New Valley Journal of Agricultural Science
Published by Faculty of Agriculture, New Valley University, Egypt
DOI: 10.21608/NVJAS.2023.198555.1180
Research Article Open Access

Effect of Fertilization and Biostimulants on Growth and Productivity of Black Cumin (*Nigella Sativa* L.) Plants

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Received: 07/03/2023
Revised: 19/03/2023
Accepted: 22/03/2023
Published: 22/03/2023

Abstract

During two growing seasons, 2020–2021 and 2021–2022, a field study was carried out to investigate the impact of fertilization. (poultry manure at 0, 5, 10, and 15 m^3/fed. plus half of the recommendations of NPK fertilizer) and bio-stimulants (mixed bacterial seed inoculation of *Azotobacter chroococcum*, *Bacillus megatherium* var. *Phosphaticum*, and *Bacillus circulans* alone or even in conjunction with a foliar spray of seaweed extract), When plants received 15 m3 / fed of poultry manure, the best results among all of the metrics were noted. For plants that were inoculated with bacteria alone or even in conjunction with a foliar spray of seaweed extract, all treatments were found to result in a significant increase in plant height, branch number, fresh and dry plant weight, capsule number, seed production, and fixed and volatile oil. The greatest results of the aforementioned attributes were obtained when the seeds were treated with a bacterial mixture and seaweed liquid extract as foliar spray. GC-MS analysis of volatile and fixed oils identified twenty-one the volatile oils' constituents. Generally, the most successful combination was applying the highest rate of poultry manure (15 m3 / fed), seed inoculation with mixed bacteria and application of seaweed extract to the leaf.

Keywords: Poultry manure, Organic fertilizer, Biostimulants, Seaweed extract, *Nigella sativa* L.

Introduction

Plants with therapeutic properties have been used for decades in different ways, including by humans for sustenance, flavoring, health, and medicine. Black cumin (*Nigella sativa* L.) is a member of a Ranunculaceae family and blooms annually. An important seed crop with its roots in the eastern Mediterranean, it is now widely cultivated in the Middle East, Europe, and Asia. Many areas around the world are responsible for cultivating the crop. **(Aggarwal *et al.*, 2008; Bayram *et al.*, 2010; Mohamed *et al.*, 2017)**. An important nation that produces *Nigella sativa* include Egypt, India, Bangladesh, Sri Lanka, Pakistan, Iraq, Afghanistan, Iran, Syria, Turkey, and Ethiopia. The black cumin seed, when mature, has the following composition: 4.34% ash, 23% protein, 7% moisture, 4.99% starch, 0.39% fat, and 5.44% raw fiber. Furthermore, seeds are rich in lipids, fiber, minerals such as calcium, iron, salt, copper, zinc, and phosphorus, as well as vitamins such as folic acid, ascorbic acid, thiamine, and niacin. **(Takruri and Dameh, 1998; Mozaffari *et al.*, 2000 and Sultana *et al.*, 2018).** Additionally, *Nigella sativa* seeds contain 30-35% fixed oil and 0.5-1.5% essential oil both of which have extensive use in the culinary and pharmaceutical industries. The seeds also contain a bitter chemical called nigeline. Protein, alkaloids (nigellicines and nigelledine), and saponin (-hederin) are other components found in black cumin seeds. (**Ashraf *et al.*, 2005 and Özel *et al.*, 2009)**. Using natural organic fertilizers as just a practical method for agricultural production to meet the nutrient needs of crops has increased in recent years. Although organic manures only contain small amounts of nutrients, they boost soil fertility and production because they contain growth-promoting agents, including enzymes and hormones. The application of organic manure to soil improved its capacity to retain water, which increased the access of crops access to nutrients. Furthermore, organic manures significantly improve the circumstances of the root rhizobacteria (structure, humidity, etc.), which encourages plant development by increasing the number of microbes. **(Bhuma, 2001; Shaheen *et al.*, 2007; Dada** and **Fayinminnu, 2010 and Gwari *et al.*, 2014)**. As a result of the fat that nutrients in farm manure being delivered more gradually and remain in the soil for a very long time, succeeding crops will continue to benefit from them. **(Ginting *et al.*, 2003)**. Additionally, it can be found in considerable amounts locally and is a less expensive way to increase soil fertility. Organic manur was added to fine sand, which had been lacking in organic compounds, had physical and biological properties, and had increased nitrogen leakage, to increase crop production. **(Awosika *et al.*, 2014)**. According to **Norman (2004)** and **Parnes (2013)**, organic manure can appear from both plant and animal sources. Vegetation, algae, cover plants, leftover crops, biologically fixing nitrogen, mulch, and compost is one example of plant sources. The manure of sheep, pigs, goats, cattle, horses, and poultry are some examples of animal sources. Major and minor nutrients can be found in organic manure from both sources. Environmentally sustainable farming methods for food security require the use of organic and biofertilizers **(Islam *et al.*, 2017)**. Farmers can profit economically and environmentally from using biofertilizers instead of chemical fertilisers by improving the fertility and health of their soil, given its enormous potential, biofertilizer technology now encompasses all significant varieties of bacterial fertilizers **(El-Zeiny *et al.*, 2001)**. Moreover, they are economical and environmentally favorable as natural fertilizers, bio-fertilizers shield the environment from contaminants. Despite the importance of mineral fertilizer, a number of drawbacks have been resolved, such as negative effects on public health and the environment, increased production

costs, and decreased soil fertility. **(Boraste *et al*., 2009)**. Rhizobacteria promote plant growth and fix nitrogen present in the rhizosphere **(Hassan *et al*., 2012)**. Furthermore, the bulk of the growth and production traits of many plants used for medicinal and aromatic purposes increased to their maximum values when nutrient bacteria and phosphorus bacteria were combined. **(Hellal *et al*., 2011; Sakari *et al*., 2012; Hassan *et al*., 2012 and Ali and Hassan, 2014)**. A variety of apiaceous volatile oil and primary ingredient content increased as a result of the application of biofertilizers. Furthermore, leaf nitrogen, phosphate, and potassium content as well as percentage of carbohydrates were also enhanced **(Hassan *et al*. 2012 and Ali and Hassan, 2014)**. The seaweed extract is abundant of the primary minerals phosphate and potassium, in addition to secondary vitamins such as magnesium and calcium, and trace elements zinc, copper, iron, and manganese. The significant concentration, of organic material, microelements, enzymes, essential fats, and growth conditions including auxins, cytokinin, and gibberellins, found in seaweed extract, made it crucial for a variety of crops. **(Crouch and Van Staden, 1994).** Thus, the objective of this study is to examine the impact of fertilization (poultry manure and NPK) and bio-stimulants (seed inoculation with mixed bacteria; (*A. chroococcum*, *B. megatherium* var. *Phosphaticum* and *B. circulans*) alone or even in conjunction with a foliar spray of seaweed extract, as well as their interactions on the growth, yield, fixed and essential oil of black cumin plants under the conditions of the New Valley Governorate.

