Stimulative effect of chitosan with amino acid to enhance growth, essential oil, and some physiochemical characteristics of two *Mentha* cultivars

Tamer M. Abdel-Razik[a], Bakry A. Bakry[b], Mervat Sh. Sadak[c]

[a]Department of Medicinal and Aromatic Plants Research, Pharmaceutical and Drug Industries Research Institute, National Research Centre, Dokki, Giza, Egypt, [b]Department of Field Crops Research, Agricultural and Biological Research Institute, National Research Centre, Dokki, Giza, Egypt, [c]Department of Botany, Agricultural and Biological Research Institute, National Research Centre, Dokki, Giza, Egypt

Correspondence to Tamer M. Abdel-Razik, Associate professor, Department of Medicinal and Aromatic Plants Research, Pharmaceutical and Drug Industries Research Institute, National Research Centre, El-Behowth Street, PO Box 12311, Dokki, Cairo, Egypt.
Tel: +20 100 774 9925; fax: +20 233 370 931; e-mail: tamernrc81@gmail.com

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Background
Mint plants (*Mentha* spp.) are a member of the Lamiaceae family and it has long been used in medicine. Its applications include carminative, anti-inflammatory, antispasmodic, antiemetic, diaphoretic, analgesic, stimulant, emmenagogue, and anticatarrhal. Adding chitosan to medicinal plants has a major role in the process of secondary metabolism, as its addition stimulates the production of chemical compounds or essential oils in the plant. Amino acids are one of the possible strategies for increasing agricultural productivity. They are organic nitrogen polymers that are used as the building blocks of proteins and enzymes.
Objective
This study aimed to determine how chitosan, in combination with or without the foliar application of amino acids, affected the growth and physiological traits of two cultivars of mint.
Materials and methods
Two pot trail investigation studies were carried out during the two consecutive seasons 2021 and 2022 under the natural conditions of the greenhouse of the National Research Center (NRC), Dokki, Giza, Egypt. To study the effect of two levels of chitosan (1.5 and 3.0 g/l with amino acid at rates of 50 and 100 mg/l) as foliar application on growth, essential oil, and some physiochemical characteristics of two *Mentha* (*Mentha viridis and Mentha longifolia* L.) cultivars.
Results and conclusion
The results show significant differences between two mint cultivars in the growth parameters of mint plants. Plants of *M. viridis* variety were characterized by the highest significant values of herb fresh weight, number of branches/plants, essential oil (%), flavonoid content, and protein %, while the *M. longifolia* variety was superior in plant height, herb dry weight, chlorophyll a, chlorophyll b, carotenoids, total pigments, indole acetic acid, phenol, carbohydrates %, free amino acids, flavonoid, and proline contents. Using chitosan as foliar treatments at different concentrations with or without amino acid significantly increased all studied traits. The interaction between two cultivars and foliar treatments of high rates of chitosan and amino acid gave the maximum significant increase of plant height, photosynthetic pigments and indole acetic acid, phenol, protein, free amino acid, and proline contents as well as antioxidant activities (DPPH%).

Keywords:
mint cultivars, chitosan, amino acid, growth, biochemical, essential oil

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Introduction

Mint plants (*Mentha* spp.), including peppermint, are commonly known by various names such as mint, spearmint, American mint, brandy mint, lamb mint, or lam mint. Peppermint is a member of the Lamiaceae family of herbs and its branches can range in color from dark green to purple-violet. Worldwide, mint is grown for its taste, aroma, medical properties, and potential uses in pharmaceuticals. One of the most often manufactured and used essential oils is peppermint oil [1–3]. Mint has long been used in medicine. Its applications include carminative, anti-inflammatory, antispasmodic, antiemetic, diaphoretic, analgesic, stimulant, emmenagogue, and anticatarrhal. Mint is also used in cuisine, herbal tea preparations, and confectioneries. In addition, it is used to treat liver issues, anorexia, pneumonia, flatulence, nausea, and ulcerative colitis. Mint essential oils are commonly applied externally to treat neuralgia, myalgia, headaches, and migraines, and for antipruritic, astringent, rubefacient, antibacterial, and antimicrobial reasons [2–4]. As pointed out by Singh *et al*. [5] the market for mint oil has grown

rapidly along with the rise in menthol use worldwide; around the world, mint oils are manufactured. One of the most significant mint products is essential oil, which is used extensively in the food and beverage sector, medicine, relaxation, and fragrance industries. Fever, cough, and stomach issues can all be treated with mint essential oils and extracts [6–8]. Some species of mints have antioxidant and anticancer properties because they include phytosterols, phenolic compounds, and unsaturated fatty acids [9–11]. Because of their overall phenolic content, antioxidant capability, and ability to inhibit acetylcholinesterase and histone deacetylase, mints may offer a significant foundation for future research into the therapy of Alzheimer's disease, according to Hanafy *et al.* [12]. The distillation process yields essential oils rich in a variety of chemicals from the shoots of *M*entha *arvensis* [13].

In terms of mint crop production, elicitor-chitosan treatment is one of the cultivation tactics being used to boost secondary metabolite synthesis in plants [14]. On an experimental basis, chitosan has been used as a substitute for artificial plant growth regulators [15]. Seafood shells are the natural source of chitosan, which was first, used commercially [16]. In addition to being biodegradable and ecologically benign, chitosan is a low-toxic, inexpensive material with a variety of uses in agriculture. Individual traits such as bioactivity and biocompatibility are taken into consideration while evaluating chitosan [17]. A biopolymer belonging to the carbohydrate group, chitosan is primarily formed from a free amino group at carbon number two on the glucose ring. Nevertheless, its use increases as amino groups rise [18]. Furthermore Hidangmayum *et al.* [19], have reported that chitosan plant elicitation is a great method for acquiring bioactive plant chemicals as it induces alterations in secondary metabolism. Certain aromatic plants increased the amount of chemical compounds or essential oils by applying chitosan. For example, *Mentha piperita* cultivated in the greenhouse and open field showed better production quality and quantity when foliar chitosan treatment was combined with 400 mg/l of citric acid [20].

