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Effect of Biostimulators as Foliar Application on Eggplant "*Black Beauty* Cultivar" Growth, Yield and Chemical Composition in Multi-Stressed Loamy Sand Soil

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Abstract: This study examines the potential of natural biostimulants to mitigate environmental stress and enhance growth, yield, and quality in eggplant (*Solanum melongena* L., cv. Black Beauty) grown in loamy sand soil. Eggplants were treated with foliar applications of ascorbic acid (AA) at 300 mg/L, chitosan (Ch) at 200 mg/L, and moringa oil (MO) at 1000 mg/L as natural biostimulants. Results indicated significant increases in plant height, branch number, leaf chlorophyll content, fruit count, and total yield per feddan (0.42 ha) with the AA, Ch, and MO treatments compared to untreated controls. Treated plants also displayed enhanced fruit characteristics, including increased weight, diameter, length, and size. Biochemical analyses revealed elevated levels of fruit dry matter, ascorbic acid content, total phenols, flavonoids, and antioxidant activity. Untreated plants, in contrast, showed significantly lower values across all measured parameters, indicating higher susceptibility to environmental stressors and reduced growth and fruit quality. These findings underscore the effectiveness of AA, Ch, and MO as biostimulants in enhancing eggplant growth, yield, and fruit quality under loamy sand conditions. Furthermore, the use of biostimulants could be extended to other crops, offering a sustainable approach to improving food security and sustainability in agricultural practices.

Keywords: eggplant; moringa oil; antioxidant activity; total phenols; chitosan; ascorbic acid; flavonoids

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1. Introduction

Eggplant (*Solanum melongena* L.) is a widely consumed vegetable worldwide. It belongs to the Solanaceae family and is low in calories and rich in essential nutrients (potassium, manganese, magnesium, phosphorus, and copper), folate, and dietary fibers, and it is considered a good source of vitamin C, K, B6, and thiamin [1]. Eggplant contains a variety of bioactive phytochemicals, including flavonoids, ascorbic acid, and phenolic compounds, which are potent antioxidants with significant health benefits [2]. It has been proven that eggplant extracts effectively suppress the development and growth of tumors and lung cancer [3], as well as inhibit inflammation [4] and cardiovascular diseases [5]. Due to these health benefits, eggplant has gained increasing interest among consumers and researchers and is ranked among the top 10 vegetables in terms of anti-inflammatory potential [6].

Therefore, it is a priority to use safe substances to improve plant growth, flowering, fruit setting, and yield, as well as to mitigate the negative effects of environmental stress conditions such as salinity, heavy metals, hydrocarbons, and cold stress [7].

Ascorbic acid (vitamin C) functions as a co-enzyme in the metabolism of carbohydrates, fats, and proteins. Vitamin C increases nucleic acid content, particularly ribonucleic acid (RNA), and plays a significant role in delaying senescence and protecting cells from damage. Vitamin C functions as an antioxidant, enzyme cofactor, and growth regulator factor, having an important role in photosynthesis, photoprotection, cell wall growth, and cell expansion resistance to environmental stresses through the synthesis of ethylene, gibberellins, anthocyanins, and hydroxyproline [8]. Foliar application of ascorbic acid significantly increased eggplant's vegetative growth, including plant height, leaf number, branch number, and plant fresh weight, leading to higher yields compared to untreated controls [9]. Additional trials showed that foliar application of ascorbic acid enhanced fruit characteristics, including fruit length, fruit diameter, fruit freshness, and dry weight, as well as early yield and total yield compared to untreated controls. It also increased the fruit content of carbohydrates, total phenols, acidity, anthocyanin, tannin, and ascorbic acid [1]. The application of ascorbic acid has also been shown to improve growth parameters, total yield, and phytochemical composition in other crops, including tomato [10], potato [11], pepper [12], and pea [13]. Similarly, a study by Midan and Sorial in 2011 [14] found that ascorbic acid application positively influenced lettuce plant height, number of leaves, leaf area/plant, fresh and dry weights of leaves, as well as stem length and diameter, increasing total chlorophyll content, carbohydrates, phenols, and total soluble solids (TSS).

Meanwhile, chitosan has potential applications in agriculture as a plant growth promoter and elicitor. Its application can support food security and safety by increasing crop yield, reducing post-harvest losses, and helping to maintain biodiversity by decreasing reliance on hazardous chemicals in farming practices. Foliar application of chitosan at the early growth stage of eggplant improved plant growth and yield in addition to increasing total protein, dry matter, total soluble solids, vitamin C, and total phenols content [15]. In another study, chitosan foliar spray increased plant height, stem diameter, number of leaves per plant, and total yield [16]. Similarly, early growth-stage chitosan foliar application in tomato resulted in increased plant height, branching number, number of leaves per plant, and leaf area plant and quality attributes, such as chlorophyll content and dry matter content [17,18]. The benefits of chitosan foliar applications have also been observed in other crops, including soybean [19], okra [20] and sunflower, where it positively influenced growth and yield characteristics [21].

Additionally, moringa oil is highly valued for its high concentration of bioactive compounds and has a range of applications in agriculture, including post-harvest quality management, fertilization, and as a growth promoter [22–24].

Finally, there are limited studies on the application of ascorbic acid (AA), chitosan (Ch), and moringa oil (MO) application in eggplant cultivation, and in contrast to chemical fertilizers, which are major contributors to environmental and human pollution and require significant financial investment for raw materials, these natural biostimulants offer a more sustainable and cost-effective alternative. Therefore, the objectives of this study are to obviate the environmental pollution associated with the excessive use of chemical fertilizers through the application of AA, Ch, and MO as an alternative to these harmful chemicals and to improve eggplant (*Black Beauty* cultivar) growth, yield, and the chemical composition of the fruits, as well as the antioxidant activity, and increase both plant quantity and the quality of yield.

2. Materials and Methods

2.1. Field Experiment

Field trials were carried out at the Faculty of Agriculture farm, Al-Azhar University at Sadat City, Menofiya Governorate, Egypt (30°32′37.28″ E, 30°25′14.43″ N), as shown in Figure 1. Eggplant seeds were planted on 15 August, and the planting process was

carried out on 3 and 7 October of the 2021–2022 and 2022–2023 seasons, respectively, with a distance of 30 cm between plants within the rows. Plants were sprayed twice with 360 L/ha at an interval of 20 days, starting 40 days after planting, with the following substances: ascorbic acid at concentrations of (100, 200, and 300 mg/L), chitosan (100, 150, and 200 mg/L), and moringa oil (500, 750, and 1000 mg/L), as shown in Table 1. Meteorological conditions such as temperature, wind speed, relative humidity, and rainfall averages through the two planting seasons are described in Table 2. Physical and chemical analyses were conducted before the planting according to Flick et al. (1978) [25], shown in Table 3, which established that the experimental soil was classed as loamy sand. The experimental site posed multiple stress factors impacting eggplant growth, including slightly saline soil (EC 1–2 dS/m), mild heavy metal contamination (15 mg/kg lead and 1.5 mg/kg cadmium), and hydrocarbon pollution (75 mg/kg), all of which could affect plant health and soil quality. Additionally, suboptimal minimum temperatures during the early growth stage (6.40–18.47 °C) introduced cold stress, potentially stunting development and delaying growth, as eggplants prefer warmer conditions (21–30 °C). These stress factors were inherent to the experimental site and underlined the challenging conditions faced by the plants.