Materials and Methods

This experiment was carried out on the farm of the Faculty of Agriculture, New Valley University, El-Kharga, Egypt during two successive seasons 2020/2021 and 2021/2022 to study the effect of fertilization (poultry manure in rats 0, 5, 10 and 15 m^3/fed. plus, half the recommended dose of NPK fertilizer) and biostimulants (seed inoculation with a mixture of bacteria; *A. chroococcum*, *B. megatherium* var. *Phosphaticum*, *B. circulas* and/or seaweed extract), as well as the effects of their interactions on the fixed, volatile and fixed yield of black seed. The seeds of black cumin were acquired from the Department of Aromatic and Medicinal Plants in Giza, Egypt. In addition, some poultry dung was collected from the poultry farm at New Valley University, Egypt. The bacterial mixture was obtained from the Egypt National Research Center's Microbiology Division. The UAD Company in Egypt produces a commercial seaweed extract (Oligo-X) that is rich in minerals (K 18.5%, Ca 0.17%, N 1%, Fe 0.06%, Mg 0.42%, and S 2.2%) as well as plant hormones produced by the UAD, Company, Egypt. **Black (1965) and Page *et al*. (1982)** methodologies were used to analyze the physical and chemical parameters of five randomly selected soil samples from the surface of the experimental soil prior to, cultivation, (0-30 cm depth). The results of soil analysis are listed in Table (1).

Table (1): Physicochemical characteristics of the experimental soil during the growing seasons 2020/2021 and 2021/2022.

Soil Properties		Season	
		2020/2021	2021/2022
Physical analysis	Particle size distribution (%)		
	Coarse sand	5.74	5.20
	Fine sand	75.04	75.28
	Silt	13.25	12.90

	Clay	5.97	6.62
	Texture class	Loamy Sandy	Loamy Sandy
Chemical analysis	EC. dsm^{-1} (1:1 ex.)	0.91	0.88
	pH (1:1 w/v)	7.82	7.92
	Organic matter (%)	0.75	0.84
	Saturation capacity (%)	27.49	27.85
	Available nutrients (mg/kg)		
	N	50.4	52.3
	P	6.22	6.85
	K	87.8	85.6

The New Valley University poultry farm provided the poultry manure used in this study. The Nutritional content was determined using the recommended analytical methods and the results are shown in Table (2).

Table (2): Chemical analysis of poultry manure during the 2020/2021 and 2021/2022 seasons.

No.	Component	Poultry manure	
		2020/2021	**2021/2022**
1	EC.dsm^{-1}(1:2 ex.)	3.95	3.83
2	pH (1:2 w/v)	6.42	6.30
3	O.M %	69.39	69.89
4	O.C %	40.26	40.55
5	N %	8.81	8.95
6	C/N ratio	4.56	4.53
7	P %	1.40	1.45
8	K %	3.00	3.05

Experimental design

In this study, a randomized complete block design (RCBD) with three replicates was used to carry out the experiment. The main plot was poultry manure rates (PM0=0, PM1=5, PM2=10, PM3=15 m^3/fed. and NPK in the half recommended doses as follows; ammonium nitrate (33.5%) at 150 kg/fed., calcium superphosphate (15.5% P_2O_5) at 200 kg / fed, and potassium sulfate (48% K_2O) at 37.5 kg / fed). while biotimulants treatments (Control, *A. chroococcum*, *B. megatherium* var. *Phosphaticum*, *B. circulas)* and seaweed extract were assigned as subplots. The black cumin were sowed on 5 November of bath season. Each sub-plot of 3.0 x 2.5 m contained 3 rows and 60 cm apart. Seeds planted at 25 cm spacing. The plants were cut to two plants per hill after 35 days following planting. The seaweed extract was sprayed on the plants three times: 60 days, 75 days and 90 days after cultivation. The seeds were soaked in bacterial solutions for 60 minutes

before being cultivated. All other standard farming practices were followed. Plant height (cm), number of branches/plants, fresh and dry weight of plants (g), number of capsules/plant, seed yield/plant (g) and seed yield/fed. The percentage of fixed oil, the fixed oil yield / plant (ml), and the fixed oil yield / fed were all recorded during the first week of May. Furthermore, percentage of volatile oil, as well as the volatile oil yields/plant in (ml), the volatile oil yield/ fed. (l), and the fixed and volatile oil components.

The mineral fertilizer was derided four repeat times at three-week interval, stated 35 days later. Mature poultry manure was added and mixed with soil before sowing during the preparation of the soil for cultivation.