The effectiveness of chitosan treatment depends on several factors, including the substance's concentration, substrate type, water content, temperature, root stage, and plant development during drought stress. Chitosan treatment enhanced chlorophyll, the number of nodes, and root development in grapevine plants under water stress [21]. In maize and bean crops, chitosan has been observed to affect higher root and shoot dry weight, germination, leaf area index, and chlorophyll content [22]. In milk thistle [23] it has been found that the use of chitosan led to improved physiological traits, soluble sugars, and enhanced the capacity of antioxidant enzyme activities. Chitosan application may lead to several metabolic changes, a decrease in transpiration, and a yield increase [24,25]. Moreover, the application of chitosan enhanced the activity of three important nitrogen metabolism-related enzymes: glutamine synthetase, protease, and nitrate reductase. This enhanced nitrogen transmission in the workable leaves led to an increase in plant growth and development. In tomato root exudates, chitosan promotes plant hormones, lipid signaling, and protective chemicals such as phenolic compounds [26]. Furthermore, according to Singh *et al.* [5] and Lopez-Moya *et al.* [27], chitosan encourages the accumulation of auxin at the apex of plant roots and metabolic pathways involved in the synthesis of phenolic compounds.

Amino acids are one of the possible strategies for increasing agricultural productivity. They are organic nitrogen polymers that are used as the building blocks of proteins and enzymes [28] and stronger bioregulators of plant growth [29]. The foliar spray of amino acids increased the total phenol content by 1.22–3.51 times, depending on the variety of mint and treatment [7]. Amino acids are also significant because they have a frequent role in the production of aspartate, vitamins, coenzymes, purine and pyrimidine bases, pigments, and other nonproteinic nitrogenous compounds. Glutamate, glutamine, and aspartate are the primary amino acids that are synthesized by plant cells; they can be combined to create additional amino acids [30]. Plants use amino acids for a wide range of purposes, such as hormone precursors, stress relief, and nitrogen uptake [31,32]. Given that molecules may act as signals for a variety of important plant biochemical processes, exogenous administration of amino acids by foliar treatment enhanced plant growth and productivity in this situation [33–35]. By increasing plant mineral absorption and optimizing nutrient use efficiency, external application of different amino acids as bioregulators can lower fertilizer consumption and increase crop output [36]. Amino acids promote the growth of the aboveground plant components and aid in the creation of the root system [35,37]. Prior research has demonstrated that amino acids enhance photosynthesis, respiration, and the water cycle in plants when applied as fertilizer. These amino acids also increased the amounts of ascorbic acid, sped up the manufacture of proteins, and enhanced the growth and

yield of plants. Moreover, amino acids have a chelating role in micronutrients by changing the permeability of cell membranes, which promotes their uptake and movement throughout plants [38]. The α-amino acid that helps in protein synthesis and promotes plant development is glutamic acid. Under adverse conditions, it had a beneficial impact, reducing physiological destruction by encouraging the growth of the chlorophyll particle, the anabolism of carbohydrates, the release of plant hormones, and the activity of antioxidant enzymes. Plant development is supported by glutamic acid [39]. Under conditions of stress, glutamic acid is proposed to be the precursor of proline and γ-aminobutyric acid [40]. This study aimed to determine how chitosan, in combination with or without the foliar application of amino acids, affected the growth and physiological traits of two cultivars of mint.

Materials and methods

In two consecutive seasons, 2021 and 2022, two pot trail investigation studies were conducted in the open field of the National Research Center's (NRC) greenhouse in Dokki, Giza, Egypt. Two cultivars of mint, *Mentha viridis and Mentha longifolia* L. produced seedlings measuring 12 cm in length and 10 cm in root length, derived from three-node rhizomes. On February 15 of each season, the seedlings were transplanted into pots (30 cm in diameter and 50 cm in depth). Every pot held three seedlings and was placed in direct sunlight. Ten kilograms of air-dried soil were inside the pots. Jackson [41] and Cottenie *et al*. [42] were consulted to determine the mechanical and chemical parameters of the soil. The physical composition of the sandy soil was as follows: 81.50% sand, 13.23% silt, 5.2% clay, and 0.45% organic matter (OM). The pH was 7.92, E C was 0.72 mmohs/cm, total nitrogen was 0.1%, accessible phosphorus was 1.9 mg/100 g, potassium was 21.1 mg/100 g, and iron was 20.8 ppm according to the findings of the soil chemical study. Then after 1 month from transplanting, two levels of chitosan (1.5 and 3.0 g/l with amino acid at rates of 50 and 100 mg/l) were applied as foliar application at two equal portions at 60 and 90 days from transplanting.

A factorial experiment with four replications in a complete randomized block design was used as the experimental design. Five pots were included in each replicate, with each pot sowing three plants. The factor studies of this experiment are as follows: *Mentha* varieties (*M. viridis* and *M. longifolia*) and foliar applications.

Foliar fertilizers

The foliar treatments were administered were as follows:

T1-control.
T2-chitosan at a rate of 1.5 g/l.
T3-chitosan at a rate of 1.5 g/l)+amino acid (50 ml/l).
T4-chitosan at a rate of 1.5 g/l+amino acid (100 ml/l).
T5-chitosan at a rate of 3.0 g/l.
T6-chitosan at a rate of 3.0 g/l+amino acid (50 ml/l).
T7-chitosan at a rate of 3.0 g/l+amino acid (100 ml/l).

Chitosan and amino acids which were used in this study were supplied by Sigma-Aldrich Co (Chemie Gmbh Export Department Eschenstrabe 5, 82024 Taufkirchen Germany). The purity was 98 wt.%. All other agriculture practices and operations other than experimental treatments were carried out as usual.

Samples were taken for growth characters and chemical constituent determinations after 120 days from transplanting. The following traits were studied.

The height of the plant (cm).
Branches number/plant.
Fresh and dry weights of herb/plant (g).

Essential oil percentage of the air fresh herb.

Chemical determinations

Photosynthetic pigment

The approaches of Li and Chen [43] were used to estimate and quantify the levels of carotenoids, chlorophyll a, and chlorophyll b using a mortar and pestle, and the fresh tissue was mashed with 80% acetone. Concentrations of photosynthetic pigment are measured in mg/g fresh weight.

Determination of indole acetic acid content

Three times at 0°C, a known weight of the fresh samples was obtained and extracted using 85% cold methanol (v/v). The blended extracts were collected and mixed with cold methanol to a predetermined amount. The PDAB reagent (para-dimethylamino benzoic acid 1 g diluted in 50 ml HCl, 50 ml of 95% ethanol) was then combined with 1 ml of the methanolic extract and left for 60 min at 30–40°C. At 530 nm, the developing color was evaluated using spectrophotometry [44].

Determination of phenol contents

Following the extraction of indole acetic acid (IAA), 0.5 ml of the extract was added to 0.5 ml of Folin, shaken, and let to stand for 3 min. After adding 1 ml of

saturated sodium carbonate to each tube, the distilled water was agitated and left to stand for 60 min. According toGonzalez *et al.* [45], the optical density was measured at a wavelength of 725 nm using a spectrophotometer.

