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The Effect of Microbial Inoculants, Humic Acid and Phosphorus on the Production and Quality Characteristics of *Nigella sativa* plants.

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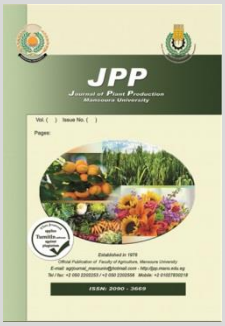

ABSTRACT

This investigation was carried out throughout the two successive seasons of 2021 /2022 and 2022 /2023 at the Sids Horticultural Research Station, ARC, Egypt, to explore the response of *Nigella sativum* L. plants to some microbial inoculants, humic acid and phosphorous on productivity and quality characteristics. Treatments were inoculated seeds with (*Bacillus megatherium* and Mycorrhizae) and addition of H.A. plus three rate (50, 100 and 200 kg) of calcium super phosphate. It was observed that the plant growth, root length, seed yield, volatile and fixed oil % and yield as well as chemical constituents were improved by treated plants with microbial inoculants and humic acid plus phosphate. The highest values of the growth characters, seed yield and volatile and fixed oil of *Nigella sativum* L. resulted from inoculated seeds with microbial inoculants, humic acid at 4 kg/fed. and andaddition 100 kg calcium super phosphate.

Keywords: Nigella sativa, microbial inoculants, humic acid, phosphate

INTRODUCTION

Nigella sativa L. is considered a medicinal and aromatic plant that belongs to the Ranunculaceae family. It is grown as a winter cropin Mediterranean region. It is grown to produce seeds that extract volatile and fixed oils, which are useful for the pharmaceutical sector and foods (Ustun *et al.*, 1990). It has been commonly used for flavoring food and as a traditional remedy for certain illnesses (Cheikh-Rouhou *et al.*, 2007). The seeds contain several characteristics like insecticidal anti bacterial, antifungal and antimalarial. In addition, the seeds are noted to have digestive, carminative, diuretic, antispasmodic, and antiseptic properties (Ali *et al.*, 2008).

The global cultivation of medicinal plants has gained attention as a means of preventing the negative health effects of chemotherapy (Hassan *et al.*, 2012). Fertilization is the most important factor influencing medicinal plant yield. Using chemical fertilizers resulted in a decline in soil fertility, raising production costs and having negative consequences on the ecosystem and public health (Borast*et al.*, 2009).

Recently, incorporating microbial inoculants into agricultural practices as sustainable fertilizers can drastically reduce the usage of pesticides and chemical fertilizers, resulting in a more sustainable and eco-friendly approach to crops production. Microbial inoculants have the potential to enhance soil quality, reduce plant diseases, promote the growth of crops, and raise crop production and quality (Yu *et al.*, 2022). Direct application of microbial inoculants follows light industrial manufacturing. Additionally, they are created by concentrating on two or more target microbes (Wang *et al.*, 2022). Oleńska *et al.* (2020) employed three different *Bacillus* species to fix nitrogen and stave off disease. Also, *Pseudomonas putida* has the ability to dissolve phosphorus.

All soils contain mycorrhizal fungi, which frequently colonize the roots of a wide variety of plant species. The mycorrhizal fungus receives carbon from the plant, and the plant receives water and nutrients from the fungus (Ortas *et al.*, 2001). These fungi improve nutrient uptake, which can lead to increased plant growth and production.

On the other hand, humic acid (HA) can improve vegetative growth and nutrient uptake, so HA has been the subject of several studies in a variety of domains, including soil chemistry, fertility, plant physiology, and environmental sciences (Paksoy *et al.*, 2010). Humic substances are organic compounds that are either black or brownish, possess huge molecular weights, and have intricate structures that are formed by the decay of plant and animal remnants (Lee *et al.*, 2004). According to previous reports, HA significantly increases the amount of organic matter in the soil, which promotes plant development and productivity (Mahmoud and Hafez, 2010). Adding the HA increases nutrient uptake, acts as a source of mineral nutrients, and controls nutrient release, all of which help plants to grow, yield more, and have higher quality (Bakry *et al.*, 2015). Moreover, HA influences sugar content, respiratory mechanism, and amino acid content (Boehme *et al.*, 2005). Bakry *et al.*, (2015) discovered that adding HA treatment enhanced the concentration of the fixed and volatile oil.

Phosphorus fertilizers are sprayed on the soil in large quantities to increase agricultural production, however only 15–30% of these fertilizers are utilized by the crops in the year that it is applied (Veneklaas *et al.*, 2012). Over time, fertilizing leads to buildup extraordinarily phosphorus concentrations in soils, which eutrophicates water ways (Johnston *et al.*, 2014). Thus, increasing crop P-uptake and usage efficiency is crucial to lower dependency on natural resources and minimize the damaging effects of excessive P fertilizer application on the environment (Cong *et al.*, 2020).

