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Stimulatory Influence of Organic and Bio-Fertilizers on Yield, Seed Oils and Fatty Acid Fractions of *Nigella sativa* L. Grown In Calcareous Soil

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Abstract: In order to investigate the stimulatory effects of amending calcareous soil by organic and biofertilizers on growth, yield and oil content of *Nigella sativa* L; an experiment conducted during the two successive seasons 2011/2012 and 2012/2013 in complete randomized design in three replications with five treatments. The studied treatments consisted of diatomaceous earth (D.E.), sewage effluent (S.E.), *Nostoc*, liquid Yeast-Plus and modified Hoagland solution, each at five successively increasing concentrations. The statistical analysis indicated that soil amendment significantly increased *N. sativa* growth and yield parameters, expressed as fresh and dry weight per plant, number of capsules/plant and number of seeds/capsule at most of the used concentrations. Six g/5kg of diatomaceous earth, 60% sewage, 1 mg/L *Nostoc*, 32 ml/L (10^{12} cells/ml) of liquid yeast and 1/4x Hoagland were the most stimulatory concentrations on growth and yield characters. The results showed that application of diatomaceous earth (6 g/5kg) and sewage (60%) significantly increased the production of fixed oils compared with the control (31.6%, 22.7% and 16.1% respectively).The highest protein content of seeds was obtained using 6 g/5kg D.E. and 60% sewage while the highest carbohydrates were detected in 60% sewage. Unsaturated fatty acids were found to be the major fraction in total fatty acids. The overall results suggest that organic and biofertilizers improved the characteristics of calcareous soil as it clearly enhanced growth, yield and oil percentage of *N. sativa* L.

Key words: Medicinal Plants · Bioremediation · Biomolecules

INTRODUCTION

Agreat attention recently focused on exogenous application of biofertilizers and organic matter to improve growth and productivity of crop plants, in poor soils especially the calcareous soil. Nutrient management in calcareous soils differs from that in non-calcareous soils because of the effect of alkaline soil pH on nutrient availability and on the chemical reactions that affect the loss or fixation of nutrients. For example, both native and applied P is tied up in highly insoluble Ca and Mg phosphates, rendering the added P only sparingly available for plant uptake and Iron, Zn, Mn and Cu deficiencies are also common in soils that have high $CaCO_3$ contents due to their reduced solubility at alkaline pH value [1]. Furthermore, calcareous soils tend to be low in organic matter and available nitrogen [1]. Chlorotic symptoms are usually observed on plants grown in calcareous soils, but potential productivity may be very high where adequate nutrients and water can be supplied [2].

To improve physico-chemical characteristics of calcareous soils, plant growth and hence productivity of planted crops, there are several approaches often applied

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such as application of organic materials, supplementation and management of essential nutrients and application of bio-fertilizers. Biofertilizers are products containing living cells of different types of microorganisms that have the ability to convert nutritionally important elements from unavailable to available form through biological processes [3-5]. Biological fertilizers contain micro-organisms that enhance soil fertility, plant growth and yield crop [5]. They are of low-cost, renewable sources of plant nutrients, which supplant chemical fertilizers and a good substitute fertilizer for inorganic compounds [6]. Recently, the use of bio-fertilizers has been gaining momentum due to increasing emphasis on maintenance of soil health, minimizing environmental pollution and reducing the use of chemicals in agriculture [7]. Cyanobacteria attract the attention of scientists due to their nitrogen fixing capacity and hence their role in maintaining soil fertility is well-documented [8]. Algae, mainly represented by green and blue-green species, are sources of organic substances in the soil [9-12]. Algalization increases the fertilizer use efficiency of crop plants, reduces the loss of nitrogen fertilizers and above all, provides these benefits in a recurring manner [13]. Diatomaceous earth is a naturally occurring substance, it is the fossilized remains of salt or fresh water organisms called diatoms. Amorphous diatomaceous earth is known to be agood source of silicon [14]. Although silicon has not been listed among the essential elements for higher plants however, the beneficial role of silicon in stimulating the growth and development of many plant species has been generally recognized especially when plants are submitted to biotic or abiotic stresses.The main roles of Si-mediated alleviation of salt stress include: improvement of plant growth, stimulation of antioxidant systems in plants, improvement of photosynthesis, improvement of ion balance under salt stress and increase of root activity [15]. Hence, diatomaceous earth has been applied as a biofertilizer. There is an increasing interest in the agricultural application of sewage sludges (obtained in waste water treatment plants) due to the possibility of recycling valuable components: organic matter, N, P and other plant nutrients [16,17]. Studies on sludges are important due to the economic and environmental implications of widespread application of these materials to agricultural lands [18]. Yeasts supply plants with vitamins (B complex) and growth regulating hormones [19-22], produce organic acids and chelating agents which increase mineral (P, Fe, Zn, Mn) nutrient uptake [23,24] and act as bio-control agent against certain plant diseases [25].

Nigella sativa, commonly known as black seed is a dicotyledon of the buttercup family (Ranuculaceae), native to south Europe, north Africa and south west Asia [26]. This annual is a herbaceous plant about 20-40 cm in height, has finely divided linear leaves (but not thread like) and delicate pale-blue or white flowers. Its seeds represent the main useful product are angular, small sized, dark grey or black colored and contain saturated and unsaturated fatty acids, amino acids, vitamins, minerals and traces of isoquinoline and pyrazolic alkaloids [27]. Black seed has proved itself as a forceful tool against many ailments [28]. A wide spectrum of medicinal uses can be mentioned, such as anti-histamine, anti-tumer, anti-bacterial and anti-inflammatory and antiviral [29, 30] and these actions alone provide relief for a multitude of ailments and disorders. In this study, the effect of biological fertilizers on growth and yield of black seed grown in calcareous soil were investigated to assess the possibilities of using the studied biofertilizers for improving calcareous soils in agriculture systems.

