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Response of black cumin (*Nigella sativa* L.) plants to the addition of natural fertilizers and the inoculation by bacteria mix and seaweed liquid extract

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Abstract

This experiment was carried out during the two successive seasons of 2017/2018 and 2018/2019 aiming to study the effect of natural fertilizers namely mixed minerals ores; control, 200, 400 and 600 Kg/fed. (fed. = feddan = 0.420 hectares = 1.037 acres) and half recommended dose of NPK fertilizers and the inoculation of mixed of bacteria namely (*Azotobacter chroococcum*, *Bacillus megatherium* var *Phosphaticum* and *Bacillus circulans*) alone or with seaweed liquid extract , as well as, their interactions on vegetative growth, yield, fixed oil (percentage & yield) and fixed oil components of black cumin (*Nigella sativa* L.) plants. The highest values of all parameters were observed when the plants received the high rate of mixed minerals ores (600 kg /fed.). In regard to the inoculation with bacteria alone or with seaweed liquid extract, it was noticed that all treatments led to a significant increase in plant height, branch number, shoot dry weight, capsules number, seed yield , fixed oil (percentage & yield). The highest values of these previous characters were obtained when the inoculation with bacteria plus seaweed liquid extract. Generally, the combined effect among mixed minerals ores, the inoculation with bacteria and seaweed liquid extract treatments on *Nigella sativa* L., plants parameters was statistically significant. In most cases, the addition of the high rate of mixed minerals ores (600 kg /fed.) plus the inoculation with bacteria plus seaweed liquid extract was the most effective treatment in increasing these parameters. Results of GC-MS analysis of fixed oil revealed that the main fatty acids offered were myristic acid, palmatic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and arachidic acid. The elevated percentages between the previous components were oleic acid followed by Stearic acid and arachidic acid. The treatments of mixed mineral ores and the inoculation with bacteria with seaweed liquid extract increased the former fatty acids in the fixed oil paralleled to untreated control.

Keywords: natural fertilizers, mixed minerals ores**,** *Azotobacter chroococcum, Bacillus megatherium, Nigella sativa*.