Therefore, this investigationwas aimed to study the effect of microbial inoculants and humic acid and their

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interaction on improving systemic phosphorus to increase the production and quality characteristics of *Nigella sativa* plants.

MATERIALS AND METHODS

The present investigation was carried out during the two successive seasons of 2021 /2022 and 2022 /2023 in the Experimental Farm of Horticultural Research Station at Sids, Beni-Suef Governorate to explore the response of *Nigella sativum* L. plants to some microbial inoculants (*Bacillus megatherium* and Mycorrhizae), humic acid and phosphorous on productivity and quality characteristics. Soil samples were collected from the experimental area before planting at the depth of 30 cm. and physical and chemical properties are shown in Table (A) which were determined according to Jakson (1958) at the soil laboratory of Sids Agricultural Research Station.

Table A. Physical and chemical characteristicsof the experimental soil at 2021/2022 and 2022/2023.

Seasons	Particle size distribution			Textural Class	Chemical properties								
	Clay %	Silt %	Sand %		OM %	EC, dSm^{-1} (at 25°C)	Available (ppm) N	P	K	Fe	Mn	Zn	pH
2021/2022	48.78	33.11	18.11	Clay	1.68	1.12	41.00	11.18	210.3	2.40	0.60	0.20	7.7
2022/2023	48.10	33.61	18.29	Clay	1.80	1.03	36.00	10.60	224.6	2.20	0.65	0.30	7.8

Seeds of *Nigella sativa* plants, were obtained from the Sids Horticultural Research Station and were sown on 1st of November in both consecutive seasons. Every experimental plot measured 3.5 by 3.0 m, with hills and rows spaced 30 cm and 70 cm apart, respectively. There were 5 rows and 50 hills in each plot. The seeds were manually planted in the soil. The plants were thinned to two plants per hill after one month of seeding. Every other agricultural procedure was carried out as normal.

Complete randomized block design (CRBD) was applied in this investigation. Calcium super phosphate treatments (50, 100 and 200 Kg/ fed. P_2O_5 15.5%) were done before sowing date, microbial vaccines treatments with (*Bacillus megatherium* and Mycorrhizae by 800 g per fed. for both of them) were mixed with seeds before sowing while HA treatment by 4 Kg per fed. was administered 45 days after sowing the seed, and it was repeated twice at intervals of fifteen days during the two growing seasons. Furthermore, in both seasons, all experimental units, including the control group, were supplied with ammonium sulphate at the prescribed rate of 300 kg/fed as recommended by A.R.C. (2005). These doses were applied in two parts: half on December 1st (one month after the planting time) and the other half one month after the initial dosage. Also, 100 kg of potassium sulphate (48% K_2O) fertilizer were supplied in two equal dosages at the start of the flowering phase and 3 weeks later. The crops were harvested through the 3rd week of April.

Measurements:

Properties of vegetative growth: plant height (cm), stem diameter (mm), branch number /plant, plant weight (g /plant) and length of root (cm) were recorded at harvesting time.

Yield and yield component parameters:

Number of capsule / plant, weight of capsules g /plant, weight of 1000 seed g /plant, weight of seeds g /plant and weight of seeds kg /fed. were determined.

Chemical constituents:

Pigments content (mg/g F.W.)

At flowering stage, leaf samples were collected to estimate photosynthetic pigments Chlorophyll a, Chlorophyll b and Carotenoidsmg /g F.W.according to A.O.A.C. (1985).

Nutrient elements determination:

Nitrogen (N), phosphorus (P), and potassium (K) analyses were carried out in the herb as follows: N % was measured by the microkeldahl method according to Black *et al*., (1965)., P % was colourimetrically determined according to Chapman and Pratt (1975).and K % was measured with flame-photo-meter according to Brown and Lilleland (1946).

Oil determinations:

Volatile oil percentage and yield (litre/fed.) and fixed oil percentage and yield (litre /fed.) were measured.

The experiment ended for two consecutive seasons during the 3rd week of April, when various vegetative growth traits, yield components, and yield were recorded. Extraction of essential oil from *Nigella sativa* seeds was done according to Guenther (1961).; Fixed oil percentage on seeds and fixed oil yield /fed. were recorded. Samples of dry seeds were taken to determine the percentage of fixed oil using Soxhlet apparatus according to Johnson and Davenpart (1997).

Statistical analysis:

The MSTAT-C (1985) program was used for the statistical analysis of the data, and means were compared using Duncan's multiple range test as published by Duncan (1955).