Determination of flavonoid contents

Chang *et al.* [46] used the aluminum chloride colorimetric technique to determine the crude extract's flavonoid concentration. To summarize, 4 ml of distilled water, 50 µl of crude extract (1 mg/ml ethanol), and 0.3 ml of 5% $NaNO_2$ solution were combined. After incubating for 5 min, 0.3 ml of 10% $AlCl_3$ solution was added, and the mixture was left to stand for 6 min. After that, 2 ml of a 1 mol/l NaOH solution was added, and double-distilled water was used to bring the mixture's final volume to 10 ml. After the combination had stood for 15 min, the absorbance at 510 nm was determined. A calibration curve was used to determine the total flavonoid content, which was then reported as milligrams of rutin equivalent per gram of dry weight.

Determination of antioxidant activity (2,2,-diphenyl-2-picryl-hydrazyl)

A plant sample with a defined weight was homogenized with methanol and subsequently filtered [47]. The filtrate was used to measure the amount of free radical scavenging activity using the 2,2,-diphenyl-2-picryl-hydrazyl (DPPH) technique at an optical density of 517 nm. The following formula was used to determine the antioxidant activity (%):

The sample (517 nm)/the control (517 nm)×100.

Determination of total carbohydrate

The measurement of total carbohydrates was done in accordance with Albalasmeh *et al.* [48]. A measure of 10 ml of sulfuric acid (1 N) was added to a test tube containing a known mass (0.2–0.5 g) of dry tissue. After sealing, the tube was kept in a 100°C oven for the whole night. After filtering the mixture into a 100 ml measuring flask, distilled water was added to bring it up to the proper level.

Determination of proline content

The proline was measured by Kalsoom *et al.* [49]. After grinding 0.5 g of leaves using 10 ml of 3% sulphosalicylic acid, the leaves were centrifuged at 10 000*g* for 10 min. A measure of 2 ml of the supernatant and 2 ml of recently made acid ninhydrin reagent were combined. For 30 min, the mixture was incubated at 90°C in a water bath. The reaction was then stopped by cooling the mixture in an ice bath. To extract the reaction, 5 ml of toluene was added, and the mixture was vortexed for 15 s. The toluene and aqueous phases were then separated by 20 min in darkness. After gathering the toluene phase, the color's absorbance was measured at 520 nm using proline as a reference, and the result was stated as µg/g fresh weight.

Total free amino acids

The technique described by Sorrequieta *et al.* [50] was used to determine the total free amino acids (FAA). After homogenizing the leaves in 80% ethanol, they were boiled for 10 min, and then centrifuged for 10 min at 2000*g*. After mixing and boiling for 15 min in a water bath, 0.05 ml of the resulting supernatant and 2 ml of ninhydrin reagent were combined. After adding distilled water to get the mixture up to 10 ml, it was allowed to cool at room temperature. Using glycine as a reference and a spectrocolorimeter (VEB Carl Zeiss), the color produced was measured at 570 nm.

Essential oil isolation

Using a Clevenger-type equipment and hydro-distillation for 3 h, the complete plants (100 g) of the various mint fresh samples of *Mentha* variety plants were used to extract the essential oil [51]. Anhydrous sodium sulfate was used to separate and dry the resulting oily layer. Until additional examination, all of the essential oils were stored in airtight, sealed glass vials that were wrapped with aluminum foil and kept at 4°C.

Gas chromatography-mass spectrometry

Samples of all treatments of the two *Mentha* cultivars were analyzed using an Agilent 8890 gas chromatography system, which was connected to an Agilent 5977B gas chromatography/mass spectrometer and fitted with an HP-5MS fused silica capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness). The temperature of the oven was kept at 50°C at first, then programmed to rise to 200°C at a rate of 5°C/min and then to rise to 280°C at a rate of 10 °C/min. Finally, it was held at 280°C for 7 min. The carrier gas, helium, was used at a flow rate of 1.0 ml/min. A split ratio of 1 : 50 was used to inject 1 µl of the dissolved essential oil (20 µl essential oil/ml diethyl ether) into the gas chromatograph. The injection temperature was 230°C. Mass spectra were acquired at 70 eV in the electron impact mode (EI) scanning a range of 39–500 amu in m/z. By comparing the isolated peaks with information from the mass spectra library (National Institute of Standard and Technology, NIST), the isolated peaks were found.

Statistical analysis

The field experiment data was statistically analyzed using the MSTAT-C software program [52] and the factorial experiment in a complete randomized block design [53]. The combined analysis of variance was performed after completing Bartlett's homogeneity test [54]. The test of least significant differences was used to identify any noteworthy distinctions between the means of the treatments that were evaluated [54].

Results and discussion

Varietal differences

The results in Table 3 showed significant variations between the two *Mentha* varieties (*M. viridis* and *M. longifolia*) in growth characteristics, some physiological constituents as well as oil% and carbohydrates% in the plants. Data clearly show significant differences between the two mint cultivars in growth parameters of plant height, number of branches, and the fresh and dry weight of mint plants (Table 1). Plants of the *M. viridis* variety were characterized by the significant higher values of herb fresh weight, number of branches/plants, essential oil (%), flavonoids, and protein % contents. However, the *M. longifolia* variety was superior in plant height, herb dry weight, chlorophyll a, chlorophyll b, carotenoids, total pigments, IAA, phenol, carbohydrates %, FAA, and proline contents. The results are in agreement with many research reports. The essential oil in aromatic plants is affected by several environmental and agricultural variables, including climatic fluctuations, nutritional availability, soil microflora, genetic factors, and geographical variables that influence the expression of genes controlling growth and development [55–57].

Effect of chitosan and amino acid foliar treatments on changes in growth and photosynthetic pigments

The impact of different chitosan concentrations (1.5 and 3 mg/l with or without amino acid at rates of 50 and 100 mg/l) on the parameters related to oil percentage, photosynthetic pigments, and growth of mint plants is shown in Table 2. The findings showed that applying various doses of chitosan as foliar treatments with or without amino acid significantly improved every characteristic under investigation. The maximum significant ($P<0.05$) increase percentage was obtained in plant height (33 cm) by 62.06%, number of branches/plant (3.67) by 48.93%, herb fresh (103.83 g) by 80.53% and dry weight (24.78 g) by 76.91%, essential oil (0.08%) by 133.33%, chlorophyll a (0.72) by 75%, chlorophyll b (0.4) by 70.17%, carotenoid (0.22) by 68.75%, and total pigment contents (1.35) by 73.37% were reported at 3.0 g/l chitosan with 100 mg/l amino acid as compared with the untreated plants (control).