MATERIALS AND METHODS

Culture Technique, Treatments and Experimental Design: Apot experiment was carried out in open air under natural conditions at the green house of the Faculty of Science, Assiut University during two successive winter seasons (2011/2012 and 2012/2013).To study the effect of the different treatments on *Nigella sativa* plants grown in 50 cm diameter pots containing 5 Kg calcareous soil (physical and chemical characteristics of the soil are shown in Table 1). Seeds were sown directly in the soil on the first of November of both seasons (five plants/pot). The experiment was set up in a completely randomized design with three replicates for eachtreatment. They were immediately watered; treatments were added after 21 days of sowing. Control and treated plants received only tap water twice a week up to the field capacity.

Treatments:

- Diatomaceous earth (D.E.), bought from "Growth Promoters World, Egypt" for trading, import and supplies and applied at concentrations of 1.5, 3, 6,12, 24 g/5kg.
- Sewage effluent (S.E.) brought from "Al-Arbaeen Sewage Treatment Station" (Assiut city) and applied at concentrations of 20%, 40%, 60%, 80% and 100%.

Table 1: The physical and chemical characteristics of the soil obtained from the experimental farm of Arab EL-Awamer, Assiut, Egypt

Soil properties	Values	Soil properties	Values
Particle size distribution		Soluble cations (meq/L)	
Sand (%)	96.72	Ca^{++}	1.73
Silt (%)	2.12	Mg^{++}	1.00
Clay (%)	1.169	Na^{+}	0.56
Field capacity (%)	9.92	K^{+}	0.17
		Total $CaCO_3$(%)	35.18
Soil texture	Sandy	Water saturation (%)	20.58
Total nitrogen (%)	0.003	EC mm hos/cm(1:1)	0.35
Organic matter (%)	0.24	pH(1:1watersuspension)	8.65

- *Nostoc*, grown in Botany and Microbiology Department, Faculty of Science, Assiut University, applied at concentrations of 0.2, 0.4, 0.6, 0.8 and 1 mg/L.
- Liquid yeast–Plus, (10^{12} cells/ml), bought from the "Unit of biofertilizers, Faculty of Agriculture, Ein Shams University" and applied at concentrations of 4, 8, 16, 32 and 64 ml/L.
- Modified Hoagland solution, applied at concentrations of 1/10, 1/8, 1/6, 1/4 and 1/2x [31].

Sampling: At the end of growth stage, plants were collected from each pot (in May for the two seasons); then the following agronomic characters were estimated: plant height, number of branches/plant, fresh and dry weights of plants, number of capsules/plant, number of seeds/capsule and percentage of fixed oil in seeds.

Determination of Soluble Carbohydrates in Seeds: The anthronesulphuric acid method [32,33] was used for the determination of carbohydrates in aqueous extracts.

Determination of soluble proteins in seeds: Protein contents were determined in the seeds extractby Folin reagent according to Lowry et al. in aqueous extracts [34].

Extraction and analysis of fixed oils: Definite weights of air-dried and finely ground *N. sativa* seeds were extracted with chloroform-methanol mixture (1:2 v/v) in Falcon tubes in a water bath at 40°C for half an hour. The lower phase (chloroform) contains the fixed oils, which has been drawn off from the tube with a long syringe and transferred into dry test tube, evaporated and then weighed in aluminum foil cups and the percentage of oil content was calculated [35].

Analysis of Free Fatty Acids by GC-Mass: The esterified oils were analyzed by GC-Mass spectroscopy (GC/MS (7890A/5975B)) and column (GD-5MS) in the Analytical Chemistry Unit (ACAL), Facullty of Science, Assiut University. The results were used to analyze the free fatty acids composition and percentage of oil samples.

Statistics Analysis: Data obtained were subjected to one way analysis of variance (ANOVA), using the SPSS statistical package. For comparison of the means, the Duncan s multiple range tests ($p<0.05$) were used.

RESULTS AND DISCUSSION

Effect of Biofertilizersin Soil Amendments on *Nigella sativa* L.

Vegetative and Reproductive Growth: The response of *N. sativa* plants grown in calcareous soil to the application of diatomaceous earth (D.E.), sewage (S.E.), *Nostoc*, yeast and Hoagland solution during 2011/2012 and 2012/2013 seasons is Tables (2, 3, 4, 5 and 6).

Effect of Diatomaceous Earth (D.E.): In the first season, raising D.E. doses from 3 to 12 g/5kg significantly increased plant fresh and dry weights, number of capsules per plant and number of seeds per capsule (Table 2). The highest fresh and dry mass at 3 g/5kg and 6 g/5kg; then,they began to decrease with increasing D.E. concentration. However, analysis of the results indicated that there was no significant differences in branch numbers and plant height. The number of capsules per plant in the first season showed significant increase at 3 g/5kg compared with the control. However, seeds number per capsule recorded statistically significant increase at all studied concentrations of D.E.; the highest number was observed at 1.5 g/5kg (59.8 seeds versus 35.9 seeds/capsule for the control plants). In the second season, variable response of fresh and dry weight to all concentrations was observed; significant induction in plant dry masscompared with the control plants was recorded only at 3 g/5kg D.E. The data also revealed that in 2012/2013, the number of main branches at 24 g/5kg D.E. was significantly higher than that in control plants (Table 2). Plant height was significantly enhanced at D.E. concentrations of 12 and 24 g/5kg relative to the control.