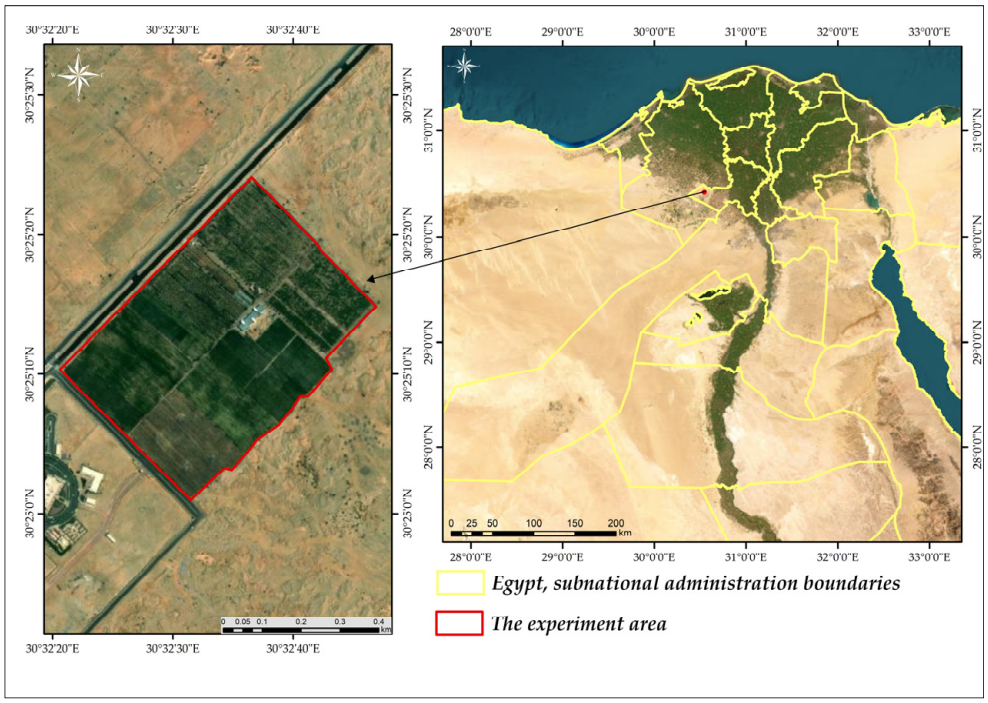

Figure 1. Location of the experiment area.

Table 1. Abbreviations of the treatments used in the experiment.

Treatments	Description
Control	Foliar spraying with water only (control)
AA1	Foliar spraying with 100 mg/L ascorbic acid solution (AA)
AA2	Foliar spraying with 200 mg/L ascorbic acid solution (AA)
AA3	Foliar spraying with 300 mg/L ascorbic acid solution (AA)
Ch1	Foliar spraying with 100 mg/L chitosan solution (Ch)
Ch2	Foliar spraying with 150 mg/L chitosan solution (Ch)
Ch3	Foliar spraying with 200 mg/L chitosan solution (Ch)
MO1	Foliar spraying with 500 mg/L moringa oil solution (MO)
MO2	Foliar spraying with 750 mg/L moringa oil solution (MO)
MO3	Foliar spraying with 1000 mg/L moringa oil (MO)

Table 2. Meteorological data during the 2021–22 and 2022–23 seasons for the experiment site.

Month	2021–2022								2022–2023							
	Temperature (°C)			Wind Speed (m s^{-1})	Rainfall (mm day^{-1})	Relative Humidity (%)			Temperature (°C)			Wind Speed (m s^{-1})	Rainfall (mm Day^{-1})	Relative Humidity (%)		
	Max.	Mini.	Average			Max.	Mini.	Average	Max.	Mini.	Average			Max.	Mini.	Average
October	32.24	18.47	25.36	4.225	0.06	36.99	19.92	28.46	31.51	18.66	25.09	4.375	0.13	41.2	17.66	29.43
November	28.27	15.80	22.06	3.475	1.23	44.7	19.16	31.93	26.24	14.29	20.27	3.395	0.08	39.18	21.1	30.14
December	19.89	10.05	14.97	4.075	0.72	38.76	30.45	34.61	23.84	12.13	17.99	3.3	1.07	39.55	26.37	32.96
January	17.34	6.40	11.87	4.02	1.14	42.41	26	34.21	21.29	9.26	15.28	3.63	0.91	47.1	22.16	34.63
February	19.79	7.29	13.54	4.01	0.32	48.48	18.85	33.67	19.81	7.80	13.81	3.69	0.83	46.51	20.89	33.7
March	21.27	7.76	14.52	4.475	0.82	43.68	18.72	31.2	25.96	11.42	18.69	4.32	0.37	40.83	15.1	27.97

Table 3. Physical and chemical properties of experiment soil.

Physical Proprieties								
Field Capacity (%)		**Permanent Wilting Point (%)**		**Available Water (%)**	**Bulk Density (mg m^{-1})**		**Total Porosity (%)**	
12.97		5.4		7.57	1.7		35.8	
Particles Size								
Coarse sand (%)		Fine sand (%)		Silt (%)	Clay (%)		Texture class	
7.08		78.88		4.42	9.62		Loamy sand	
Chemical Proprieties								
Soluble cations (mmolc L^{-1})					Soluble anions (mmolc L^{-1})			
EC	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	$CO_3{}^{2-}$	HCO^{3-}	Cl^{-}	$SO_4{}^{2-}$
1.45	1.65	1.78	2.98	0.43	0	1.86	4.27	0.71

A randomized complete blocks experimental design with three replications was used. The experimental area was 324 m^2 divided into 10 plots, and each plot included 3 replicates. Each replicate comprised 2 rows. Each plot had an area of 32.4 m^2 and consisted of 6 rows, each 6 m long and 90 cm wide, with 35 cm in-row spacing. The recommended fertilizer rates were applied, and the recommended agricultural practices for commercial eggplant production in a net greenhouse were followed.

