J. Mod. Res. 6 (2024) 92-97

Improving the Productivity of *Nigella sativa* Plants Via Chitosan and Some Amino Acids

Mohmed M. A-H.*, F. S. Badran, E. T. Ahmed, E. M. Ahmed
Department of Horticulture, Faculty of Agriculture, Minia University
* Correspondence: mmahmohamed@gmail.com; Tel: + 01062892355;

Article information
Received:17 April 2024
Revised: 23 May 2024
Accepted: 29 May 2024

Keywords
Black cumin seeds,
Glycine,
Tryptophan,
Chitosan

Abstract

Seeds of *Nigella sativa*, are considered as one of the vital forms of available healing treatment. A field experiment was carried out to investigate the effect of chitosan (0.0, 1.0, 2.0, and 3.0 g/l) and two individual amino acids; glycine and tryptophan (0.0, 1.0, 2.0, and 3.0 g/l) application on plant growth and productivity. Results showed a significant effect of both investigated factors in herb day weight, seed yield, and proximate analysis. The highest seed yields (44.1 and 42.7 g/plant) with insignificant difference between them was for plants treated with 1.0 g/l of chitosan + (1 g/l tryptophan) or (3.0 g/l glycine) respectively. Whereas the minimum seed yield of about 21.2 and 21.7 g/plant was estimated for non-amino acid treated plants + 2.0 or 3.0 g/l of chitosan. The higher seed moisture content (5.37%) was for plants treated with 2 g/l of tryptophan in addition to 1.0 g/l of chitosan. Generally, increasing chitosan over 2.0 g/l caused a reduction in seed moisture content. Non-amino acid-treated plants that received 3.0 g/l of chitosan had the lowest ash (3.18%), but the highest value (4.63%) was for plants treated with 1.0 g/l chitosan + 1.0 g/l tryptophan. The lowest and highest lipids (28.11 and 30.75) were for untreated plants, and those treated with (2.0 g/l chitosan + 2.0 g/l tryptophan), respectively. Therefore, the study suggested that N. sativa plants could be treated with 1.0 g/l chitosan in addition to 1.0 g/l tryptophan to achieve the highest seed yield. However, for higher lipids content plants should be treated with 2.0 g/l of chitosan and 2.0 g/l tryptophan.

1. Introduction

Nigella sativa Linn (black cumin) plants which belong to the Ranunculaceae family, is a small annual herb native to North Africa, Southern Europe, and Southeast Asia [1]. However, it is cultivated in numerous region and countries like the Middle East, the Mediterranean region, Saudi Arabia, India, and Pakistan [2 and 3]. The prophet Muhammad peace be upon him considered it as one of the vital forms of available healing treatment. The use of black cumin seeds has been cited by Ibne–Sina [4 and 5]. They are used as astringent, stimulant, bitter, diuretic, anthelmintic, emmenagogue, jaundice, dyspepsia, paralysis, piles, skin diseases, etc. [6 and 7]. There are hundreds of evidences for this miraculous medicinal herb, using different parts extracts, seed oil, volatile oils, fatty acids, and other isolated compounds [8, 9, and 10]. Black cumin seeds contain about 8.1–11.6% moisture and 4.8% ash. However, they are a rich source of protein (26.7%), fatty oil (35.6–41.5%), and carbohydrates (24.9%). They also contain volatile oils (0.5–1.6%), crude fiber (8.4%), and cellulose (6.8–7.4%) [11 and 12]

Chitosan is widely used in agricultural production as a potential and fascinating biocatalyst agent [13] *via* stimulating plant immune system by regulating many genes [14]. Moreover, it might increase the activity of many antioxidant enzymes which would protect plants from damage via free radical groups. These responses could help plants to retain water, absorb nutrients, and produce stress-tolerant proteins. On this basis, there is increasing attention on its agricultural applications to improve crop production [15]. Recent literature indicates the efficiency of chitosan application as soil and/or foliar usage in the rise of growth and secondary metabolites biosynthesis in numerous species [16]. *Nigella sativa* plants which were treated with nanoparticles of chitosan had significantly higher vegetative growth and seed yield compared to untreated ones [17]. Chitosan improved plant height, leaf area index, water efficiency, leaf temperature, and stomatal conductance of *N. sativa* [18].