RESULTS AND DISCUSSION

Results

A- Vegetative growth criteria:

The present data in Table (1) for plant height, branches number /plant, plant weight at harvest, stem diameter and root length of *Nigella sativa* illustrated that treatments, which are called microbial vaccines (*Bacillus megatherium* and Mycorrhizae), humic acid and phosphorous fertilization caused a significant improvement in every tested vegetative property in both seasons compared to control. The treated plants yielded the greatest overall values with (Bac.+ Myc.+ HA.+ 100 kg P) treatment followed by (Bac. + Myc. + HA.+ 200 kg P) treatment in both seasons. The rise in plant height, branches number /plant, plant weight at harvest, stem diameter and root length due to (Bac. + Myc. + HA.+ 100 kg P) treatment in comparison with control treatment reached 59.05, 48.45, 129.91, 55.56 and 114.56 % , respectively, in the first season and by 35.07, 40.58, 125.58, 46.15 and 98.00 %, respectively, in the second one.

B- Yield components:

Data in Table (2) noted that there were significant variations among all treatments (microbial vaccines, humic acid and phosphorous) when compared to control; except for the (*Bacillus megatherium* plus 50 kg P_2O_5) treatment, for weight of capsules g /plant. The best tested treatment which gave high yield components was treated plants with (*Bacillus megatherium* and Mycorrhizae), 4 kg humic acid and 100 kg phosphorous fertilization. Notice no significant differences between (Bac. + Myc. + HA.+ 100 kg P) treatment and (Bac. + Myc. + HA.+ 200 kg P) treatment at weight of 1000 seed

(g /plant), weight of seeds (g /plant) and weight of seeds (kg /fed.) in both seasons. The high increment in number of capsules /plant, weight of capsules (g /plant), weight of 1000 seed (g /plant), weight of seeds (g /plant) and weight of seeds (kg /fed.) in comparison with check treatment, in order the increments were 62.92, 78.48, 25.93, 49.01 and 49.28 % respectively,in the first season and 49.02, 63.15, 22.88, 53.89 and 53.44 % respectively, in the second season.

Table 1. Effect of microbial inoculants, humic acid and phosphorus on plant height (cm), branch number, plant weigh at harvest (g /plant), stem diameter (cm) and length of root (cm) of *Nigella sativa* plants during the two successive growing seasons 2021 /2022 and 2022 /2023.

Treatments	Plant height (cm)		Branch number /plant		Plant weigh at harvest(g /plant)		Stem diameter (cm)		Length of root (cm)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	55.33 j	63.67 g	11.00 f	10.67 e	45.67 k	44.33 l	0.90 k	0.91 k	13.67 g	14.33 j
Bac. + 50 kg P	57.67 i	66.33 f	12.33 e	12.33 cd	49.33 j	47.67 k	0.93 j	0.94 j	17.00 f	16.67 i
Bac.+ 100 kg P	62.67 h	70.00 e	12.67 de	13.00 cd	50.33 j	51.33 j	0.93 j	0.94 j	17.33 f	17.00 i
Bac.+ 200 kg P	70.33 f	71.33 e	13.00 de	13.33 bcd	55.00 i	52.00 j	1.03 h	0.98 i	19.33 ef	18.00 hi
Bac.+HA.+50 kg P	75.33 e	76.00 d	14.33 bc	14.00 bc	66.67 gh	60.00 h	1.07 g	1.01 h	22.00 de	20.67 g
Bac.+HA.+ 100 kg P	76.00 e	80.33 c	13.00 d	14.33 b	57.67 i	58.33 h	1.07 g	1.04 g	23.00 cd	22.33 ef
Bac.+ HA.+200 kg P	76.67 e	80.33 c	13.33 cd	14.67 ab	64.33 h	58.67 hi	0.97 i	1.00 h	23.33 c	22.67 de
Myc.+ 50 kg P	65.67 g	70.67 e	13.67 cd	14.33 b	64.33 h	56.33 i	0.97 i	0.98 i	18.67 f	20.00 gh
Myc.+ 100 kg P	70.33 f	71.00 e	14.67 b	14.33 b	75.33 e	70.00 f	1.10 f	1.08 f	23.00 cd	21.33 fg
Myc.+ 200 kg P	72.00 f	80.33 c	13.67 c	14.67 ab	68.67 fg	65.33 g	1.07 g	1.06 fg	22.33 d	22.67 def
Myc.+HA.+ 50 kg P	78.67 cd	81.00 c	14.33 bc	14.67 ab	71.33 ef	70.00 f	1.03 h	1.05 g	22.33 d	23.00 def
Myc.+HA.+100 kg P	78.33 d	80.00 c	14.67 b	15.00 ab	74.00 e	72.33 f	1.07 g	1.05 g	23.67 cd	23.33 def
Myc.+HA.+200 kg P	82.67 b	85.33 a	14.67 b	15.33 ab	86.00 c	87.00 d	1.10 f	1.20 e	24.00 cd	23.67 de
Bac.+Myc.+ 50 kg P	75.67 e	76.33 d	15.00 b	15.33 ab	95.33 b	90.33 c	1.27 c	1.22 de	25.67 bc	24.33 cde
Bac.+Myc.+100 kg P	78.67 cd	80.00 c	14.33 bc	15.00 ab	77.00 e	80.67 e	1.20 e	1.22 de	23.00 cd	24.00 cd
Bac.+Myc.+200 kg P	80.67 bc	82.33 bc	14.33 bc	15.33 ab	82.67 d	82.00 e	1.20 e	1.23 cd	23.33 cd	24.67 cd
Bac.+ Myc.+ HA. + 50 kg P	82.33 b	83.67ab	15.33 ab	15.67 a	95.00 b	90.67 c	1.23 d	1.25 c	25.33 bc	26.00 bc
Bac.+ Myc. + HA.+ 100 kg P	88.00 a	86.00 a	16.33 a	15.00 ab	105.00 a	100.00 a	1.40 a	1.33 a	29.33 a	28.33 a
Bac.+ Myc.+ HA.+ 200 kg P	86.67 a	85.33 a	14.33 bc	15.33 ab	88.33 c	96.33 b	1.33 b	1.30 b	28.00 ab	28.00 ab

