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EFFECT OF SOME SAFE AGRICULTURAL TREATMENTS ON GROWTH AND PRODUCTIVITY OF *NIGELLA SATIVA* L. PLANTS UNDER SOUTH SINAI CONDITIONS

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A field experiment was conducted in a private farm at Tour Sinai, South Sinai, during two consecutive winter seasons of 2019/2020 and 2020/2021 to study the influence of bio-fertilization treatments (with and without bio-fertilizer) and bio-stimulant treatments, which contain 'marine algae extract, humic acid and some micro elements', on vegetative and yield traits as well as chemical constituents of black cumin (*Nigella sativa* L.) plants. The bio-stimulants were applied as control, 5 ml/l and 10 ml/l as foliar spray, 5 ml/l and 10 ml/l as soil drench. From the results it was observed that, all growth traits, yield components, oil production as well as chemical components in seeds were affected significantly by the bio-fertilizer treatment. Also, all bio-stimulant treatments showed significant differences in this regard in comparison with control treatment. The superior interaction treatment in this respect was that the combination between the bio-fertilizer and bio-stimulant at 10 ml/l as foliar spraying, which recorded a significant increase and the highest values in this concern. Also, scored the major components of the essential oil which were lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid and eicosanoid acid, respectively.

Keywords: *Nigella sativa*, bio-fertilizers, bio-stimulant, growth, seed and oil yields, fixed oil composition

INTRODUCTION

Nowadays, there is a trend in an increment of herbal therapy by substituted medicine. So, the production of medicinal plant all over the world became an essential goal to meet to avoid the side effects of chemical therapy on human health through using of the medical herbs. Also, the use of Good Agricultural Practices (GAP) in sowing such crops could provide higher income to the producers, in comparison with several traditional crops (Hassan et al., 2012).

Black cumin (*Nigella sativa*, L.) is one of fundamental medicinal plants used in this regard, it belongs to Ranunculaceae family. The main products of back cumin are seeds and its fixed oil which reached to 30-35% and used for some of pharmaceutical and food industries (Ramadan, 2007 and Lutterodt et al., 2010). *Nigella sativa* seeds and its fixed oil show an extensive range of antitumor, antibacterial, anti-inflammatory, muscle relaxant, hypoglycemic as well as immune-stimulant activities (Shabnam et al., 2012).

Although the importance of chemical fertilizers, several limitations have been raised such as their adverse influences on the public health, environment, raising the cost of production as well as deterioration of soil fertility (Boraste et al., 2009). So, it is very essential to find different methods for providing nutrients to the growing plant to challenge the previous problems. Nowadays, numerous researchers reveal the utilization of organic and bio-fertilization as promising alternative nutrition which utilized without any harmful effects on environment with increasing the soil fertility (Yasin et al., 2012).

Plant Growth Promoting Rhizobacteria (PGPR) are a group of bacteria that actively colonize plant root and improving plant growth (Deshwal et al., 2011). The mechanisms of PGPR directly related to the plant growth are production of phytohormone, enhancing plant nutrition by solubilizing of minerals such as phosphorus and iron, siderophores production as well as enzymes, decreasing of ethylene levels and induction of systemic resistance. The indirect benefit of PGPR on plant growth by the bio-control of deleterious microorganisms or root pathogens that prevent plant growth, parasitism, including antibiotic and production of hydrogen cyanide, competition for nutrients and places within the rhizosphere, synthesis of extracellular enzymes to hydrolyze the fungal cell wall and decreasing pollutant toxicity (Bhattacharyya and Jha, 2012).

Agricultural bio-stimulants contain microorganisms and many ingredients that improve plant growth when addition to plants or the rhizosphere, promotion natural processes to raise nutrient uptake, nutrient efficiency, tolerance to abiotic stress as well as crop quality as clear by EBIC (European Biostimulants Industry, 2012a). Bio-stimulants increase plant growth and improvement throughout several mechanisms including: developing yield and improved crop quality; raising plant tolerance, recovery from abiotic stresses, easing nutrient uptake, translocation and utilize, improving some of the soil physicochemical properties and promoting the development of complementary soil micro-organisms (European Biostimulants Industry, 2012b).

Marin algae are multi-cellular, macroscopic organisms produce in coastal, marine ecosystems and are a rich source of polyunsaturated fatty acids (PUFAs), polysaccharides, enzymes as well as bioactive peptides among others (Shukla et al., 2016 and Okolie et al., 2018). In the same time, marine algae resources are essential sources to bio-stimulants of plant and are generally used to improve crops productivity (Du Jardin, 2015 and Van Oosten et al., 2017).

Mora et al. (2014) found that, humic can also counteract environmental stress, reducing the absorption of toxic elements and promoting the absorption of essential nutrients and minerals under stressful conditions. Recently, humic acid-rich materials have been widely used as soil conditioners and growth stimulator.

Tour Sinai is a promising land in Egypt, so the application of GAP is crucial for production of agriculture there. To date, little information is available on the agricultural practices of black cumin plants under South Sinai conditions. Therefore, this study aimed to investigate the effect of bio-fertilization and bio-stimulation treatments on growth, seeds yield, fixed oil yield, fixed oil components and some chemical constituents of *Nigella sativa* plants under South Sinai conditions.

MATERIALS AND METHODS

The present study was conducted in a private farm, Tour Sinai, South Sinai Governorate, during the two successive seasons of 2019/2020 and 2020/2021 to study the influence of bio-fertilization [without bio-fertilizer (F0) and with bio-fertilizer (F1)] and bio-stimulant which contain 'marine algae, humic acid and some micro elements' at two concentrations (5 and 10 ml/l) as a foliar spraying or soil drench on vegetative and yield characters as well as chemical constituents of *Nigella sativa* (black cumin) plants.

Seeds of *N. sativa* were obtained from Department of Medicinal and Aromatic Plants, Agricultural Research Center (ARC), Dokky, Giza, Egypt and sown directly in soil on 5th and 7th November during the first and second seasons, respectively.