Volatile and fixed oil %

The **British Pharmacopoeia (1963)** describes a hydro distillation procedure that was used to extract volatile oils from seeds. with the following equation: volatile oil content = oil content in the measuring ÷ tube sample weight x 100

Just before distillation, 50 g of materials were pulverized and added to the extraction device. The proportion of volatile oil was calculated along with the oil output per plant and fed. Using anhydrous sodium sulfate, the volatile oil was dried, and then stored cold and dark until GC-MS analysis. According to the Organization of Official Agricultural Chemists, fixed oil was instead calculated using the Soxhlet apparatus with petroleum ether (BP 40-60 ° C) **(AOAC. 1980).**

The **AOCS (1990)** method was used to extract the fixed oil content of black cumin seeds. Powder was made by grinding the samples at 70 ° C. After separating for 24 hours, 10 grams of material were added to 300 milliliters of diethyl ether in a Soxhlet apparatus. The targeted solvent was recovered from the oil by rotating it after a six-hour. The oil was stored in amber glass vials after separation so that its chemical composition could be identified. Black cumin fixed oil was analyzed using gas chromatography mass spectrometry (GC-MS) as described by **Rezaei-Chiyaneh *et al*. (2021)**. The following formula was used:

$$\text{Seed fixed oil \%} = \left[\frac{\text{Weight of oil}}{\text{Weight of sample}}\right] \times 100 - \%\ \text{seed moisture.}$$

Statistical analysis:

All data obtained were tabulated and statistically analyzed according to **MSTATE-C (1986),** and the means were compared using the LSD test at 5% according to **Mead *et al*. (1993).**

Results and Discussion

1- Characteristics of vegetative growth

According to Table (3), the addition of poultry manure to black seed plants dramatically altered their height, branch number / plant, both fresh and dry weight/plant in the two growth seasons. However, in all seasons, plants that have been treated with half the NPK for the recommended dose grew noticeably taller, had more branches per plant, and had a greater fresh and dry weight/plant compared to control treatment grown without any fertilizers. Clearly, the greatest improvement in plant growth attributes were achieved by applying poultry manure fertilizer according to of 15 m^3/fed., with increases in plant height, number of branches/plant and fresh and dry weight/plant of 76.0-72.4, 34.6-29.3, 25.9-27.6 and 35.9-33.9%, respectively, compared to untreated plants (control) during both growth seasons. For black cumin, **Musa *et al*. (2012) and Shaalan (2005)** have both shown how beneficial organic fertilizer is in boosting growth metrics.

According to data in Table (3) the effects of combined treatments with seaweed extract and bacteria considerably improved plant height, branch number/plant, and both fresh and dry weight/plant of black cumin in contrast to plants that were left untreated (control)

during both seasons. It was discovered that mixed bacteria (*A.chroococcum*., *B. megatherium* var. *Phosphaticum*, *B. circulas*) and spraying seaweed extract according to 2 ml/l on black cumin plants resulted in plants that were taller, had more branches /plant, both fresh and dry weight / plant as ranged 28.2-30.3, 46.7-38.3, 15.1-13.9, and 22.4-15.4% than controls in both seasons, respectively. For black cumin, **Mahdi *et al*. (2012)**, **Ali and Hassan (2014)**, and **Mohamed *et al*. (2020)** found that biofertilization had a beneficial effect on boosting growth indices.

Regarding the impact of the interaction between the components in the study the results of Table (3) showed that plant height, branch count, and the fresh and dry weight/plant of black cumin plants were considerably higher in treated plants than in (control) plants during both growth seasons. It was discovered that cultivating black cumin plants and treating them with poultry manure according to 15 m^3/fed. and inoculating seeds with mixed bacteria and spraying the plant with 2 ml/l of seaweed extract giving the tallest, plants, the highest number of branches / plant and the highest fresh and dry weight/plant such range 133.3-126.5, 120.0-105.8, 55.6-53.8 and 71.5 -64.5% in both seasons, respectively, over the control. Growing characteristics were improved by using organic and biostimulants, as was seen by **Shaalan (2005)**, **Valadabadi and Farahani (2011)**, **Sen *et al*. (2018)** and **Dashti *et al*. (2020)** on black cumin.

This advantageous result may be due to nutrients being delivered consistently and steadily at various periods during the crop growth season. Organic manures applied to the soil improve its quality by feeding the biofertilizers, which promote their growth and help them perform their function more efficiently. By mixing fertilizers, **Iman and Pariari (2007)** and **Farooqui *et al*. (2009)**.

Table (3). Effect of fertilizer in poultry manure and NPK (A) and bio-stimulants seed inoculation with mixed bacteria and foliar spray with seaweed extract (B), on growth parameters of black cumin plants during the 2020/2021 and 2021/2022 seasons.

Factor (A)	2020/2021 season					2021/2022 season				
	Factor (B)									
	Control	SW	MB	MB+SW	Mean	Control	SW	MB	MB+SW	Mean
	Plant height (cm)									
Control	32.00	36.00	38.00	40.00	36.50	34.00	38.00	40.33	43.33	38.91
NPK HR	50.00	53.33	56.66	61.66	55.41	51.00	54.66	59.66	65.33	57.66
PM (1)	43.33	47.66	52.66	57.00	50.16	45.00	50.00	54.66	59.66	52.33
PM (2)	51.66	55.33	58.7	62.33	57.00	53.00	56.66	61.66	67.66	59.75
PM (3)	53.66	62.00	66.66	74.66	64.25	57.33	64.33	69.66	77.00	67.08
Mean	46.13	50.86	54.53	59.13		48.06	52.73	57.20	62.60	
L.S.D at 5%	A: 2.09	B: 0.72		AB: 4.19		A: 2.00	B: 0.68		AB: 4.00	
	Number of branch/plants									
Control	10.00	13.00	15.00	17.00	13.75	11.66	15.00	17.00	19.00	15.66
NPK HR	14.00	16.00	19.00	19.66	17.16	16.00	18.00	19.00	21.00	18.50
PM (1)	13.00	15.00	17.00	19.00	16.00	15.00	16.00	18.00	20.00	17.25
PM (2)	15.00	16.66	19.00	20.66	17.83	17.00	19.00	20.00	22.00	19.50
PM (3)	15.00	18.00	19.00	22.00	18.50	17.00	19.00	21.00	24.00	20.25
Mean	13.40	15.73	17.80	19.66		15.33	17.40	19.00	21.20	
L.S.D at 5%	A: 0.76	B: 0.46		AB: 1.53		A: 0.55	B: 0.46		AB: 1.10	
	Plant fresh weight (g)									
Control	50.45	58.01	60.31	63.49	58.06	51.33	59.02	60.58	62.85	58.44
NPK HR	62.86	65.67	67.86	71.48	66.97	63.91	66.44	68.47	72.16	67.74
PM (1)	61.53	63.28	64.99	67.74	64.38	62.40	64.33	66.02	68.29	65.26
PM (2)	64.95	68.23	69.91	73.90	69.25	65.65	68.30	70.40	74.23	69.64