Means followed by the same letters within each column do not differ significantly according to Duncan's multiple range test at the 5% level.Bac, =*Bacillus megatherium* Myc. =Mycorrhizae HA, = Humic acid P = Phosphorus

Table 2. Effect of microbial inoculants, humic acid and phosphorus on number of capsules /plant, weight of capsules (g /plant), weight of 1000 seed (g /plant), weight of seeds (g /plant) and weight of seeds (kg /fed.), of *Nigella sativa* plants during the two successive growing seasons 2021 /2022 and 2022 /2023.

Treatments	Number of capsules /plant		Weight of capsules g /plant		Weight of 1000 seed g /plant		Weight of seeds g /plant		Weight of seeds kg /fed.	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	50.33 n	51.67 j	31.00 g	31.67 k	2.70 f	2.71 g	17.67 k	17.33 k	670.33 l	658.54 l
Bac. + 50 kg P	51.67 m	54.00 i	33.00 fg	32.00 gk	2.73 ef	2.74 fg	18.67 j	18.67 j	709.33 k	709.46 k
Bac.+ 100 kg P	51.67 m	55.33 hi	34.33 ef	34.00 ig	2.73 ef	2.75 fg	19.33 ij	19.00 j	734.67 jk	722.00 jk
Bac.+ 200 kg P	52.67 lm	56.33 h	35.67 ef	36.33 h	2.87 def	2.77 fg	20.33 h	20.67 i	772.67 i	785.46 i
Bac.+HA.+50 kg P	55.33 k	58.67 g	38.00 d	40.00 fg	2.90 def	2.81 efg	22.00 g	22.33 h	836.00 h	848.54 h
Bac.+HA.+ 100 kg P	58.67 j	58.67 g	32.33 g	37.33 h	2.73 ef	2.75 fg	22.67 fg	23.00 gh	861.33 fgh	874.00 gh
Bac.+ HA.+200 kg P	60.33 i	61.00 f	33.00 fg	37.00 h	2.77 ef	2.76 fg	23.00 ef	23.33 fh	874.00 efg	886.54 fgh
Myc.+ 50 kg P	61.00 i	61.00 f	34.67 ef	38.67 gh	2.87 def	2.82 efg	23.00 ef	23.67 ef	874.00 efg	899.46 efg
Myc.+ 100 kg P	63.00 h	62.33 f	42.67 cd	40.00 fg	3.03 cd	2.90 defg	24.00 cd	23.67 ef	912.00 cd	899.46 efg
Myc.+ 200 kg P	66.00 g	64.67 e	35.33 ef	39.67 fg	2.73 ef	2.92 cdef	22.33 e	23.00 gh	848.67 gh	874.00 g
Myc.+HA.+ 50 kg P	67.00 fg	65.00 e	36.67 e	41.33 fg	2.83 def	2.96 cde	22.67	24.00 defg	861.33 fgh	912.00 def
Myc.+HA.+100 kg P	67.67 f	65.33 e	38.33 de	41.67 ef	2.90 def	3.00 cde	23.33 d	24.00 defg	886.67 def	912.00 def
Myc.+HA.+200 kg P	68.00 ef	67.67 d	40.33 d	42.33 de	2.97 c d	3.02 bcde	24.67 c	25.00 cde	937.33 c	950.00 bcd
Bac.+Myc.+ 50 kg P	71.67 d	70.00 c	44.67 c	44.00 cd	3.17 bc	3.08 bcd	24.67 c	24.33 cdef	937.33 c	924.54 cdef
Bac.+Myc.+100 kg P	67.67 f	69.33 cd	37.33 e	43.33 de	2.90 def	3.00 cde	24.67 c	24.67 cde	937.33 c	937.46 cde
Bac.+Myc.+200 kg P	68.00 ef	69.00 cd	38.67 de	42.00 c	3.00 cd	3.11 bc	25.33 bc	25.00 bcd	962.67 bc	950.00 bcd
Bac.+ Myc.+ HA. + 50 kg P	74.67 c	72.33 b	41.33 d	44.67 c	3.17 bc	3.11 bc	25.33 bc	25.40 bc	962.33 bc	960.54 bc
Bac.+ Myc. + HA.+ 100 kg P	82.00 a	77.00 a	55.33 a	51.67 a	3.40 a	3.33 a	26.33 a	26.67 a	1000.67 a	1010.46 a
Bac.+ Myc.+ HA.+ 200 kg P	80.33 b	74.33 b	51.00 b	47.67 b	3.30 ab	3.21 ab	25.67 a	26.00 ab	975.33 a	988.00 ab