The obtained value of these results was in agreement withZayed *et al*. [58] that using chitosan improved the morphological characteristics of common beans [59]. Sheikha and Al-Malki discovered that the enhanced growth features of beans with chitosan might be attributed to an improvement in photosynthetic machinery. Furthermore Ke *et al*. [60], it has been found that applying carboxymethyl chitosan increased nitrogen metabolism enzyme activity,

Table 1 Comparison between two *Mentha* cultivars in growth, mint oil %, photosynthetic pigments, and some physiochemical characteristic

Characteristic	Mentha cultivars		$LSD_{0.05}$
	Mentha viridis	*Mentha longifolia*	
Plant height (cm)	55.14	89.52	5.46
No. of branches/plant	11.24	9.24	1.63
Herb fresh weight (g)	191.24	160.98	14.38
Herb dry weight (g)	42.95	44.24	1.20
Essential oil (%)	0.11	0.10	0.01
Chlorophyll a (mg/g fresh wt.)	1.28	1.41	0.05
Chlorophyll b (mg/g fresh wt.)	0.79	0.85	0.01
Carotenoids (mg/g fresh wt.)	0.40	0.47	0.01
Total pigments (mg/g fresh wt.)	2.47	2.73	0.05
IAA (mg/100 g fresh wt.)	32.19	55.61	1.04
Phenols (mg per 100 g fresh wt.)	307.71	326.80	1.87
Flavonoids (mg per 100 g fresh wt.)	206.95	203.97	0.86
Protein %	20.55	24.91	0.21
Carbohydrates % (mg/100 g fresh wt.)	56.07	48.76	0.84
Free amino acids (mg/100 g fresh wt.)	286.04	353.12	5.41
Proline (mg/100 g fresh wt.)	53.42	55.82	0.91
DPPH % (μmol per g fresh wt.)	47.40	55.68	1.12

The data are the mean of the two seasons.

Table 2 Effect of chitosan and different concentrations of amino acid on growth, oil %, and photosynthetic pigments of mint plants

Treatments (g/l)	Plant height (cm)	No. of branches/ plant	Herb fresh weight (g)	Herb dry weight (g)	Essential oil (%)	Chlorophyll a (mg/g fresh wt.)	Chlorophyll b (mg/g fresh wt.)	Carotenoids (mg/g fresh wt.)	Total pigments (mg/g fresh wt.)
Control	53.17	7.50	128.92	32.22	0.06	0.96	0.57	0.32	1.84
Chitosan 1.5	70.17	10.00	131.83	33.91	0.09	1.10	0.70	0.37	2.18
Chitosan 1.5+50 A	73.17	10.50	164.92	40.65	0.09	1.37	0.86	0.44	2.67
Chitosan 1.5+100 A	77.83	10.83	189.25	46.33	0.11	1.50	0.95	0.49	2.95
Chitosan 3	71.00	10.67	184.50	45.42	0.11	1.28	0.77	0.41	2.46
Chitosan 3+50 A	74.83	11.00	200.58	49.60	0.13	1.52	0.90	0.47	2.90
Chitosan 3+100 A	86.17	11.17	232.75	57.00	0.14	1.68	0.97	0.54	3.19
LSD 0.05	2.55	1.05	16.25	3.81	0.01	0.03	0.02	0.01	0.03

The data are the mean of the two seasons.

Table 3 Effect of chitosan and different concentrations of amino acid on indole acetic acid, phenol, protein %, carbohydrates %, free amino acids, and proline, flavonoids and antioxidant activity (DPPH %) contents of mint plants

Treatments (g/l)	IAA (mg/ 100 g fresh wt.)	Phenol (mg per 100 g fresh wt.)	Flavonoids (mg per 100 g fresh wt.)	Protein %	Carbohydrates % (mg per 100 g fresh wt.)	FAA (mg per 100 g fresh wt.)	Proline (mg per 100 g fresh wt.)	DPPH % (μmol per g fresh wt.)
Control	24.85	269.49	153.06	20.14	49.89	267.15	29.64	34.15
Chitosan 1.5	33.17	293.98	179.00	21.58	51.27	287.66	39.65	40.18
Chitosan 1.5+50 A	40.63	319.24	198.86	22.47	52.25	316.77	51.09	47.65
Chitosan 1.5+100 A	50.45	336.40	221.17	23.29	52.95	340.09	63.59	55.73
Chitosan 3	40.32	306.62	200.30	22.55	52.49	308.72	52.60	49.41
Chitosan 3+50 A	54.67	336.98	235.48	24.07	53.61	346.08	64.41	63.27
Chitosan 3+100 A	63.19	358.08	250.33	25.00	54.43	370.58	81.36	70.38
LSD 0.05	0.79	2.65	0.25	0.25	0.35	3.65	1.13	1.06

which in turn boosted photosynthesis and improved plant development characteristics. Applications of chitosan improve plant growth characteristics by increasing the availability of nutrients and water uptake. These changes in the environment affect cell osmotic pressure, elongation and division, protein biosynthesis, and the induction of the antioxidant defense system [61]. In addition, chitosan promotes stomata closure, which lowers transpiration, or it activates endogenous plant hormone production [62,63].

The chitosan foliar treatments of mint plants resulted in a significant increase in photosynthetic pigments. This increase may have been caused by the increased availability of amino compounds released from chitosan, improved cytokinin contents that promote chlorophyll synthesis, or prevention of the reduction in light-harvesting pigment–protein complexes [64]. Chitosan might improve the plant defense system by boosting photochemistry and enzymes related to photosynthesis [65]. Chitosan improves the efficiency of photosynthesis and the buildup of organic matter in mint plants. A rise in the overall amount of carbohydrates may be the cause of this. According to Abdallah *et al.* [66,67] applying chitosan topically to sunflower plants greatly improved their growth indices, photosynthetic pigments, and carbohydrate components.