Table 2: Effect of diatomaceous earth (1.5, 3, 6, 12, 24 g/5 kg) on vegetative and reproductive growth of *Nigella sativa* L. during (2011/2012- 2012/2013) seasons

Treatment	Whole plant fresh weight(g)	Whole plant dry weight(g)	Number of main branches	Plant height (cm)	Number of capsules/plant	Number of seeds/capsule
			First season (2011/2012)			
Control	207.5±21.9^{AB}	24.0±0.4^{C}	6.9±0.4^{A}	40.6±6.4^{A}	11.3±0.6^{BC}	35.9±1.8^{B}
1.5	208.2±9.4^{AB}	27.7±1.0^{B}	7.3±0.7^{A}	43.0±0.9^{A}	11.7±0.6^{B}	59.8±8.5^{A}
3.0	222.6±3.8^{A}	29.2±0.9^{A}	6.9±0.4^{A}	40.8±1.9^{A}	13.7±1.2^{A}	56.9±3.1^{A}
6.0	222.0±2.2^{A}	29.2±0.2^{A}	7.333±0.7^{A}	42.3±0.3^{A}	11.7±0.6^{B}	56.4±3.4^{A}
12.0	216.5±9.9^{A}	28.4±1.0^{AB}	6.9±0.4^{A}	42.3±0.9^{A}	9.7±0.6^{D}	52.7±4.6^{A}
24.0	188.8±10.2^{B}	24.7±0.9^{C}	6.7±1.2^{A}	45.0±2.3^{A}	10.0±1.0^{CD}	55.0±0.5^{A}
F test	3.634*	26.50**	0.50 n.s.	0.923 ns	10.03**	11.31**
			Second season (2012/2013)			
Control	198.5±15.2^{A}	23.0±0.5^{B}	6.4±0.8^{AB}	38.3±1.5^{B}	10.0±2.0^{CD}	26.8±5.9^{C}
1.5	143.2±7.9^{B}	19.5±0.7^{C}	6.4±0.4^{AB}	40.1±1.1^{AB}	12.3±0.6^{BC}	37.0±2.0^{BC}
3.0	188.0±2.7^{A}	24.7±1.0^{A}	5.8±0.4^{B}	39.7±0.3^{AB}	17.3±1.2^{A}	39.9±6.6^{B}
6.0	145.1±6.1^{B}	19.1±0.8^{C}	5.8±0.4^{B}	40.1±0.8^{AB}	14.0±1.0^{B}	39.0±4.1^{BC}
12.0	137.0±5.8^{B}	18.0±0.6^{C}	6.2±0.8^{AB}	41.2±1.2^{A}	8.3±1.5^{D}	42.5±3.0^{AB}
24.0	105.7±11.5^{C}	13.8±0.5^{D}	7.3±1.2^{A}	41.0±2^{A}	10.5±2.5^{CD}	53.3±13.0^{A}
F test	42.64**	94.82**	2.01 n.s.	2.00 n.s.	12.33**	4.81**

Values are means ± SE (n=3). Different letters indicate significant differences (P □ 0.05) according to one way analysis of variance (ANOVA)

Table 3: Effect of sewage effluent (20, 40, 60, 80 and 100%) on vegetative and reproductive growth of *Nigella sativa* L. during (2011/2012-2012/2013) seasons

Treatment	Whole plant fresh weight(g)	Whole plant dry weight(g)	Number of main branches	Plant height (cm)	Number of capsules/plant	Number of seeds/capsule
			First season (2011/2012)			
Control	207.5±21.9^{BC}	24.0±0.4^{C}	6.9±0.4^{A}	40.6±6.4^{A}	11.3±0.6^{B}	35.9±1.8^{D}
20%	195.2±11.8^{C}	27.3±1.3^{B}	7.1±0.8^{A}	44.0±1.2^{A}	14.7±2.3^{A}	54.4±4.6^{B}
40%	223.0±2.9^{AB}	28.4±0.6^{AB}	6.9±1.0^{A}	39.2±7.1^{A}	15.3±2.1^{A}	62.8±3.1^{A}
60%	245.7±11.8^{A}	30.2±0.6^{A}	6.7±0.7^{A}	46.8±1.0^{A}	17.0±1^{A}	53.5±7.4^{B}
80%	233.8±9.4A	29.9±1.8^{A}	7.1±0.4^{A}	44.9±0.7^{A}	16.7±1.5^{A}	42.8±2.0^{CD}
100%	239.0±7.3^{A}	30.0±1.0^{A}	7.6±1.0^{A}	10.0±2.0^{AB}	16.3±0.6^{A}	44.6±2.9^{C}
F test	7.492**	15.07**	0.486 n.s.	1.446 n.s.	5.775**	16.75**
			Second season (2012/2013)			
Control	198.5±15.2^{B}	23.0±0.5^{B}	6.4±0.8^{AB}	38.3±1.5^{D}	10.00±2^{AB}	26.8±5.9^{E}
20%	107.7±10.9^{D}	15.1±1.7^{C}	5.7±0.3^{BC}	43.4±1.0^{C}	9.5±1.5^{AB}	34.8±3.6^{DE}
40%	114.1±4.8^{D}	14.5±0.7^{C}	4.9±0.4^{C}	42.4±0.4^{C}	7.5±2.5^{B}	84.0±0^{A}
60%	241.2±13.4^{A}	29.7±0.7^{A}	7.0±0.3^{A}	51.3±1^{A}	8.5±1.5^{B}	67.9±5.5^{B}
80%	169.6±14.7^{C}	21.7±2.2^{B}	6.2±0.4^{AB}	50.8±2.5^{A}	9.5±0.5^{AB}	54.7±8.7^{C}
100%	167.1±11.5^{C}	21.0±1.6^{B}	7.0±0.3^{A}	48.2±0.2^{B}	12.0±2^{A}	38.9±9.2^{D}
F test	51.13**	48.4**	9.85	45.43**	2.18 n.s.	36.25**

Values are means ± SE (n=3). Different letters indicate significant differences (P □ 0.05) according to one way analysis of variance (ANOVA)

Table 4: Effect of *Nostoc* at 0.2, 0.4, 0.6, 0.8 and 1 mg/L on vegetative and reproductive growth of *Nigella sativa* L. during (2011/2012-2012/2013) seasons.