Ascorbic acid (AA) solution preparation: Amounts of 100, 200, and 300 mg of AA were added to 250 mL of distilled water in a glass beaker. The mix was stirred by a hot plate stirrer until all the AA was dissolved (about 15 min). The dissolved AA was transferred to a volumetric flask. Distilled water was added to the volumetric flask until the total volume reached 1000 mL and was kept under continuous stirring until complete homogeneity occurred; then, the solution was stored temporarily in a dark glass bottle for later use.

Chitosan (Ch) solution preparation: Amounts of 100, 150, and 200 mg of Ch were added to 1000 mL of 0.1 M acetic acid solution, and the mixture was heated at about 40–50 °C with continuous stirring for 2 h. The mixture was cooled and stored temporarily in a dark glass bottle for later use.

Preparation of moringa oil (MO) solution: Air-dried *Moringaoleifera* seeds were crushed into small pieces and placed into the compartment (500 mL) of the Soxhlet apparatus. Then, 300–350 mL hexane was added to the compartment. The Soxhlet apparatus was set up at 60 °C for 5 h and heated in a mantle. Hexane solvent was removed from the oil extract by distillation in rotary evaporation. The mixture was washed with 15 mL of cold saturated NaCl solution 2 to 3 times, and the upper layer was collected. Amounts of 500, 750, and 1000 mg of extracted MO were added to 1 L of water.

2.2. Plant Measurements

2.2.1. Plant Growth Measurements

Plant growth measurements were recorded 75 days after transplanting. A total of 16 plants from each plot were divided into 4 replicates; each replicate consisting of 4 plants was used to perform growth measurements. Plant height (cm) was measured using a measuring tape, starting from the soil level up to the plant apex; branch number per plant was manually counted, and the leaf total chlorophyll content was measured using a Handheld Chlorophyll Meter (SPAD 502, Konica Minolta, Minolta Corp., Ramsey, NJ, USA) at two points on each leaf to calculate the average chlorophyll concentration values for each plant.

2.2.2. Fruits' Physical Measurements

The total number of fruits per plant, were determined by accumulating the number of fruits during the harvest period (60 days), and a total of 24 fruits were taken from each plot and then divided into 4 replicates; each replicate consisting of 6 fruits was used to perform physical measurements. Fruit weight (g) was measured using an electronic balance, fruit diameter and length (cm) were determined using a vernier caliper, and fruit size (cm^3) was determined using the water displacement method (wherein the fruit was immersed in a container filled with water, and the volume of water displaced was measured using a graduated jar, 2000 mL (ASTM E1272, Eisco Labs, Rochester, NY, USA)) at each harvest season.

2.2.3. Fruit Chemical Measurements

A total of 20 fruits were taken from each plot and then divided into 4 replicates; each replicate consisting of 5 fruits was used for chemical measurements. Dry matter (*D.M* %) was determined as g 100 g^{-1} fresh weight (*F.W*) by subjecting 100 g of the fresh weight to 70 °C in an oven until constant dry weight (*D.W*) was reached. Calculations used the following Equation (1):

$$D.M = \frac{D.W}{F.W} \times 100 \quad (1)$$

Ascorbic acid (mg g^{-1} *F.W*) was determined as (mg g^{-1} *F.W*) was determined using the methodology of the titration with 2–6 dichlorophenol indophenol (DCPIP) according to Equation (2), which was outlined by Bassi et al., 2018 [26].

$$Ascorbic\ acid\left(\frac{\text{mg}}{\text{g}}F.W\right) = \frac{V2 \times 500 \times 25}{V1 \times 5 \times 5} \quad (2)$$

where $V1$ is the volume of dye used up with 500 µg of ascorbic acid standard, and $V2$ is the volume of dye used up with 5 mL of test sample.

The total phenolic content (mg 100 g^{-1} *F.W.*) was determined calorimetrically following the method defined by Thaipong et al., 2006 [27], with modifications in step with Helaly et al., 2015 [28]. Fresh tissue samples (50 mg) from 10 replicate samples of each accession were submerged in 2.5 mL of 95% ethanol at 0 °C for 48 h. After the extraction duration, the samples were homogenized and centrifuged at 13,000× *g* for 10 min to separate the tissue from the extract.

A 1 mL aliquot of the ensuing supernatant was transferred into a 16 mm × 100 mm test tube and combined with 1 mL of 95% ethanol and 5 mL of distilled water. Subsequently, 0.5 mL of 50% Folin–Ciocalteu reagent was added, and the combination was allowed to react for 5 min. To stop the reaction, 1 mL of 5% sodium carbonate (Na_2CO_3) solution was added by blending and left for 60 min at room temperature.

The reaction mixture absorbance was spectrophotometrically measured at 725 nm using blank ethanol (95%). Gallic acid solutions were prepared as standards, and the sample's total phenolic content was expressed as gallic acid equivalents (GAE) by comparing the sample's absorbance with a standard calibration curve.

According to Rohman et al., 2010 [29], the colorimetric aluminum chloride method modified by Helaly et al., 2017 [30], total flavonoid content (mg 100 g^{-1} *F.W.*) was determined using 50 mg of fine plant tissue sample that was powdered and mixed with 10 mL of methanol (80%) in a Falcon tube (15 mL); this mixture was shaken at room temperature for 1 h to facilitate extraction. After extraction, 2 mL of the solution was centrifuged at 4000 rpm for 15 min, and 0.8 mL of the resulting supernatant was mixed with an equal volume (0.8 mL) of distilled water and 0.8 mL of aluminum chloride ($AlCl_3$, 10%) solution. This mixture was incubated at room temperature for 5 min after 4 mL of distilled water was added. The absorbance was measured at 415 nm using a spectrophotometer (CHNSpec DS60/62/64, Hangzhou, China), and the flavonoid content solution (mg per 100 g of *F.W.*) was determined by comparison to a standard calibration curve prepared using quercetin.

2.3. Antioxidant Activities

The determination of DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity (EC_{50}, μg mL^{-1}) was performed according to the method described by Brand-Williams et al. (1995) [31]. A DPPH solution (2 mL) was mixed with aqueous eggplant extract (150, 300, 450, 600, 750, and 900 μL volumes) and measured by spectrophotometer at 517 nm. AA was used as the reference antioxidant, and the extract's antioxidant capacity was expressed as EC_{50}, and defined as the concentration of extract required to reduce the initial DPPH concentration by 50%.

A determination of ABTS [2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity (EC_{50}, μg mL^{-1}) was performed to evaluate the total antioxidant capacity of the extracts. The ABTS radical was generated by reacting ABTS with AAPH (2,2′-Azobis (2-amidinopropane) dihydrochloride). Each extract (150 μL, 25 mg mL^{-1}) was mixed with 2.85 mL of the ABTS radical solution. The absorbance of the mixture was measured at 734 nm at 1-min intervals for a total duration of 6 min using a spectrophotometer. The radical scavenging activity after 6 min was calculated as the percentage of ABTS discoloration, according to the method described by Kang et al., 2017 [32].