Changes in indole acetic acid, phenolics, and free amino acid contents

The results in Table 3 show the changes in IAA, phenol, protein %, carbohydrates %, FAA and proline, flavonoids, and DPPH % contents of mint plants in response to different concentrations of chitosan (1.5 and 3 g/l with or without amino acid at rates of 50 and 100 mg/l) . Increasing chitosan concentration from 1.5 to 3 g/l and with an amino acid concentration from 50 to 100 mg/l) increased gradually and significantly the traits of IAA, phenol, protein %, carbohydrate %, FAA and proline, flavonoids and antioxidant activity (DPPH%) contents compared with control plants. Moreover, chitosan (3.0 g/l with amino acid at rates 100 mg/l) shows the maximum highest significant increases in IAA, phenol, protein %, carbohydrates %, FAA and proline, flavonoids and antioxidant activity (DPPH %) contents compared with the other treatments (Table 3). These results are in accordance with those

Table 4 Effect of interaction between the two *Mentha* cultivars and chitosan with or without amino acid foliar treatments at different levels on growth parameters and essential oil (%) (The data are the mean of the two seasons)

Mentha cultivars	Treatments (g/l)	Plant height (cm)	No. of branches/ plant	Herb fresh weight (g)	Herb dry weight (g)	Essential oil (%)
Mentha viridis	Control	47.67	8.67	131.50	30.14	0.08
	Chitosan 1.5	53.33	9.67	133.50	31.49	0.10
	Chitosan 1.5+50 A	55.00	10.67	175.00	38.91	0.11
	Chitosan 1.5+100 A	58.00	11.67	214.33	47.99	0.12
	Chitosan 3	54.00	12.33	212.33	47.59	0.12
	Chitosan 3+50 A	58.00	13.00	223.50	49.83	0.13
	Chitosan 3+100 A	60.00	12.67	248.50	54.67	0.14
Mentha longifolia	Control	58.67	6.33	126.33	34.31	0.05
	Chitosan 1.5	87.00	10.33	130.17	36.32	0.07
	Chitosan 1.5+50 A	91.33	10.33	154.83	42.40	0.08
	Chitosan 1.5+100 A	97.67	10.00	164.17	44.67	0.10
	Chitosan 3	88.00	9.00	156.67	43.25	0.10
	Chitosan 3+50 A	91.67	9.00	177.67	49.37	0.13
	Chitosan 3+100 A	112.33	9.67	217.00	59.34	0.14
$LSD_{0.05}$		3.61	1.48	22.98	n.s	0.01

reported by Abdallah *et al.* [66,67] and claimed that foliar application of chitosan, greatly boosted growth metrics, photosynthetic pigments, and carbohydrate components in 49 sunflower plants. According to Farouk and Ramadan [68] and Mohamed *et al.* [69], foliar chitosan treatment at 250 ppm on cowpea plants or 100 and 150 ppm on sour orange seedlings resulted in a considerable increase in leaf total carbohydrates.

Because of their hydroxyl groups, phenols are significant constituents with scavenging potential. These groups may also directly contribute to their antioxidant activities, which set off a series of secondary metabolites shaped by malonic or shikimic acid cycles. Regarding this issue, Abdallah *et al.* [66] observed that the use of chitosan markedly increased phenolic compounds concurrently with a decrease in lipid peroxidation.

Changes in growth parameters and essential oil (%)

Significant increases ($P \leq 0.05$) were listed in the traits of plant height, number of branches/plant, herb fresh weight (g), herb dry weight (g), and essential oil (%) in *Mentha* plants of the two cultivars in response to different levels of chitosan (1.5 and 3 g/l with or without amino acid at rates of 50 and 100 mg/l) are presented in Table 4. The interaction between *M. longifolia* and foliar treatments of chitosan (3.0 g/l with amino acid at rates of 100 mg/l) significantly increased plant height and essential oil (%) content with no significant difference between the same treatment with the two *Mentha* cultivars as compared with other interactions. In addition, the interaction between *M. viridis* and foliar treatments of chitosan (3.0 g/l with amino acid at rates of 100 mg/l) significantly increased herb fresh weight, and the same interaction gave the highest value of number of branches/plant with no significant difference between the interaction of the same variety and the foliar treatments of chitosan (3.0 g/l only or with amino acid at rates of 50 and 100 mg/l).

These results are in agreement with those obtained byAbu-Muriefah [70], who found that using foliar application of chitosan at 100 and 200 mg/l improved all plant growth parameters (plant height, number of leaves, leaf area, shoot fresh, and dry weights) compared with untreated control plants. The foliar application of chitosan increased the shoot dry weight of soybean [71]. Also Pal and Saharan [72], mentioned that foliar application of chitosan nanoparticles improved vegetative growth traits and oil seed content. Numerous studies have shown that aspartic and glutamic acid concentrations in plants affect how much mineral nutrition is absorbed by root sections [73]. Prior research has demonstrated that amino acids enhance photosynthesis, respiration, and the water cycle in plants when applied as a fertilizer. These amino acids also enhanced plant growth and productivity, increased ascorbic acid levels, and sped up protein production [38]. Furthermore, through altering the permeability of cell membranes, amino acids have a chelating impact on micronutrients, facilitating their absorption and transport inside plants. Marschner [74] and El-Bassiouny *et al.* [75] observed that foliar treatment of chitosan significantly increased the growth parameters in wheat plants.

Changes in photosynthetic pigments and endogenous indole acetic acid

The results in Table 4 show a significant increase in photosynthetic pigments and endogenous IAA with increasing chitosan levels (from 1.5 to 3 g/l with or without amino acid at rates of 50 and 100 mg/l). The interaction between *M. longifolia* and foliar treatments of chitosan (3.0 g/l with the amino acid at rates of 100 mg/l) gave the maximum significant increase of chlorophyll a, chlorophyll b, carotenoids, total pigments, and IAA as compared with all other interactions. These results are in agreement with those obtained by Gornik *et al.* [21], who observed that chitosan application improved the chlorophyll content, number of nodes, and root establishment in grapevine plants under drought stress. Moreover, chitosan with amino acid treatments improved the photosynthetic pigments of *Mentha* plants of both cultivars under the conditions of this trial. These increases might be attributed to the role of chitosan in enhancing cytokinin contents that stimulated chlorophyll synthesis and/or to increase the availability of amino compounds released from chitosan [64]. Likewise, chitosan increased chlorophyll and carotenoids of plants by activating the expression of genes responsible for the biosynthesis of photosynthetic pigments Chibu and Shibayama [64] and El-Bassiouny *et al.* [75], who observed that foliar treatment of chitosan at different concentrations significantly increased the growth parameters, photosynthetic pigments and different endogenous phytohormones auxins (IAA) in wheat plants (Table 5).