Means followed by the same letters within each column do not differ significantly according to Duncan's multiple range test at the 5% level.
Bac, =*Bacillus megatherium* Myc. =Mycorrhizae HA, = Humic acid P = Phosphorus

C- Oil productivity:

Results in Table (3) showed that the percentage of volatile oil and oil yield per fed. were progressively and significantly enhanced by application of microbial inoculants (*Bacillus megatherium*and and Mycorrhizae) and humic acid plus phosphorous fertilizers compared to the control in the two consecutive seasons, except the first treatment (Bac. + 50 kg P) without significant differences compared to the control foroil percentage. Treated black cumin plants with (Bac.+ Mic.+ HA. + 100 kg P) treatment led to the highest significant in volatile oil percentage and oil yield per fed. by 39.53 and 109.06 % in the 1st season and by 32.56 and 104.96 % in the

2nd season respectively, compared to control. A similar trend was noted for the percentage of fixed oil and oil yield / fed. The greatest values of fixed oil percentage and fixed oil yield per feddan were 33.33 % and 333.26 litter per feddan in the 1st season respectively, and 33 % and 334.65 L /fed. in the 2nd season, resulted by treating plants with *Bacillus megatherium*, Mycorrhizae,humic acid plus 100 kg phosphorus fertilization.

Table 3. Effect of microbial inoculants, humic acid and phosphorus on fixed oil %, fixed oil yield (litre/fed.), volatile oil % and volatile oil yield (litre/fed.) of *Nigella sativa* plants during the two successive growing seasons2021 /2022 and 2022 /2023.

Treatments	Volatile oil %		Volatile oil yield litre/fed.		Fixed oil %		Fixed oil yield litre/fed.	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	0.43 i	0.43 i	2.87 k	2.82 n	26.00 h	27.00 f	174.17 h	177.71 m
Bac. + 50 kg P	0.44 hi	0.44 hi	3.11 j	3.13 m	27.67 g	28.33 e	196.46 g	200.67 l
Bac.+ 100 kg P	0.45 h	0.46 gh	3.31 ij	3.16 m	28.33 fg	28.00 e	208.49 fg	202.23 l
Bac.+ 200 kg P	0.45 h	0.46 gh	3.47 i	3.54 l	29.67 ef	28.67 de	229.14 ef	225.15 k
Bac.+HA.+50 kg P	0.48 fg	0.46 gh	4.00 h	3.91 k	29.67 ef	29.33 de	247.89 de	249.00 j
Bac.+HA.+ 100 kg P	0.47 g	0.45 gh	4.06 h	3.94 k	30.00 de	29.67 d	255.61 d	259.60 ij
Bac.+ HA.+200 kg P	0.48 fg	0.47 fg	4.21 gh	4.17 ijk	30.33 cde	30.00 d	261.19 d	265.74 ghij
Myc.+ 50 kg P	0.47 g	0.47 fg	4.11 h	4.24 ghij	30.33 cde	30.00 d	269.80 d	269.74 fghij
Myc.+ 100 kg P	0.50 de	0.48 efg	4.56 ef	4.31 fghij	30.00 de	30.33 cd	268.91 d	272.81 efghi
Myc.+ 200 kg P	0.47	0.47 fg	3.99 h	4.11 jk	30.33 cde	30.33 cd	257.39 d	265.11 hij
Myc.+HA.+ 50 kg P	0.47	0.46 fg	4.04 h	4.20 hijk	30.33 cde	30.67 bc	261.31 d	279.82 defghi
Myc.+HA.+100 kg P	0.49 ef	0.50 de	4.34 fg	4.57 ef	30.33 cde	31.00 bc	268.68 d	282.71 defgh
Myc.+HA.+200 kg P	0.49 ef	0.51 cd	4.60 e	4.85 cde	31.00 bcde	31.00 bc	290.70 c	294.72 cde
Bac.+Myc.+ 50 kg P	0.51 d	0.52 cd	4.78 e	4.81 de	31.33 bcd	31.33 bc	290.57 c	289.77 cdef
Bac.+Myc.+100 kg P	0.54 c	0.52 cd	5.07 d	4.85 cde	31.67 bc	31.33 bc	296.91 bc	293.71 cde
Bac.+Myc.+200 kg P	0.55 c	0.53 bc	5.30 c	5.04 cd	32.33 ab	31.67 b	311.47 bc	300.76 cd
Bac.+ Myc.+ HA. + 50 kg P	0.57 b	0.53 bc	5.55 b	5.11 c	32.33 ab	32.00 a	315.65 abc	308.11 bc
Bac.+ Myc. + HA.+ 100 kg P	0.60 a	0.57 a	6.00 a	5.78 a	33.33 a	33.00 a	333.26 a	334.65 a
Bac.+ Myc.+ HA.+ 200 kg P	0.58 b	0.55 ab	5.67 b	5.44 b	33.33 a	32.67 a	325.53 ab	322.71 ab