Treatment	Whole plant fresh weight(g)	Whole plant dry weight(g)	Number of main branches	Plant height (cm)	Number of capsules/plant	Number of seeds/capsule
			First season (2011/2012)			
Control	24.0±0.4^{C}	207.5±21.9^{B}	6.9±0.4^{AB}	40.6±6.4^{B}	11.3±0.6^{C}	35.9±1.8^{C}
0.2	29.5±0.8^{B}	253.1±10.4^{A}	6.4±0.4^{B}	49.4±1.8^{A}	12.7±1.5^{BC}	64.6±6.7^{A}
0.4	29.2±0.5^{B}	231.9±3.5^{A}	5.3±0^{C}	47.6±1.6^{A}	12.0±1^{C}	53.4±1.1^{B}
0.6	29.4±1.5^{B}	231.6±6.1^{A}	6.9±1.0^{AB}	46.9±1.0^{A}	14.3±0.6^{AB}	47.3±3.4^{B}
0.8	31.5±0.4^{A}	237.5±8.3^{A}	7.8±0.4^{A}	49.3±0.3^{A}	16.0±1.0^{A}	54.9±8.4^{AB}
1.0	28.0±1.6^{B}	249.5±6.0^{A}	6.9±0.4^{AB}	48.3±2.4^{A}	12.3±1.2^{C}	47.4±8.7^{B}
F test	6.645**	10.45**	7.072**	3.705*	8.547**	7.970**
			Second season (2012/2013)			
Control	23.0±0.5^{D}	198.5±15.2^{C}	6.4±0.8^{AB}	38.3±1.5^{D}	10.0±2.0^{B}	26.8±5.9^{D}
0.2	25.4±1.4^{BC}	218.2±4.9^{B}	6.3±0.3^{B}	47.7±0.3^{B}	11.0±1.0^{B}	58.4±2.1^{B}
0.4	24.0±0.2^{CD}	190.0±5.1^{C}	6.7±0.7^{AB}	45.6±1.6^{B}	17.5±0.5^{A}	42.0±2.5^{C}
0.6	28.2±0.3^{A}	193.6±4.4^{C}	7.0±1^{AB}	41.9±1.7^{C}	18.5±1.5^{A}	41.2±2.0^{C}
0.8	27.0±0.6^{AB}	202.9±5.4^{C}	7.7±0.3^{A}	46.8±1.4^{B}	19.0±1.0^{A}	48.7±8.7^{BC}
1.0	28.0±1.6^{A}	209.5±6.0^{C}	7.3±0.7^{AB}	51.1±1.1^{A}	11.0±1.0^{B}	77.7±14.3^{A}
F test	16.83**	24.39**	1.82 n.s.	40.04**	16.47**	34.11**

Values are means ± SE (n=3). Different letters indicate significant differences (P □ 0.05) according to one way analysis of variance (ANOVA)

Table 5: Effect of Liquid yeast-plus at 4, 8, 16, 32 and 64 ml/L on vegetative and reproductive growth of *Nigella sativa* L.during (2011/2012-2012/2013) seasons

Treatment	Whole plant fresh weight(g)	Whole plant dry weight(g)	Number of main branches	Plant height (cm)	Number of capsules/plant	Number of seeds/capsule
			First season (2011/2012)			
Control	207.5±21.9^{C}	24.0±0.4^{D}	6.9±0.4^{A}	40.6±6.4^{B}	11.3±0.6^{C}	35.9±1.8^{B}
4	230.0±18.5^{BC}	27.3±1.3^{C}	6.2±0.8^{A}	47.6±0.4^{A}	12.0±1.7^{BC}	53.3±5.6^{A}
8	254.2±28.7^{B}	29.4±0.7^{ABC}	6.0±0.7^{A}	45.8±4.0^{AB}	13.7±0.6^{AB}	48.7±2.2^{A}
16	256.1±12.5^{B}	31.4±1.1^{A}	6.9±0.4^{A}	48.1±2.5^{A}	15.3±0.6^{A}	55.5±11.2^{A}
32	289.7±3.1^{A}	29.1±0.7^{BC}	7.1±0.8^{A}	49.3±0.9^{A}	12.7±0.6^{BC}	57.1±6.3^{A}
64	262.2±17.6^{AB}	29.5±2.1^{AB}	6.4±0.4^{A}	48.1±0.5^{A}	14.0±1.7^{AB}	55.3±7.8^{A}
F test	8.386**	14.30**	1.657 n.s.	2.818 n.s.	5.209**	4.289*
			Second season (2012/2013)			
Control	198.5±15.2^{B}	23.0±0.5^{C}	6.4±0.8^{B}	38.3±1.5^{D}	10.0±2^{C}	26.8±5.9^{B}
4	193.5±10.8^{B}	23.0±0.4^{C}	6.7±0.7^{AB}	44.4±1.7^{BC}	10.0±2^{C}	53.3±5.6^{A}
8	217.5±15^{B}	24.87±0.378^{B}	6.22±1.018^{B}	48.11±0.192^{A}	15.00±3^{AB}	48.7±2.2^{A}
16	218.3±22.7^{B}	26.70±1.4^{A}	6.0±0.7^{B}	46.8±1.2^{A}	16.0±4.0^{A}	59.2±12.9^{A}
32	256.1±4.4^{A}	25.7±0.7^{AB}	8.0±0.7^{A}	43.7±1.5^{C}	18.0±2^{A}	55.0±7.2^{A}
64	208.3±7.5^{B}	23.4±1.0^{C}	6.7±0.7^{AB}	46.4±0.2^{AB}	11.0±1^{BC}	57.9±9.0^{A}
F test	8.225**	11.28**	2.61 n.s.	24.89**	5.62**	7.02**

