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Effect of a Natural Growth Stimulant (Ascobein) on Growth and Yield of Seeds and Oil of *Nigella sativa* Plants

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The importance of *Nigella sativa* plant is increasing day by day in many medical and nutritional fields, and the demand for it is increasing in foreign markets. Therefore, we chose the nigella plant to study the effect of spraying with a natural growth stimulant (Ascobein) at concentrations of zero, 50, 100 and 150 ppm on the productivity of seed and oil, as well as its effect on the activity of antioxidants. The results showed that the concentration of 100 ppm gave the highest values of vegetative growth traits; (Plant height (86.3 and 89.5cm), Number of branches (21.7 and 25.1), Fresh and Dry weight per plant (118.43 and 143.85gm, 23.86 and 28.29gm/plant) respectively for both seasons. Also, results revealed that foliar application of 100 ppm (Ascobein) improved the seed yield/plant (16.95 and 19.74gm), Oil % (45.73 and 48.02%), DPPH% (99.04 and 102.39%), TPC (447.17 and 450.54 mg GAE/g) and TFC (110.19 and 114.43 mg QE/g) for both seasons 20/2021 and 21/2022. The percentage of major fatty acids in nigella seeds oil has been estimated (Linoleic, Oleic, Palmitic and Stearic).

Keywords: Nigella sativa L.; Ascobein; fixed and essential oil; GC-MS; TPC; TFC; DPPH%.

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1. INTRODUCTION

The black seeds (*Nigella sativa*) plant is a member in Ranunculaceae family, native to the Mediterranean region and its cultivation spread in many countries of the world. It has many medicinal uses and folk medicine in many different countries. In Arabic it is called the black seed, and in English it is called black cumin.

The seeds of Nigella have been used as spices from ancient times in India when preparing pickles, as one of the ingredients, has the properties of a preservative. India is known to be the largest producer of Nigella in the world. T he other producing countries are Sri Lanka, Bangladesh, Nepal, Egypt, Iraq and Pakistan.

“Nigella Seeds Market Analysis by Form (Organic Nigella Seeds and Conventional Nigella Seeds), by Distribution Channel (Business to Business (B2B) and Business to Consumer (B2C) (Hypermarkets & Supermarkets, Specialty Stores, and Online Channels)), and by Region (North America, South America, Europe, Asia Pacific, and Middle East & Africa) - Size, Share, Outlook, and Opportunity Analysis, 2022-2028” [1].

“Many spices and seasonings can be used to enhance the flavor and aroma of food and drinks second hand. Medicinal plants are commonly used as such natural sources (Reddy et al., 2018)” [2]. Requires the use of essential oils in addition to use in dry or powder form increased recently, because it is rich in bioactive ingredients such as phenolic acid, essential oils of flavonoids and aromatics act as the best antioxidants and antibacterial agents, as active substance in food. These properties are finding increasing use in food preparation and classified [3].

“Most spices are understood to have a wide range of biological and pharmacological properties antimicrobial and antioxidant activities most commonly applied to foods pharmaceutical formulation. The rich phenolic compounds in *Nigella sativa* (black cumin) seeds keep them intact acts as a stabilizer, antioxidant and antibacterial agent and can help with this extends the shelf life of food and beverages. India and Egypt have the highest black seed producers all over the world, other countries such as Sri Lanka, Iraq, Nepal and Pakistan also make them small. Production of black cumin seeds and oil is often used to treat chronic diseases cough and bronchial asthma” [4].

Respiratory diseases, diabetes, infectious diseases, paralysis, eczema, blisters, and arthritis [5]. “Various connections are contained in seeds that contribute to various potential effects of black seed as a food like medicine. Black cumin seeds plus other parts of the plant such as roots” [6].

“The effects of this spice, including its antioxidant properties, are antibacterial, anti-inflammatory, and anticancer effects are also emphasized. Literature of ancient Egypt, Greece and Rome. In Islam's “Alsuna Alnabwia”, nigella seeds and oil are cures all diseases except the death” [7].