Table (1) shows the mechanical and chemical properties of the used soil according to Page et al. (1984). The experiment irrigation system was drip irrigation with the drippers (4 l/h) for one hour twice every week. The chemical analysis of the used water is shown in Table (2). The experimental unit contains four rows each of 7.5 m length and 50 cm width and the distance between hills within the row was 30 cm, where the area of each plot was 15 m^2. Plants were thinned after complete germination (21 days after sowing) leaving two plants / hill.

Table (1). Physical and chemical properties of the experimental soil.

Soil depth (cm)	Texture class	pH of soil paste	EC (dSm^{-1})	Soluble anions (me/l)			Soluble cations (me/l)			
				HCO_3^-	SO_4^{--}	Cl^-	Ca^{++}	Mg^{++}	Na^+	K^+
0-30	Sandy loam	7.24	4.36	2.10	17.65	23.64	8.23	12.56	20.2	2.4

Table (2). Chemical analysis of the irrigation water.

pH	EC (dSm^{-1})	Soluble anions (me/l)			Soluble cations (me/l)			
		HCO_3^-	SO_4^{--}	Cl^-	Ca^{++}	Mg^{++}	Na^+	K^+
7.88	0.77	1.44	1.89	4.37	2.63	3.86	0.78	0.46

The experiment included 10 treatments which were the combination between two bio-fertilization treatments [without bio-fertilizer (F0) and with bio-fertilizer (F1)] and five bio-stimulants [control, 5 ml/l as foliar spray (B1), 10 ml/l as foliar spray (B2), 5 ml/l as soil drench (B3) and 10 ml/l as soil drench (B4)]. The biological fertilizer and bio-stimulant were obtained from Department of Soil Fertility and Microbiology, Desert Research Center.

The used bio-fertilizer consists of the mixture between *Azotobacter chroococcum* as nitrogen fixer, *Bacillus megaterium* for phosphate solubilizing and *Bacillus circulans* for potassium solubilizing, strains were used for plants inoculation at the rate of 10^8 colony forming units (cfu/ml). The bio-fertilizer was added as a soil drench after 30, 60 and 90 days of sowing date.

The used bio-stimulant consists of the mixture between marine algae extract, humic acid at 1.5 ml/l and some of micro elements at 0.5 mmol/l for each of Fe, Zn and Mn. Bio-stimulant was used as a foliar spray or soil drench in three times after 45 days of sowing date and repeated three times every 21 days. The chemical analysis of the used algae extract contents of bio-stimulant are shown in Table (3). All agricultural practices were carried out as recommended.

Table (3). Chemical analysis of marine algae extract.

Parameters	**N**	**P**	**K**	**S**	**Mg**	**Fe**	**Mn**
Concentration (ppm)	19.3	9.6	25.1	3.2	19	14	15.9
Parameters	**Zn**	**Cu**	**Auxin**		**Gibberellin**	**Cytokinin**	
Concentration (ppm)	4.1	2.5	4.3		10.2	21.1	

Recorded data were as follows:

1. Growth Traits

At harvesting stage, from each treatment, ten random plants were used as samples to determine the following growth traits: plant height (cm), number of branches / plant and shoot fresh and dry weights/plant (g).

2. Yield Characters

Capsules number /plant, yield of seeds /plant (g) and yield of seeds / feddan (kg).

3. Fixed Oil Components

- Fixed oil percentage of black cumin seeds was determined by Soxhlet Manufacture using petroleum ether (BP 40-60°C) as solvent according to the Association of Official Agricultural Chemists (A.O.A.C., 1984). Regarding the fatty acid composition of the fixed oil, the methyl ester of fatty acids was prepared using benzene: methanol: concentrated sulfuric acid (10: 8: 4) and methylation was carried out for one hour at 80-90ºC.
- Fixed oil yield/plant (ml) was calculated by multiplying fixed oil percentage by seed yield per plant.
- Fixed oil yield/feddan calculated by oil yield/plant × number of plants/feddan.
- The fixed oil of black cumin seeds obtained from treatments during the second season was analyzed by using Gas liquid chromatograph (GLC) / PYE unicam PRO-GC equipped with a flame ionization detector for separation of fixed oil constituents:

The analysis conditions were as follows:

Column: OV-17 (methyl phenyl silicone). Dimensions 1.5X4 mm. Temperature programing: Initial time 5 min, final temperature 200°C, initial temperature 70°C, final temperature 20°C /min. Rate 8°C / min. Injector 250°C. Detector 300°C. Gases flow rate: N_2 30 ml/min. H_2 33 ml/min. Air 330 ml/min. Chart speed was 0.4 cm/min.

4. Chemical Constituents

Total chlorophyll content (mg/g) was estimated in fresh leaves according to Sadasivam and Manickam (1992). Extraction in acetone was repeated until all pigments were extracted. The absorbance of extracts was determined by a spectrophotometer. Total carbohydrates in black cumin seed were estimated (Chaplin and Kennedy, 1994). Total nitrogen, total protein, total phosphorus and potassium percentages were estimated in seeds according to James (1995), Hucker and Catroux (1980) and Cottenie et al. (1984), respectively.

Design and statistical analysis

The experimental layout was factorial experiment between bio-fertilization and bio-stimulant treatments in split plot design (Main plots were consisted of bio-fertilization and sub plots contained the bio-stimulant) with three replicates. Data of this experiment were statistically analyzed and considered significant when the differences between the means of the treatments were more than L.S.D. at the 5% level by using computer program of Statistix version 9 (Analytical Software, 2008).

RESULTS

1. Effect of Bio-fertilization, Bio-stimulant and Their Interaction Treatments on Growth Traits

The results in Table (4) indicate that, the bio-fertilizer treatments recorded significant differences in all parameters under the study. However, black cumin plants which treated with bio-fertilizer gave higher values in plant height, number of branches as well as shoots fresh and dry weights per plant than untreated plants during both seasons. On the other hand, all growth parameters decreased significantly in untreated plants.

In addition, all bio-stimulant treatments [5 ml/l (B1) and 10 ml/l (B2) as foliar spray, 5 ml/l (B3) and 10 ml/l (B4) as soil drench] significantly increased the above-mentioned traits compared with control treatment. The treatment of bio-stimulant at 10 ml/l as foliar spray (B2) was the superior in this respect and gave significant increase in plant height and shoots fresh and dry weight /plant, which recorded 59.33, 60.50 cm; 47.47, 52.31 g and 14.41, 15.87 g in the first and second seasons, respectively. But, branches number/plant gave insignificant effects between all of bio-stimulant treatments.