PM (3)	**68.84**	**70.51**	**74.52**	**78.51**	**73.09**	**69.56**	**73.54**	**76.34**	**78.93**	**74.59**
Mean	**61.72**	**65.14**	**67.52**	**71.02**		**62.57**	**66.32**	**68.36**	**71.29**	
L.S.D at 5%	**A: 1.21**		**B: 0.54**		**AB: 2.42**	**A: 0.97**		**B: 0.54**		**AB: 1.95**
Plant dry weight (g)										
Control	**12.66**	**14.50**	**15.01**	**15.40**	**14.39**	**12.86**	**14.35**	**14.84**	**15.98**	**14.51**
NPK HR	**14.98**	**16.75**	**17.29**	**17.90**	**16.73**	**16.10**	**16.72**	**16.89**	**18.04**	**16.94**
PM (1)	**14.70**	**16.21**	**17.60**	**18.27**	**16.69**	**15.27**	**15.88**	**16.51**	**16.88**	**16.13**
PM (2)	**15.92**	**17.28**	**18.16**	**19.06**	**17.60**	**16.21**	**16.75**	**17.26**	**18.24**	**17.11**
PM (3)	**17.21**	**19.14**	**20.13**	**21.71**	**19.55**	**17.75**	**18.57**	**20.25**	**21.15**	**19.43**
Mean	**15.09**	**16.78**	**17.64**	**18.47**		**15.64**	**16.45**	**17.15**	**18.05**	
L.S.D at 5%	**A: 0.52**		**B: 0.44**		**AB: 1.04**	**A: 0.40**		**B: 0.41**		**AB: 0.81**

2- Yield and its components

2-1- Seed yield

The results of Table (4) showed that poultry manure had a substantial impact on, the number / plant capsule, the seed yield/plant, and seed yield / feed. These effects increased these values during the two growing seasons in contrast to unfertilized plants (the control).

In addition, the highest amount of capsule number/plant, seed yield / plant, and seed yield/fed were generated by treated plants with poultry manure. Furthermore, in both seasons, plants treated with half the required dose of NPK produced noticeably more capsules/plant, seeds/plant, and seeds / feed than plants that were left untreated (control). The highest values of these qualities were obtained from the recorded data when poultry manure was used fertilizer according to the 15 m^3/fed. These traits increased over the control by 36.6–35.4, 39.5–39.4 and 39.3–39.1% respectively of the two growing seasons. The results of the use of organic fertilizers in boosting production metrics were consistent with those reported **Shaalan (2005), Musa *et al*. (2012) on** black cumin.

As shown in Table (4), it is worth noting that the biostimulant therapies have a big impact on capsule number/plant, seed yield / plant, and seed yield/fed. Black cumin seed inoculated with a mixture of bacteria (*A. chroococcum*., *B. megatherium* var. *Phosphaticum*, and *B. circulas*) and using seaweed according to 2 ml/l resulted in increases in capsule number/plant, seed yield/plant and seed yield / feed. an increase of 11.5-11.5, 11.4-12.6, and 11.3-12.3% throughout the course of both growth seasons versus the control plants, respectively.

Similar results on improving yield characteristics were found by **Mahdi *et al*. (2012)**, **Ali and Hassan (2014)**, and **Mohamed *et al*. (2020)** on black cumin.

As shown in Table (4) on the impact of the interaction between seaweed extract treatments, mixed bacterial seed, inoculation, and poultry manure treatments showed that the number of capsules/plants, seed yield / plant and seed yield / feed. of black cumin plants were significantly higher compared to untreated controls in both seasons. The tallest plants, capsule number/plant, seed yield/plant, and seed yield/fed. increased by 59.3-50.0, 63.9-61.3 and 55.6-53.8 over control in two growing seasons, respectively, when poultry manure was applied according to 15 m^3/fed. with a mixture of bacteria and seaweed extract at 2 ml/l. These findings on black cumin were in agreement with those of **Shaalan (2005)**, **Valadabadi and Farahani (2011)**, **Sen *et al*. (2018)**, and **Dashti *et al*. (2020)** and demonstrated the beneficial effects of organic and biostimulant on boosting yield indices.

The beneficial role of organic matter in promoting the growth of black cumin, which is also is evident in an increase in seed output, can be used to explain these outcomes. **El-Sharkawy and Abdel-Razzak (2010)** discovered that HCl includes cytokinins, a chemical that resembles a plant hormone that can help to maintain the balance of nutrients necessary to support growth and production. These

conclusions are confirmed by other papers showing an increase in yield brought on by organic matter **(Abdel-Razzak and El-Sharkawy, 2013; Bakri *et al*., 2015)**. Since organic matter has beneficial effects on growth, biofertilizers have also increased the features of seed production. Logically this improvement the outcome of this. Similar outcomes they have recorded **(Akhani *et al*., 2012; Hassan *et al*., 2012** and **Ali and Hassan, 2014)**.

Table (4). Effect of fertilizer in poultry manure and NPK (A) and bio-stimulants seed inoculation with mixed bacteria and foliar spray with seaweed extract (B), on the yield characters of black cumin plants during the 2020/2021 and 2021/2022 seasons.