2.4. Statistical Analysis

Data were processed using Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA) and statistically analyzed using the analysis of variance (ANOVA) method (IBM SPSS 20.0 Statistics, IBM Corporation, Armonk, NY, USA) [33], with significance at $p \leq 0.05$. The means of the treatments were separated using Duncan's multiple range test (DMRT) to assess differences between treatment means at a given level of significance. Pearson's correlation analysis was conducted to evaluate relationships between variables using OriginPro 2021 (Origin Lab Corporation, Northampton, MA, USA).

Biostimulators' effect on eggplant "*Black Beauty* cultivar" growth, yield, and chemical composition in loamy sand soil is represented in Figure 2.

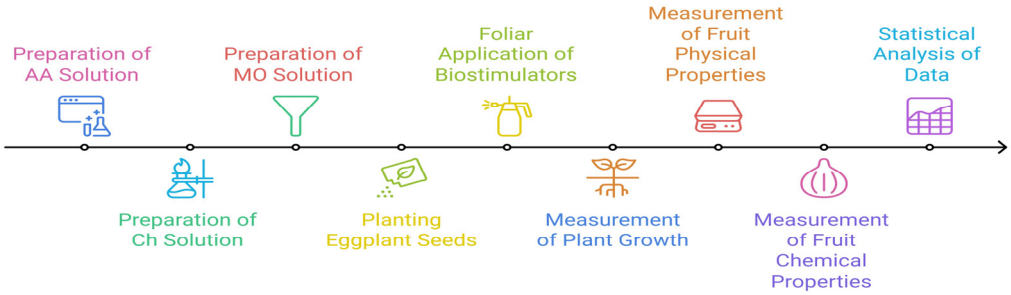

Figure 2. Methodology flowchart of biostimulators' application on eggplant growth, yield, and chemical composition.

3. Results

3.1. Plant Growth Parameters

Figure 3 displays the data regarding changes in plant growth parameters, including plant height, number of branches per plant, and leaf total chlorophyll as affected by biostimulant concentrations. The results demonstrate that, in comparison to different concentrations and the control in general, the external applications of AA, Ch, and MO solution concentrations had a positive impact on all plant growth indicators. In more detail, AA treatment resulted in a significant increase in plant height in all concentrations, with 300 mg/L AA exhibiting the maximum plant height values (78.14 and 76.15 cm), but the minimum values resulted from the control treatment (60.67 and 58.9 cm).

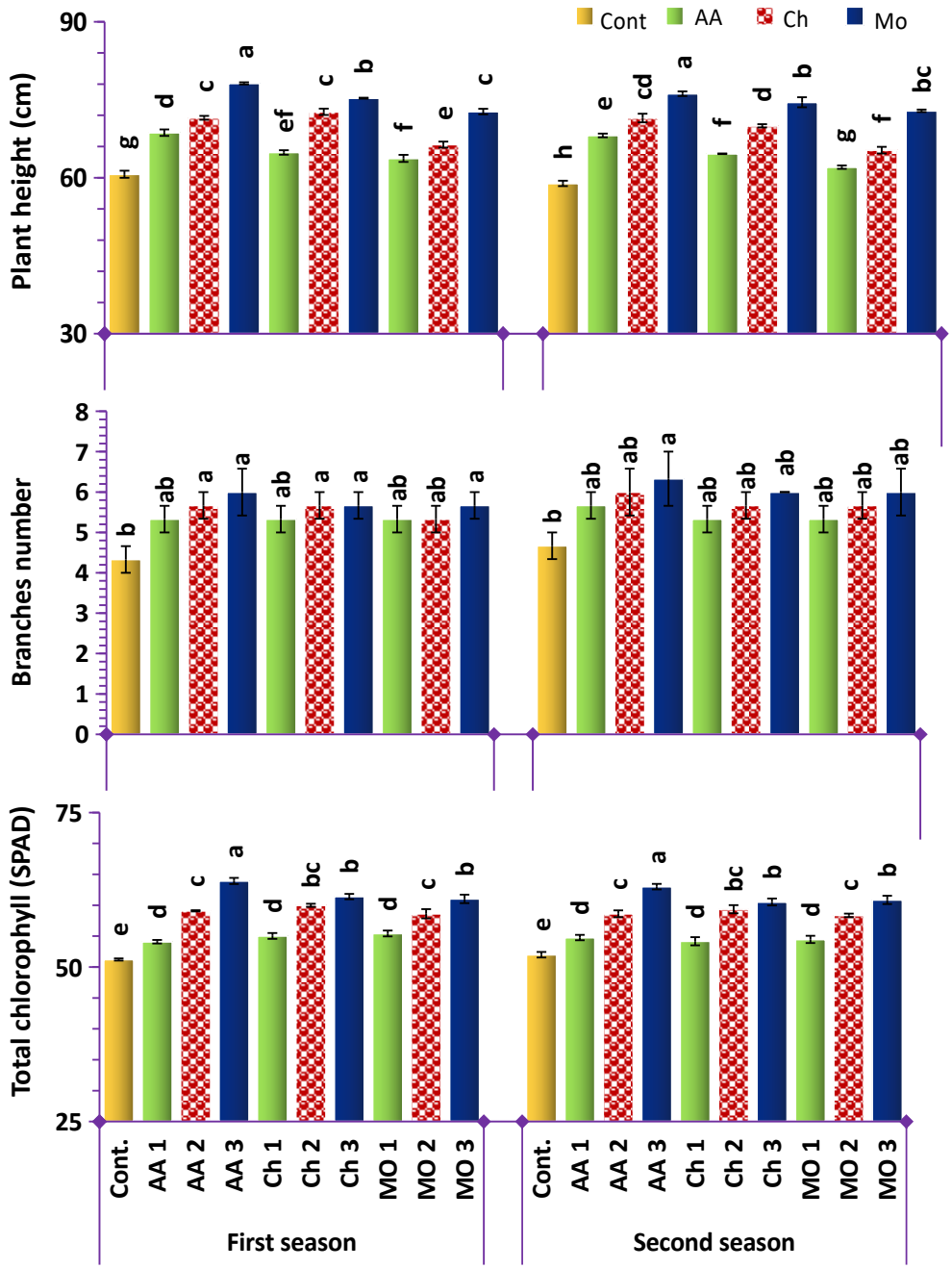

Figure 3. Effect of AA, Ch, and MO as a foliar spray on eggplant plant growth parameters during the 2021–2022 and 2022–2023 seasons. For each season characteristic, means followed by different letters differ by Duncan's test ($p < 0.05$).