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1. 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 are 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). In the winter, viral infection spreads, so many scientists are looking to strengthen the immune system of a person in a safe natural way to combat various diseases. Among the most important plants that strengthen the immune system. In ancient medicine, they used black cumin to increase the efficiency of the immune system through several recipes in order to avoid infection with many viral and immune diseases such as adding it to honey or yogurt and eating it daily or drinking it in the form of tea with the addition of fenugreek seeds and fennel seeds once a day. This works to strengthen the immune system. It can also be boiled with a glass of water and inhale the resulting steam to strengthen the respiratory system or add it to foods and baked goods in order to increase its nutritional value. Likewise, black seed oil can be placed on a cup of water and eaten on an empty stomach. As for modern medicine, studies have proven that *Nigella sativa* is used for 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 (Ali and Blunden, 2003; Burites and Bucar, 2000; EL-Dakhakny *et al*., 2000; Houghton *et al.*, 1995; Nickavar *et al*., 2003; Zaoui *et al*., 2002). Mixed minerals ores are major essential macronutrients for plant growth, soluble P and K fertilizers are commonly applied to replace removed minerals and to optimize yield. When phosphate is added into soils as a fertilizer in relatively soluble and plant available forms, it is easily converted especially in alkaline soil like in Egyptian soil, into insoluble complexes. Consequently, to achieve optimum crop yields, soluble phosphate, fertilizers have to be applied at high rates which cause unmanageable excess of phosphate application and environmental and economic problems (Brady, 1990). On the other hand, K deficiencies become a problem because K decreases easily in soils due to crop uptake, runoff, leaching and soil erosion (Sheng and Huang, 2002). Direct application of mixed minerals ores may be agronomically more useful and environmentally more feasible than soluble N, P and K (Rajan *et al*., 1996). Mixed minerals ores materials are cheaper sources of P, K and micronutrients however, most of them are not readily available to a plant because the minerals are released slowly and their use as fertilizer often causes insignificant yield increases of current crop (Zapata and Roy, 2004). PSB have been used to improve rock P value because they convert insoluble rock P into available soluble forms for plant growth (Bojinova *et al* 1997). The promoting effect of mixed minerals ores treatments on growth and yield was studied by Hassan (2015) on dill (*Anethum graveolens*, L.) plants and Ahmed (1997) on *Nigella sativa*, Sharma and Israel (1991), Abdel-Gawad (2001) and Ali *et al* (2003) on coriander (*Coriandrum sativum*), 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 as well as P % and P uptake of yarrow plants. Omar (1996) revealed that plant height, branch number and shoot dry weight of guar were augmented of due to fertilizing the plants with safaga or sabaiaa rock phosphate. Ali (2001) on *Calendula officinalis*, emphasized that safaga rock phosphate led to an augment in plant height, branch number and herb dry weight. 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 the leaves and flowers of *Tagetes minuta*. Biological activities are markedly enhanced by microbial interactions in the rhizosphere of plants (Tilak and Reddy, 2006). Such syntrophic associations are of ecological importance with implied significance agricultural. The plant growth promoting rhizobacteria (PGPRs) can influence on plant growth directly through the production of phytohormones and indirectly through nitrogen fixation and production of bio-control agents against soil-borne phytopathogens (Glick, 2003). *Azospirillum* species are nitrogen-fixing organisms, on the other hand *Bacillus megaterium* var. *phosphaticum* is known for its ability to solubilize rock P material (Han and Lee, 2005). The same authors showed that KSB are able to solubilize rock K mineral powder, such as micas, illite and or thoclases. It was shown that KSB such as *Bacillus mucilaginosus and B. circulans* increased K availability in soils and increased mineral content in plant (Sheng *et al* 2002). El-Shafie *et al*. (2009) postulated that inoculating *Ammi visnaga* seeds with different N fixing bacteria (*Azospirillum* and/or *Azotobacter*) led to an increase in fruit yield. Azzaz *et al*. (2009) cleared that the application of Bio-fertilizer mixtures (*Azotobacter sp., Bacillus megatherium var Phosphaticum,* and *Bacillus circulans*) increased the response of all growth parameters, yield, essential oil and crude oil of Fennel (*Foeniculum vulgare L.*).Hassan *et al*. (2009) postulated that inoculating *Nigella sativa* seeds with *Bacillus megatherium var. phosphaticum* plus vesicular arbuscular mycorrhizal fungi led to an increase in growth, yield, fixed oils percentage and yield / plant and / fed. Hassan *et al*. (2009) on khella (*Ammi visnaga*) utilized phosphate and potassium fertilizers namely: calcium superphosphate at the recommended dose (200 kg / fed.), rock phosphate at the rates of (128& 256 kg / fed.), Potassium fertilizer namely: potassium sulphate at the recommended dose (50 kg / fed.), feldspar at the rates of (240 & 480 kg / fed.) and biofertilization with *Bacillus megaterium* BF2 and their interactions, and biofertilization with *B. circulans* F5 and their interactions. All parameters were observed when the plants received phosphate and potassium fertilize at the high rate. In regard to biofertilizer treatments, all of them led to a significantly increase in the growth. The application of seaweed extract for different crops was a great importance due to contain high levels of organic matter, micro elements, vitamins , fatty acids and also rich in growth regulators such as auxins, cytokinin and gibberellins (Crouch and Van Staden, 1994). Extracts derived from seaweeds are biodegradable, non-toxic, non-polluting and non-hazardous to humans, animals and birds. These fertilizers are often found to be more successful than chemical fertilizers (Booth, 1969). However, the application of seaweed extract increased chlorophyll content (Thirumaran *et al*., 2009; Whapham *et al*., 1993). Turan and Köse (2004) on grapevine, Mancuso *et al*. (2006) and Rathore *et al*. (2009) on soybean observed increasing yield as well as N, P and K with application of seaweed extract. Gajewski *et al*. (2008) on Chinese cabbage revealed that application of Goteo (an organic-mineral fertilizer which contains algae extract *Ascophyllum nodosum* with addition of phosphorus) increased yield, marketable heads as well as vitamin C content compared to the untreated cabbagewhereas, slightly higher nitrate content was noted. Zodape *et al*. (2008) on okra (*Abelmoschus esculentus*), Arthur *et al*. (2003) on pepper (*Capsicum annuum*) and Zodape *et al*. (2010) on mung bean (*Vigna radiate*), indicated that application of seaweed extract significantly increased seed yield and pod weight as well as improved nutritional values of seeds, *i.e*., protein and carbohydrates. Also, Eyszkowska *et al*. (2008) reported that an organic fertilizer which contains amino acids and short peptide chains such as goteo and amino insignificantly increased nitrate in lettuce of examined cultivars. Abdel Mawgoud *et al*. (2010) cleared that the application of seaweed extract at the concentrations of 1, 2 and 3 g/L increased the response of all growth parameters and yield of watermelon. The aim of this study was to evaluate the potential of the direct application of natural fertilizers and the inoculation by bacteria (*Azotobacter chroococcum, Bacillus megatherium var Phosphaticum*, and *Bacillus circulans*) and seaweed liquid extract as well as their interactions on vegetative growth, yield, fixed oil (percentage & yield) and fixed oil components of black cumin (*Nigella sativa* L.) plants.