Changes in antioxidant compounds and carbohydrates %

The data in Table 6 shows the changes in some antioxidant compounds and carbohydrates % of *Mentha* leaves of the interaction between two tested varieties in response to different treatments of chitosan (1.5 and 3 g/l with or without amino acid at rates of 50 and 100 mg/l) were presented in Table 4. The interaction between *M. longifolia* and foliar treatments of chitosan (3.0 g/l with amino acid at rates of 100 mg/l) resulted in maximum significant increase of phenolic, protein, FAA, and proline contents as well as antioxidant activities (DPPH%), compared with all other interactions. Moreover, the interaction between *M. viridis* and foliar treatments of chitosan (3.0 g /l with amino acid at a rates of 100 mg/l) gave the maximum significant increase of flavonoid content compared with all other interactions. These findings concur with those of El-Bassiouny *et al.* [75], who discovered that foliar treatment of chitosan significantly increased the growth parameters concurrently with an increase in the nutritional value of wheat plants in terms of minerals, phenol, flavonoids, total soluble sugar, proline, FAA, total carbohydrates, and antioxidant activities. When compared with untreated plants, wheat plants treated with chitosan at varying dosages dramatically boosted several endogenous phytohormones, including auxins (IAA), abscisic acid (ABA), gibberellins (GAs), and cytokinins (Cyt). In addition, grain yield, nutritional values, protein percentage, carbohydrate percentage, antioxidant compound percentage, and macronutrient percentage all increased noticeably with increasing chitosan concentrations.

Essential oil composition

A total of 23 compounds were identified in the essential oils extracted from two *Mentha* cultivars (*M. viridis* and *M. longifolia*) plants (7). The identified oil compounds represented 98.01–100% of the total oil compositions. Data in Table 7 represent

Table 5 The effect of interaction between the two *Mentha* cultivars and chitosan with or without amino acid foliar treatments at different levels on photosynthetic pigments and endogenous indole acetic acid (the data are the mean of the two seasons)

Mentha cultivars	Treatments (g/l)	Chlorophyll a (mg/ g fresh wt.)	Chlorophyll b (mg/ g fresh wt.)	Carotenoids (mg/ g fresh wt.)	Total pigments (mg/ g fresh wt.)	IAA (mg/ 100 g fresh wt.)
Mentha viridis	Control	0.88	0.56	0.33	1.77	18.51
	Chitosan 1.5	1.03	0.64	0.37	2.05	23.42
	Chitosan 1.5+50 A	1.35	0.84	0.40	2.59	29.89
	Chitosan 1.5+100 A	1.43	0.95	0.41	2.79	36.34
	Chitosan 3	1.24	0.74	0.40	2.37	29.85
	Chitosan 3+50 A	1.44	0.89	0.43	2.75	40.97
	Chitosan 3+100 A	1.58	0.92	0.44	2.94	46.34
Mentha longifolia	Control	1.03	0.57	0.30	1.90	31.19
	Chitosan 1.5	1.18	0.76	0.37	2.31	42.92
	Chitosan 1.5+50 A	1.39	0.88	0.49	2.76	51.37
	Chitosan 1.5+100 A	1.58	0.96	0.57	3.11	64.56
	Chitosan 3	1.33	0.81	0.42	2.55	50.80
	Chitosan 3+50 A	1.61	0.91	0.52	3.04	68.36
	Chitosan 3+100 A	1.79	1.02	0.63	3.44	80.04
$LSD_{0.05}$		0.05	0.02	0.01	0.05	1.12

Table 6 The effect of interaction between the two *Mentha* cultivars and chitosan with or without amino acid foliar treatments at different levels on antioxidant compounds and carbohydrates %

Cultivars	Treatments (g/l)	Phenol (mg per 100 g fresh wt.)	Flavonoids (mg per 100 g fresh wt.)	Protein %	Carbohydrates % (mg per 100 g fresh wt.)	FAA (mg per 100 g fresh wt.)	Proline (mg per 100 g fresh wt.)	DPPH % (μ mole per g fresh wt.)
Mentha viridis	Control	254.88	151.70	17.50	53.51	247.54	27.62	35.13
	Chitosan 1.5	279.97	187.35	19.49	55.15	266.11	37.15	38.88
	Chitosan 1.5+50 A	311.52	199.39	20.22	55.93	284.24	49.92	43.27
	Chitosan 1.5+100 A	333.07	219.29	20.94	56.24	297.91	63.25	49.01
	Chitosan 3	294.62	204.32	20.40	56.31	281.77	51.06	44.38
	Chitosan 3+50 A	328.60	233.82	22.26	57.33	301.71	64.04	59.01
	Chitosan 3+100 A	351.34	252.80	23.03	58.04	323.00	80.91	62.13
Mentha longifolia	Control	284.11	154.43	22.78	46.27	286.75	31.66	33.17
	Chitosan 1.5	308.00	170.66	23.67	47.40	309.21	42.15	41.49
	Chitosan 1.5+50 A	326.97	198.34	24.72	48.58	349.29	52.26	52.02
	Chitosan 1.5+100 A	339.72	223.04	25.63	49.66	382.26	63.92	62.46
	Chitosan 3	318.62	196.29	24.70	48.68	335.67	54.15	54.44
	Chitosan 3+50 A	345.37	237.14	25.88	49.90	390.45	64.78	67.53
	Chitosan 3+100 A	364.81	247.87	26.96	50.82	418.17	81.81	78.63
$LSD_{0.05}$		3.74	2.43	0.35	n.s	5.16	1.59	1.50

The data are the mean of the two seasons.

the obtained compounds from two *Mentha* cultivars (*M. viridis* and *M. longifolia*) herb essential oil under chitosan (1.5 and 3 g/l with or without amino acid at rates of 50 and 100 mg/l) compared with control treatment.

From the same table, it is evident that chitosan treatments (1.5 and 3 g/l with or without amino acid at rates of 50 and 100 mg/l) gave the highest values of (–)-Carvone and cis-Muurola-4(15),5-diene concentration in the *M. viridis* cultivar compared with the control. The treatment involving chitosan at a concentration of 3 g /l with amino acid at a rate of 100 mg/l with *M. viridis* cultivar increased the (–)-Carvone content by 118.7% compared with the control. The chitosan treatment at 1.5 g/l gave the highest values of α-Pinene, Sabinene, β-Myrcene, and Eucalyptol contents. However, chitosan treatment at 3.0 g/l gave the highest values of cis-p-Mentha-2,8-dien-1-ol and Carveol acetate content and the same treatment of chitosan with 100 mg amino acid gave the highest value of cis-Muurola-4 (15),5-diene content in the essential oils extracted from the cultivar *M. viridis* compare with other treatments. In addition, chitosan treatment at 1.5 g/l with amino acid at 50 mg/l) gave the highest values of Dihydrocarveol, cis-Carveol, Carveol, Dihydrocarvyl acetate, cis-Carvyl acetate, (–)-β-Bourbonene, Bicyclogermacrene, and Calamenene contents in the essential oils extracted from the cultivar *M. viridis* compare with the other treatments. However, the control treatment gave the highest values of D-limonene, isomenthone, l-menthone, and pulegone from the cultivar *M. viridis* compared with the other treatments.