Means followed by the same letters within each column do not differ significantly according to Duncan's multiple range test at the 5% level.
Bac, =*Bacillus megatherium* Myc. =Mycorrhizae HA, = Humic acid P = Phosphorus

D- Chemical characteristics:

1- Photosynthetic pigments:

The response of photosynthetic pigment content in the fresh leaves to various concentrations of microbial inoculants (*Bacillus megatherium*and Mycorrhizae) and humic acid plus phosphorous fertilizers shown in Table (4), showed that in both seasons, the treatments were proven to have beneficial effects when compared to the control. The treated plants with (Bac.+ Mic.+ HA. + 100 kg P) treatment had the highest concentrations of photosynthetic pigments which were 1.181, 0.617 and 0.778 mg /g F.W. compared with control 1.018, 0.427 and 0.517 mg /g F.W. chlorophyll a, chlorophyll b and carotenoids respectively, in the first season and by 1.173, 0.611 and 0.676 mg /g F.W. compared with untreated plants 1.022, 0.432 and 0.523 mg /g F.W., consecutively, in the second season.

Table 4. Effect of microbial inoculants, humic acid and phosphorus on the content of Chlorophyll a mg /g F.W., Chlorophyll b mg /g F.W. and Carotenoids mg /g F.W. of *Nigella sativa* plants during the two successive growing seasons 2021 /2022 and 2022 /2023.

Treatments	Chlorophyll amg /g F.W.		Chlorophyll b mg /g F.W.		Carotenoids mg /g F.W.	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	1.018 g	1.022 g	0.427 i	0.432 g	0.517 n	0.523 k
Bac. + 50 kg P	1.032 fg	1.036 fg	0.449 h	0.451 f	0.526 lm	0.533 j
Bac.+ 100 kg P	1.022 g	1.025 g	0.444 h	0.447 f	0.523 mn	0.532 j
Bac.+ 200 kg P	1.048 f	1.044 ef	0.452 gh	0.450 f	0.533 l	0.545 i
Bac.+HA.+50 kg P	1.054 ef	1.051 de	0.453 fgh	0.452 f	0.558 k	0.571 h
Bac.+HA.+ 100 kg P	1.064 de	1.060 de	0.464 efg	0.456 f	0.566 j	0.580 g
Bac.+ HA.+200 kg P	1.068 de	1.064 d	0.468 e	0.457 f	0.574 i	0.582 g
Myc.+ 50 kg P	1.070 de	1.065 d	0.472 e	0.455 f	0.584 h	0.588 g
Myc.+ 100 kg P	1.066 de	1.064 d	0.462 efg	0.454 f	0.588 h	0.601 f
Myc.+ 200 kg P	1.067 de	1.065 d	0.464 efg	0.455 f	0.600 g	0.603 ef
Myc.+HA.+ 50 kg P	1.073 de	1.066 d	0.474 e	0.456 f	0.612 f	0.609 ef
Myc.+HA.+100 kg P	1.176 a	1.116 c	0.516 d	0.490 e	0.774 a	0.645 c
Myc.+HA.+200 kg P	1.075 de	1.111 c	0.584 c	0.543 c	0.649 e	0.577
Bac.+Myc.+ 50 kg P	1.085 d	1.090 c	0.584 c	0.540 c	0.657 d	0.611 e
Bac.+Myc.+100 kg P	1.091 c	1.095 c	0.593 c	0.511 d	0.676 c	0.634 d
Bac.+Myc.+200 kg P	1.145 b	1.150 b	0.600 bc	0.581 b	0.680 c	0.633 d
Bac.+ Myc.+ HA. + 50 kg P	1.170 a	1.161 ab	0.610 ab	0.601 a	0.772 a	0.670 a
Bac.+ Myc. + HA.+ 100 kg P	1.181 a	1.173 a	0.617 a	0.611 a	0.778 a	0.676 a
Bac.+ Myc.+ HA.+ 200 kg P	1.168 a	1.160 ab	0.616 a	0.606 a	0.770 b	0.658 b