Also, plant height, number of branches / plant as well as shoots fresh and dry weights/plant were significantly affected by the combination between bio-fertilization and bio-stimulant applications. Generally, the maximum values of all growth parameters were achieved when treated black cumin plants by bio-fertilizer (F1) with bio-stimulant at 10 ml/l as foliar spraying (B2), followed by F1 with bio-stimulant at 10 ml/l as soil drench (F4). The minimum values in this respect were recorded in interaction treatment between F0 (without bio-fertilizer) and control, the same behavior was recorded in both seasons.

Table (4). Effect of bio-fertilizer and bio-stimulator levels as well as their interaction treatments on growth traits of black cumin (*Nigella sativa*) during the two seasons (2019/2020 and 2020/2021).

Treatments	*F0	F1	Means	F0	F1	Means
	First season			Second season		
	Plant height (cm)					
Control	***50.17i	52.33i	51.25E	51.33i	52.67h	52.00E
****B1**	54.67g	59.00c	56.83C	55.50f	62.17c	58.83C
B2	56.33e	62.33a	59.33A	56.50e	64.50a	60.50A
B3	53.47h	58.33d	55.75D	54.67g	58.00d	56.33D
B4	55.50f	59.50b	57.50B	56.17e	62.67b	59.42B
Means (F)	53.97B	58.30A		54.83B	60.00A	
	Number of branches					
Control	9.33e	10.67cd	10.00D	9.67g	10.00fg	9.83C
B1	11.67c	13.33b	12.50AB	11.83cd	12.67bc	12.25A
B2	10.67cd	15.33a	13.00A	10.67ef	13.67a	12.17A
B3	10.33de	11.67c	11.00C	10.67ef	11.33de	11.00B
B4	10.67cd	13.67b	12.17B	12.83ab	12.67bc	12.75A
Means (F)	10.53B	12.93A		11.13B	12.07A	
	Shoots fresh weight /plant (g)					
Control	26.58J	30.61i	28.59E	28.50j	31.51i	30.00E
B1	33.51g	46.68c	40.09C	36.49g	52.08c	44.28C
B2	42.83e	52.11a	47.47A	45.34e	59.29a	52.31A
B3	32.30h	45.40d	38.85D	33.22h	49.89d	41.55D
B4	41.37f	48.68b	45.02B	43.89f	53.42b	48.65B
Means (F)	35.32B	44.69A		37.48B	49.24A	
	Shoots dry weight /plant (g)					
Control	8.58i	9.56h	9.07E	9.20j	9.84i	9.52E
B1	10.44f	14.34c	12.39C	11.38g	15.82c	13.60C
B2	13.39d	15.43a	14.41A	14.17e	17.56a	15.87A
B3	10.14g	14.09c	12.11D	10.42h	15.43d	12.93D
B4	12.99e	14.67b	13.83B	13.77f	16.33b	15.05B
Means (F)	11.11B	13.62A		11.79B	14.99A	

*F0= without bio-fertilizer, F1= with bio-fertilizer, **B= bio-stimulant B1= 5 ml/l as foliar spray, B2= 10 ml/l as foliar spray, B3= 5 ml/l as soil drench, B4= 10 ml/l as soil drench, ** *Means having the same letter(s) within the same column are not significantly different according to LSD for all pairwise comparisons test at 5% level of probability.

2. Effect of Bio-fertilization, Bio-stimulant and Their Interaction Treatments on Yield Components

The obtained data in Table (5), demonstrate that, black cumin capsules number/plant as well as yield of seeds/plant and /feddan recorded significantly increased by the application of bio-fertilization compared to without bio-fertilization treatment in the two seasons. Seeds yield/feddan were 33.07 and 27.23% in 1st and 2nd seasons, respectively. On the other hand, all bio-stimulant treatments significantly increased all recorded yield parameters (capsules number/plant and seeds yield/ plant as well as /feddan) as compared to control treatment in both seasons. The highest value in capsules number/plant observed from the treatment of B2 which recorded 50.33 and 52.33 in the two seasons, respectively, and gave significant increase compared with the other treatments under this study. In addition, seeds yield/plant and feddan recorded the maximum values from the same treatment (B2) followed by B4 during the two seasons.

The interaction treatment between bio-fertilizer (F1) and 10 ml/l bio-stimulant as foliar spray (B2) was the superior treatment in this respect and showed significant increase in comparison with other interaction treatments under this study in the first and second seasons. Under each bio-fertilization treatment, capsules number/plant and yield of seeds/plant and feddan were increased by increasing concentrations of bio-stimulant. Capsules number per plant recorded the highest values (59.33 and 60.33) in both seasons from the interaction treatment between F1 and B2. Furthermore, yield of seeds per plant and feddan obtained the maximum numbers (16.83, 18.35 g/plant and 942.67, 1128.00 kg/feddan) from the interaction treatment between F1 and B2 during the first and second seasons, respectively.

3. Effect of Bio-fertilization, Bio-stimulant and Their Interaction Treatments on Fixed Oil Production

It was clear from data in Table (6) that, the treatments of bio-fertilization (F1) tended to an increase in all fixed oil parameters (fixed oil percentage as well as fixed oil yield/plant and fixed oil yield/feddan) compared to F0 treatment. The averages of percentage increment of fixed oil yield/feddan were 42.92 and 37.01 in both seasons, respectively.

Meanwhile, all treatments of bio-stimulant application (B1, B2, B3 or B4) led to increase in this regard compared with untreated plants during both seasons. The largest values of these parameters were obtained from the treatment of foliar application by bio-stimulant at 10 ml/l in both seasons in comparison with the other treatments under study. Furthermore, all bio-stimulant treatments gave significant increase in fixed oil percentage as well as fixed oil yield per plant and feddan compared with control treatment during the two seasons.