Superior compound of black cumin, Thymoquinone is highly valued for its bioactive properties, but is rich in many other substances active ingredients [8].This review is important bioactive compounds, therapeutic potential, and food applications of *Nigella sativa* seeds or oils.

“Nigella seeds usually contain an yellowish volatile oil, a fixed oil, proteins, amino acids such as valine, lysine, leucine, isoleucine, phenylalanine, glycine, alanine, cystine, threonine, glutamic acid, aspartic acid, proline, tryptophan, tyrosine and serine; reducing sugars, mucilage, alkaloids, organic acids, tannins, resins, toxic glucoside, metarbin, bitter principles, glycosidal saponins, melanthin resembling helleborin, melanthigenin, moisture and arabic acid. The seeds also found to possess crude fiber, minerals like Fe, Cu, Zn, Ca, P, and Na and vitamins like ascorbic acid, thiamine, niacin, pyridoxine and folic acid” [9].

In general fatty acids are known as important nutrients in both human and animal diets, and also possess various health benefits [10, 11] and are used in the pharmaceutical industry [12]. “Saturated and unsaturated fatty acids from nigella seed oil source showed good antioxidant activities” [13].

Ascobein as one of the products Egyptian Ministry of Agriculture as a natural stimulant consisting of a mixture of ascorbic and citric acid 38%, (25% ascorbic acid, 13% citric acid), hence the importance of Ascobein as a natural growth stimulant for plants sourced from the presence of both ascorbic and citric acids.

"Ascorbic acid (vitamin C) is as essential to plants as it is to animals. Ascorbic acid functions as a major redox buffer and as a cofactor for enzymes involved in regulating photosynthesis, hormone biosynthesis, and regenerating other antioxidants. In general, ascorbic acid regulates and promotes of the growth plant by cell division and it is main involved in signal transduction in the cells plant" [14].

"Different plants, fruits of citrus such as lemons and oranges, contain large amounts of citric acid, which is ubiquitous in nature as it is an intermediate in aerobic metabolism through the tricarboxylic acid cycle (TCA cycle) whereby carbohydrates are oxidized to carbon dioxide. The citric acid (CA) or TCA cycle is a pivotal element of carbon metabolism in higher plants, which provides electrons for oxidative phosphorylation in the inner mitochondrial membrane, intermediates for amino-acid biosynthesis, and oxaloacetate for gluconeogenesis from succinate derived from fatty acids via the glyoxylate cycle in glyoxysomes, among others" [15].

The purpose of this study was evaluation of the effects of Ascobein on growth and fixed oil constituents and antioxidants activity of *Nigella sativa* plants.

2. MATERIAL AND METHODS

The experiments study was conducted at the Horticulture Research Station Farm at Sides, Beni-Suef Governorate during the two successive seasons of 2020/2021 and 2021/2022. Nigella seeds were obtained from Horticulture Research Station at Sides, Beni-Suef Governorate.

Nigella seeds were sown in a clay soil on the 30th of October for both seasons. Soil samples were obtained from a depth of 30 cm from the used soil surface and some physical and chemical properties of the soil were done according to the methods described by Jackson [16] and Black [17] as shown in Table (1).

Nigella seeds were sown in 3 x 3.50 m contained five rows. The planting distance was 30 cm between plants. After 30 days from planting the plants were thinned to two plants per hill. The experimental design was randomized complete block design (RCBD) four treatments including with three replicates as follows: Four levels of Ascobein at 0, 50, 100 and 150 ppm were sprayed three times after 15, 30, 45 days from the thinned. Ascobein is a natural growth stimulant produced by General Authority of Agriculture budget fund of the Egyptian Ministry of Agriculture. The chemical composition is 38% of citric acid and ascorbic acid.

2.1 Data Recorded

2.1.1 Vegetative growth characteristics

Plant height, number of branches per plant, and fresh and dry weight of each plant were estimated.

2.2 Seeds Yield

Capsules number per plant, seed yield per plant and weight of 100 seeds, were estimated.