Means followed by the same letters within each column do not differ significantly according to Duncan's multiple range test at the 5% level.
Bac, =*Bacillus megatherium* Myc. =Mycorrhizae HA, = Humic acid P = Phosphorus

2-N, P and K percentages

Different treatments of microbial inoculants**,** humic acid and phosphorous fertilizers had a substantial impact on the percentages of N, P and K in the dry herb of *Nigella sativa* plants, in two consecutive seasons (Table,5). Plants treated with *Bacillus megatherium*, Mycorrhizae and humic acid plus100 kg calcium super phosphate gave the highest percentages of the three elements over those of the control by 29.43, 89.47 and 14.08 % in the 1st season and by 18.51, 90.44 and 20.49 % in the 2nd one, respectively for N, P and K %, consecutively.

Table 5. Effect of microbial inoculants, humicacid and phosphorus onN ,Pand K percentage of *Nigella sativa* plants during the two successive growing seasons2021 /2022 and 2022 /2023.

Treatments	N %		P %		K %	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	1.135 k	1.129 l	0.266 l	0.251 l	1.776 l	1.669 k
Bac. + 50 kg P_2O_5	1.148 j	1.132 l	0.277 k	0.261 k	1.788 k	1.711 j
Bac.+ 100 kg P_2O_5	1.153 ij	1.150 k	0.278 k	0.260 k	1.793 jk	1.723 ij
Bac.+ 200 kg P_2O_5	1.161 i	1.159 j	0.289 j	0.267 jk	1.803 j	1.735 i
Bac.+HA.+50 kg P_2O_5	1.163 i	1.158 j	0.292 j	0.270 j	1.850 i	1.801 h
Bac.+HA.+ 100 kg P_2O_5	1.182 h	1.162 j	0.310 i	0.298 i	1.888 h	1.834 g
Bac.+ HA.+ 200 kg P_2O_5	1.183 h	1.177 i	0.314 i	0.307 h	1.921 g	1.900 f
Myc.+ 50 kg P_2O_5	1.202 g	1.193 h	0.332 h	0.311 h	1.944 f	1.906 ef
Myc.+ 100 kg P_2O_5	1.205 g	1.200 g	0.336 h	0.310 h	1.950 e	1.911 def
Myc.+ 200 kg P_2O_5	1.212 g	1.206 g	0.356 g	0.322 g	1.963 d	1.922 d
Myc.+HA.+ 50 kg P_2O_5	1.243 f	1.233 f	0.366 f	0.338 f	1.972 d	1.920 d
Myc.+HA.+100 kg P_2O_5	1.443 d	1.312 b	0.429 d	0.401 e	1.997 b	2.000 ab
Myc.+HA.+ 200 kg P_2O_5	1.449 cd	1.300 c	0.490 b	0.437 b	1.977 c	1.987 c
Bac.+Myc.+ 50 kg P_2O_5	1.424 e	1.280 d	0.491 b	0.445 b	1.984 c	1.990 bc
Bac.+ Myc.+ 100 kg P_2O_5	1.429 e	1.270 e	0.411 e	0.405 e	1.998 b	2.004 a
Bac.+ Myc.+ 200 kg P_2O_5	1.455 bc	1.303 c	0.419 e	0.415 d	1.995 b	1.987 c
Bac.+ Myc.+ HA. + 50 kg P_2O_5	1.464 ab	1.330 a	0.445 c	0.427 c	2.019 a	2.005 a
Bac.+ Myc. + HA.+ 100 kg P_2O_5	1.469 a	1.338 a	0.504 a	0.478 a	2.026 a	2.011 a
Bac.+ Myc.+ HA.+ 200 kg P_2O_5	1.466 a	1.336 a	0.501 a	0.476 a	2.024 a	2.010 a

Means followed by the same letters within each column do not differ significantly according to Duncan's multiple range test at the 5% level.
Bac, =*Bacillus megatherium* Myc. =Mycorrhizae HA, = Humic acid P = Phosphorus

Discussion

Using microbial inoculants such phosphate solubilizing bacteria (*Bacillus megaterium*) gives phytohormones that have a positive impact on the rhizosphere of active roots by accelerating cells replication and elongation, which aids in root proliferation and enhances nutrient uptake, leading to improved vegetative growth. Improvements in growth characteristics are associated with the beneficial effects of bio-fertilizers on plant nutrient uptake (Gad, 2001). Addition of *Bacillus megaterium* affects the synthesis of vitamins, amino acids, organic acids, IAA, and GA, all of which improved plant growth and yield characteristics (Prasad *et al.,* 2018).