The main higher constituents of *M. longfolia* essential oil as detected by gas chromatography were α-pinene, sabinene, β-pinene, β-myrcene, eucalyptol, l-menthone, isomenthone, isopulegone, α-terpineol, cis-pulegone oxide, pulegone, piperitone, –iperitenone, and β-caryophyllene. The chitosan treatment at 1.5 g/l gave the highest values of isopulegone and α-terpineol, as well as chitosan treatment at 1.5 g/l with the amino acid at 50 mg/l gave the highest values of cis-Pulegone Oxide, Pulegone, Piperitone, and Piperitenone, while chitosan treatment at 1.5 g/l with the amino acid at 100 mg/l gave the highest values of α-pinene, sabinene, and trans-isopulegone contents compared with the other treatments. However, the highest value of sabinene, β-pinene, and β-myrcene contents were obtained by chitosan treatment at 3.0 g/l with amino acid at 50 mg/l, while, the highest value of (–)-menthol, piperitone, piperitenone, and β-caryophyllene contents were obtained by chitosan treatment at 3.0 g/l with amino acid at 100 mg/l compared with the other treatments.

Pearson correlation coefficient heat map matrix, with significance levels

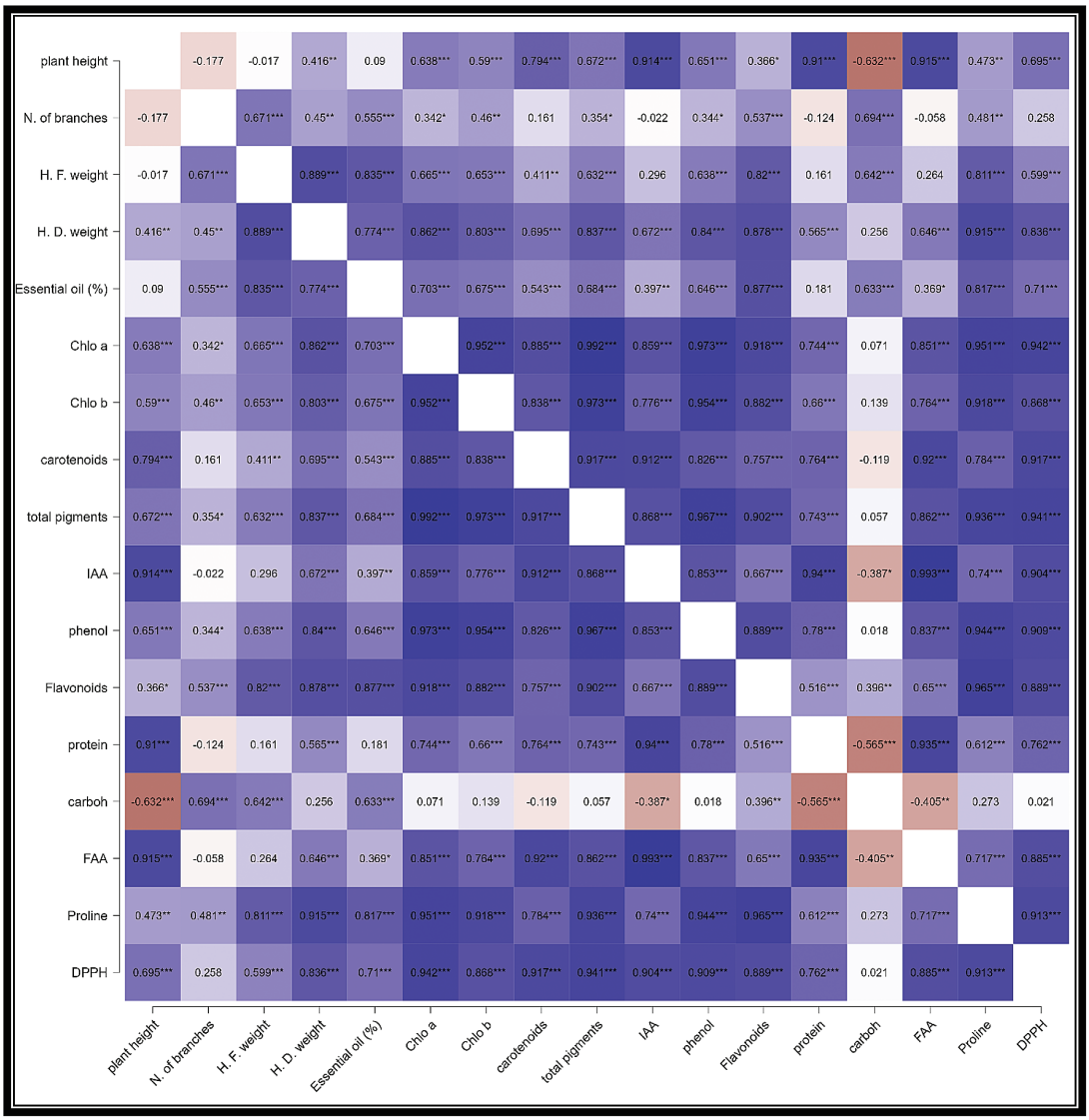
Figure 1 represents the correlation coefficients between various traits and variables related to mint plants. Correlation coefficients range from −1 to 1, with −1 indicating a strong negative correlation, 1 indicating a strong positive correlation, and 0 indicating no correlation. The summary of the correlations was that plant height has moderate positive correlations with many other variables, such as herb dry weight, chlorophyll a, chlorophyll b, carotenoids, t. pigments, IAA, phenol, protein, FAA, proline, and DPPH%.

Table 7 The effect of chitosan at different levels with or without amino acid foliar treatments on essential oil composition of two *Mentha* cultivars