Amino acids are considered precursors of proteins and other nitrogen compounds such as nucleic acids [19 and 20]. They are well known as plant biostimulants which have a significant effect on plant growth, more abundant growth, and more uniform flowering, yield, and significantly mitigate the injuries caused by abiotic stresses [21 and 22]. Therefore, they have a great importance in plant nutrition for obtaining higher yields and quality and shortening of the productive cycle with better biomass [23]. Plants can biosynthesis amino acids, but this is an extremely energy consuming phenomena. So, the application of ready amino acids for uptake might save plant energy and increase their development, especially during the critical times of their development [24 and 25]. There are many studies regarding the plant growth response to foliar application of amino acids. The influence of amino acids on the vegetative growth, yield, and chemical constituents of different medicinal and aromatic plants and many other crops has been widely examined [25 and 26]. So that, this study aimed to improve seed and lipids of N. sativa using natural biostimulants; chitosan and amino acids

2. Materials and methods

2.1. Experimental design

A randomized complete block design field experiment in split plot arrangement [27] with three replicates was conducted during the seasons of 2020/2021 and 2021/2022 at the Farm of Hort. Dept., Fac. of Agric., Minia Univ. The physical and chemical analysis [28] of the experimental soil is shown in Table 1. Black cumin (*Nigella sativa*) seeds were sown on 15th Oct. in plots (3×4 m) consisting of 7 rows with a 60 cm distance between the rows and 30 cm between the hills within the row. The main plot included 4 concentrations of chitosan (0.0, 1.0, 2.0, and 3.0 g/l) whereas the subplot included the two amino acids glycine and tryptophan under seven treatments {control (0.0), and 1.0, 2.0 or 3.0 g/l for both types of amino acid). Hence, the interaction between both studied factors included 28 treatments.

Table (1): Physical and chemical analysis of the experimental soil (average of both seasons).

Soil characters	Value	Soil characters		Value
Soil type	Clayey loam	Avail. P (%)		15.40
Sand (%)	28.59	Exch. K (mg/100g)		2.45
Silt (%)	30.29	Exch. Ca (mg/100g)		31.43
Clay	41.12	Exch. Na (mg/100g)		2.46
Organic Matter (%)	1.65		Fe	8.39
$CaCO_3$ (%)	2.10	DTPA	Cu	2.04
pH (1:2.5)	7.79	Ext.(ppm)	Zn	2.81
EC (mmhos/cm)	1.06		Mn	8.19
Total N (%)	0.08			

Chitosan (Itan Biotech Limited, India), Glycine (Biosntha, UK) and tryptophan; (AgriBegari, India) were applied as a foliar application using a hand spray. Chitosan was dissolved in acetic acid then the volume was completed to the final one. The plants were sprayed till runoff using liquid soap as a wetting agent. Control plants were sprayed with water containing a wetting agent. The treatment commenced after 45 days of transplanting and was repeated three times every 30-day intervals.

All plants were fertilized with ammonium sulphate (20.6 % N), 200 kg/fed. of calcium superphosphate (15.5% P2O5) and 50 kg/fed. of potassium sulphate (48% K2O). The amount of N fertilizer was divided into two batches, added at three-week intervals, starting Nov. 15th, while the K fertilizer was added with the first batch of N fertilizer. Whereas P fertilizer was added during preparing the soil for cultivation in both experimental seasons. All other agriculture practices were carried out following farmer habitat.

2.2. Harvesting and estimation of plant growth and seed yield

During the 1^{st} week of May in both seasons, plants were cut just above ground surface. The plants were air-dried for several days to remove moisture before recording the herb dry weights. Seed yield/plant was manually extracted from each plant and weighted.

2.3. Seed proximate analysis

A sample of 10 g seeds was randomly taken from each season and mixed. The standard methods of the Association of Official Analytical Chemists [29] were followed to assess moisture, ash, and crude lipids.

Moisture %: Milled seed samples were precisely weighed in an aluminum dish and then dried overnight at 72° C. Therefore, the dish was covered and cooled in a desiccator before being weighed. The sample was further dried for 2 h and reweighed until estimated at a constant weight.