Data in the same table clearly show that, fixed oil percentage, fixed oil yield per plant as well as per feddan of black cumin plants significantly increased by all interaction treatments between bio-fertilization (F0 or F1) and bio-stimulant (B1, B2, B3 or B4) compared to the control during the two seasons. The highest values in this regard were resulted from the interaction treatment between F1 by B2 which recorded 26.98, 27.31% and 4.54, 5.02 ml/plant as well as 254.41, 280.82 l/feddan in the two seasons, respectively.

Table (5). Effect of bio-fertilizer and bio-stimulator levels as well as their interaction treatments on yield components of black cumin (*Nigella sativa*) during the two seasons (2019/2020 and 2020/2021).

Treatments	Number of capsules /plant					
	*F0	F1	Means	F0	F1	Means
	First season			Second season		
Control	32.00j	33.33i	32.67E	30.00i	35.66h	32.83E
****B1**	38.00g	48.33c	43.16C	39.00g	49.66c	44.33C
B2	41.33e	59.33a	50.33A	44.33e	60.33a	52.33A
B3	35.33h	45.67d	40.50D	36.66h	47.00d	41.83D
B4	39.67f	50.33b	45.00B	42.00f	52.00b	74.00B
Means (F)	37.27B	47.40A		38.40B	48.93A	
	Yield of seeds / plant (g)					
Control	7.79h	8.70gh	8.25E	9.11j	9.87i	9.49E
B1	10.04f	13.37c	11.71C	11.00g	14.24c	12.62C
B2	11.47d	16.83a	14.15A	13.41e	18.35a	15.88A
B3	9.46g	12.05d	10.75D	10.71h	13.91d	12.31D
B4	10.63e	14.77b	12.70B	11.93f	15.09b	13.51B
Means (F)	9.88B	13.14A		11.23B	14.29A	
	Yield of seeds / fed.(kg)					
Control	436.43h	487.39g	467.91	510.20j	552.70i	531.44
B1	562.43f	748.53c	655.48	616.40g	797.40c	706.91
B2	642.13d	942.67a	792.40	751.30e	1028.00	889.65
B3	529.57g	674.80d	602.19	599.90h	779.10d	689.55
B4	595.09e	826.93b	711.01	668.10f	845.20b	756.65
Means (F)	553.13	736.06A		629.18	800.50A	

*F0= without bio-fertilizer, F1= with bio-fertilizer, **B= bio-stimulant B1= 5 ml/l as foliar spray, B2= 10 ml/l as foliar spray, B3= 5 ml/l as soil drench, B4= 10 ml/l as soil drench, ** *Means having the same letter(s) within the same column are not significantly different according to LSD for all pairwise comparisons test at 5% level of probability.

Data in Table (7) indicate that, the fixed oil constituents were affected by the interaction treatments between bio-fertilizer with bio-stimulant at 5 and 10 ml/l as foliar spraying or soil drench. Nine components were identified in the fixed oil of black cumin under different treatments (lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid and eicosanoic acid). Linoleic acid, oleic acid and palmitic acid were the major components which recorded 37.47%, 28.87 and 12.99 as the highest values in this study. Linoleic acid obtained the highest value (37.47%) from the interaction treatment between F1 and B2 but the lowest value (35.10%) was recorded from control treatment. Meanwhile, the maximum value for oleic acid (28.87%) was observed from

F1×B3 treatment while, the minimum value (26.17%) was showed by F1×B1 treatment. Also, palmitic acid reached to highest percentage (12.99%) from control treatment and the lowest percentage (11.24) at F1×B4 (combination between bio-fertilizer and bio-stimulant at 10 ml/l as soil drench) treatment. Other components were present in amounts less than 5% in most treatments.

Table (6). Effect of bio-fertilizer and bio-stimulator levels as well as their interaction treatments on fixed oil production of black cumin (*Nigella sativa*) during the two seasons (2019/2020 and 2020/2021).

Treatments	Fixed oil percentage					
	*F0	F1	Means	F0	F1	Means
	First season			Second season		
Control	***22.65j	23.18i	22.92E	22.38j	22.87i	22.62E
****B1**	24.36g	26.18c	25.27C	24.50g	26.78c	25.64C
B2	25.22e	26.98a	26.10A	25.58e	27.31a	26.45A
B3	23.52h	25.59d	24.55D	24.21h	26.31d	25.26D
B4	24.58f	26.56b	25.57B	24.93f	26.98b	25.95B
Means (F)	24.07B	25.70A		24.32B	26.05A	
	Yield of fixed oil / plant (ml)					
Control	1.77h	2.02gh	1.89E	2.04j	2.25i	2.14E
B1	2.45f	3.50c	2.98C	2.69g	3.81c	3.25C
B2	2.89d	4.54a	3.72A	3.43e	5.02a	4.22A
B3	2.22g	3.08d	2.65D	2.59h	3.66d	3.12D
B4	2.61e	3.92b	3.27B	2.97f	4.07b	3.52B
Means (F)	2.39B	3.41A		2.76B	3.76A	
	Yield of fixed oil / fed. (L)					
Control	98.86h	112.98gh	105.92E	114.20j	126.46i	120.33E
B1	137.01f	195.97c	166.49C	151.01g	213.59c	182.30C
B2	161.96d	254.41a	208.19A	192.24e	280.82a	236.53A
B3	124.57g	172.69d	148.63D	145.26h	205.05d	175.16D
B4	146.31e	219.63b	182.97B	166.59f	228.07b	197.33B
Means (F)	133.74B	191.14A		153.86B	210.80A	

*F0= without bio-fertilizer, F1= with bio-fertilizer, **B= bio-stimulant, B1= 5 ml/l as foliar spray, B2= 10 ml/l as foliar spray, B3= 5 ml/l as soil drench, B4= 10 ml/l as soil drench, ** *Means having the same letter(s) within the same column are not significantly different according to LSD for all pairwise comparisons test at 5% level of probability.

Table (7). Effect of bio-fertilizer and bio-stimulator levels as well as their interaction treatments on fixed oil components of black cumin seeds (*Nigella sativa* L.) during the second season (2020/2021).