Factor (A)	2020/2021 season					2021/2022 season				
	Factor (B)									
	Control	SW	MB	MB+SW	Mean	Control	SW	MB	MB+SW	Mean
	Number of capsules/plants									
Control	59.00	65.00	66.33	69.00	64.83	64.66	66.66	67.66	72.00	67.75
NPK HR	73.00	76.33	79.00	81.00	77.33	75.66	80.00	82.66	86.00	81.08
PM (1)	71.00	72.00	74.00	76.00	73.25	72.00	77.66	79.00	79.33	77.00
PM (2)	80.00	82.00	84.33	88.00	83.58	84.00	88.00	88.66	93.00	88.41
PM (3)	83.00	86.33	91.00	94.00	88.58	87.00	89.00	94.00	97.00	91.75
Mean	73.20	76.33	78.93	81.60		76.66	80.26	82.40	85.46	
L.S.D at 5%	A: 1.10		B: 0.40	AB: 2.21		A: 0.83		B: 0.66	AB: 1.66	
	Seed yield/plant (g)									
Control	19.65	21.52	23.14	23.82	22.03	20.37	21.98	23.08	24.37	22.45
NPK HR	25.89	27.09	27.72	28.47	27.29	27.09	29.08	29.47	30.17	28.95
PM (1)	24.97	25.22	26.41	26.90	25.87	24.5	25.33	26.61	27.34	25.94
PM (2)	27.99	29.18	29.91	31.11	29.55	28.13	29.69	30.70	31.63	30.03
PM (3)	29.41	30.13	31.17	32.21	30.73	29.86	30.67	31.78	32.85	31.29
Mean	25.58	26.63	27.67	28.50		25.99	27.35	28.33	29.27	
L.S.D at 5%	A:0.27		B: 0.22	AB: 0.54		A: 0.22		B: 0.19	AB: 0.45	
	Seed yield/fed. (kg)									
Control	632.0	688.8	740.4	762.3	705.9	652.0	707.0	738.6	779.9	719.4
NPK HR	828.5	866.9	887.2	911.0	873.4	866.9	930.6	943.0	965.4	926.5
PM (1)	799.25	807.0	845.2	860.8	828.0	784.0	810.5	851.7	874.8	830.2
PM (2)	895.6	933.8	957.3	995.8	945.6	910.8	950.1	973.4	1012	961.6
PM (3)	941.2	964.2	997.4	1030	983.4	955.5	981.6	1017	1051	1001
Mean	819.3	852.2	885.5	912.1		833.8	876.0	904.8	936.7	
L.S.D at 5%	A: 8.35		B : 7.77	AB : 16.70		A :6.63		B: 5.56	AB: 13.27	

MB= mixed bacteria (*A. chroococcum, B. megatherium* var. *Phosphaticum, B. circulas*).
SW= seaweed extract (2 ml/l).
NPK_{HR} = NPK at half recommended dose.
PM (1)= 5, PM (2)= 10 and PM (3)= 15 m^3/fed. of Poultry manure.

2-2- Fixed oil yield

The results of Table (5) clearly demonstrated that treatments using poultry manure and just half the recommended amount of NPK fertilizer had a substantial influence on the percentage of fixed oil, fixed oil/plant, and fixed oil / feed. of black cumin plants throughout the two growing seasons. It appears that applying half the allowed amount of NPK and using to fertilize plants poultry manure in plants at all rates brought about a substantial significantly higher the percentage of fixed oil, fixed oil/plant, and fixed oil/fed in all seasons when compared to untreated plants (control). According to results, it can be shown that applying poultry manure at a high rate (15 m 3 / feed.) increased the fixed oil percentage, fixed oil/plant, and fixed oil / feed, which varied between 32.8-30.5,

83.3-81.9, and 83.2-81.9%, control in each of the two experimental seasons. The effectiveness of using organic fertilizers to increase fixed oil parameters supported with those reported with **Shaalan (2005)**, **Valadabadi and Farahani (2011)**, **Ali and Hassan (2014)**, **Sen *et al*. (2018)**, **Dashti *et al*. (2022)** and **Shnrwe and Ahmed (2022)** on black cumin.

According to the findings in Table (5), the combined treatments with seaweed extract and bacteria significantly increased fixed oil %, fixed oil / plant, and fixed oil/fed. of black cumin plants over the course of two seasons compared to the control group (untreated). In general, inoculating black cumin seeds with a mixture of bacteria (*A. chroococcum*, *B. megatherium* var. *Phosphaticum*, and *B. circulas*) and spraying plants with seaweed extract according to 2 ml/l increased the fixed oil %, fixed oil/plant, and fixed / fed. by 28.6 - 26.2, 42.8 - 43.0 and 42.8 - 43.1% over control in each of the two experimental seasons.

Similarly, to the result achieved by bio-stimulants, bio-stimulants had a favourable impact on improving fixed oil parameters by **Valadabadi and Farahani. (2011)**, **Harb *et al*. (2011)**, **Fravani *et al*. (2012)**, **Al-mohammedi *et al*. (2016)**, **Sen *et al*. (2019)**, **Mohamed *et al*. (2020)** and **Moradzadeh *et al*. (2021)** on black cumin.

The results shown in Table (5) regarding the interactions between the treatments with poultry manure, a combination of, bacteria and seaweed extract showed that, in both seasons, the fixed oil %, fixed oil/plant. and fixed oil/fed of black cumin plants rose considerably in comparison to untreated (control) plants. Additionally, the tallest plants, fixed oil %, fixed oil yield/plant, and fixed oil/fed. as ranged from 73.7-70.7, 179.7-175.0, and 179.8-175.1% increase in control in each of the two experimental seasons, respectively, were produced by applying poultry manure according to 15 m^3/fed., inoculating seeds with mixed bacteria, and spraying plants with seaweed extract at 2 ml/l. Our findings on black cumin agreed with those of **Shaalan (2005)**, **Valadabadi and Farahani (2011)**, **Sen *et al*. (2018)**, and **Dashti *et al*. (2020)** about the positive impacts of organic and biostimulants on increasing fixed oil metrics.

Table (5). Effect of fertilizer in poultry manure and NPK (A) and bio-stimulants seed inoculation with mixed bacteria and foliar spray with seaweed extract (B), on fixed oil characteristics of black cumin plants during 2020/2021 and 2021/2022 seasons.