Moisture matter content % = (Final weight- Primary weight)/ (Weight of the sample) X 100

Ash %: A sample of about 5 g was precisely weighed into a porcelain crucible and heated over a low flame till completely charred before being heated in a muffle furnace till a constant weight to warrant complete conversion to ash.

Ash content % = (Ash weight)/ (Weight of the sample) X 100

Total crude lipids %: The crude lipids were assessed by extracting the sample using petroleum ether for 16 h in a Soxhlet apparatus. The solvent was evaporated, and the residue was weighed to estimate the crude fat content.

2.4. Statistical analysis

The obtained data were tabulated and subjected to proper statistical analysis [27] using the statistical program MSTAT-C.

3. Results and discussion

3.1. Herb dry weights

Results showed that *N. sativa* herb dry weights were significantly affected following chitosan and amino acids application in both seasons (Table 2). Moreover, the interaction between both factors was significant. In the 1st one, the lowest weight (50.7 g/plant) was for plants that were not treated with any of these substances. However, the highest value (84.5 g/plant) was assessed for plants treated with 1.0 g/l chitosan + 2.0 g/l glycine. But there was no significant difference between this value and these for plants treated with the same concentration of chitosan + 1.0, 2.0, or 3.0 g/l glycine.

Under any concentration of glycine or tryptophan increasing chitosan over than 1.0 g/l caused a reduction on the herb weight with no significant difference in most cases between 0.0, 2.0, and 3.0 g/l of chitosan. Regarding the effect of tryptophan (Table 2) shows that the highest herb dry weights were achieved when plants treated with 3.0 g/l tryptophan + 0.0, 1.0, or 2.0 g/l chitosan since 2.0 g/l tryptophan was not significantly better. Similar observations were assessed in the 2^{nd} season (Table 2).

Table (2): Effect of chitosan and amino acids (tryptophan and glycine) on herb dry weights of *Nigella sativa* during two seasons.

Amino Acids (g/l) (B)		Chitosan (g/l) (A) 0	1	2	3	Mean (B)
First season						
Control		50.7	58.4	51.4	47.3	**52.0**
Glycine	**1**	57.0	80.3	59.2	52.9	**62.4**
	2	51.4	84.5	56.38	61.6	**63.5**
	3	59.7	83.7	52.9	61.6	**66.7**
Tryptophan	**1**	59.7	65.6	52.88	50.0	**57.1**
	2	62.1	62.4	64.0	62.7	**62.7**
	3	66.9	65.9	66.9	58.7	**64.6**
Mean (A)		**58.2**	**71.2**	**58.6**	**56.3**	
LSD 5 %		**A:1.5**		**B:3.2**		**AB: 6.4**
Second season						
Control		46.3	57.8	51.2	51.5	**51.7**
Glycine	**1**	53.2	73.8	52.2	50.0	**57.3**
	2	56.4	83.9	51.5	60.9	**63.7**
	3	60.1	85.8	58.6	59.5	**66.0**
Tryptophan	**1**	61.8	67.5	53.8	50.8	**58.5**
	2	63.6	62.6	66.2	62.1	**63.6**
	3	68.1	66.4	68.7	61.1	**66.1**
Mean (A)		**56.5**	**71.1**	**57.5**	**52.7**	
LSD 5 %		**A: 2.6**		**B:2.8**		**AB:5.6**

3.2. Seed yield

Black cumin seed weights were significantly affected by both investigated factors. Moreover, there was a significant interaction between them with a similar trend in both seasons (Table 3). In the 1st one non amino acids-treated plants had the lowest yield with no significant difference among 0.0, 2.0, and 3.0 g/l of chitosan treatments but, these treated only with 1.0 g/ chitosan had significantly higher yield (26.7 g/plant). All amino acid applications significantly improved seed yield however, the response was a significant varied depending on the type and concentration. Plants that were treated with 1.0 g/l chitosan in addition to 1.0, 2.0, or 3.0 g/l of glycine or tryptophan had significantly the highest yield than all other chitosan concentrations. However, all amino acids treated plants had significantly higher yields than non-treated ones regardless of the chitosan concentration. An augmentation of seed yield was observed under any concentration of chitosan by increasing amino acid concentrations. However, the highest concentration of amino acids is not preferable as no significant difference was detected between 2.0 and 3.0 g/l under the same concentration of chitosan.