Treatments		Lauric acid	Myristic acid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Arachidic acid	Eicosanoic acid
Bio-fertilizer (F)	**Bio-stimulant (B)**									
***F0**	**Control**	0.68	0.39	12.99	0.78	27.09	35.10	4.17	4.68	1.96
	****B1**	0.51	0.44	12.72	0.71	26.98	35.52	4.48	4.67	1.88
	B2	0.53	0.49	12.74	0.72	27.11	36.89	4.52	4.72	1.52
	B3	0.54	-	12.96	-	28.19	36.17	4.64	4.52	1.64
	B4	-	0.52	12.78	-	27.23	36.23	4.52	4.57	1.52
F1	**Control**	0.54	0.46	12.11	-	28.42	36.21	4.59	4.59	1.67
	B1	-	0.51	12.24	0.76	26.17	37.24	4.67	4.64	1.72
	B2	0.58	0.54	12.87	0.81	28.18	37.47	4.72	3.53	-
	B3	0.61	0.57	12.22	-	28.87	35.95	4.52	4.62	1.62
	B4	-	0.63	11.24	-	28.57	36.89	4.74	3.67	1.74

*F0= without bio-fertilizer, F1= with bio-fertilizer, **B= bio-stimulant, B1= 5 ml/l as foliar spray, B2= 10 ml/l as foliar spray, B3= 5 ml/l as soil drench, B4= 10 ml/l as soil drench

4. Effect of Bio-fertilization, Bio-stimulant and Their Interaction Treatments on Chemical Constituents

It is clear from data in Tables (8 and 9) that, total chlorophyll, carbohydrate, protein, nitrogen, phosphorus as well as potassium in *Nigella sativa* seeds were significantly affected by different treatments of bio-fertilizer in the two seasons. Moreover, all above parameters were significantly increased by using the treatment of F1 in comparison with F0 treatment during the two tested seasons.

On the other hand, using bio-stimulant as foliar spraying or soil drench gave significant differences in this regard compared to control treatment. Meanwhile, B2 treatment was the superior in this respect followed by B4, B1 then B3, respectively. In addition, the highest values of total chlorophyll, total carbohydrate and total protein in *Nigella sativa* seeds (1.385, 1.406 ml/g; 19.332, 18.858% and 10.813, 10.970%) were obtained with foliar spraying by bio-stimulant at 10 ml/l. Also, the maximum values of total nitrogen, total phosphorus as well as potassium percentages in seeds of black cumin (1.730, 1.755; 0.316, 0.322 and 2.205, 2.311%) were given from the same treatment in the first and second seasons, respectively.

The data given in the same Tables (8 and 9) suggest that total chlorophyll (mg/g) content, total carbohydrates % in seeds, total protein %, total nitrogen %, and total phosphorus % as well as potassium in seeds of black cumin recorded significant differences by using the interaction between bio-fertilizer and bio-stimulant. Also, under each treatment of bio-fertilization, all these parameters increased by increasing the concentration of bio-stimulant. The superior treatment in this regard was that interaction between F1 with B2 which gave a significant increase compared to other interaction treatments.

Table (8). Effect of bio-fertilizer and bio-stimulator levels as well as their interaction treatments on chemical constituents of black cumin (*Nigella sativa*) during the two seasons (2019/2020 and 2020/2021).

Treatments	Total Chlorophyll (mg/g)					
	*F0	F1	Means	F0	F1	Means
	First season			Second season		
Control	***1.148i	1.201h	1.175E	1.150j	1.236i	1.193E
****B1**	1.243f	1.368c	1.305C	1.275g	1.376c	1.325C
B2	1.291e	1.480a	1.385A	1.345e	1.467a	1.406A
B3	1.221g	1.325d	1.273D	1.248h	1.361d	1.304D
B4	1.276e	1.420b	1.348B	1.3277f	1.438b	1.383B
Means (F)	1.236B	1.359A		1.269B	1.376A	
	Total carbohydrates in seeds					
Control	13.823h	14.497g	14.160E	13.600j	14.477i	14.038E
B1	16.883e	18.087c	17.485C	16.283g	18.263c	17.273C
B2	17.920cd	20.743a	19.332A	17.150e	20.567a	18.858A
B3	15.670f	17.843d	16.757D	15.403h	17.377d	16.390D
B4	16.710e	19.780b	18.245B	16.837f	19.313b	18.075B
Means (F)	16.201B	18.190A		15.855B	17.999A	
	Total protein % in seeds					
Control	8.797j	8.920i	8.858E	8.690j	8.900i	8.795E
B1	9.480g	10.690c	10.085C	9.270g	10.750c	10.010C
B2	10.063e	11.563a	10.813A	10.063e	11.877 a	10.970A
B3	9.083h	10.377d	9.730D	9.107h	10.520d	9.813D
B4	9.857f	11.357b	10.607B	9.540f	11.083b	10.312B
Means (F)	9.456B	10.581A		9.334B	10.626A	

*F0= without bio-fertilizer, F1= with bio-fertilizer, **B= bio-stimulant, B1= 5 ml/l as foliar spray, B2= 10 ml/l as foliar spray, B3= 5 ml/l as soil drench, B4= 10 ml/l as soil drench, ** *Means having the same letter(s) within the same column are not significantly different according to LSD for all pairwise comparisons test at 5% level of probability.

Table (9). Effect of bio-fertilizer and bio-stimulator levels as well as their interaction treatments on NPK percentages in black cumin seeds (*Nigella sativa*) during the two seasons (2019/2020 and 2020/2021).