In the same Figure 3, the number of branches per plant increased with increased concentrations of the applications, but the differences did not rise to a significant level during the two seasons; in contrast, the minimum values were produced from control plants (6 and 6.33).

Additionally, the results in Figure 3 show that foliar spraying with biostimulant concentrations significantly increased the amount of chlorophyll in the leaves, and the maximum amount (63.93 and 63.01) was at 300 mg/L of AA followed by Ch at 200 mg/L (61.41 and 60.53) and MO solution at 1000 mg/L. At the same time, the results for the untreated plants were noticeably lower.

3.2. Yield Parameters

The data in Figure 4 show that foliar spraying of eggplant with AA, Ch, and MO significantly affects the crop yield. The results demonstrated that using biostimulant concentrations significantly increased the number of fruits per plant and total yield per feddan. The largest amounts were exerted from foliar spraying at 300 mg/L AA (15.67 and 15.33 plant^{-1}) and (37.73 and 36.46 ton fed^{-1}) in the first and second seasons, compared to 200 mg/L Ch and 1000 mg/L MO in the second season. The untreated plants exhibited the least significance of the previously noted traits.

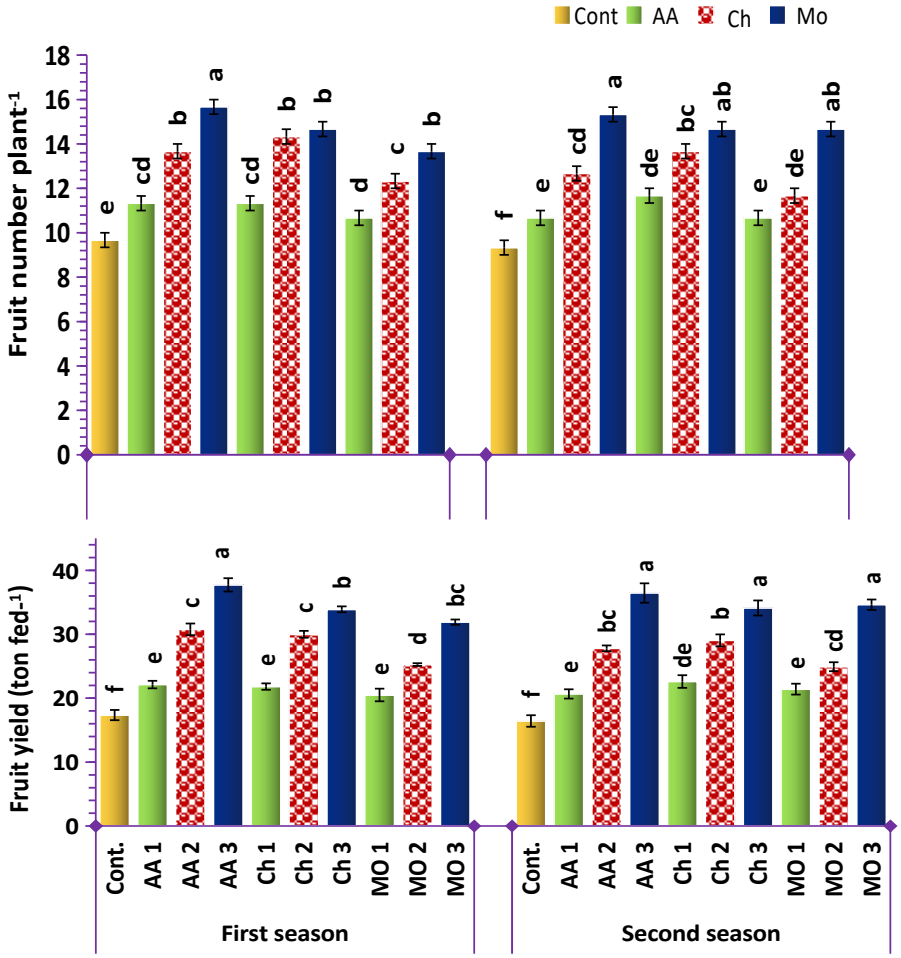

Figure 4. Effect of AA, Ch, and MO as a foliar spray on eggplant yield parameters during the 2021–2022 and 2022–2023 seasons. For each season characteristic, means followed by different letters differ by Duncan's test ($p < 0.05$).

3.3. Fruit's Physical Parameters

Data from the growth seasons of 2021–2022 and 2022–2023 revealed a considerable impact of foliar spraying of AA, Ch, and MO solutions on the physical characteristics of eggplant (Figure 5). According to the findings, there was a significant rise in fruit weight, diameter, length, and size with advancing concentrations from low and high doses. When the first solution (AA) was increased up to 300 mg/L, the highest values were achieved for fruit weight (270.89 and 267.24 g), diameter (10.06 and 10.65 cm), length (21.20 and 21.06 cm), and size (575.65 and 557.35 cm^3) in both seasons. As the concentration of the second solution (Ch) was raised to 200 mg/L, the highest values were seen for fruit weight (261.5) in the second season, diameter (10.08 and 10.31 cm), and length (21.02 and 20.73 cm) during the two seasons, and size (557.72 cm^3) in the first season. When it came to foliar spraying with the third solution (MO), the results showed that this solution caused an

important rise in all attributes, especially at 1000 mg/L. However, the untreated plants produced the lowest significance levels of fruit weight, diameter, length, and size.