2.2.1 Extract of nigella fixed oil

The crude oil was obtained by mechanical cold pressing of the seeds with a screw press machine (CARVER model 2759S/N 2759-595 FREDS CARVER INC.). The oil percentage, some physical properties (Refractive index, Iodine value, Acid value and Saponification value) and chemical characteristics of the cold pressed oil were determined by official methods (AOAC) [18].

2.2.2 Extract of nigella oils

It was done by adding one ml of methanol to 1 ml of crude oil (Fixed oil and Essential oil) in a glass centrifuge tube with cover. Vortex mix for 2 mins, the methanol top layer was transferred to a small glass vial [19].

Table 1. Physical and Chemical properties of the experimental soil at 2020 and 2021

Season	Particle size distribution			Texture Grade	Chemical properties								
	Clay %	Silt %	Sand %		pH	Ec dS/m	OM %	Available (ppm) N	P	K	Fe	Zn	Mn
2020	48.30	33.50	17.20	Clay	7.7	1.15	1.70	40.00	15.10	261.40	2,50	0.27	0.60
2021	49.60	32.40	17.00	Clay	7.8	1.20	1.80	39.00	13.60	246.22	2.25	0.30	0.65

2.2.3 GC-MS analysis of nigella essential oil

GC-MS was used to determine the main component of Nigella seeds fixed oil (Fatty acids: Linoleic, Oleic, Palmitic and Stearic), and essential oils (α-Pinene, Camphene, Dithymoquinone and Thymoquinone). Essential and fixed oils analyzes were performed in a Central Laboratory Network, National Research Centre, and Cairo, Egypt. Nigella oils extract (Fixed and Essential oils) was carried out by GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A). Samples were diluted with hexane (1:19, v/v). The GC- MS was equipped with HP-5MS column (40 m x 0.25 mm internal diameter and 0.25 µm film thickness). The analysis was performed using helium as carrier gas, a flow rate of 1.0 ml/min, a split ratio of 1:10, an injection volume of 1 µl, and the following temperature program. 1 minute at 40°C. Heat up to 150°C at 4°C/min and hold for 6 minutes. Ramp at 4.5°C/min to 220°C and hold for 1 minute. The injector and detector were maintained at 280°C and 220°C, respectively. Mass spectra were acquired by electron ionization (EI) at 70 eV. A spectral range of m/z 50-550 and a solvent delay of 5 min. The identities of the various components were determined by comparing the spectral fragmentation patterns with those stored in Wiley and NIST mass spectral library data.

2.3 Antioxidant Activities

2.3.1 DPPH% (1, 1-diphenyl-2- picrylhydrazyl) radical scavenging assay

Free radical scavenging activity of the seed oil was measured in terms of radical scavenging ability using the stable free radical DPPH 16. Different concentrations (10, 20, 30, 40 and 50µl) of sample were taken and 50µl of 0.659 mM DPPH dissolved in methanol solution was added to make up to one using double distilled water. The tubes were incubated at 25ºC for 20 minutes. The absorbance value was recorded at 510 nm using shimadzu UV 1800 spectrophotometer. The same procedure was followed for control without the sample [20].

DPPH Scavenging ability (%) = $[A_{control} - A_{sample} / A_{control}] \times 100$

2.3.2 Determination of total Phenolics and flavonoids content in seed extract

Seeds (3 g) were mechanically ground and treated at 80%. Aqueous methanol for 60 min at 30 0C. The supernatants were filtered through a whatman grade (1) filter paper. The volumes of the seeds extract were adjusted to 50 ml and 100 ml by adding the appropriate volume of aqueous methanol (80 %), respectively. The extracts were stored at 4 ^{o}C for analysis. Concentrations of total phenol in extracts were determined using the Folin-Ciocalteu method described by Kim [21]. The total phenols content (TPC) were determined as gallic acid equivalents (GAE) per gram of each extract. Gallic acid was prepared 400-1000 mg/l, and the values are presented as means of triplicate analyses. The total flavonoids content (TFC) in the seeds extracts was measured according to Subhasree [22]. The measurement was based on reaction with $AlCl_3$ and spectrophotometrical technique. All determinations were performed in triplicate. The total flavonoids content was expressed as mg pyrocatechol (Quercetin) equivalents (QE) per g of seeds extract.