Treatments	*F0	F1	Means	F0	F1	Means
			Total nitrogen %			
	First season			Second season		
Control	***1.407j	1.427i	1.417E	1.390j	1.422i	1.406E
**B1	1.517g	1.710c	1.613C	1.483g	1.720c	1.601C
B2	1.610e	1.850a	1.730A	1.610e	1.900a	1.755A
B3	1.453h	1.660d	1.557D	1.456h	1.683d	1.570D
B4	1.577f	1.817b	1.697B	1.526f	1.773b	1.650B
Means (F)	1.513B	1.693A		1.493B	1.700A	
			Total phosphorus %			
Control	0.223j	0.235i	0.229E	0.221j	0.237i	0.229E
B1	0.263g	0.312c	0.287C	0.268g	0.337c	0.302C
B2	0.282e	0.350a	0.316A	0.2910e	0.353a	0.322A
B3	0.252h	0.287d	0.269D	0.258h	0.323d	0.291D
B4	0.277f	0.333b	0.305B	0.282f	0.351b	0.316B
Means (F)	0.259B	0.303A		0.264B	0.320A	
			Potassium %			
Control	1.660j	1.750i	1.705e	1.676g	1.770f	1.723D
B1	1.867g	2.300c	2.083c	1.850e	2.276c	2.063C
B2	2.110e	2.500a	2.205a	2.140d	2.483a	2.311A
B3	1.810h	2.250d	2.030d	1.836e	2.243c	2.040C
B4	2.040f	2.407b	2.223b	2.120d	2.356b	2.238B
Means (F)	1.897B	2.241A		1.924B	2.226A	

*F0= without bio-fertilizer, F1= with bio-fertilizer, **B= bio-stimulant, B1= 5 ml/l as foliar spray, B2= 10 ml/l as foliar spray, B3= 5 ml/l as soil drench, B4= 10 ml/l as soil drench, ** *Means having the same letter(s) within the same column are not significantly different according to LSD for all pairwise comparisons test at 5% level of probability.

DISCUSSION

Constructing the impacts of bio-fertilization on stimulating plants growth may be due to increment in nitrogenase activity and synthesis of growth-regulation ingredients by phosphate solubilizing and N fixation bacteria. The bacteria which solubilizing phosphorus play a great role in phosphorus nutrition by improving its availability to plants by converting from inorganic and organic soil P pools through solubilization and mineralization processes (De et al., 2012). Also, KSB are able to solubilize of rock K mineral powder, such as micas, illite and or thoclases. KSB such as *Bacillus muciloginosus* and *Bacillus circulans* improved content of elements in plants (Sheng et al., 2002). So, it is the aim why adding bio-fertilization to the soil increase plant height and other features of plant traits.

Bio-fertilizers have shown superior potential as application, renewable and environmentally friendly sources of plant nutrients. These promote growth by increment the availability of essential nutrients and/or growth promotion to the aim crop when added to seeds, plant surfaces, and/or soil (Mishra and Dash, 2014). Moreover, bio-fertilization strains promote plant growth directly (nitrogen fixation, phosphate solubilization, iron chelation as well as production of phytohormones) or indirectly by increasing the tolerance of plants against plant pathogens and environmental stresses (Gopalakrishnan et al., 2015).

Furthermore, the enhancing influences of bio-fertilization on vegetative growth may be revealed to increasing the seeds yield. Bio-fertilizer has an excessive tendency to produce ingredients such as gibberellins, indole acetic acid (IAA), vitamin B complex as well as phytohormones having excessive potential increment of the growth and finally enhancing the yield of crops (Yasin et al., 2012). These products rise the surface area/unit root length and enhance the root hair branching and increase the absorption of nutrients and adsorption of water from the soil that ultimately yield greater in several cases, more productivity plants (Dobbelaere et al., 2001).

The stated results before reveal that, bio-fertilization is a promoting growth parameter, yield and fixed oil as well as its components of black cumin plants compared to the control. The highest values in this respect were obtained when the bio-fertilizer were applied. These results coincide with those reported by Hassan and Ali (2013) on anise; Ali and Hassan (2014) and Mohamed et al. (2020) on black cumin; Hashem et al. (2017) on roselle; Abd-Elghany et al. (2017) on fenugreek and Hamed et al. (2017) on lemongrass plants.

Meanwhile, the essential oil content and its main components of several medicinal and aromatic plants were increased by the addition of bio-fertilizers. In addition, carbohydrate percentage, total nitrogen, total phosphorus and potassium content were also enhanced. These results are in harmony with those found by Hassan and Ali (2013) on anise; Ali and Hassan (2014) and Mohamed et al. (2020) on black cumin; Abd-Elghany et al. (2017) on fenugreek and Hamed et al. (2017) on lemongrass.

The improving in growth, yield of seeds and fixed oil yield as well as chemical constituents of black cumin plants may be attributed to the effect of using bio-stimulant as growth promoters, which contains marine algae extract, humic acid as well as some of micro elements (Fe, Zn and Mn).

So, the promoting influence of bio-stimulant which contains the marine algae extract on plants may be due to its high contents of macro and micro elements as well as free amino acids. In addition, having natural pigments as carotene and xanthophyll, which are the richest natural source of vitamin B_{12}. While the presence of high levels of natural plant hormones such as auxins and cytokinins that are important for increasing plant productivity and raising tolerate of plants to environmental stresses.

As observed from the results of this experiment, adding the bio-stimulant which contains marine algae extract as foliar spraying or soil drench led to an increase in all studied black cumin growth traits, yield in addition to promote its chemical composition similar to what reported by Khater (2016) on *Cyamopsis tetragonoloba*; Moghith (2016) on *Origanum vulgar*e; El-Mahrouk et al. (2018) on *Cymbopogon citratus*; Mohamed et al. (2020) on black cumin and Abd El-Aleem et al. (2021) on curly parsley.

Generally, it can be recognized that humic acid as a component of bio-stimulant have various beneficial impacts on soil structure and soil microorganism as well as raising of modified mechanisms involved in plant growth promotion, cell permeability and nutrient absorption producing increases growth traits, essential oil, chemical constituents of plants (Rahmat et al., 2010). Using bio-stimulant as spraying or soil addition contribute to increase the plant growth, yield and oil yield as well as chemical parameters of *Nigella sativa* plant. These results are in harmony with those found by Mohammadipour et al. (2012) on marigold; Bakry et al. (2015) on flax; Noroozisharaf and Kaviani (2018) on *Thymus vulgaris* and Aiyafar et al. (2015) and Mazrou (2019) on *Nigella sativa*.

CONCLUSION

It could be concluded that, the addition of bio-fertilizer combined with bio-stimulate contain marine algae extract, humic acid and some of micro elements at 10 ml/l as foliar spraying or soil drench is suitable for increasing the productivity of black cumin (*Nigella sativa*) plant and its chemical constituents under South Sinai conditions.