Values are means ± SE (n=3). Different letters indicate significant differences (P □ 0.05) according to one way analysis of variance (ANOVA)

Table 6: Effect of different Hoagland concentrations (1/10, 1/8, 1/6, 1/4 and 1/2x) on vegetative and reproductive growth of *Nigella sativa* L. during (2011/2012-2012/2013) seasons

Treatment	Whole plant fresh weight(g)	Whole plant dry weight(g)	Number of main branches	Plant height (cm)	Number of capsules/plant	Number of seeds/capsule
			First season (2011/2012)			
Control	207.5±21.9^{AB}	24.0±0.4^{E}	6.9±0.4^{B}	40.6±6.4^{AB}	11.3±0.6^{B}	35.9±1.8^{c}
1/10	189.1±32.5^{B}	26.1±0.8^{D}	6.2±0.4^{BC}	34.0±2.5^{B}	11.3±1.2^{B}	47.7±2.2^{B}
1/8	191.5±12.7^{B}	27.4±0.6^{C}	4.9±0.4^{C}	36.6±4.0^{AB}	11.3±0.6^{B}	48.9±3.0^{B}
1/6	224.1±7.8^{A}	28.7±0.6^{B}	6.0±1.2^{BC}	40.3±2.9^{AB}	11.3±2.3^{B}	55.1±6.3^{AB}
1/4	239.4±6.23^{A}	30.3±1.2^{A}	6.0±0.7^{BC}	41.0±1.7^{A}	14.7±1.2^{A}	56.6±4.8^{A}
1/2	229.1±2.2^{A}	29.1±0.5^{AB}	8.2±1.0^{A}	39.9±1.4^{AB}	14.3±0.6^{A}	40.2±4.7^{C}
F test	4.268*	29.93**	6.836**	1.884 n.s.	5.370**	11.79**
			Second season (2012/2013)			
Control	198.5±15.2^{B}	23.0±0.5^{A}	6.4±0.8^{A}	38.3±1.5^{C}	10.0±2.0^{B}	26.8±5.9^{C}
1/10	129.4±20.5^{B}	17.8±0.4^{B}	5.6±0.4^{A}	48.3±0.7^{A}	6.7±0.6^{C}	37.6±6.1^{B}
1/8	116.5±9.7^{B}	16.6±0.7^{CD}	6.7±1.2^{A}	42.8±1.2^{B}	8.5±0.5^{BC}	26.3±3.5^{C}
1/6	125.8±4.2^{B}	16.1±0.7^{D}	6.7±0.7^{A}	45.0±2.7^{B}	7.5±2.5^{BC}	39.5±0.5^{AB}
1/4	136.4±6.5^{A}	17.3±0.6^{BC}	6.0±0.7^{A}	44.4±1.3^{B}	9.5±1.5^{BC}	46.8±7.8^{A}
1/2	126.5±4.6^{B}	16.1±0.7^{D}	5.7±0.3^{A}	43.2±2.8^{B}	16.0±2.0^{A}	19.49±1.2^{C}
F test	19.54**	58.91**	1.44 n.s.	9.33**	11.65**	12.75

Values are means ± SE (n=3). Different letters indicate significant differences (P □ 0.05) according to one way analysis of variance (ANOVA)

The number of capsules showed significant increasesat D.E. concentrations of 3 and 6 g/5kg compared with the control. Seeds number per capsuleat D.E. levels of 3, 12 and 24 g/5 kg was significantly higher than control plants. The highest yields were observed at 1.5 g/5kg (59.8 seeds/capsule compared with 35.9 seeds and 45 cm compared with 40.6 cm for the control). In agreement with these results, Ashraf [36] reported that cane yield and yield attributes such as cane height, internodes length and number of tillers per plant were significantly higher (p= 0.05) when silicate was added. The results suggested that Si interacted with Na^{+}, reduced its uptake and transport to shoots and consequently improved cane yield under salt stress. Bernal reported that the applied magnesium silicate at low rates of 100 to 300 Kg/ha to acidic or basic soils increased yields of different crops such as sugarcane and rice by 5-20%, indicating a positive response In yield and other parameters with the application of increasing levels of Mg silicate to agricultural soils [37].