Mycorrhizae colonization could promote the synthesis of chlorophyll, improve root activity and the root absorption area. Furthermore, it encourages the uptake and movement of nutrients and water, which boosts photosynthesis and plant growth (Baslam *et al.,* 2013). Large amounts of hyphae are produced by mycorrhizal fungi, which increase the surface area of the plant's roots. The hyphae allow the nutrients to be transported to the plant from a distance (Marschner, 1993).

One possible explanation for the enhanced *Nigella sativa* growth is the function of humic acid in enhancing the physical and chemical properties of soil, hence augmenting the adsorption of nutrients (Bakry *et al.,* 2015). Furthermore, potassium humate influences the availability of micronutrients needed for growth and development as well as the intake and transportation of nutrients (Karakurt *et al.,* 2009). El-Sharkawy and Abdel-Razzak (2010) reported that plant hormone-like substances called cytokinins are found in humic acid, which may have helped maintain the balance of nutrients that in turn promotes growth and yield.

Humic acid application boosted phosphorus absorption, which is essential for the synthesis of phosphoprotein, phospholipids, coenzyme such as triphosphate of adenosine and nucleic acids. The energy can be transferred to several processes including activation absorption and the synthesis of different organic substances, like fixed and volatile oil (El-Ghadban *et al.,* 2003). The beneficial impact of humic acid on chlorophyll concentration may be attributed to higher rates of photosynthetic activity and CO_2 absorbing which in turn enhanced the plant's ability to absorb minerals (Ameri and Tehranifar, 2012). It is believed that humic acid stimulates microbial activity, which raises the concentration of total nitrogen and soluble phosphorus (Busato *et al.,* 2012).

Concerning, using phosphorus fertilization led to improving vegetative growth and yielding of Black cumin plants, this improvement could be the result of increased protein and photosynthesis, higher chlorophyll concentration, and increased nutrient uptake by the root system following fertilizer application (Rana *et al.,* 2012).We have observed that enhanced enzymatic activity and elevated metabolite levels may be the reason of the rising quantities of seeds in the Nigella sativa capsules (Shah and Samiullah, 2007).

CONCLUSION

In this study it observed that the highest values of the growth characters, seed yield and volatile and fixed oil of *Nigella sativa*, L. plants, resulted for inoculated seeds with some microbial inoculants (*Bacillus megatherium* and

Mycorrhizae) and humic acid at 4 kg fed., as well as, addition 100 kg calcium super phosphate.

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تأثير الملقحات الميكروبية وحامض الهيوميك والفوسفور في الصفات الإنتاجية والنوعية لنبات حبة البركة

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الملخص

تم إجراء هذا البحث خلال الموسمين المتتاليين 2022/2021 و2023/2022 بالمزرعة التجريبية بمحطة بحوث البساتين بسدس بمحافظة بني سويف لمعرفة استجابة نباتات حبة البركة لبعض الملقحات الميكروبية وحامض الهيوميك و الفسفور على زيادة الإنتاجية وخصائص الجودة. تم تلقيح بالبذور ببكتريا الباسيلس ميجاثيرم وفطر الميكورهيزا واضافة حامض الهيوميك بالإضافة إلى ثلاثة معدلات (50، 100، 200 كجم) من سماد سوبر فوسفات الكالسيوم. وقد لوحظ أن النمو الخضري وطول الجذور ومحصول البذور ونسبة الزيوت الطيارة والثابتة والمحتوى والمكونات الكيميائية قد تحسنت عند معاملة النباتات بالملقحات الميكروبية وحامض الهيوميك بالإضافة إلى الفوسفات. وكانت افضل المعاملات اللتي أدت الي أعلى القيم لصفات النمو ومحصول البذور والزيت الطيار والثابت لنباتات حبة البركة هي تلقيح البذور ببكتريا الباسيلس ميجاثيرم وفطر الميكورهيزا معاً واضافة حامض الهيوميك بمعدل 4 كجم / فدان مع التسميد بـ 100 كجم سوبر فوسفات الكالسيوم.