REFERENCES

A.O.A.C. (1984). In: “Official Methods of Analysis”. 12th Ed., Association of Official Analysis Chemists, Washington D.C., USA.

Abd El-Aleem, W.H., E.S. Hamed and W.I.M. Toaima (2021). Effect of blue green algae extract on three different curly parsley varieties under Sinai conditions. Bulg. J. Agric. Sci., 27 (5): 887–895.

Abd-Elghany, G.G., M.M. El-Shazly and Hashem, H.A. (2017). Water management for the fenugreek plant and its response to bio-fertilization in North Sinai. Egypt. J. Appl. Sci., 32 (12 B): 494-515.

Aiyafar, S., H. Poudineh and M. Forouzandeh (2015). Effect of humic acid on qualitative and quantitative characteristics and essential oil of black cumin (*Nigella sativa* L.) under water deficit stress. DAV International Journal of Science, 4 (2): 89-102.

Ali, E. and F. Hassan (2014). Bio-production of *Nigella sativa* L. seeds and oil in Taif area. Int. J. Curr. Microbiol. App. Sci., 3 (1): 315-328.

Analytical Software (2008). Statistix Version 9, Analytical Software, Tallahassee, Florida, USA.

Bakry, A.B., M. Sadak and M.F. El-karamany (2015). Effect of humic acid and sulfur on growth, some biochemical constituents, yield and yield attributes of flax grown under newly reclaimed sandy soils. ARPN Journal of Agricultural and Biological Science, 10 (7): 247-259.

Bhattacharyya P. and D. Jha (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J. Microbiol. Biotechnol., 28 (4): 1327-1350.

Boraste, A., K.K. Vamsi, A. Jhadav, Y. Khairnar, N. Gupta, S. Trivedi, P. Patil, G. Gupta, M. Gupta, A.K. Mujapara and B. Joshi (2009). Biofertilizers: A novel tool for agriculture. Int. J. Microbiol. Res., 1 (2): 23-31.

Chaplin, M.F. and J.F. Kennedy (1994). In: “Carbohydrate Analysis, A Practical Approach”. 2nd Ed., Oxford University Press, Oxford, New York, Tokyo, 324 p.

Cottenie, A., M. Verlso, L. Kilkens, G. Velghe and R. Camerlynck (1984). In: “Chemical Analysis of Plants and Soils”. Lab. Agroch. State Univ. Gent, Belgium.

De, T., T. Sarkar, M. De, T. Maity, A. Mukherjee and S. Das (2012). Abundance and occurrence of phosphate solubilizing bacteria and phosphatase in sediment of Hooghly estuary, northeast coast of bay of Bengal, India. J. Coastal Develop., 15 (1): 9-16.

Deshwal, V.K., M.S. Devi, N. Bhajanka, J. Mistri, A. Bose and N. Saini (2011). *Pseudomonas aeruginosa* strains and their role in plant growth promotion in medicinal plant. Global J. Res. Appl. Agric., 1 (1): 49-55.

Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek and J. Vanderleyden (2001). Responses of agronomically important crops to inoculation with *Azospirillum*. Aust. J. Plant Physiol., 28: 871-879.

Du Jardin, P. (2015). Plant biostimulants: definition, concept, main categories and regulation. Scientia Horticulturae, 196: 3-14.

El-Mahrouk, E.M., A.I. Abido, F.I. Radwan, E.S. Hamed and E.E. El-Nagar (2018). Vegetative growth and essential oil productivity of lemongrass (*Cymbopogon citratus*) as affected by NPK and some growth stimulators. International Journal of Botany Studies, 3 (6): 48-55.

European Biostimulants Industry Council (2012a). EBIC: Promoting the biostimulant industry and the role of plant biostimulants in making agriculture more sustainable. Available online: http://www.biostimulants.eu/

European biostimulants industry Council (2012b). What are biostimulants? Available online: http://www.biostimulants.eu/about/whatare-biostimulants-benefits/

Gopalakrishnan, S., A. Sathya, R. Vijayabharathi, R.K. Varshney, C.L.L. Gowda and L. Krishnamurthy (2015). Plant growth promoting rhizobia: challenges and opportunities. 3 Biotech, 5: 355-377.

Hamed, E.S., W.I.M. Toaima and M. El-Shazly (2017). Effect of planting density and biofertilization on growth and productivity of *Cymbopogon citratus* (DC.) Stapf. (Lemongrass) plant under Siwa Oasis conditions. Journal of Medicinal Plants Studies, 5 (2): 195-203.

Hashem, H.A.E.A., El-Hadidy, A.E. and E.A. Ali (2017). Impact of some safe agricultural treatments on insect pests, vascular wilt disease management and roselle *(Hibiscus sabdariffa* L.) productivity under Siwa Oasis conditions. Int. J. Environ., 6 (4): 139-162.

Hassan, F.A.S., E.F. Ali and S.A. Mahfouz (2012). Comparison between different fertilization sources, irrigation frequency and their combinations on the growth and yield of coriander plant. Aust. J. Basic Appl. Sci., 6 (3): 600-615.

Hassan, F.A.S. and E.F. Ali (2013). A comparative study between traditional mineral nutrition and other alternative sources on anise plant. Eur. J. Sci. Res., 106 (2): 201-212.

Hucker, T.W.G. and G. Catroux (1980). Phosphorus in sewage ridge and animal waster slurries. Proceeding of the EEC Seminar, Haren (Gr), Groningen Netherlands 12, 13 June.

James, C.S. (1995). In: “Analytical Chemistry of Foods”. Blackie Academic and Professional Publisher, An Imprint of Chapman and Hall, 178 p.

Khater, R.M.R. (2016). Effect of sowing dates and foliar spray with algae extract on cluster bean (*Cyamopsis tetragonoloba* L.). International Journal of PharmTech Research, 9 (9): 75-84.

Lutterodt, H., M. Luther, M. Slavin, J.J. Yin, J. Parry, J.M. Gao and L.L. Yu. (2010). Fatty acid profile, thymoquinone content, oxidative stability and antioxidant properties of cold-pressed black cumin seed oils. LWT Food Sci. Technol., 43 (9): 1409–1413.