2. Materials and methods

2.1 Experimental plan and location

This experiment was carried out in Experimental Farm of Faculty of Agriculture, Al-Azhar University, Assiut, Egypt during the two successive seasons of 2017 / 2018 and 2018 / 2019 to study the effect of natural fertilizers namely mixed minerals ores; control, 200, 400 and 600 Kg/fed. (fed. = feddan = 0.420 hectares = 1.037 acres) and half recommended dose of NPK fertilizers "NPK_h" and the inoculation of mixed of bacteria namely *(Azotobacter chroococcum* , *Bacillus megatherium* var *Phosphaticum*, and

Bacillus circulans (MB)) alone or with seaweed liquid extract (SW) and their combinations on vegetative growth, yield, fixed oil (percentage & yield), of black cumin (*Nigella Sativa* L.) plants. The seeds of black cumin (*Nigella sativa* L.) were obtained from Medicinal and Aromatic Plants Department, Horticulture Research Institute Giza, Egypt. Mixed Minerals Ores sources were obtained from El-Ahram Company for Mining and Natural Fertilizers Maadi, Cairo, Egypt. The mixed of bacteria used were obtained from Agric. Microbiology Dep. National Research Center, Egypt. A commercial seaweed extract product (OLIGO- X) contains N (1%), K (18.5%), Ca (0.17%), Mg (0.42%), Fe (0.06%), S (2.2%), alganic acids (10-12%) and plant hormones (600 ppm).

2.2 Experimental design and measured characters

A split plot design with three replications, mixed minerals ores (Control, MMO_1=200, MMO_2=400, MMO_3=600 and NPK_{Hh}=225 kg /fed.) was the main plots, bio-fertilizers treatments (Control, MB, SW and MB+SW) were assigned as the sub plots. Black cumin seeds were sown on November 5^{th} of the two seasons. The experimental plot was 3.0 × 2.5 m and contained 4 rows, 60 cm apart. The distances between the hills were 25 cm. and the plants were thinned 35 days later to two plant / hill. Physical and chemical properties of the soil are shown in Table (1). Before cultivation, the seeds, except control were, treated with bacterial solutions by immersion for one hour and also, SW was used as sprayed on plants three times, after 50 , 65 and 80 days from planting. All agricultural practices were performed as usual. At the end of the experiment, the following data were recorded: plant height, number of branches / plant, shoot dry weight (g) / plant, number of capsules / plant, seed yield (g) /plant, and seed yield (kg) /fed. Was calculated, fixed oil percentage, fixed oil yield (ml) /plant, and oil yield (L) /fed. was and main fatty acids of fixed oil. Statistical analysis was carried out according to the method of Gomez and Gomez (1984).