Factor (A)	2020/2021 season					2021/2022 season				
	Factor (A)									
	Control	SW	MB	MB+SW	Mean	Control	SW	MB	MB+SW	Mean
	Fixed oil percentage									
Control	19.96	20.70	21.03	23.43	21.28	21.40	22.50	23.30	24.70	22.97
NPK HR	22.16	22.90	23.13	28.96	24.29	22.90	23.93	24.70	29.43	25.24
PM (1)	21.63	21.93	25.63	25.70	23.47	22.63	23.40	26.30	27.06	24.85
PM (2)	23.20	26.90	26.16	30.13	26.60	24.36	29.03	28.30	30.26	27.99
PM (3)	24.20	25.83	28.30	34.66	28.25	26.03	28.30	29.03	36.53	29.97
Mean	22.23	23.65	24.65	28.58		23.46	25.43	26.32	29.60	
L.S.D at5%	A :0.38		B :0.37		AB: 0.77	A :0.38		B: 0.22		AB: 0.77
	Fixed oil yield/plant (ml)									
Control	3.990	4.456	4.866	5.596	4.727	4.363	4.943	5.380	6.020	5.176
NPK HR	5.740	6.206	6.436	8.246	6.657	6.136	6.963	7.280	8.940	7.330
PM (1)	5.403	5.526	6.506	6.913	6.087	5.533	5.956	6.963	7.400	6.463
PM (2)	6.400	7.753	7.856	8.963	7.743	6.936	8.620	8.690	9.576	8.455
PM (3)	7.090	7.886	8.510	11.160	8.662	7.746	8.680	9.230	12.000	9.414
Mean	5.724	6.366	6.835	8.176		6.143	7.032	7.508	8.787	
L.S.D at5%	A: 0.14		B: 0.10		AB: 0.29	A: 0.09		B : 0.08		AB : 0.19
	Fixed oil yield/fed. (l)									

Control	**127.7**	**142.5**	**155.7**	**179.1**	**151.3**	**139.6**	**158.3**	**172.0**	**192.7**	**165.6**
NPK $_{HR}$	**183.6**	**198.5**	**205.9**	**263.9**	**213.0**	**196.3**	**222.7**	**232.9**	**286.1**	**234.5**
PM (1)	**172.9**	**177.0**	**208.2**	**221.2**	**194.8**	**176.9**	**190.6**	**222.8**	**236.8**	**206.8**
PM (2)	**204.8**	**248.1**	**251.3**	**286.7**	**247.7**	**221.9**	**275.8**	**278.0**	**306.3**	**270.5**
PM (3)	**226.9**	**252.3**	**272.2**	**357.3**	**277.2**	**247.9**	**277.8**	**295.3**	**384.0**	**301.2**
Mean	**183.2**	**203.7**	**218.7**	**261.6**		**196.5**	**225.0**	**240.2**	**281.2**	
L.S.D at$_{5\%}$	**A: 4.64**		**B: 3.29**	**AB: 9.28**		**A :4.23**		**B: 2.83**		**AB: 8.47**

MB= mixed bacteria (*A. chroococcum, B. megatherium* var. *Phosphaticum, B. circulas*).
SW= seaweed extract (2 ml/l).
NPK$_{HR}$ = NPK at half recommended dose.
PM (1)= 5, PM (2)= 10 and PM (3)= 15 m^3/fed. of Poultry manure.

2-3-Volatile oil yield

The recorded information shown in Table (6) demonstrated that during the two growth seasons, the volatile oil %, volatile oil/plant, and volatile oil / feed. of black cumin plants were significantly affected by fertilization treatments using poultry manure and half the recommended amount of NPK fertilizer. It seems that the practice of fertilizing plants with poultry manure at all rates and applying only half the necessary amount of NPK led to a noticeably higher percentage of volatile oil, volatile oil/plant, and volatile oil/fed when compared to untreated plants over both seasons. According to results, this can be shown that the application of poultry manure at a high rate (15 m^3/fed.) led to increases in the percentage fixed oil, fixed oil/plant, and fixed oil/fed., which varied between 94.7-81.8, 169.0-147.1 and 169.2-150.0% in the two seasons, respectively, over control. According to studies on black cumin conducted by **Shaalan (2005)**, **Valadabadi and Farahani (2011)**, **Ali and Hassan (2014)**, **Sen *et al*. (2018)**, **Dashti *et al*. (2022)**, and **Shnrwe and Ahmed (2022)**, organic fertilizers are efficient in increasing volatile oil characteristics.

According to data in Table (6), inoculation of black cumin seeds with a mixture of bacteria and seaweed extract at 2ml/l treatments resulted in significant increases in volatile oil %, volatile oil/plant, and volatile oil/fed. of the treated seeds over the course of two successive seasons. The amount of volatile oil %, volatile oil/plant, and volatile oil/fed increased by 64.38 - 59.38, 84.21 - 76.74, and 82.55 - 77.78 % over control in both seasons, respectively, when black cumin seeds were given a bacteria, inoculation and seaweed extract was sprayed on the plants. Similar to a result reported by **Valadabadi and Farahani (2011)**, **Harb *et al*. (2011)**, **Fravani *et al*. (2012)**, **Al-mohammedi *et al*. (2016**), **Sen *et al*. (2019)**, **Mohamed *et al*. (2020)**, and **Moradzadeh *et al*. (2021)** biostimulants had a favourable impact on improving volatile oil metrics on black cumin.

According to the information in Table (6), the volatile oil %, volatile oil/plant, and volatile oil/fed. of black cumin were much higher compared to those who aren't treated (control) the two seasons as a result of the interaction between poultry manure, inoculation of seeds with mixed bacteria, and plants that were sprayed with seaweed extract treatments. It was found that cultivating black cumin plants and treating them with poultry manure at a rate of 15 m^3/fed., inoculating seeds with mixed of bacteria and black cumin plants sprayed with seaweed extract at 2 ml/l gave the tallest plants, volatile oil %, volatile oil yield/plant and volatile oil/fed as ranged 297.59-233.01, 562.50-433.33 and 542.08-246.24% increase in control in both seasons, respectively. Our findings on black cumin agreed with those of **Shaalan (2005)**, **Valadabadi and Farahani (2011)**, **Sen *et al*. (2018)**, and **Dashti *et al*. (2020)** about the beneficial effects of organic and bio-stimulants on increasing growth metrics.

Table (6). Effect of fertilizer in poultry manure and NPK (A) and bio-stimulants seed inoculation with mixed bacteria and foliar spray with seaweed extract (B), on volatile oil characteristics of black cumin plants during 2020/2021 and 2021/2022 seasons.