2.4 Statistical Analysis

All the data recorded throughout the study was exposed to the analysis of variance techniques according to the design (RCBD) used by the CoStat software package for Windows. Treatment means were separated and compared using the L.S.D test at 0.05 level of significance according to Snedecor and Cochran [23].

3. RESULTS

The results obtained in Table (2) indicate that the concentration of 100 ppm of Ascobein gave the highest value in the studied traits; (Plant height (83.3 and 89.5 cm / plant, number of branches (21.7 and 25.1 / plant), fresh weight (118.43 and 143.85 g / plant) and dry weight (23.86 and 28.29 g / plant, respectively for both seasons 2021 and 2022 compared to other treatments. Also, the results obtained in Table (3) indicate that the concentration of 100 ppm of Ascobein gave the highest value for fruit number / plant (49.79 and 52.82), weight of 100 seeds (0.318 and 0.312 g) and seeds yield / plant (16.95 and 19.74 g) respectively for both seasons 2021 and 2022.

The results showed that the percentage of Nigella seeds fixed oil was as follows (100 ppm gave 45.73 and 48.02%,), (150 ppm gave 42.09 and 46.57%), (50 ppm gave 39.96 and 46.52%) and finally the control treatment gave the lowest value of Nigella seeds oil percentage (38.81 and

42.30%) for both seasons 2021 and 2022 (Table 4). On the other hand, GC-MS analysis of the fixed oil of Nigella showed that linoleic acid (38.66 and 39.91%), oleic acid (24.06 and 24.84%), palmitic acid (13.26 and 14.05%) and stearic acid (6.04 and 6.38%) respectively, were a direct result of the 100 ppm Ascorbin treatment for both seasons 2021 and 2022 (Table 4), then came the following treatments 150 ppm and 50 ppm and finally the control treatment gave the lowest value for the percentage of the four fatty acids.

GC-MS analysis of the essential oil of nigella in Table (5) showed that the main compounds percentage; α-Pinene (17.82 and 19.33%), Camphene (8.94 and 9.79%), Dithymoquinone (10.16 and 11.33%) and Thymoquinone (2.09 and 2.16%) respectively, were a direct result of the 100 ppm Ascorbin treatment for both seasons 2021 and 2022. Also, came the following treatments 150 ppm and 50 ppm and finally the control treatment gave the lowest value for the percentage of the four main compounds α-Pinene (14.17 and 13.92%), Camphene (7.60 and 8.94%), Dithymoquinone (8.94 and 9.36%) and Thymoquinone (1.81 and 1.89%) for both seasons 2021 and 2022,respectively.

The results shown in Table (6) that there are no significant differences for the three physical characteristics of the fixed oil of Nigella (Saponification value and Iodine value and Refractive index 20^0C) for all treatments.

Generally, the results obtained in Table (7) indicate that the concentration of 100 ppm of Ascobein gave the highest value antioxidant activity (DPPH% (99.04 and 102.39%), TPC (447.17 and 450.54 mg GAE/g) and TFC (110.19 and 114.43 mg QE/g), respectively for both seasons. On the other hand, the results shown in Table (7) that there are no significant differences between 50 ppm and 150 ppm treatments for DPPH% and TFC in both seasons 2021 and 2022. Control treatment gave the lowest value for DPPH%, TPC and TFC in both seasons 2021 and 2022.

Table 2. Effect of different concentrations of Ascobein on *Nigella sativa* growth (PH, Br.No, FW and DW/ Plant) for both seasons 2021 and 2022

Treatment	Plant Height (cm)		Branches No. / Plant		Fresh Weight / Plant (gm)		Dry Weight / Plant (gm)	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	65.1 d	70.3 d	11.2 d	12.6 d	65.84 d	69.96 d	12.91 c	13.83 d
50 ppm	83.9 b	87.7 b	17.9 c	18.6 c	92.53 c	107.29 b	18.13 b	19.96 b
100 ppm	86.3 a	89.5 a	21.7 a	25.1 a	118.43 a	143.85 a	23.86 a	28.29 a
150 ppm	79.8 c	83.4 c	19.6 b	21.2 b	96.36 b	99.37 c	18.31 b	18.79 c
L.S.D $_{0.05}$	1.36	1.29	1.02	1.05	2.67	2.88	1.01	1.03