Table (1): Some physical and chemical properties of the experimental soil (average of the two seasons).

Texture	PH (1:2.5)	E.C. (m.mo hs/cm)	$CaCo_3$ (%)	O.M. (%)	Total N (%)	Available		Water soluble ions (meg/l) in the soil paste				
						P (ppm)	K (mg/100g soil)	Ca	Mg	Co_3+ Hco_3	Cl	So_4
Loamy	7.5	2.2	2.53	0.50	0.12	0.14	3.5	3.4	1.9	2.9	2.2	6.6

2.3 Analysis condition of fixed oil

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 fixed oil was analyzed using DSChrom 6200 Gas Chromatograph equipped with a flame ionization detector for separation of fixed oil constituents. The analysis conditions were as follows:

The chromatograph apparatus was fitted with capillary column DE-VAX 122-7032 Polysillphenylene - Siloxane 30 mx 0.25 mm D. 0.25 um film (Table 2). Temperature program ramp is increase with a rate of 50"C / min from 100 to 220°C. Flow rates of gases were nitrogen at 1 ml /min, hydrogen at 30 ml/min and 330 ml/min for air. Detector and injector temperatures were 280 and 250 °C, respectively. The obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of main fatty acids of fixed oil.

Table (2): Chemical analysis for mixed minerals ores.

Item	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	MnO	MgO	CaO	Na_2O	K_2O	P_2O_5	SO_3	L.O.I
(%)	39.36	0.81	7.68	4.05	0.68	3.20	15.07	1.76	4.24	7.33	5.83	8.08

3. Results and Discussion

3.1 Vegetative growth parameters

Obtained data in Table (3) clearly revealed that plant height (cm), number of branches/plant and shoot dry weight (g/plant) of black cumin (*Nigella sativa* L.) plants were significantly influenced by mixed minerals ores fertilizers treatments in the two growing seasons. It is appear that fertilizing the plants with mixed minerals ores at all levels, besides the half recommended dose of NPK led to a significant increase in plant height, number of branches/plant and shoot dry weight compared to untreated plants in both seasons. From the recorded data, it is noticed that the addition of the high level of mixed minerals ores fertilizer gave the highest values of these traits, whereas increased plant height, the number of branches/plant and the shoot dry weight / g/plant by 10.14 & 9.16, 29.79 & 24.20 and 64.17 & 69.54 % over untreated plants in the two experimental seasons, respectively. Our results agreement with the results of Hassan *et al.* (2009) on black cumin plants, Hassan (2015) on dill plants, Khalil (2018) on caraway plants and Hassan *et al*. (2009) on khella (*Ammi visnaga*) plants where the role of some natural fertilizers had been shown on enhancement vegetative growth. Concerning the effect of mixed of bacteria and seaweed liquid extract treatments, data in Table (3) showed that plant height (cm), number of branches/plant and shoot dry weight (g /plant) of black cumin were significantly increased in comparison with untreated ones in the two consecutive seasons. It was found that receiving black cumin plants mixed of bacteria plus seaweed liquid extract gave the tallest plants, highest the number of branches /plant and heaviest the shoot dry weight /g/plant as ranged 3.69 & 5.50,18.37 & 21.85 and 14.35 & 13.96 % over control in the first and second seasons, respectively. The positive effect of biofertilization on enhancing plant growth was observed by Mahdi *et al* (2012) and Ali and Hassan (2014) on black cumin (*Nigella sativa* L.) plants, Hassan *et al*. (2015), Abdullah *et al*. (2012) and Leithy *et al*. (2006) on rosemary (*Rosmarinus officinalis* L.) plants, El-Hindi and El- Boraie (2005) and Ghallab and El-Gahdban (2004) on marjoram plants, Hendawy *et al*. (2010) on *Thymus vulgaris* plants and Gharib *et al*. (2008) on marjoram (*Majorana hortensis*) plants. According to the interaction between mixed mineral ores and mix of bacteria with seaweed liquid extract treatments, it had a significant effect on the shoot dry weight (g / plant) of black cumin in both seasons.