Factor (A)	2020/2021 season					2021/2022 season				
	Factor (B)									
	Control	SW	MB	MB+SW	Mean	Control	SW	MB	MB+SW	Mean
	Volatile oil (%)									
Control	0.083	0.130	0.146	0.166	0.131	0.103	0.140	0.163	0.186	0.148
NPK HR	0.150	0.176	0.213	0.236	0.194	0.163	0.186	0.230	0.250	0.207
PM (1)	0.123	0.153	0.170	0.193	0.160	0.133	0.163	0.190	0.210	0.174
PM (2)	0.166	0.200	0.240	0.276	0.220	0.176	0.213	0.250	0.286	0.231
PM (3)	0.210	0.223	0.260	0.330	0.255	0.223	0.240	0.270	0.343	0.269
Mean	0.146	0.1766	0.206	0.240		0.160	0.188	0.220	0.255	
L.S.D at5%	A :0.008		B :0.003		AB: 0.016	A :0.005		B: 0.003		AB: 0.011
	Volatile oil yield /plant (ml)									
Control	0.016	0.028	0.033	0.040	0.029	0.021	0.031	0.037	0.045	0.034
NPK HR	0.039	0.048	0.059	0.069	0.053	0.044	0.053	0.067	0.075	0.060
PM (1)	0.030	0.038	0.044	0.052	0.041	0.032	0.041	0.050	0.057	0.045
PM (2)	0.046	0.058	0.072	0.086	0.065	0.050	0.063	0.076	0.090	0.070
PM (3)	0.061	0.067	0.081	0.106	0.078	0.066	0.074	0.086	0.112	0.084
Mean	0.038	0.048	0.058	0.070		0.043	0.052	0.063	0.076	
L.S.D at5%	A: 0.002		B: 0.001		AB: 0.005	A: 0.002		B: 0.001		AB: 0.004
	Volatile oil yield/fed. (l)									
Control	0.530	0.893	1.060	1.273	0.939	0.686	0.983	1.206	1.460	1.084
NPK HR	1.243	1.533	1.900	2.206	1.720	1.423	1.696	2.170	2.430	1.930
PM (1)	0.960	1.226	1.420	1.663	1.317	1.043	1.330	1.610	1.840	1.455
PM (2)	1.493	1.870	2.306	2.756	2.106	1.606	2.026	2.456	2.903	2.248
PM (3)	1.966	2.150	2.593	3.403	2.528	2.126	2.353	2.746	3.610	2.709
Mean	1.238	1.534	1.856	2.260		1.377	1.678	2.038	2.448	
L.S.D at5%	A: 0.08		B: 0.02		AB: 0.17	A :0.08		B: 0.03		AB: 0.16

MB= mixed bacteria (*A. chroococcum, B. megatherium* var. *Phosphaticum, B. circulas*).
SW= seaweed extract (2 ml/l).
NPK_{HR} = NPK at half recommended dose.
PM (1)= 5, PM (2)= 10 and PM (3)= 15 m^3/fed. of Poultry manure.

3- Fixed oil components

Based on the results of the GC-MS analysis presented in Table (7), the most abundant fatty acids in the fixed oil samples extracted from black cumin plants were palmitic acid, myristic acid, oleic acid, stearic acid, linoleic acid, arachidic acid and linolelaidic acid. The preceding fixed oil components were primarily composed of oleic acid, followed by arachidic acid and stearic acid. A higher concentration of pre-fatty acids was found in the fixed oil component of black cumin plants when seaweed extract was sprayed on them and they were treated with poultry manure.

Table: (7) Effect of the interaction between poultry manure and NPK, and seed inoculation with mixture of bacteria and foliar spray with seaweed extract on fixed oil components of the black cumin as the beginning of bath seasons.

Fatty acids	Relative concentration (%)				
	Control	PM (1) + MB+SW	PM (2) + MB+SW	PM (3) + MB+SW	NPK HR + SW
Linoleic acid (C18:2c)	52.77	55.73	53.83	57.85	56.38
Oleic acid (C18:1c)	18.55	22.11	19.06	22.94	22.68
Palmitic acid (C16:0)	11.57	13.35	12.23	13.38	12.60

γ- Linolenic acid (C18:3)	**0.00**	**0.00**	**5.32**	**0.13**	**0.29**
Stearic acid (C18:0)	**2.33**	**2.87**	**2.48**	**2.42**	**2.59**
Arachidic acid (C20:0)	**2.22**	**2.77**	**2.66**	**2.33**	**2.89**
Linolelaidic acid (C18:2t)	**0.00**	**0.00**	**2.06**	**0.00**	**0.05**
Palmitoleic acid (C16:1)	**0.00**	**0.00**	**1.93**	**0.00**	**0.18**
Heptadecanoic acid (C17:0)	**0.00**	**1.80**	**0.00**	**0.00**	**0.95**
Elaidic acid (C18:1t)	**0.00**	**0.00**	**0.44**	**0.98**	**1.03**
Myristic acid (C14:0)	**0.0**	**0.77**	**0.0**	**0.0**	**0.15**
Cis-10-Heptadecanoic acid (C17:1)	**0.19**	**0.59**	**0.00**	**0.00**	**0.21**

MB= mixed bacteria (*A. chroococcum, B. megatherium* var. *Phosphaticum, B. circulas*).SW= seaweed extract (2 ml/l).
NPK_{HR} = NPK at half recommended dose.
PM (1)= 5, PM (2)= 10 and PM (3)= 15 m^3/fed. of Poultry manure

4- Volatile oil components

Twenty-one components were identified in volatile oil extracted from black cumin seeds, which accounted as a percentage of total components and belong to two major chemical groups. Monoterpene hydrocarbons (MH) were the main class of the arranged (80.96 to 91.54%), and sesquiterpene hydrocarbons (SH) were from (8.48 to 19.04%), as shown in (Table 5). The main constituents of volatile oils in black cumin seed detected by GC/MS were p-cymene from (29.05 to 35.89%), γ-Terpinene from (5.15 -to 13.32%), β-Thujene analysis (10.67 to 13.59%), trans-4-methoxy thujane (1.42 to 8.32%), longifolene from (420 to 6.28%), carvacrol (3.97 to 5.44%), and d-limonene from (3.39 to 8.72%). Furthermore, the highest value of the main components of volatile oil was obtained from poultry manure at rate (5m^3/fed.) and seed with mixed bacteria and sprayed with seaweed extract PM (1) + MB + SW compared to other volatile oil at the same treatment, p-Cymene (35.89%).