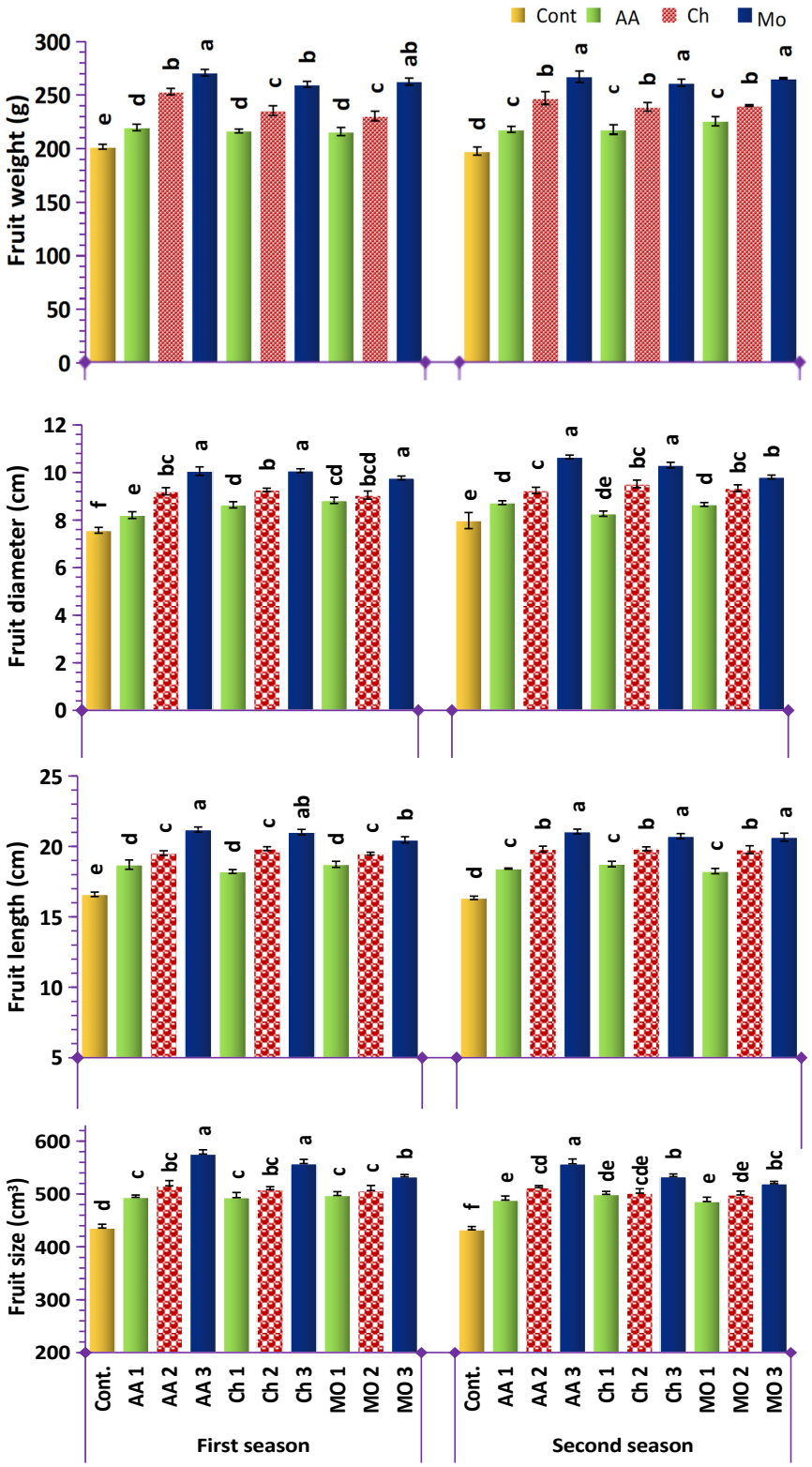

Figure 5. Effect of AA, Ch, and MO as a foliar spray on eggplant fruit physical parameters during the 2021–2022 and 2022–2023 seasons. For each season characteristic, means followed by different letters differ by Duncan's test ($p < 0.05$).

3.4. Fruit's Chemical and Biochemical Parameters

As shown in Figure 6, the results reflect changes in the chemical and biochemical traits of eggplant fruits that are significant for fruit quality, including dry matter, ascorbic acid, total phenols, and total flavonoid contents. The data show that the increment of spraying with AA, Ch, and MO concentrations up to 300, 200, and 1000 mg/L led to a significant increase in fruit dry matter content, with values of 6, 5.92, 5.84, and 5.96, 5.85, 5.78% in both seasons, respectively. At the same time, fruit AA content significantly increased with advancing foliar spraying concentrations of biostimulants, and the concentration of 300 mg/L AA produced the highest values (6.69 and 6.27 mg g^{-1} F.W).

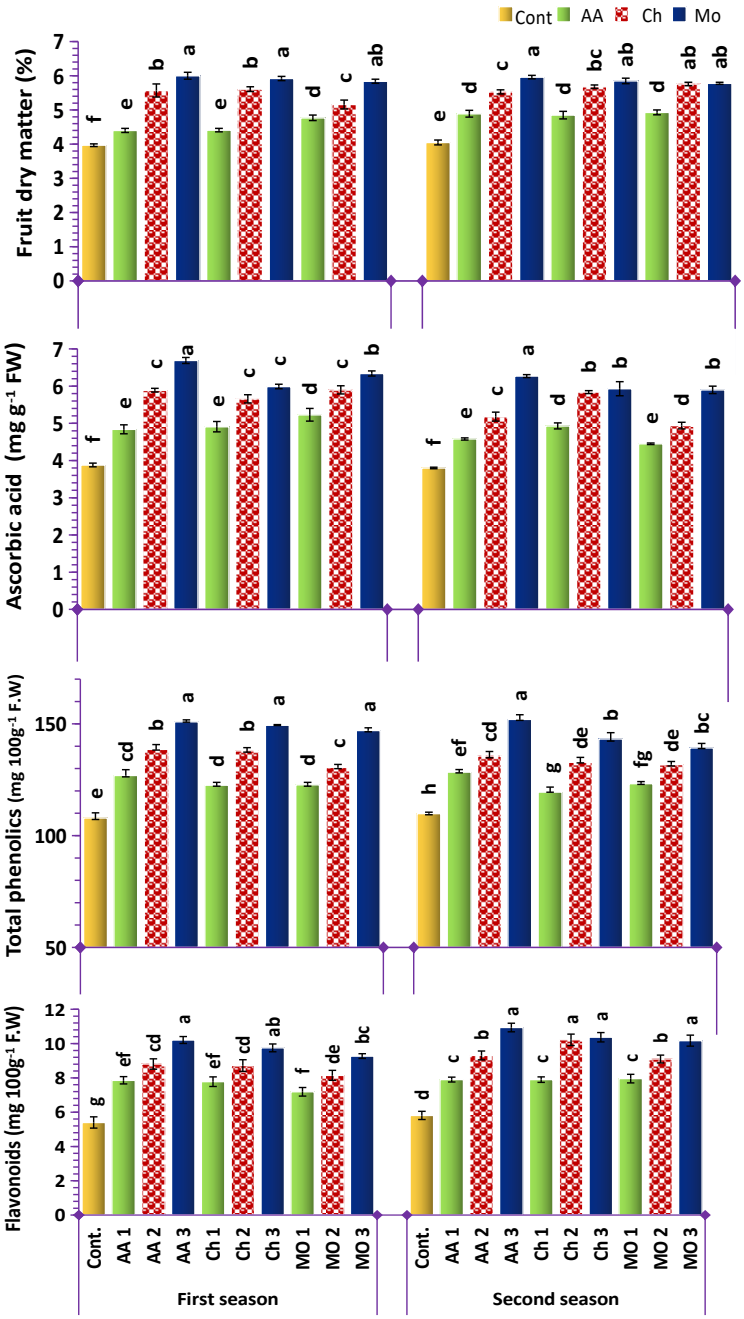

Figure 6. Effect of AA, Ch, and MO as a foliar spray on eggplant fruit chemical and biochemical parameters during the 2021–2022 and 2022–2023 seasons. For each season characteristic, means followed by different letters differ by Duncan's test ($p < 0.05$).