Effect of Sewage Effluent (S.E.): The results in Table (3) showed that sewage treatment augmented growth and yield parameters compared with control plants. Treatment of soil with sewage effluent resulted in a significant enhancement in plant fresh weight at all used concentrations. The highest fresh weight (245.7 g) was recorded at 60% S.E. in the first season while in the second season, the significant increase of fresh weight relative to the control was observed only at 60% concentration.The same results were detected in dry mass in both of the two cultivated seasons. It was found that no significant enhancement in the number of branches per plant at any of the used concentrations in both of seasons. All sewage concentrations significantly improved the number of capsules per plant in the first season. The highest number of capsules was detected at 60% (17.0 capsules compared with 11.33 capsules for control). Amendment of calcareous soil with sewage effluent significantly increased seeds number per capsule at all used doses; the highest number of seeds was

recorded at 40%S.E. in both cultivation seasons. Also, a significant increase was shown in plant height at all concentrations in the second season, the tallest plants were detected at 60 and 80%. Teresa et al. [18] observed that the application of organic materials enhanced soil fertility and the crop yield in the amended soils was higher than in the control. In agreement with the present investigation, Dowdy *et al*. [38] reported that the increase of crop yield by sludge application often exceeded that of a well-managed fertilized control. Growth of *Brassica* rapa *Chinensis* was better on sewage sludge than on pol-compost, the improved growth was due to the higher nitrogen content and continuous mineralization in sewage sludge, which is essential for plant growth [39].

Effect of *Nostoc*: Data in Table (4) clearly indicated that soil amended with *Nostoc* exhibited significant increase in fresh weight of *Nigella* plants at all concentrations in the first season and most of the studied concentrations in the second season compared with the control.The highest fresh weight was detected at 0.8 mg/L in the first season but at 0.6 mg/L in the second one. In the case of dry mass, a significant increase was observed at all used concentrations compared with the control plants in the season of 2011/2012, while in 2012/2013 it has been significantly increased only at 0.2 mg/L relative to the control.At both study seasons, there was no significant enhancement compared with the control in the number of branches at all concentrations exept that at 0.8 mg/L in the first season. Assessment of the number of capsules per plant revealed that application of *Nostoc* resulted ina significant increase of number ofcapsules per plant at 0.6 and 0.8 mg/L atboth seasons; the highest number of capsules was recorded at 0.8 mg/L (16.0 capsules in the first season and 19.0 in the second one). Seeds number per capsule has been influenced by *Nostoc* application, it increased significantly compared with the control at all concentrations at both cultivation seasons.The highest number of seeds was shown in the second season at 1 mg/L (77.7 seeds per capsule) while the lowest seeds number per capsule belonged to the non-treated control (26.8 seeds per capsule). These results are in agreement with El-Nakip who reported that application of cyanobacteria fertilizers significantly increased the vegetative growth criteria and crop yield of treated plants [40]. The same results were revealed by Rizvi and Sharma; they observed stimulatory effects of algae on vegetative growth of tomato [41]. Rodgers et al. stated that filamentous cyanobacteria, which was shown to produce compounds that stimulate plant growth included *Anabaena* sp., *Nostoc* sp. and *Nodularia* sp. [42]. Abd-Alla et al. studied the effect of inoculants of cyanobacteria on wheat grown in sandy soil and other soils and recorded that living inoculants of cyanobacteria significantly increased dry mass of plants; the increase in growth parameters could be attributed to the substantial increase of N_2-fixation due to the nitrogenase activity of cyanobacteria [43]. Glass explained that the increase of fresh and dry weights is due to the increase of nutrient uptake and growth enhancement is induced by hormones from the used algal extracts [44]. Gupta and Pandher reported that cyanobacteria synthesize and excrete growth promoting substances like indole acetic acid, vitamins, sugars and free amino acids to benefit the crop; they also liberate biopolymers and improve the soil aggregation status which help in maintaining soil productivity [45]. In the same way, Kabli *et al*. reported that *Nostoc* and *Anabaena* could supply wheat plants with atmospheric nitrogen to maintain its vegetative growth [46]. Therefore,these species of cyanobacteria could be used as biofertilizers instead of utilizing expensive industrial chemical manures.

Effect of Liquid Yeast-Plus: Adding yeast-plus at the rates of 4,8,16, 32and 64 ml/L (each ml contains 10^{12} yeast cells) increased growth parameters of *N. sativa* plants compared with control treatment. At the first season, increasing the dose of liquid yeast from 4-8 ml to 64 ml/L showed statistically significant differences in the mean values of fresh weight per plant while the significant enhancement was recorded at 32 ml/L in the second season. As well, dry weight per plant showed similar significant increases (Table 5). Yeast treatments exerted no statistically significant effect on branches number of *N. sativa* plants. Plant height was significantly increased by all concentrations at the first and second seasons. The tallest plant recorded at the first season was 49.3 cm at 32 ml/L compared with 40.6 cm in control plants. Concerning the number of capsules per plant, the significant increase began at 8 ml/L and increased with increasing the concentrations at bothe seasons; the highest number was 18 capsules per plant at 32 ml/L at the second season.All the used doses of yeast exerted statistically significant enhancement on the number of seeds/capsule in the two seasons. The highest number of seeds per capsule (59.2) was achieved by 16 ml/L compared with 26.78 in control plantsat the second season. These results were coinciding with those of Ezz El-Dinand Hendawy; they showed that increasing the dose of dry yeast from 2 g/L to 4 g/L exhibited significant

differences in the mean values of fresh weight of aerial parts, number of suckers, seed weight and oil percentage of *Borago officinalis* plant [47]. Heikal reported that active dry yeast as foliar fertilizer enhancedthe growth, plant nutritionof thyme plants [48]. Rosella plants sprayed with dry yeast showed the highest yield of calyxes as revealed by Ahmed et al. [49]. Moreover, several investigators studied the response of other plants to the application of dry yeaste.g.on coriander [50,51] and on black cumin [52]. The positive effect of yeast in enhancing growth and yield of *N. sativa* plants could be attributed to its high content of minerals particularly N,P and K and certain natural hormones, beside high amount of vitamins, especially B which plays an important role in improving growth [53]. Khedr and Farid demonstrated that the effect of dry yeast is due to its capability of inducing endogenous hormones like GA3 and IAA [54]. The same results were observed by Safaa et al.; they reported that foliar application of 90 mg/L dry yeast caused the most promoting effect for increasing fresh and dry weights of geranium plants and the variation in dry matter accumulation could be attributed to seasonal changes in climatic conditions [55].