Means with similar letters of the alphabet are not significantly different according to the least significant difference test at the 0.05 probability level

Table 3. Effect of different concentrations of Ascobein on *Nigella sativa* growth (Fruit No, Weight of 100 seeds and Seed yield / Plant) for both seasons 2021 and 2022

Treatment	Capsule No. / Plant		Weight of 100 seeds		Seeds yield / Plant (gm)	
	2020	2021	2020	2021	2020	2021
Control	25.28 d	27.87 d	0.263 c	0.265 c	7.52 d	10.39 d
50 ppm	48.05 b	50.82 b	0.295 b	0.281 b	12.17 c	15.18 c
100 ppm	49.79 a	52.94 a	0.318 a	0.312 a	16.95 a	19.74 a
150 ppm	44.25 c	46.63 c	0.297 b	0.299 b	14.73 b	18.62 b
L.S.D $_{0.05}$	1.62	1.46	0.018	0.019	0.84	0.88

Means with similar letters of the alphabet are not significantly different according to the least significant difference test at the 0.05 probability level

Table 4. Effect of different concentrations of Ascobein on Nigella fixed oil and some fatty acids % (Linoleic, Oleic, Palmitic and Stearic) for both seasons 2021 and 2022

Treatment	Nigella oil %		Fatty acids %							
			Linoleic		Oleic		Palmitic		Stearic	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Control	38.81 d	42.30 c	34.18 d	36.51 d	21.60 d	22.03 d	11.82 c	12.01 d	4.58 d	4.46 d
50 ppm	39.96 c	46.52 b	36.68 c	37.59 c	22.99 b	23.37 c	12.07 b	13.81 c	5.67 b	5.91 c
100 ppm	45.73 a	48.02 a	38.66 a	39.91 a	24.06 a	24.84 a	13.26 a	14.05 a	6.04 a	6.38 a
150 ppm	42.09 b	46.57 b	38.01 b	38.14 b	23.01 b	24.28 b	13.10 a	13.49 b	5.07 c	6.24 b
L.S.D $_{0.05}$	0.83	0.82	0.46	0.41	0.37	0.39	0.23	0.29	0.08	0.12

Means with similar letters of the alphabet are not significantly different according to the least significant difference test at the 0.05 probability level

Table 5. Effect of different concentrations of Ascobein on main compounds percentage of Nigella essential oil for both seasons 2021 and 2022

Treatment	The main compounds percentage of Nigella essential oil							
	α-Pinene		Camphene		Dithymoquinone		Thymoquinone	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	14.17 d	13.92 d	7.60 c	8.01 c	8.94 c	9.36 d	1.81 d	1.89 c
50 ppm	16.23 c	17.03 c	8.25 b	9.21 b	9.42 b	10.05 c	1.98 b	2.04 b
100 ppm	17.82 a	19.33 a	8.94 a	9.79 a	10.16 a	11.33 a	2.09 a	2.16 a
150 ppm	17.02 b	18.61 b	8.17 b	8.97 b	10.04 a	10.81 b	1.87 c	2.03 b
L.S.D $_{0.05}$	0.78	0.62	0.49	0.48	0.42	0.46	0.04	0.06

Means with similar letters of the alphabet are not significantly different according to the least significant difference test at the 0.05 probability level

Table 6.Effect of different concentrations of Ascobein on Physical properties of Nigella fixed oil for both seasons 2021 and 2022

Treatment	Physical properties					
	Saponification value		Iodine value		Refractive index 20 C^0	
	2020	2021	2020	2021	2020	2021
Control	196.21	196.25	112.40	112.38	1.471	1.471
50 ppm	196.21	196.25	112.40	112.38	1.471	1.471
100 ppm	196.21	196.25	112.40	112.38	1.471	1.471
150 ppm	196.21	196.25	112.40	112.38	1.471	1.471
L.S.D $_{0.05}$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