Table (3): The interaction effect of mixed minerals ores with biofertilizers on vegetative parameters of black cumin plants during 2017/2018 and 2018/2019 seasons.

Mixed Minerals Ores(A)	Biofertilizers (B)									
	Plant height (cm)									
	Control	MB	SW	MB+SW	Mean (A)	Control	MB	SW	MB+SW	Mean (A)
	First season					Second season				
Control	91.00	91.00	91.00	92.00	91.25	91.00	93.00	92.00	95.00	92.75
NPK $_{HR}$	94.67	96.00	97.33	99.00	96.75	96.00	100.00	99.33	102.00	99.33
MMO (1)	92.00	95.00	93.00	96.00	94.00	92.00	95.00	93.00	96.00	94.00
MMO (2)	95.00	99.00	97.00	99.00	97.50	96.00	100.00	98.00	102.00	99.00
MMO (3)	98.00	101.00	101.00	102.00	100.50	98.00	102.00	101.00	104.00	101.25
Mean (B)	94.13	96.40	95.87	97.60		94.60	98.00	96.67	99.80	
L.S.D0.05	A :1.47 B :1.06 AB :N.S					A :1.03 B :0.93 AB:N.S				
	Number of branches									
Control	9.00	10.00	9.00	10.00	9.50	9.00	10.00	10.00	11.00	10.00
NPK $_{HR}$	10.67	11.67	11.33	12.00	11.42	11.00	11.33	11.33	12.33	11.50
MMO (1)	9.00	10.67	10.00	10.67	10.08	9.00	11.00	11.00	11.33	10.50
MMO (2)	9.67	12.00	11.67	11.33	11.17	10.33	12.00	12.00	12.67	11.83
MMO (3)	10.67	13.00	11.67	14.00	12.33	11.00	11.67	11.67	14.00	12.42
Mean (B)	9.80	11.47	10.73	11.60		10.07	11.20	11.20	12.27	
L.S.D0.05	A :0.39 B :0.78 AB :N.S					A :0.75 B:0.67 AB:N.S				
	Shoot dry weight (g/plant)									
Control	28.37	29.87	29.37	31.43	29.76	28.73	30.57	29.80	31.57	30.17
NPK $_{HR}$	43.77	47.47	44.80	49.80	46.46	44.43	48.07	44.73	50.57	46.95
MMO (1)	29.30	30.90	29.63	33.37	30.80	29.93	31.33	29.93	34.43	31.41
MMO (2)	42.37	46.23	44.10	48.97	45.42	42.70	46.27	44.50	49.47	45.73
MMO (3)	45.10	50.37	47.53	52.43	48.86	47.53	52.30	50.43	54.33	51.15
Mean (B)	37.78	40.97	39.09	43.20		38.67	41.71	39.88	44.07	
L.S.D0.05	A:1.20 B:0.58 AB:1.31					A:0.76 B:0.68 AB:1.68				

Data indicated that the most effective treatments were obtained due to the high level of mixed mineral ores plus mixture of bacteria with seaweed liquid extract compared to other combination complex treatments, while the effect of the interaction between the two factors was not significant on plant height and number of branches/ plant during the two experimental seasons, as clearly shown in Table (3).