Overall, the highest seed yield 44.1 and 42.7 g/plant for the 1st season were for plants treated with 1.0 g/l of chitosan + (3.0 g/l tryptophan) and (3.0 g/l glycine) respectively. Whereas the minimum seed yield (21.2 and 21.7 g/plant) was estimated for non-amino acid treated plants + all chitosan concentrations except 1.0 g/l.

Our current study showed a pronounced effect of chitosan on *N. sativa* herb weight and seed yield. Interestingly the increment in plant herb weights was highest at 1.0 g/l of chitosan then a negative relationship was seen between both characters and chitosan concentration. Similarly, the herb fresh and dry weights of *Pelargonium garveolens* [30] and *M. arvensis* [31] was correlated with the concentration of chitosan. The enhancing effect of chitosan on plant growth is subject to its deacetylation degree, molecular weight, and concentration [32]. Chitosan could improve plant physiological properties by stimulating plant immune system [13] that could be attributed to its content of amino acids, vitamins, and growth hormones as cytokinin and auxin which encourage a range physiological response, such as biomass [33]

The observed encouraging effects of amino acids application on black cumin seeds plant was similar to many achievements on medicinal and aromatic plants as reviewed by [16] Sun et al. (2024). The relationship between plant nitrogen utilization, and concentrations amino acids application had been found [34 and 35]. They suggested that the main role of amino acids on plant growth could be related to regulation the nitrate uptake and assimilation, but not as sources of reduced nitrogen. Amino acids application might affect nitrate reductase enzyme which occupying a control point in the pathway of nitrate assimilation.

3.3. Seed proximate analysis

3.3.1. Moisture content

The moisture content of black cumin seeds was significantly varied due to both investigated factors as well as the interaction between them (table 4). In the 1st one, untreated plants had the minimum value (4.04%) which did not significantly vary under various concentrations of chitosan. However, the highest seed moisture contents (5.70 and 5.44%) were for plants treated with 1.0 g/l of tryptophan or glycine in addition to 1.0 g/l of chitosan, respectively. Mostly, increasing chitosan over 2.0 g/l caused a reduction in seed moisture content. Also, the 1.0 g/l of any of the applied amino acids was preferable to the other concentrations (table 4). A similar observation was achieved in the 2nd season.

Table (3): Effect of chitosan and amino acids (tryptophan and glycine) on seed yield of *Nigella sativa* during two seasons.

Amino acids (g/l)(B)		Chitosan (g/l) (A) 0	1	2	3	Mean (B)
		First season				
Control		21.2	26.7	21.7	21.6	**22.6**
Glycine	1	31.6	39.3	32.5	32.5	**23.6**
	2	34.2	42.3	32.6	32.6	**36.0**
	3	36.31	42.7	35.0	35.0	**37.9**
Tryptophan	1	30.1	38.5	28.8	28.8	**32.7**
	2	34.9	41.6	28.8	32.7	**36.8**
	3	36.0	44.1	34.4	34.4	**37.9**
Mean (A)		**32.0**	**39.2**	**33.2**	**31.1**	
LSD 5 %		**A:1.2**		**B:1.6**		**AB:3.2**
		Second season				
Control		21.04	20.72	21.25	21.18	**21.05**
Glycine	1	33.06	40.94	29.04	34.28	**34.33**
	2	33.50	43.78	28.24	34.29	**34.95**
	3	34.08	43.59	26.72	32.10	**34.12**
Tryptophan	1	32.93	47.36	34.80	31.33	**36.60**
	2	32.21	35.67	38.84	34.89	**35.40**
	3	42.08	35.37	32.23	30.37	**35.01**
Mean (A)		**30.8**	**39.9**	**33.2**	**31.1**	
LSD 5 %		**A: 1.3**		**B:1.4**		**AB:2.8**

Table (4): Effect of chitosan and amino acids (tryptophan and glycine) on seed moisture % of *Nigella sativa* during two seasons.