(n.s.) no significant differences

Table 7. Effect of different concentrations of Ascobein on DPPH %, TPC and TFC in Nigella fixed oil for both seasons 2021 and 2022

Treatment	DPPH %		Total phenol content (TPC) mg GAE/g		Total flavonoid content (TFC) mg QE/g	
	2020	2021	2020	2021	2020	2021
Control	87.54 c	92.41 c	410.43 d	413.81 d	104.11 c	105.78 c
50 ppm	94.34 b	98.67 b	431.29 c	433.91 c	106.06 b	109.55 b
100 ppm	99.04 a	102.39 a	447.17 a	450.54 a	110.19 a	114.43 a
150 ppm	95.11 b	98.05 b	443.24 b	445.29 b	107.27 b	108.04 b
L.S.D $_{0.05}$	2.04	2.41	3.81	3.92	1.89	1.97

Means with similar letters of the alphabet are not significantly different according to the least significant difference test at the 0.05 probability level

4. DISCUSSION

Ascobein as a natural stimulant consisting of a mixture of ascorbic and citric acid 38%. Ascobien compound, (25% ascorbic acid, 13% citric acid and 62% organic materials). The importance of Ascobien comes in that it contains ascorbic, citric acids, where the ascorbic and citric acid play an important role in physiological processes such as regulating growth and metabolism of the plants. Noctor [24] and Smirnoff [25] showed that ascorbic acid acts as a primary substrate in the cyclical pathway for detoxification and neutralization of superoxide radicals and singlet oxygen. Also, it is a small, water-soluble molecule. In this context, Abdossi [26] stated that ascorbic, citric acids application maximized cell division, growth, and preservation of cell, which affected on the regulation of growth flowering, physiological processes and chemical components on carnation plant. Initial metabolites like amino acids, carbohydrates, fatty acids, and organic acids are involved in growth, respiration and photosynthesis, hormone synthesis, and protein synthesis [14,15].

Our experiment showed that plants were sprayed with Ascobein at 50, 100 and 150 ppm resulted increasing in the studied vegetative characteristics and seeds yield per plant. A report illustrated that ascorbic acid and α-tocopherol affected the growth and some chemical compounds of *Hibiscus rosa sineses* [27]. Ascorbic acid (AA) is an antioxidant that, combined with other components of the antioxidant system, protects plants from oxidative damage as a resulting from photosynthesis, aerobic metabolism and some of pollutants like heavy metal, ozone and saline stress. Plus, it's more than just an antioxidant. It also functions as a cofactor for several metabolic enzymes involved in fundamental developmental processes in plants and as a well-known cellular reducing agent with narrow- and wide-range roles in responses to environmental stresses. Several studies have also suggested that endogenous AA are involved in promoting plant growth and development by engaging in a complex set of plant hormone-mediated signaling networks associated with various environmental stresses [28].

“Ascorbic acid can be a regulator on cell division and differentiation and has an important role in a wide range of functions such as antioxidant defense, regulation of photosynthesis and growth” [29]. Ascobein at 100 ppm caused an increase in all vegetative parameters (plant height, number of branches, fresh and dry weigh per plant) of *Nigella sativa* plant. These results are in harmony with those reported by Gahory [30] on Nigella sativa and Ali [31] on fennel with respect to ascorbic acid.

“The presence of this fatty acid in significant amounts together with the other unsaturated fatty acids was characterized to be a specific chemotaxonomic criterium for *Nigella spp.”* [32, 33]. In this study, the main saturated fatty acids (palmitic and stearic acids) accounted for 19.30, 20.43 % (at 100 ppm Ascobein) of total fatty acids for both seasons. These results are in agreement with previously published data [34, 35, 36].

Our results were confirmed the antioxidant potential of Nigella oil in terms of radical scavenging assay. In general, *Nigella sativa* oil showed significantly increased DPPH radical scavenging activity, TPC and TFC showed a significant increase. Therefore, it is possible that the antioxidant activity of the extracts is due to high presence of TPC and TFC in Nigella oil [37].