3.2 Yield parameters

Data recorded in Table (4) indicated that the main effect of mixed minerals ores and half recommended dose of NPK fertilization treatments in the two growing seasons on capsules number/plant, seed yield (g)/ plant and seed yield (kg) /feddan of black cumin (*Nigella sativa* L.) plants was statistically significant. From the obtained results it could be noticed that by increasing the mixed minerals ores fertilization treatments the capsules number/plant, seed yield (g) /plant and seed yield (kg) /feddan was significantly augmented. Therefore, the maximum value of capsules number/plant, seed yield (g) /plant and seed yield (kg)

/feddan was observed when receiving the plants high rate of mixed minerals ores as ranged 27.00 & 27.17, 28.53 & 24.52 and 27.48 & 24.13 over the check treatment in the first and second seasons, respectively. The beneficial effect of some natural fertilizers on yield components was emphasized by Hassan *et al*. (2009) on black cumin plants, Hassan (2015) on dill plants, Khalil (2018) on caraway plants and Hassan et al. (2009) on khella (*Ammi visnaga*) plants. It is worthy that all bacteria and seaweed liquid extract treatments led to a significant increase in capsules number/plant, seed yield (g) /plant and seed yield (kg) /feddan in the two consecutive seasons. It was found that the highest value of capsules number/plant, seed yield (g) /plant and seed yield (kg) /feddan was detected due to treating black cumin plants with mixed of bacteria plus seaweed liquid extract which they increased these traits over unsprayed control by 5.73 & 6.39, 10.12 & 14.52 and 9.90 & 14.48 in the two seasons, respectively, as clearly revealed in Table (4). The positive effect of biofertilization on enhancing yield parameters was observed by Mahdi *et al*. (2012) and Ali and Hassan (2014) on black cumin (*Nigella Sativa* L.), Hassan (2015), Abdullah *et al*. (2012) and Leithy *et al*. (2006) on rosemary (*Rosmarinus officinalis* L.), El-Hindi and El- Boraie (2005), Ghallab and El-Gahdban (2004) and Gharib *et al*. (2008)on marjoram (*Majorana hortensis*), and Hendawy *et al*. (2010) on *Thymus vulgaris*.

Table (4): The interaction effect of mixed minerals ores with biofertilizers on yield parameters of black cumin plants during 2017/2018 and 2018/2019 seasons.

Mixed Minerals Ores(A)	Biofertilizers (B)									
	Capsules number /plant									
	Control	MB	SW	MB+SW	Mean (A)	Control	MB	SW	MB+SW	Mean (A)
	First season					Second season				
Control	85.00	87.67	86.00	88.33	86.75	85.33	88.67	85.67	90.00	87.42
NPK $_{HR}$	98.67	100.67	100.00	101.67	100.25	99.00	102.67	99.67	105.00	101.58
MMO (1)	88.00	90.67	89.67	92.00	90.08	90.33	91.33	90.00	92.00	90.92
MMO (2)	94.00	98.67	96.00	101.00	97.42	95.00	100.00	98.00	103.00	99.00
MMO (3)	105.33	111.33	109.00	115.00	110.17	105.00	114.33	110.33	115.00	111.17
Mean (B)	94.20	97.80	96.13	99.60		94.93	99.40	96.73	101.0	
	A :1.11 B :0.65 AB :1.45					A :1.07 B :0.77 AB:1.17				
	Seed yield (g/plant)									
Control	19.50	20.50	20.33	21.00	20.33	20.00	21.17	20.00	22.00	20.79
NPK $_{HR}$	22.47	24.00	22.50	25.33	23.58	22.00	23.33	23.83	24.33	23.38
MMO (1)	20.17	21.00	21.00	23.17	21.33	20.33	20.97	20.17	24.17	21.41
MMO (2)	21.00	22.67	21.63	23.00	22.08	21.17	21.17	21.00	24.83	22.04
MMO (3)	25.50	26.00	25.83	27.17	26.13	24.67	25.00	25.40	28.50	25.89
Mean (B)	21.73	22.83	22.26	23.93		21.63	22.33	22.08	24.77	
L.S.D0.05	A :0.45 B :0.36 AB :0.80					A :0.61 B:0.52 AB:1.16				
	Seed yield (Kg/fed.)									
Control	748.3	786.9	780.3	809.3	781.2	767.5	812.3	767.5	844.3	797.9
NPK $_{HR}$	863.8	924.2	841.6	959.4	897.3	844.2	895.3	914.5	933.7	896.9
MMO (1)	773.9	805.9	793.1	888.9	815.5	780.3	804.6	773.9	927.4	821.6
MMO (2)	805.9	869.7	830.1	882.5	847.1	812.3	812.3	805.9	953.0	845.9
MMO (3)	971.9	991.1	984.7	1035.9	995.9	946.6	952.7	974.8	1093.7	991.9
Mean (B)	832.8	875.6	846.0	915.2		830.2	855.5	847.3	950.4	
L.S.D0.05	A:23.32 B:16.49 AB:36.88					A:23.30 B:19.88 AB:44.45				

