للبروتين والكربوهيدرات قد زاد نتيجة معاملات التسميد الفوسفاتي. وأظهرت النتائج أن أعلى القيم لتلك الصفات المذكورة تم الحصول عليها من إستخدام سماد سوبر فوسفات الكالسيوم أو المعدل العالي من سماد الفوسفات الصخري. أما جميع معاملات السماد الحيوي فقد أدت إلى زيادة النمو والمحصول والنسبة المئوية للزيت الطيار والثابت ومحصولهما وكذا النسبة المئوية لعنصر الفوسفور وإمتصاصه وأيضا النسبة المئوية للكربوهيدرات والبروتين. وتلقيح النباتات بالبكتيريا المذيبة للفوسفات (باسللس ميجاتيريم) + فطريات الميكورهيزا أعطى أفضل النتائج لجميع الصفات تحت الدراسة.

وفي معظم الحالات كان التداخل بين العاملين معنويا على تلك الصفات وبصفة عامة إتضح أن تلقيح النباتات ببكتيريا باسللس ميجاتيريم + فطريات الميكورهيزا مع التسميد بسوبر فوسفات الكالسيوم أو المعدل العالي من الفوسفات الصخري قد سجل أكبر التأثير على صفات النمو والمحصول و المكونات الكيميائية.