Constituents	*Mentha viridis*						
	Control	Chitosan 1.5	Chitosan 1.5+50 A	Chitosan 1.5+100 A	Chitosan 3.0	Chitosan 3.0+50 A	Chitosan 3.0+100 A
α-Pinene	1.74	4.24	0.39	1.99	0.0	0.52	0.0
Sabinene	2.38	9.09	0.4	5.15	0.0	0.0	0.0
β-Pinene	2.33	2.34	0.83	1.69	0.8	0.89	0.0
β-Myrcene	2.02	2.98	0.47	2.73	0.0	0.0	0.0
D-Limonene	20.46	16.31	4.49	12.69	6.6	6.94	4.71
Eucalyptol	11.16	12.2	4.48	6.83	4.58	6.80	3.18
Isomenthone	5.65	0.64	0.0	0.0	0.0	2.76	0.0
l-Menthone	4.57	0.0	1.34	0.0	0.0	2.97	0.0
Terpinen-4-ol	0.85	0.0	0.54	0.0	3.5	0.93	5.8
Dihydrocarveol	0.0	2.46	9.02	1.9	2.21	6.59	2.47
cis-Carveol	0.0	1.94	2.42	1.44	0.0	2.41	0.0
Carveol	0.0	0.0	2.85	1.77	2.16	1.56	1.51
cis-p-Mentha-2,8-dien-1-ol	0.0	0.0	3.33	0.0	3.55	1.68	1.8
Pulegone	16.11	0.0	2.32	0.0	3.31	2.57	2.54
(–)-Carvone	29.79	40.77	45.02	54.17	63.39	61.55	65.14
Dihydrocarvyl acetate	0.95	1.11	3.49	1.49	1.28	1.26	2.11
cis-Carvyl acetate	0.0	0.94	2.29	2.72	0.0	0.0	0.0
Carveol acetate	0.0	0.0	1.01	0.0	2.29	0.93	1.35
(–)-β-Bourbonene	0.0	1.44	2.53	1.28	0.89	0.0	0.0
β-Caryophyllene	0.0	1.42	2.72	1.68	1.29	0.0	1.9
cis-Muurola-4(15),5-diene	0.0	1.03	4.49	1.30	2.01	0.0	4.71
Bicyclogermacrene	0.0	0.0	2.5	0.0	0.0	0.0	1.39
Calamenene	0.0	0.0	1.64	0.0	1.13	0.0	1.37
Total Iden. Com.	98.01	98.91	98.57	98.83	98.99	100.0	99.98
Constituents				*Mentha longifolia*			
α-Pinene	3.12	3.6	3.62	4.59	1.61	3.86	3.18
Camphene	0.77	0.61	1.13	4.55	0.0	0.0	0.0
Sabinene	2.48	2.99	2.36	2.02	1.80	3.60	2.77
β-Pinene	4.22	5.09	4.30	3.41	3.14	5.54	4.58
β-Myrcene	1.68	1.89	1.52	1.21	1.50	2.16	1.80
Î -Terpinyl acetate	0.0	0.0	0.0	0.39	0.0	0.0	0.0
D-Limonene	1.69	0.0	2.23	0.0	1.02	0.0	0.0
Eucalyptol	16.21	21.92	14.59	25.37	17.87	26.44	19.95
Sabinene hydrate	0.0	0.25	0.0	0.0	0.0	0.0	0.0
l-Menthone	24.20	33.33	22.51	20.79	26.97	24.26	23.16
p-Menthan-3-one	14.25	0.0	0.0	2.25	0.0	0.0	0.0
Isomenthone	0.54	0.0	13.98	7.56	15.99	13.43	14.37
. (–)-Menthol	0.0	0.45	0.0	0.0	0.72	0.28	0.96
Isopulegone	1.89	1.97	1.85	1.50	1.88	1.29	1.54
α-Terpineol	0.69	1.20	0.75	1.00	0.97	0.65	1.04
cis-Pulegone Oxide	0.7	1.04	1.28	0.58	0.59	0.47	0.78
trans-Isopulegone	0.0	0.0	0.0	7.22	0.0	0.0	0.0
Pulegone	23.0	23.65	25.51	14.06	23.45	17.27	20.62
Piperitone	1.00	0.66	1.06	0.32	0.87	0.33	0.87
Piperitenone	1.07	0.85	1.88	0.62	0.58	0.0	0.98
β-Caryophyllene	0.97	0.49	0.56	0.94	1.04	0.42	1.83
γ-Cadinene	0.52	0.0	0.45	0.40	0.0	0.0	0.66
Caryophyllene epoxide	0.0	0.0	0.0	0.32	0.0	0.0	0.49
Total Iden. Com	99.00	99.99	99.58	99.1	100	100	99.58

This suggests that plant height has some influence on these traits and has negative correlations with many variables, including the number of branches, herb fresh weight, and number of branches plant. It has positive correlations with several variables, including herb fresh and dry weight, essential oil (%), chlorophyll a, chlorophyll b, flavonoids, carbohydrates %, and proline %, and others. It may play a role in the

Figure 1



Heatmap of Pearson correlation analysis of all investigated traits in chitosan (1.5 and 3.0 g/l) with or without amino acid at rates of 50 and 100 mg/l on two mint cultivars with significance levels (*$P<0.05$, **$P<0.01$, ***$P<0.001$). Blue and brown represent positive and negative correlations, respectively.

growth and development of the mint plant. Herb fresh weight has strong positive correlations with herb dry weight, essential oil (%), chlorophyll a, chlorophyll b, carotenoids, total pigments, phenol, flavonoids, carbohydrates %, proline %, and DPPH %, and several other variables. This suggests that herb fresh weight is closely related to these variables. Herb dry weight also shows strong positive correlations with essential oil (%), chlorophyll a, chlorophyll b, carotenoids, total pigments, IAA, phenol, flavonoids, protein %, FAA, proline %, and DPPH %, and several other variables, indicating a strong relationship with these traits. Essential oil (%) has positive correlations with several other variables, including chlorophyll a, chlorophyll b, carotenoids, total pigments, IAA, phenol, flavonoids, carbohydrates %, FAA, proline %, and DPPH %, and others essential oil (%) seems to be related to these traits. Chlorophyll a shows positive correlations with several variables, including chlorophyll b, carotenoids, total pigments, IAA, phenol, flavonoids, protein %, FAA, proline %, and DPPH %, which means that chlorophyll a may be influenced by these traits.

The chlorophyll (a and b) and carotenoid content have various correlations with other traits. Chlorophyll a and chlorophyll b are strongly positively correlated, indicating that they are closely related. The total pigment has positive correlations with several variables, including herb fresh and dry weight, essential oil (%), and others. IAA, phenol, flavonoids, and protein % traits are strongly

positively correlated with FAA, proline %, and DPPH %, and other traits. FAA has positive correlations with several variables, including proline % and DPPH %, and several other variables. Proline % and flavonoids traits show strong positive correlations with all studied characters except carbohydrates % with proline %. In summary, this table provides valuable information about the relationships between various traits related to the mint plant, including its growth, essential oil (%) content, and biochemical traits. These correlations can be used to better understand the traits that influence mint plant characteristics and make informed decisions in agricultural practices.

Conclusion

From the results, it is concluded that the treatment of chitosan (3 g/l) with amino acid at a rate of 100 mg/l) with the *M. viridis* cultivar increased the (-)-Carvone content by 118.7% compared with the control. Also, it led to increased plant height, herb dry weight, chlorophyll a, chlorophyll b, carotenoids, total pigments, IAA, phenol, carbohydrates %, FAA, flavonoid, and proline contents.

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Conflicts of interest

There are no conflicts of interest.

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