Amino acid (g/l) (B)		Chitosan (g/l) (A) 0	1	2	3	Mean (B)
		First season				
Control		4.04	4.11	4.06	4.02	**4.06**
Glycine	1	4.44	**5.44**	5.03	4.32	**4.81**
	2	4.29	5.30	4.42	4.16	**4.54**
	3	4.18	5.10	4.37	4.19	**4.49**
Tryptophan	1	4.49	**5.70**	4.74	4.38	**4.82**
	2	4.27	5.36	4.58	4.17	**4.60**
	3	4.18	5.20	4.36	4.01	**4.44**
Mean (A)		**4.26**	**5.18**	**4.51**	**4.22**	
LSD 5 %		**A:0.19**		**B:0.15**		**AB:0.27**
		Second season				
Control		4.05	4.06	4.05	4.05	**4.06**
Glycine	1	4.28	5.74	4.70	4.39	**4.82**
	2	4.46	5.1	4.46	4.22	**4.54**
	3	4.43	5.31	4.20	4.06	**4.34**
Tryptophan	1	4.28	5.73	4.80	4.32	**4.83**
	2	4.48	5.55	4.65	4.17	**4.66**
	3	4.19	5.31	4.51	4.06	**4.52**
Mean (A)		**4.30**	**5.21**	**4.48**	**4.20**	
LSD 5 %		**A: 0.18**		**B:0.13**		**AB:0.27**

3.3.2. Ash content

The ANOVA showed a significant interaction between chitosan and amino acids application regarding the seed ash content in both investigated seasons (Table 5). In the 1st one non amino acid-treated plants which received 3.0 g/l of chitosan had the lowest ash content (3.18%). On the other hand, the highest seed ash percentage (4.63%) was for plants treated with 1.0 g/l chitosan + 1.0 g/l tryptophan. Interestingly under the same concentration of chitosan, the ash percentages were gradually increased by decreasing the tryptophan or glycine. Under the same concentration of chitosan, results indicated that tryptophan was significantly more effective in most cases than glycine in increasing seed ash content. Comparable results can be observed in Table 5 for the 2nd season.

Ash content estimates the overall quantity of minerals in a diet, but the mineral content estimates the amount of specific inorganic components, such as N, P, Ca, etc. present in a food [36]. Achieved results showed that chitosan application improved ash seed content, confirming results previously reviewed [37] and investigated [38] in Amaranthus gangeticus and [39] Spinacia oleracea. Amino acids are well-known as bio-stimulants that positively affect plant growth and yield [40] due to their easy mobility and transport [41]. Amino acid application directly or indirectly increased the ash content of Pisum sativum [42]. Also, the ash content in wheat grains was significantly increased by the application of commercial amino acids foliar spray [25].

Table (5): Effect of chitosan and amino acids (tryptophan and glycine) on seed ash % of *Nigella sativa* during two seasons.

Amino Acid (g/l) (B)		Chitosan (g/l)(A) 0	1	2	3	Mean (B)
		First season				
Control		3.29	3.39	3.26	3.18	**3.28**
Glycine	1	3.78	4.58	4.44	4.38	**4.30**
	2	3.70	4.49	4.42	4.25	**4.22**
	3	3.50	4.41	4.37	4.20	**4.12**
Tryptophan	1	4.43	4.63	4.59	4.56	**4.55**
	2	4.23	4.55	4.46	4.31	**4.39**
	3	4.18	4.40	4.29	4.28	**4.29**
Mean (A)		**3.87**	**4.35**	**4.26**	**4.15**	
LSD 5 %		**A:0.08**		**B:0.11**		**AB:0.22**
		Second season				
Control		3.27	3.57	3.51	3.22	**3.79**
Glycine	1	3.83	4.46	4.38	4.31	**4.25**
	2	3.78	4.40	4.35	4.26	**4.22**
	3	3.56	4.42	4.24	4.19	**4.10**
Tryptophan	1	3.47	4.66	4.54	4.36	**4.51**
	2	4.28	4.57	4.50	4.28	**4.41**
	3	3.99	4.42	4.36	4.22	**4.25**
Mean (A)		**3.88**	**4.36**	**4.28**	**4.12**	
LSD 5 %		**A: 0.09**		**B:0.09**		**AB:0.17**

3.3.3. Lipids contents

Chitosan as well as amino acids application significantly affected seed lipid contents with a similar trend in both seasons (Table 6). In the 1st one, untreated plants had the lowest value (28.11%) while the highest ones (30.75 and 30. 66% with no significant difference between them) were for plants that were treated with 2.0 g/l chitosan + 2.0 g/l tryptophan and 1.0 g/l glycine, respectively. Regardless of the concentration of amino acids, results showed that chitosan at 2.0 g/l was the best concentration to achieve higher lipid content which did not significantly decrease at the highest concentration of chitosan.