5. CONCLUSION

The obtained results showed that spraying nigella plants with Ascobein caused a significant increase in the vegetative growth, yield characteristics and oil yield as well as main components, total phenolic contents and antioxidant activity of nigella oil compared to untreated plants. The highest values of all studied parameters were obtained by using Ascobein at 100 ppm. GC–MS analysis of the essential oils showed that the major components were α-Pinene followed by Dithymoquinone, Camphene and Thymoquinone, respectively. 100 ppm of Ascobein gave the highest values of radical scavenging activities of nigella oil. Therefore, we recommended that spraying nigella plants with 100 ppm Ascobein can be used for obtaining higher vegetative growth, seeds yield and quantity and quality of nigella oil.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAO, Statistical Database of the United Nation Food and Agriculture Organization (FAO) Statistical Division. Rome; 2021.
2. Reddy H., Adhari S., Afrah S. Studies on Phytochemical Screening - GC-MS Characterization, Antimicrobial and Antioxidant Assay of Black Cumin Seeds (*Nigella Sativa*) and Senna Alexandria (*Cassia Angustifolia*) Solvent Extracts. International Journal of Pharmaceutical Sciences and Research 2018; 9(2):490-97.
3. Shan B., Yi Zhong C., John D., Harold C. Potential Application of Spice and Herb Extracts as Natural Preservatives in Cheese. Journal of Medicinal Food. 2011; 14(3):284-90.
4. Kaskoos R. Fatty acid composition of black cumin oil from Iraq. Res. J. Med. Plant. 2011; 5(1):85-89.
5. Yimer E., Kald B., Aman K., Najeeb R., Farooq A. *Nigella Sativa L*. (Black Cumin): A Promising Natural Remedy for Wide Range of Illnesses. Evidence-Based Complementary and Alternative Medicine; 2019.
6. Bourgou S, Andre P, Brahim M, Jean L. Antioxidant, Anti-Inflammatory, Anticancer and Antibacterial Activities of Extracts from *Nigella Sativa* (Black Cumin) Plant Parts. Journal of Food Biochemistry. 2012;36(5): 539-46.
7. Ramadan M. Black Cumin (*Nigella Sativa*) Oils. Essential Oils in Food Preservation, Flavor and Safety. Elsevier Inc; 2016.
8. Amin B., Hossein H. Black Cumin (*Nigella Sativa*) and Its Active Constituent, Thymoquinone:An Overview on the Analgesic and AntiInflammatory Effects. Planta Medica. 2016;82(1-2):8-16.
9. Reza A., Ladan M. Essential Oil Constituents of *Nigella Sativa*. Pharmacology and Life Sciences Bull. Env.Pharmacol. Life Sci. 2015;4:153-155.
10. Liu J., Zschocke S., Reininger E., Bauer R. Inhibitory effects of Angelica pubescens f. biserrata on 5- lipoxygenase and cyclooxygenase. Planta Med. 1998;64(6): 525-529.
11. Singh S., Majumdar D. Evaluation of anti-inflammatory activity of fatty acids of *Ocimum sanctum* fixed oil. Indian J. Experi Biol. 1997;35(4):380- 383.
12. Weidner M. Novel pharmaceuticals, dietary supplements and cosmetics containing fatty acids Zingiber extract for the treatment or prevention of inflammation, hypersensitivity or pain. PCT Int. Appl. 2000; AN:627966.
13. Laneuville O, Breuer D, Dewitt D, Hla T, Funk C, Smith W. Differential inhibition of human prostaglandin endoperoxide H synthases-1 and-2 by nonsteroidal anti-inflammatory drugs. J. Pharmacology Exp. Therapeutic 1994;271(2):927-934.
14. Mohammad A, Sergi M, David J, Pedro D, Masayuki F. Argelia L. Ascorbic Acid in Plant Growth, Development and Stress Tolerance. Springer International Publishing AG, part of Springer Nature; 2017.
15. Alexander A. Citric acid. Springer International Publishing Switzerland; 2014.
16. Jackson, M. Soil Chemical Analysis. Prentice-Hall of Indian Private, New Delhi, India. 1973;478.
17. Black C, Evans D, Ensminger L, White J, Clark F, Dinauer R. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd Ed. Soil Sci., Soc. of Am. Inc. Publ. Madison, Wisconsin, U.S.A. 1982;1159.
18. AOAC. Official tentative methods of analysis. 12th Ed., Washington, D. C., U.S.A; 1995.
19. British Pharmacopoeia. Volatile oil in drugs. A 108–A112. The Univ. Press, Cambridge, England. 1980; II.
20. Jain P., Agrawal R. Antioxidant and free radical scavenging properties of developed mono and polyherbal formulations. Asian J. Exp. Sci. 2008; 22(3):213-220.
21. Kim D., Jeong S., Lee C. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums, Food Chemistry 2003;81:321-326.
22. Subhasree B, Baskar R, Keerthana R, Susan R, Rajasekaran P. Evaluation of antioxidant potential in selected green leafy vegetables, Food Chemistry 2009; 115:1213-1220.
23. Snedecor WC, Cochran WG. Statistical Methods 7th ed. Iowa State Univ., Press. Ames, IA; 1980.
24. Noctor G, Foyer C. Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. of Plant Physiol. and Plant Mol. Biol. 1998;49:249-279.
25. Smirnoff N. Ascrobate, tocopherol and carotenoids: mestabolism, pathway engineering and function. In: N. Smirnoff, (ed.), Antioxidants and Reactive Oxygen