Effect of Hoagland Nutritive Medium: The results in Table (6) showed that treatment of soil with Hoagland solution resulted in a significant enhancement in plant fresh weight at concentrations of 1/6,1/4 and 1/2x at the first season but only at 1/4x in the second season. As well, the five used doses of Hoagland solution significantly increased the mean of herb dry weightat both seasons; the highest dry weight was 30.3 g per plant, detected at 1/4x Hoagland at the first season. However, applying Hoagland solution showed no significant increase in the number of branches per plant relative to those obtained without treatment (control) at the two seasons. Plant height was influenced by application of Hoagland solution and recorded significant increases by all concentrations at the second season;the concentration of 1/10x produced the tallest plants. The number of capsules per plant exhibited significant increase, especially by the highest doses 1/4 and 1/2x at the first season but onlyby 1/2x at the second season. Significant increasesof seed number per capsule was recorded at all concentrations used compared with the control at the two cultivation seasons; the highest number of seeds was 56.6 achieved by applying 1/4x Hoagland medium at the first season. The total biomass and edible biomass production were as high for plants grown in half-strength Hoagland nutrient solution as for those grown in the other solutions [56].

Chemical Analysis of Seeds: Fixed oils, soluble proteins and soluble carbohydrate contentsin seeds of *N. sativa* grown at certain concentrations (6 g/5kg D.E., 60% S.E., 1 ml/L *Nostoc*, 32 ml/L yeast and 1/4x Hoagland solution) that induced the highest yield have been analyzed.

The results showed that the application of 6 g/5kg D.E. and 60% S.E. increased the proportion of fixed oils to their highest levels compared with that of the control (31.6%, 22.7% vs 16.8%, respectively (Fig. 1). The soluble protein contentsof seeds recorded a significant increase in plantseeds treated with D.E., S.E., *Nostoc* and Hoagland compared with those of the untreated (control) plants (Fig. 2). Soluble carbohydrates content in plant seeds upon treatment with S.E., yeast, *Nostoc* and Hoagland solution was significantly higher than that recorded in control seeds (Fig. 3). Lynch reported that significant improvement and performance has been consistently noted in yields, oil content after soil treatment with silicates [57].

In addition, Many investigators [58, 59, 40] reported that treatment of rice grains, wheat and canola seeds with cyanobacteria increased the protein content of the seeds. The interactive effect of the various active substances depended on algal species and the method by which soils were amended [42,60]. In agreement with our results, El-Tohamy and El-Greadly [61] revealed that dry yeast treatment (5 and 10 g/L) resulted in improving pods quality of *Phaseouls vulgaris* in terms of carbohydrates, proteinand chlorophyll.

The analyzed oil samples were taken from the oil extract of *Nigella sativa* that have been grown at certain concentrations of the studied treatments. The effect of 6 g/5kg D.E., 60% sewage, 1 mg/L *Nostoc*, 32 ml/L yeast and 1/4x Hoagland solution was studied in comparison with the control. The free fatty acids composition of the oil samples was analyzed using the GC-mass spectroscopy.

The GC/mass analysis showed that S.E. and yeast do not change oils fractions or their percentages compared with the control (Table 7). However, *Nostoc*, Hoagland and D.E. produced changes in oil fractions compared with the control. Among the five abundant fatty acids, oleic acid, palamitic acid and stearic acid could be identified at all used treatments. Azelaic acid and Hexanoic acid were included with the most abundant five fatty acids in sewage and yeast treatments with lower percentage than incontrol treatment. While in D.E., 8,11-Octadecadienoic acid, methylester, 14-methyl-Pentadecanoic acid,methyl ester and linoleic acid, methyl ester appeared within the most abundant 5 fatty acids but they were not included in the most abundant 5 fatty acids in untreated controls.

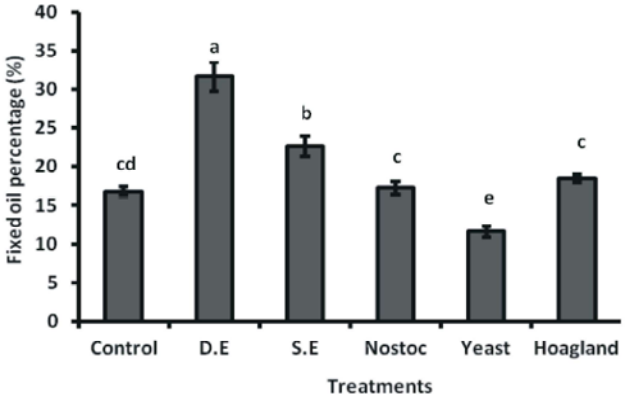

Fig. 1: Highest percentage of *Nigella sativa* seed fixed oil induced by certain concentrations [Diatoms (D.E) 6 g/5 kg, 60% sewage (S.E), 1 ml/L *Nostoc*, 32 ml/L yeast and 1/4x Hoagland] of the different biofertilizers applied (shown on the graph). Values are means of three replicates ± SE. Different letters indicate significant differences (P □ 0.05) according to one way analysis of variance (ANOVA)