Mazrou, R.M. (2019). Enhancing the growth and production of black cumin (*Nigella sativa* L.) by application of humic acid and biofertilizers. Menoufia J. Plant Prod., 4: 443-458.

Mishra, P. and D. Dash (2014). Rejuvenation of bio-fertilizer for sustainable agriculture and economic development. Journal of Sustainable Development, 11 (1): 41-61.

Moghith, W.M.A. (2016). Effect of organic and bio-fertilization on the growth, production and the chemical constituents of *Origanum vulgare* L. plants. M.Sc. Thesis, Fac. Agric., Tanta Univ., Egypt.

Mohamed, N.H., E.A. Hassan, E.H. Hamad and R.M. Khater (2020). Response of black cumin (*Nigella sativa* L.) plants to the addition of natural fertilizers and the inoculation by bacteria mix and seaweed liquid extract. Archives of Agriculture Sciences Journal, 3 (2): 1-15.

Mohammadipour, E., A. Golchin, J. Mohammadi, N. Negahdar and M. Zarchini (2012). Effect of humic acid on yield and quality of marigold (*Calendula officinalis* L.). Annals of Biological Research, 11: 5095-5098.

Mora, V., M. Olaetxea, E. Bacaicoa, R. Baigorri, M. Fuentes, A.M. Zamarreño and J.M. Garcia-Mina (2014). Abiotic Stress Tolerance in Plants: Exploring the Role of Nitric Oxide and Humic Substances. In: "Nitric Oxide in Plants: Metabolism and Role in Stress Physiology". Springer, pp. 243-264.

Noroozisharaf, A. and M. Kaviani (2018). Effect of soil application of humic acid on nutrients uptake, essential oil and chemical compositions of garden thyme (*Thymus vulgaris* L.) under greenhouse conditions. Physiol. Mol. Biol. Plants, 24 (3): 423–431.

Okolie, C.L., B. Mason and A.T. Critchley (2018). Seaweeds as a Source of Proteins for Use in Pharmaceuticals and High Value Applications. In: "Novel Proteins for Food, Pharmaceuticals and Agriculture: Sources, Applications and Advances". 1st Ed., Chapter 11, Wiley, pp. 217-237.

Page, A.L., R.H. Miller and D.R. Keeney (1984). In: "Methods of Soil Analysis". Part 2: Chemical and Microbiological Properties. Second Edition. Agronomy J. 9: 2, Am. Soc. Agron. Inc., Soil Sci. Soc. Am. Inc. Pub. Madison, Wisconsin, USA.

Rahmat, U.K., A. Rashid, M.S. Khan and E. Ozturk (2010). Impact of humic acid and chemical fertilizer application on growth and grain yield of rainfed wheat (*Truticum aestivum*, L.). Paki. J. Agric. Res., 23 (3-4): 113-121.

Ramadan, M.F. (2007). Nutritional value, functional properties and nutraceutical applications of black cumin (*Nigella sativa* L.): An overview. Int. J. Food Sci. Technol., 42 (10): 1208–1218.

Sadasivam, S. and A. Manickam (1992). In: “Biochemical Methods for Agriculture Sciences”. Wiley Eastern Limited, pp. 181-185.

Shabnam, J., A.A. Shahid, M.S. Haider, A. Umeera, R. Ahmad, S. Mushtaq (2012). Nutritional, phytochemical potential and pharmacological evaluation of *Nigella Sativa* (Kalonji) and *Trachyspermum Ammi* (Ajwain). J. Med. Plants Res., 6 (5): 768–775.

Sheng, X.F., L.Y. He and W.Y. Huang (2002). The conditions of releasing potassium by a silicate-dissolving bacterial strain NBT. Agricultural Sciences in China, 1: 662-666.

Shukla, P.S., T. Borza, A.T. Critchley and B. Prithiviraj (2016). Carrageenans from red seaweeds as promoters of growth and elicitors of defense response in plants. Frontiers in Marine Science, 3: 81-95.

Van Oosten, M.J., O. Pepe, S. De Pascale, S. Silletti and A. Maggio (2017). The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. Chemical and Biological Technologies in Agriculture, 4 (1): 5-22.

Yasin, M., K. Ahmad, W. Mussarat and A. Tanveer (2012). Bio-fertilizers, substitution of synthetic fertilizers in cereals for leveraging agriculture. Crop Environ., 3: 62-66.

تأثير بعض المعاملات الزراعية الآمنة على نمو وإنتاجية نباتات حبة البركة تحت ظروف جنوب سيناء

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أجريت تجربة حقلية في مزرعة خاصة بمدينة الطور، محافظة جنوب سيناء خلال موسمي الشتاء المتتاليين ٢٠٢٠/٢٠١٩ و٢٠٢١/٢٠٢٠ لدراسة تأثير معاملات التسميد الحيوي (بدون تسميد حيوي وبتسميد حيوي) ومعاملات المحفز الحيوي التي تحتوي على طحالب بحرية وحامض الهيوميك وبعض العناصر الصغرى والتي تم استخدامها كالتالي: كنترول، ٥ مللي/لتر و١٠ مللي/لتر كرش ورقي، ٥ مللي/لتر و١٠ ملي/لتر كإضافة أرضية، على صفات النمو ومكونات المحصول وكذلك المكونات الكيميائية لنبات حبة البركة. أوضحت النتائج أن كل صفات النمو ومكونات المحصول وإنتاج الزيت وكذلك المكونات الكيميائية في البذور تأثرت معنويًا بمعاملة التسميد الحيوي. أيضًا، كل معاملات المحفز الحيوي أظهرت اختلافات معنوية في هذا الصدد بالمقارنة بمعاملة الكنترول. كانت معاملة التفاعل بين التسميد الحيوي والمحفز الحيوي بمعدل ١٠ مللي/لتر كرش ورقي الأفضل حيث سجلت زيادة معنوية وأعلى قيم في هذا الصدد، كما سجلت المكونات الرئيسية للزيت التي كانت حمض الليوريك، حمض الميرستك، حمض البالميتيك، حمض الستيريك، حمض الأوليك، حمض الينولينيك، حمض الأراشيديك وحمض الإكوسانويد، على التوالي.