In most cases at the same concentration of amino acids, there was no significant difference in the lipid contents between the non-chitosan-treated plant and those treated with the highest concentration (3.0 g/l). Results showed that at the same concentration of chitosan seed lipids contents were decreased by increasing tryptophan or decreasing glycine concentration.

Likewise, the results of our present study regarding increasing lipids content of back cumin seeds due to chitosan application [43] on Sesamum indicum and [44] on soybean indicated chitosan as foliar plays. They suggested that chitosan could reduce stoma diameter preventing depleting cell water, which finally raises the biomass and final seed yield. Further, chitosan could contribute to an increase in photosynthesis by improve the chlorophyll content, which could be consider as one of the reasons for increasing seed and oil. Our results are in accordance with [45] on Echinacea plants, who reported that the application of some amino acids significantly increased total lipid content and glutamic acid was better than others. The same trend was reported [46] stating that spraying of amino acids successfully manipulates the total lipids yield of Urtica pilulifera.

The impact of chitosan and amino acids which are considered low-cost and environmentally safe chemicals based on secondary metabolites production of medicinal and aromatic have an increase attention [15]. The current study confirms these hypotheses. Therefore, it's recommended that N. sativa plants with 1.0 g/l chitosan in addition to 1.0 g/l tryptophan to achieve the highest seed yield. However, for higher lipids content plants should treated with 2.0 g/l of chitosan and tryptophan.

Table (6): Effect of chitosan and amino acids (tryptophan and glycine) on lipids % of Nigella sativa seeds during two seasons.

Amino Acid (g/l)(B)		Chitosan(g/l)(A) 0	1	2	3	Mean (B)
First season						
	Control	28.11	28.22	28.29	28.30	28.23
Glycine	1	28.89	30.29	30.44	29.74	29.89
	2	28.68	28.37	29.90	29.37	29.33
	3	28.60	29.14	29.62	28.66	29.01
Tryptophan	1	28.70	29.31	29.63	29.07	29.23
	2	28.91	29.67	30.75	30.56	29.95
	3	29.49	28.27	30.14	28.69	28.80
Mean (A)		28.63	29.14	29.88	29.20	
LSD 5 %		A:0.32		B:0.29		AB:0.59
Second season						
	Control	28.20	28.55	28.94	28.53	28.55
Glycine	1	28.96	30.07	30.67	29.77	29.87
	2	28.59	28.38	29.87	29.46	29.07
	3	28.61	29.12	29.19	28.67	29.90
Tryptophan	1	28.90	29.29	29.83	29.12	29.28
	2	29.74	29.60	30.73	30.55	29.89
	3	29.26	28.11	30.16	28.71	28.81
Mean (A)		28.14	29.16	29.41	29.26	
LSD 5 %		A: 0.31		B:0.28		AB:0.55

References

[1] Townsend CC. Flora of Iraq. In: Townsend CC and Guest E (Edi). Vol.4 part 2. Ministry of Agriculture-Baghdad. 1980.

[2] Gharibzahedi S M T, Mousavi S M, Moayedi A, Garavand A T, and Alizadeh S M. Moisture-dependent engineering properties of black cumin (*Nigella sativa* L.) seed. Agricultural Engineering International: CIGR Journal. 2010; 12: 194-202.

[3] Espanany A, Fallah S and Tadayyon A. Seed priming improves seed germination and reduces oxidative stress in black cumin (*Nigella sativa*) in presence of cadmium. Industrial Crops and Products. 2016;.79: 195-204.

[4] Kirtikar KR and Basu BD, Indian Medicinal Plants, L M Basu Publication, Allahabad, 1989.

[5] Ahmad A, Husain A, Mujeeb M, Khan S A, Najmi A K, Siddique N A, Damanhouri Z A, and Anwar F. A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pacific Journal of Tropical. 2013; 3: 337-352.