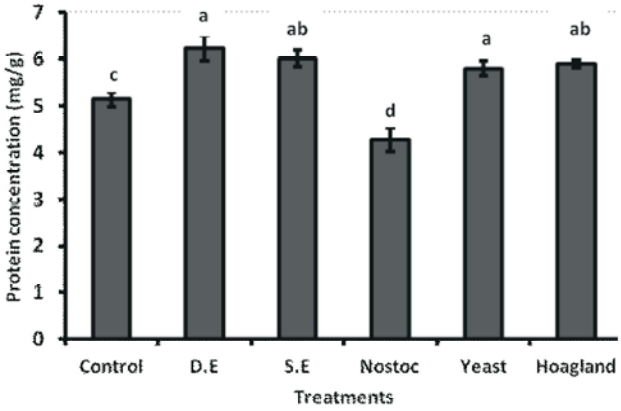

Fig. 2: Soluble proteins content of *Nigella sativa* seeds affected by certain concentrations [Diatoms (D.E) 6 g/5 kg, 60% sewage (S.E), 1 ml/L *Nostoc*, 32 ml/L yeast and 1/4x Hoagland] of the different biofertilizers applied (shown on the graph). Values are means of three replicates ± SE. Different letters indicate significant differences (P □ 0.05) according to one way analysis of variance (ANOVA)

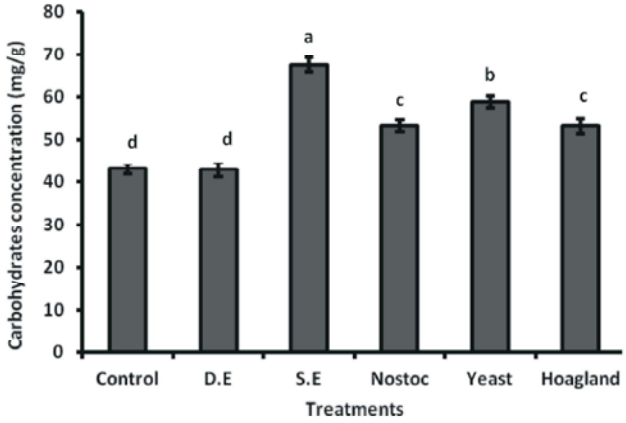

Fig. 3: Soluble carbohydrates content of *Nigella sativa* seeds affected by certain concentrations [Diatoms (D.E) 6 g/5 kg, 60% sewage (S.E), 1 ml/L *Nostoc*, 32 ml/L yeast and 1/4x Hoagland] of the different biofertilizers applied (shown on the graph). Values are means of three replicates ± SE. Different letters indicate significant differences (P □ 0.05) according to one way analysis of variance (ANOVA)

Table 7: GC-mass spectroscopy of free fatty acids (%) in seed oil samples of *Nigella sativa* L. grown on calcareous soil alone (control) andcalcareous soil treated with 6 g/5Kg diatomaceous earth (D.E.), 60% sewage (S.E.), 1 mg/L *Nostoc*, 32 ml/L yeast and 1/4x Hoagland solution

Fatty acid	Control	D.E.	S.E.	*Nostoc*	Yeast	Hoagland
Azelaic Acid	5.0	Nd	1.5	Nd	4.6	Nd
Hexanoic acid	8.6	Nd	5.3	Nd	6.1	Nd
Oleic acid	30.7	76.7	33.7	78.2	33.4	Nd
Palamitic acid	32.4	16.3	30.2	15.0	26.3	10.1
Stearic acid	3.5	Nd	4.1	0.9	3.6	1.4
Linoleic acid	Nd	Nd	Nd	Nd	Nd	80.5
Methyl linoleate	Nd	Nd	Nd	1.2	Nd	0.6
Tetradecanoic acid	Nd	Nd	Nd	0.4	Nd	Nd
Beta-Monolinolein	Nd	Nd	nd	Nd	Nd	0.6
8,11-Octadecadienoic acid,methylester	Nd	1.3	Nd	Nd	Nd	Nd
14-methyl-Pentadecanoic acid,methyl ester	Nd	0.4	Nd	Nd	Nd	Nd
Linoleic acid, methyl ester	Nd	0.5	Nd	Nd	Nd	Nd

Nd= not detected

It is interesting to note that the total unsaturated fatty acids content in *Nigella* oil represented in oleic and linoleic fatty acids with D.E., *Nostoc* and Hoagland treatments was higher than control and other treatments with percent (77, 78, 80%) while in control the percent is 30%. These results coincided with the results obtaind by Naguib and Khalil, they revealed that the mean value of the total unsaturated fatty acids was higher than that of saturated ones in fixed oil of *Nigella sativa* seeds [51]. In general, the unsaturated fatty acids are higher than saturated in all treatments and Linoleic acid was the dominant fatty acid [47].

CONCLUSION

The applied organo-fertilizers enhanced the growth and seed yield of *Nigella sativa*, being maximized by certain concentrations (6 g/5kg D.E., 60% S.E., 1 mg/L *Nostoc*, 32 ml/L yeast and 1/4x Hoagland medium).

- Fixed oil percentages have been significantly enhanced by the above-mentioned concentrations of treatments indicating the pharmaceutical and hygienic value of the seeds.
- The total unsaturated fatty acids were higher than saturated ones in fixed oil of *N. sativa* seeds. Oleic acid, palamitic acid, stearic acid, Azelaicacid and Hexanoic acid were the most abundant five fatty acids in sewage and yeast treatments. In D.E., 8,11-Octadecadienoic acid, methylester, 14-methyl-Pentadecanoic acid, methyl ester and Linoleic acid, methyl ester appeared as the most abundant 5 fatty acids while they were not included in the most abundant 5 fatty acids in untreated controls. The total unsaturated fatty acids content in *N. sativa* oil representing oleic and linoleic fatty acids with D.E., *Nostoc* and Hoagland treatments was higher than control and other treatments.

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