The highest significance values of total phenols occurred when the plants were treated with 300 mg/L AA (150.95), 200 mg/L Ch (149.35), and 1000 mg/L Mo (147.01) in the first season and 300 mg/L AA (151.99) in the second season.

Finally, the highest significance values of total flavonoid contents resulted from the plants treated with 300 mg/L AA (10.21) and 200 mg/L Ch (9.75) in the first season and 300 mg/L AA (10.94) in the second season. Contrarily, the lowest significance results in the prior parameters were obtained in untreated plants.

3.5. Antioxidant Activities

The alteration in DPPH and ABTS content as affected by the foliar spraying with AA, Ch, and MO on eggplant is discussed in Figure 7. The highest scavenging activity with both DPPH and ABTS was demonstrated when the plants were sprayed with AA at 300 mg/L (116.10 and 176.74), and the difference between 100 and 200 mg/L AA did not reach significance in DPPH. The foliar spraying in Ch induced an increase in DPPH and ABTS content, but the difference between 100 and 150 mg/L AA did not achieve significance in DPPH. MO foliar spraying significantly increased DPPH and ABTS content, with an increase in MO concentration until 1000 mg/L. Meanwhile, the untreated plants obtained the lowest significance of scavenging activity in both DPPH and ABTS.

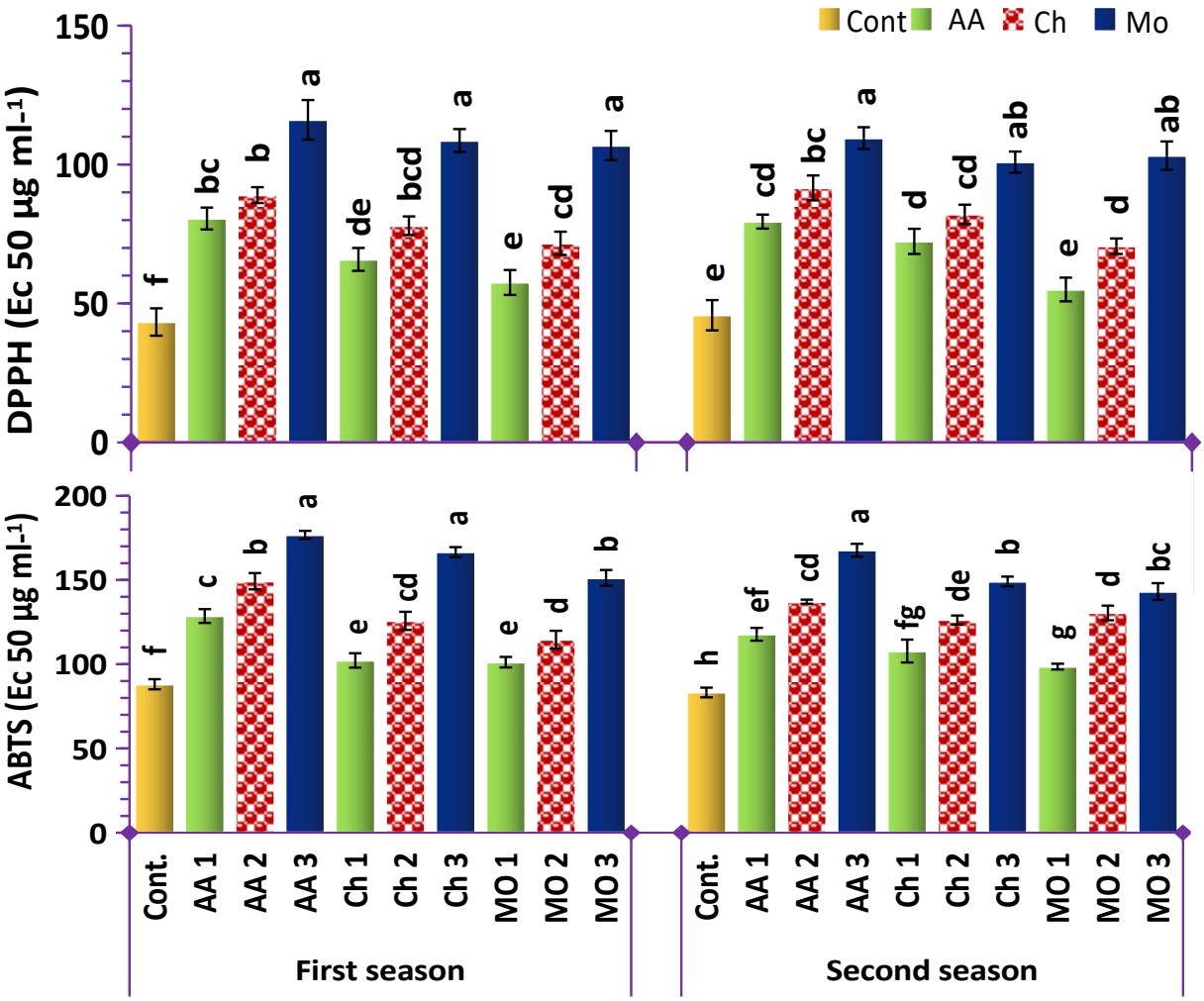

Figure 7. Effect of AA, Ch, and MO as a foliar spray on eggplant fruit antioxidant activities during the 2021 and 2022 seasons. For each season characteristic, means followed by different letters differ by Duncan's test ($p < 0.05$).

3.6. Analysis of Variance of Eggplant Different Characteristics

The results in Table 4 reflect the analysis of the different characteristics as affected by the foliar spraying with AA, Ch, and MO on eggplant plants, including plant height, number of branches per plant, leaf total chlorophyll, number of fruits per plant, total yield, fruit weight, diameter, length, size, dry matter, AA, total phenols, and total flavonoid, DPPH, and ABTS content.

Table 4. Analysis of variance for eggplant factors measurement.