The interaction effect between the two factors on capsules number/plant, seed yield (g) /plant and seed yield (kg) /feddan of black cumin was significant for the two experimental seasons. The most effective treatment was obtained when receiving the plants the high rate of mixed minerals ores with bacteria plus seaweed liquid extract compared to other combination treatments in the two seasons, as clearly illustrated in Table (4).

3.3 Fixed oil percentage and yield

Obtained data in Table (5) clearly revealed that fixed oil percentage, fixed oil yield (ml) /plant and fixed oil yield (L) /feddan of black cumin (*Nigella sativa* L.) plants was significantly influenced by mixed minerals ores and half recommended dose of NPK fertilization treatments in the two growing seasons. It is appear that fertilizing the plants with mixed minerals ores at all levels besides the half recommended dose of NPK led to a significant increase in fixed oil percentage, fixed oil yield (ml) /pant and fixed oil yield (L) /feddan compared to untreated plants in both seasons. From the recorded data, it is noticed that addition of the high level of mixed minerals ores fertilizer gave the increase the fixed oil percentage, fixed oil yield (ml) /pant and fixed oil yield (L) /feddan as ranged 30.60 & 33.10, 67.90 & 66.00 and 115.00 & 108.65 % over control in the two experimental seasons, respectively. Concerning the effect of mixed of bacteria and seaweed liquid extract treatments, data in Table (5) showed that fixed oil percentage, fixed oil yield (ml) /pant and fixed oil yield (L) /feddan of black cumin were significantly increased in comparison with untreated ones in the two consecutive seasons. It was found that receiving black cumin plants bacteria plus seaweed liquid extract gave the increased fixed oil percentage, fixed oil yield (ml) /pant and fixed oil yield (L) /feddan as by 10.65 & 11.12,21.81 & 27.17 and 34.87 & 45.00 % over control in the first and second seasons, respectively. According to the interaction between mixed mineral ores and mix of bacteria with seaweed liquid extract treatments; it had a significant effect on the fixed oil percentage, fixed oil yield (ml) / plant and fixed oil yield (L) /feddan of black cumin in both seasons. Data indicate that the most effective treatments were obtained due to the high level of mineral ores in combination with of bacteria plus to seaweed liquid extract compared to other complex treatments during two experimental seasons, as clearly shown in Table (5).

3.4 Fixed oil composition

Results of GC-MS analysis of fixed oil revealed that the main fatty acids presented were myristic acid, palmatic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and arachidic acid (Table 6). The highest percentage among the previous components was oleic acid followed by stearic acid and arachidic acid. The treatments of mixed minerals ores and the inoculation of bacteria with seaweed liquid extract increased the previous fatty acids in the fixed oil compared to untreated control especially the high level of mineral ores in addition to of bacteria with seaweed liquid extract.

Table (5): The interaction effect of mixed minerals ores with biofertilizers on fixed oil percentage, fixed oil yield (ml) / plant and fixed oil yield (L) /feddan of black cumin plants during 2017/2018 and 2018/2019 seasons.