The application of biofertilizers boosted phosphorus uptake, which is a crucial component of phospholipids, coenzymes, phosphoproteins, and nucleic acids. The most significant phosphate group one molecule with pyrophosphate linkages is adenosine triphosphate (ATP). The pyrophosphate bound in ATP is created using the energy acquired during respiration or during photosynthesis. An energy can then be transferred in this form to a variety of ongoing activities, including activation absorption and the synthesis of different chemical molecules, including volatile oils **(El-Ghadban *et al*. 2003)**.

Table: (8) Effect of the interaction of poultry manures and some biofertilizers on essential oils components of black cumin plants during the 2021/2022 season.

No	Compound	RT	Treatments			
			Control + BM	PM (1) + MB+SW	PM (2) + MB+SW	NPK_{HR} + SW
1	p-Cymene	**8.705**	**30.38**	**35.89**	**29.05**	**33.59**
2	γ-Terpinene	**9.609**	**13.32**	**5.15**	**9.24**	**13.26**
3	β-Thujene	**6.199**	**10.67**	**13.59**	**11.97**	**12.51**
4	trans-4-methoxy thujane	**11.32**	**7.89**	**8.32**	**6.05**	**1.24**
5	Longifolene	**19.153**	**6.12**	**6.28**	**4.2**	**5.46**
6	Carvacrol	**16.407**	**5.44**	**4.58**	**5.41**	**3.97**
7	D-Limonene	**8.785**	**3.39**	**4.73**	**8.72**	**3.61**

8	β-Pinene	**7.429**	**3.24**	**4.08**	**3.39**	**3.74**
9	α-Pinene	**6.365**	**2.94**	**3.87**	**3.44**	**3.34**
10	Thymoquinone	**14.976**	**2.38**	**1.34**	**0.5**	**0.59**
11	Ledol	**33.373**	**2.08**	**0.00**	**0.00**	**0.00**
12	Sabinene	**7.337**	**1.69**	**2.06**	**1.67**	**1.76**
13	β-Cyclocitral	**13.689**	**1.49**	**1.27**	**0.62**	**1.34**
14	α-Longipinene	**17.69**	**1.48**	**1.45**	**0.94**	**1.24**
15	cis-4-methoxy thujane	**10.668**	**1.47**	**1.48**	**1.04**	**0.54**
16	(-)-Carvone	**14.828**	**0.93**	**1.21**	**5.8**	**1.02**
17	Terpinen-4-ol	**12.974**	**0.86**	**1.36**	**0.86**	**0.45**
18	Hydrothymoquinone	**22.85**	**0.84**	**0.45**	**3.84**	**0.5**
19	Hydrothymoquinone	**22.85**	**0.84**	**0.45**	**3.84**	**0.5**
20	Apiol	**24.395**	**0.77**	**0.96**	**0.64**	**1.94**
21	Cosmen-2-ol	**12.63**	**0.44**	**0.57**	**0.94**	**7.63**
Total identified compounds			**100**	**100**	**100**	**100**
Monoterpene hydrocarbons			**91.54**	**80.96**	**87.77**	**91.54**
Sesquiterpene hydrocarbons			**8.48**	**19.04**	**12.23**	**8.48**

MB= mixed bacteria (*A. chroococcum, B. megatherium* var. *Phosphaticum, B. circulas*).
SW= seaweed extract (2 ml/l).
NPK_{HR} = NPK at half recommended dose.
PM (1)= 5, PM (2)= 10 and PM (3)= 15 m^3/fed. of Poultry manure.

Conclusion

The application of poultry manure at a rate of (15m^3/fed.) in combination with seed inoculation with bacteria (*A. chroococcum, B. megatherium* var. *Phosphaticum* and *B. circulas*) and sprayed with seaweed extract at a rate of (2ml/l) caused a huge rise in both vegetative growth traits and crop yield in *Nigella sativa*. The essential oils also significantly increased under New Valley conditions.

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تأثير التسميد والمحفزات الحيوية على نمو وإنتاجية نباتات حبة البركة

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أجريت هذه التجربة حقليّة خلال الموسمين المتعاقبين ٢٠٢١/٢٠٢٠-٢٠٢٢/٢٠٢١ م لدراسة تأثير التسميد (سماد الدواجن بمعدلات صفر ، ٥ ، ١٠، ١٥ م٣/ فدان بالإضافة إلي نصف المعدلات الموصى بها من الأسمدة الكيماوية) ، و المحفزات الحيوية (تلقيح البذور بالمخصبات الحيوية بخليط من بكتيريا: الازوتوباكتر كروكوكيم ، باسللس ميجاثيريم و باسللس سيركيولانس سوأ بمفردها أو مع مستخلص الطحالب البحرية بتركيز (٢ ملي/ ا لتر) ، وكذلك تفاعل بينهما علي النمو ومحصول الزيت الأساسي و الثابت علي نباتات حبة البركة .لوحظت أعلى القيم لجميع المعايير عندما حصلت النباتات علي أعلي معدل من سماد الدواجن (١٥م٣/الفدان).,فيما يتعلق بالتلقيح البذور بخليط من البكتيريا بمفرده أو مع الرش الورقي للنباتات بمستخلص الطحالب البحرية ، لوحظ أن جميع المعاملات المدروسة قد أدت إلى زيادة معنوية في طول النبات ، وعدد الأفرع ، و وزن النبات الطازج والجاف ، وعدد الكبسولات ، ومحصول البذور ومحصول الزيت الثابت و المتطاير . هذا وقد تم الحصول على أعلى قيم للصفات السابقة عند تلقيح البذور بمزيج بكتيري بالإضافة إلي مستخلص الطحالب البحرية كإضافة علي الأوراق . كشفت نتائج تحليل الزيت الثابت والمتطاير أن هناك ٢١ مكوناً تم تحديدها في الزيت المتطاير. في معظم الحالات يتم تطبيق أعلي معدل من روث الدواجن (١٥م٣/الفدان). بإضافة إلي تلقيح البذور بخليط البكتريا والتطبيق الورقي لمستخلص الطحالب البحرية كان أكثر التوليفات فاعلية.

الكلمات الإفتتاحية: روث الدواجن ، سماد عضوي ، منشطات حيوية ، مستخلص أعشاب بحرية ، حبة البركة.