[6] Seth S D and Sharma B. Medicinal plants in India. Indian Journal of Medical Research. 2004; 120: 371-3829.

[7] Warrier V P K. Indian Medicinal Plants- A Compendium of 500 species, Vol. 4, Orient Longman Pvt Ltd, Chennai, 2004.

[8] Evans W.C. Pharmacognosy. 14th edn. London: WB Saunders Company Ltd. 1996.

[9] Kapoor L D. Handbook of Ayurvedic Medicinal Plants. Boca Raton, FL: CRC Press Inc. 1990.

[10] Paarakh P M. *Nigella sativa* Linn.–A comprehensive review. Indian Journal of Natural Products and Resources. 2010; 1:409-429.

[11] Ramadan M F. Nutritional value, functional properties and nutraceutical applications of black cumin (*Nigella sativa* L.): an overview. International journal of food science & technology. 2007; 42: 1208-1218.

[12] Islam M T, Guha B, Hosen S, Riaz T A, Shahadat S, Sousa L D R and Melo-Cavalcante A A C. Nigellalogy: a review on Nigella sativa. MOJ Bioequiv Availab. 2017;): 00056.

[13] Hidangmayum A, Dwivedi P, Katiyar D, and Hemantaranjan A. Application of chitosan on plant responses with special reference to abiotic stress. Physiolo. and Molec. Biology of Plants. 2019; 25:313.

[14] Katiyar D, Hemantaranjan A and Singh B. Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review. Indian Journal of Plant Physiology. 2015; 20*:* 1-9.

[15] Azmana M, Mahmood S, Hilles A R, Rahman A, Arifin M A B, and Ahmed S. A review on chitosan and chitosan-based bionanocomposites: Promising material for combatting global issues and its applications. International J. of Biological Macromolecules. 2021; 185: 832-848.

[16] Sun W, Shahrajabian M H, Kuang Y and Wang N. Amino acids biostimulants and protein hydrolysates in agricultural sciences. Plants; 2024; 13(2): 210.

[17] Malekpoor F, Pirbalouti A G. and Salimi A. Effect of foliar application of chitosan on morphological and physiological

characteristics of basil under reduced irrigation. Research on Crops. 2016; 17: 354-359.

[18] Safaei Z, Azizi M, Aroiee H. and Davarynej G. The effect of irrigation intervals and anti- transpiration compounds on morphological traits, physiological traits, and irrigation water efficiency index in the *Nigella sativa* L. Journal of Horticultural Science. 2018*;* 32: 371-383.

[19] Goss j A. Amino acid Synthesis and Metabolism, Physiology of Plants and Their Cell. Pergamon Press INC, New York, Toronto, Oxford, Sydney, Braunschweig. 1973: p. 202.

[20] Rai V K. Role of amino acids in plant responses to stresses. Biologia Plantarum. 2002; 54:481-487.

[21] Bidwell R G S. Plant Physiology. 2nd Ed., Macmillan Publishing. Co., Inc. New York. 1979.

[22] Kowalczyk K, Zielony T. and Gajewski M. Effect of aminoplant and asahi on yield and quality of lettuce grown on rockwool. In: conf of Biostimulators in Modern Agriculture. Vegetable Crops., 2008: p. 35-43.

[23] Paleckiene R, Sviklas A and Šlinkšiene R. Physicochemical properties of a Mengel K E, Kirkby E A, Kosegarten H. and Appel T. Principles of Plant Nutrition. 5th Edtion. Kluwer Academic Publishers Dordecht. 2001; Pp. 311.

[24] microelement fertilizer with amino acids. Russian J. of Appl. Chem. 2007; 80: 352-357.

[25] Popko M, Michalak I, Wilk R, Gramza M, Chojnacka K and Górecki H. Effect of the new plant growth biostimulants based on amino acids on yield and grain quality of winter wheat. Molecules. 2018; 23:470.

[26] Huang Y, Sun L, Zhao J, Huang R, Li R, Shen Q. Utilization of different waste proteins to create a novel PGPR-containing bio-organic fertilizer. Scientific Reports. 2015; *5*(1):7766.

[27] Mead R, Currow R N, and Harted A.M. Statistical Methods In: Agricultural and Experimented Biology 2nd Ed. Chapman and Hall, London, UK. 1993; pp 472.