Mixed Minerals Ores(A)	Biofertilizers (B)									
	Oil percentage									
	Control	MB	SW	MB+SW	Mean (A)	Control	MB	SW	MB+SW	Mean (A)
	First season					Second season				
Control	25.50	25.67	25.17	25.50	25.46	25.17	26.33	25.67	26.50	25.92
NPK_{HR}	33.00	34.00	34.67	35.67	34.33	33.00	34.67	35.00	36.00	34.67
MMO (1)	23.67	24.33	25.33	26.67	25.00	24.33	25.00	25.67	26.00	25.25
MMO (2)	27.67	29.33	30.00	31.67	29.67	28.67	31.00	34.00	35.33	32.25
MMO (3)	31.00	31.67	34.00	36.33	33.25	32.67	33.67	35.67	36.00	34.50
Mean (B)	28.17	29.00	29.83	31.17		28.77	30.13	31.20	31.97	
	A :0.67 B :0.51 AB :1.15					A :0.64 B :0.56 AB:1.24				
	Fixed oil yield (ml) /plant									
Control	4.970	5.217	5.163	5.353	5.176	5.037	5.267	5.430	5.828	5.390
NPK_{HR}	7.417	7.543	8.317	8.917	8.048	7.267	8.267	8.157	8.760	8.113
MMO (1)	4.777	4.930	5.323	6.178	5.302	4.947	5.040	5.380	6.280	5.412
MMO (2)	5.810	6.350	6.807	7.287	6.563	6.070	6.513	7.193	8.777	7.138
MMO (3)	7.903	8.180	8.803	9.873	8.690	8.057	8.550	8.920	10.257	8.946
Mean (B)	6.175	6.444	6.883	7.522		6.275	6.727	7.016	7.980	
	A :0.38 B :0.27 AB :0.59					A :0.23 B:0.19 AB:0.43				
	Fixed oil yield (L) /feddan									
Control	37.46	40.73	40.69	43.18	40.52	38.67	40.47	44.12	47.79	42.76
NPK_{HR}	63.97	66.83	76.64	85.63	73.27	61.48	75.67	73.15	82.00	73.08
MMO (1)	37.06	39.31	42.99	54.96	43.58	38.61	39.03	43.36	58.25	44.81
MMO (2)	46.85	56.26	62.76	67.75	58.41	49.35	52.59	58.45	83.55	60.98
MMO (3)	77.07	80.59	88.46	102.42	87.14	76.28	83.40	85.07	112.16	89.22
Mean (B)	52.48	56.75	62.31	70.78		52.88	58.23	60.83	76.75	
	A:4.77 B:3.50 AB:7.81					A:3.93 B:3.02 AB:6.76				

Table (6): The interaction effect of some mixed minerals ores and biofertilizers on fixed oil components of black cumin plants during 2018/2019 season.

Fatty acid	RT	Relative concentration (%)				
		Control	NPK_{HR}+ MB+SW	MMO (1)+ MB+SW	MMO (2)+ MB+SW	MMO (3)+ MB+SW
Myristic acid (C14:0)	5.862	5.60	5.59	8.21	7.29	8.51
Palmitic acid (C16:0)	6.038	2.24	2.87	0.26	1.53	0.54
Stearic acid (C18:0)	7.569	10.79	6.81	14.10	14.73	14.98
Oleic acid (C18:1)	8.173	23.66	23.19	32.85	33.64	33.73
Linoleic acid (C18:2)	8.426	5.16	4.94	4.54	1.53	1.67
Linolenic acid (C18:3)	8.826	6.06	5.54	2.04	2.05	2.09
Arachidic acid (C20:0)	9.346	14.51	14.55	5.87	5.05	5.46
Total fatty acids		68.04	63.46	67.87	65.98	66.98

4. Conclusions

The final recommendation of the research is that the highest quantity of seeds per crop and acre was obtained. Moreover, the content of the plant and acre of oil was increased as a result of using the treatments of mixed mineral ores and inoculation of mixed of bacteria with seaweed liquid extract compared to untreated control. This gives us an opportunity to produce seeds and oil in a large quantity and with high quality for use in making medicines to strengthen the immune system and respiratory system, and oil can also be used to produce antifungal, bacterial, and virus medications.

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