Spexies in Plants, Blackwell Publishing Ltd, Oxford, UK. 2005;53-86.
26. Abdossi V., Danaee E. Effects of some amino acids and organic acids on enzymatic activity and longevity of *Dianthus caryophyllus* cv. Tessino at Pre-harvest stage. Journal of Ornamental Plants 2019;9(2):93-104.
27. El-Quesni F, Abd El-Aziz N., Kandil M. Some studies on the effect of ascorbic acid and α– tocopherol on the growth and some chemical composition of *Hibiscus rosa sineses L*. at Nubaria. Ozean J. App. Sci. 2009;2(2):159-167.
28. Mazid M, Khan T, Khan Z, Quddusi S, Mohammad F. Occurrence, biosynthesis and potentialities of ascorbic acid in plants. International Journal of Plant, Animal and Environmental Sciences 2011;1:167-184.
29. Blokhina O, Virolainen E, Fagerstedt K. Antioxidant, oxidative damage and oxygen deprivations stress: A Review. Ann. Bot. 2003;91(2):179-194.
30. Gahory A. Physiological studies on black cumin plant. Ph.D. Diss., Fac. of Agric., Minia Univ; 2012.
31. Ali A, Hassan E, Hamad H, Abo-Quta W. Effect of compost, ascorbic acid and salicylic acid treatments on growth, yield and oil production of fennel plant. Assiut J. Agric. Sci. 2017;(48)(1): 139-154.
32. Kokdil G, Yılmaz H, Analysis of the fixed oils of the genus *Nigella sativa L*. (Ranunculaceae) in Turkey, Biochemical Systematics and Ecology. 2005;33:1203-1209.
33. Aitzetmuller K, Werner G, Seeds oils of Nigella species and of closely related genera, OCL-Oleagineux Corps Gras Lipides. 1997;4:385-388.
34. Houghton P., Zarka R., Heras B., Hoult R. Fixed oil of *N. sativa* and derived thymoquinone inhibit eicosanoid generation in leucocytes and membrane lipid peroxidation, Planta Medica. 1995;61: 33-36.
35. Al-Jassir M. Chemical composition and microflora of black cumin (*Nigella sativa L*.) seeds growing in Saudi Arabia, Food Chemistry 1992; 45, 239– 242.
36. Ramadan M., Morsel J. Characterization of phospholipid composition of black cumin (*Nigella sativa L*.) seed oil, Nahrung/Food. 2002;46:240-244.
37. Meziti A., Meziti H., Kaouthar B., Mustapha B., Bouriche H. Polyphenolic profile and antioxidant activities of *Nigella sativa* seed extracts in vitro and in vivo, Worl academy of Science, Engineering and Technology. 2012;64: 24-32.

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