[28] Black J H. The physical state of primordial intergalactic clouds. Monthly Notices of the Royal Astronomical Society. 1981; 197:553-563.

[29] AOAC 1995. Official methods of analysis. Association of Official Analytical Chemists, Washington D.C.

[30] Bakr A, Taha R A, Botros W, and Mohamed M A E. Effect of some plant growth biostimulants on growth and volatile oils productivity of *Pelargonium graveolens* plants. Journal of Modern Research. 2024; in press.

[31] da Silva E A, Silva V N B, de Alvarenga A A and Bertolucci S K V. Biostimulating effect of chitosan and acetic acid on the growth and profile of the essential oil of *Mentha arvensis* L. Industrial Crops and Products. 2021; 171: 113987.

[32] Mujtaba M, Khawar K M, Camara M C, Carvalho L B, Fraceto L F, Morsi R E and Wang D. Chitosan-based delivery systems for plants: A brief overview of recent advances and future directions. International J. of Biolog. Macromolecules. 2020; 154: 683-697.

[33] Ali Q, Shehzad F, Waseem M, Shahid S, Hussain A I, Haider M Z. and Perveen R. Plant-based biostimulants and plant stress responses. Plant ecophysiology and adaptation under climate change: mechanisms and perspectives: General Consequences and Plant Responses. 2020; 625-661.

[34] Liu X Q, and Lee K S. Effect of mixed amino acids on crop growth. Agricultural science. 2012; 1:119-158.

[35] Baqir H A, Zeboon N H, and Al-Behadili A A J. The role and importance of amino acids within plants: A review. Plant Archives. 2019; 19: 1402-1410.

[36] Bakkali K, Martos NR, Souhail B, Ballesteros E. Characterization of trace metals in vegetables by graphite furnace atomic absorption spectrometry after closed vessel microwave digestion. Food Chem. 2009; 116: 590-594

[37 Pandey P, Verma M K and De N. Chitosan in agricultural context- A review. Bull. Environ. Pharmacol. Life Sci. 2018; *7* 87-96.

[38] Nasrin H, Zakir H M, Nipa N A, Paul N R. and Quadir Q F. Effect of foliar application of chitosan on growth, yield and nutritional qualities of red amaranth (*Amaranthus gangticus* L.). J. Exp. Agric. Int. 2022; 44:105-116.

[39] Nipa N A, Das P, Nasrin H, Zakir H M, Rahman A. and Quadir Q F. Growth, Yield and Biochemical Qualities of Spinach (*Spinacia oleracea*) being Influenced by the Foliar Application of Chitosan. Journal of Experimental Agriculture International. 2023; 45: 30-40.

[40] Calvo P, Nelson L, and Kloepper J W. Agricultural uses of plant biostimulants. Plant and Soil. 2014; 383: 3-41.

[41] Tegeder M, Rentsch D. Uptake and partitioning of amino acids and peptides. Mol. Plant Pathol. 2010; 3*:* 997–1011.

[42] Biel W, Podsiadło C, Witkowicz R, Kępińska-Pacelik J and Stankowski S. Effect of irrigation, nitrogen fertilization and amino acid biostimulant on proximate composition and energy value of *Pisum sativum* L. seeds. Agriculture, 2023; 13: 376.

[43] Varamin J K, Fanoodi F, Sinaki J M, Rezvan S and Damavandi A. Foliar application of chitosan and nano-magnesium fertilizers influence on seed yield, oil content, photosynthetic pigments, antioxidant enzyme activities of sesame (*Sesamum indicum* L.) under water-limited conditions. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2020; 48: 2228-2243.

[44] Zeng D, Luo X, Tu R. Application of bioactive coatings based on chitosan for soybean seed protection. Journal of Carbohydrate Chemistry. 2012; 12:1-5.

[45] Youssef A S M. Influence of some amino acids and micro-nutrients treatments on growth and chemical constituents of *Echinacea purpurea* plant. Journal of Plant Production. 2014; 5: 527-543.

[46] Wahba H E, Motawe H M and Ibrahim A Y. Growth and chemical composition of *Urtica pilulifera* L. plant as influenced by foliar application of some amino acids. Journal of Materials and Environmental Science. 2015; 6: 499-509.