S.O.V	df	Plant Height	Branches N.	Leaf Chlorophyll	Fruits N.	Plant Yield	Fruit W.	Fruit Diameter	Fruit Length
First season: 2021–2022									
Blocks	2	0.508 ns	0.133 ns	0.189 ns	0.433 ns	1.41 ns	14.07 ns	0.012 ns	0.057 ns
Foliar	9	92.1 ***	0.596 ns	46.0 ***	11.4 ***	132.8 ***	1681.8 ***	1.92 ***	5.7 ***
Error	18	0.813	0.429	0.672	0.322	1.46	35.29	0.053	0.119
Second season: 2022–2023									
Blocks	2	0.002 ns	0.433 ns	0.719 ns	0.1 ns	0.566 ns	11.59 ns	0.005 ns	0.004 ns
Foliar	9	94.9 ***	0.666 ns	38.1 ***	12.3 ***	136.4 ***	1611.3 ***	2.2 ***	6.2 ***
Error	18	0.941	0.544	0.907	0.359	2.93	48.64	0.075	0.125
S.O.V	**df**	**Fruit size**	**Dry matter**	**Ascorbic acid**	**Total phenolics (mg/100 g F.W)**	**Flavonoids (mg/100 g F.W)**	**DPPH (Ec50 µg/mL)**	**ABTS (Ec50 µg/mL)**	
First season: 2021–2022									
Blocks	2	9.56 ns	0.012 ns	0.019 ns	0.018 ns	0.715 *	82.87 ns	53.18 ns	
Foliar	9	4437.7 ***	1.6 ***	2.1 ***	575.7 ***	5.7 ***	1671.6 ***	2649.9 ***	
Error	18	188.42	0.028	0.032	8.38	0.15	60.64	50.68	
Second season: 2022–2023									
Blocks	2	347.21 ns	0.012 ns	0.060 ns	9.82 ns	0.101 ns	56.02 ns	48.82 ns	
Foliar	9	3244.8 ***	1.15 ***	1.8 ***	442.2 ***	7.4 ***	1303.9 ***	1895.5 ***	
Error	18	107.81	0.015	0.019	10.44	0.201	50.88	43.04	

***, *, ns: Degree of significance at 1 and 5% and non-significance, respectively.

3.7. The Correlation Between Trails

This study employed principal component analysis (PCA) to assess the correlation between different features and treatments in eggplant, including fruit yield, quality, and chemical and biochemical indicators under several treatments, which were all shown to be observed in the PCA (Figure 8). The data displayed two distinct principal component (PC) variability percentages, PC1 and PC2, which stand for 90.3% and 3.5%, respectively. Hence, the majority of the investigated parameters are in PC1, where they have a positive correlation with one another.

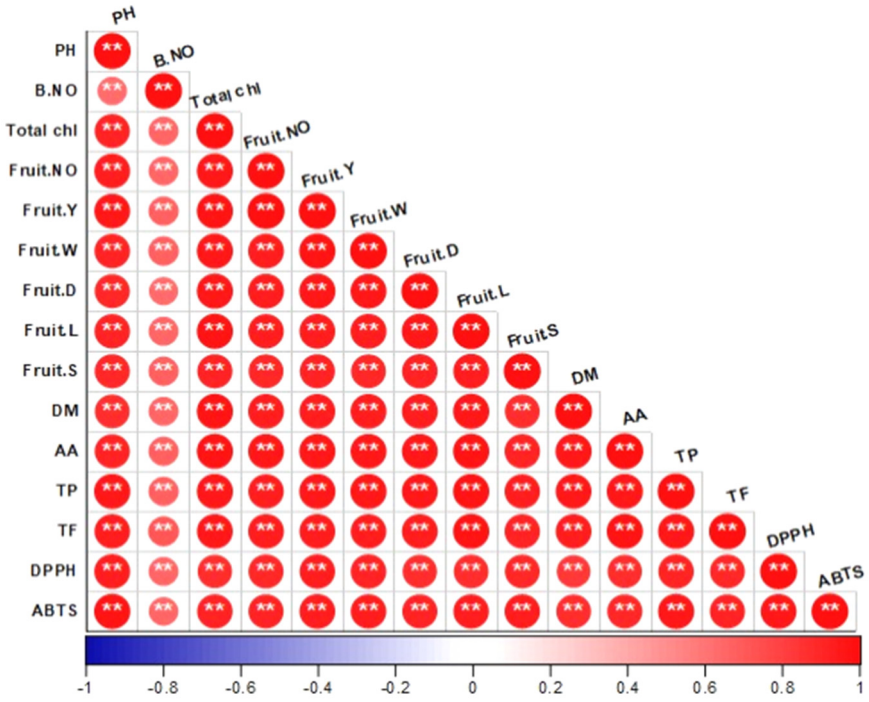

Figure 8. Correlation between different features and treatments in eggplant. ** is significantly different ($p \leq 0.05$).

4. Discussion

4.1. Plant Growth Parameters

On discussing the previous results of the changes that occurred in the plant growth parameters, it is clear that the higher concentrations of three substances used in this experiment, i.e., AA, Ch, and MO, showed the highest plant height, number of branches and leaf chlorophyll content compared with the other concentrations and control, surpassing AA in all the other substances. However, it is quite possible to say that the increase in these characteristics may be attributed to the role of AA in cell division regulation [8]. These results are in agreement with those obtained by Shaddad et al., 1990 [34], who reported that the effect of AA on plant growth may be due to the substantial role of AA in many metabolic and physiological processes, in addition to AA being involved in antioxidant defense, regulation of photosynthesis, and growth [35,36]; these results are in accordance with those reported by [1,9,37–41].

The stimulating effect of Ch on plant height, number of branches, and leaf chlorophyll content may be due to the role of Ch in increasing nitrogen metabolism enzyme activities such as nitrate reductase, protease, and glutamine synthetase and improving nitrogen transportation in the functional leaves [42,43]. Also, Ch may increase water and nutrient availability and uptake though regulating osmotic pressure and reducing free radicals accumulation by means of increasing antioxidant activity [44]. These results are in agreement with those obtained on tomato by Mondal et al., 2016, Hassnain et al., 2020, and Amerany et al., 2022 [17,18,45], who reported that the application of Ch foliar application increased plant height, number of branches, and chlorophyll content.

With respect to the effect of MO on the plant growth parameters, it was revealed that moringa leaves are rich in zeatin (naturally occurring cytokinin) hormone that enhances plant growth [46,47]. In this concern, it was reported that foliar application of MO can be used as a plant biostimulator for enhancing tomato [48], pepper [49], and cucumber [50] growth parameters and productivity.

4.2. Yield Parameters

With respect to the effect of AA, Ch, and MO on fruit number and yield, the most favorable results came from high concentrations of all three substances, with AA being superior in comparison with the low concentrations and control. These positive results with AA were associated with the indirect effect of AA in many biochemical processes in addition to root fresh and dry weights increasing. AA may cause a synergistic effect resulting from its involvement in the main metabolic processes, particularly with energy co-enzymes, carbohydrate metabolism, and improved biosynthetic activities [51]. The same attribution was reported on tomato by Abdel-Halim, 1995 [10], cantaloupe by El-Lithy and El-Greadly, 2001 [52], and potato by Arisha, 2000 [53], who found that the application of AA on plants caused a significant increase in total yield per plant. The positive effect of AA may be due to promoting cell division and enlargement as well as improving the plant's nutritional status [54–56].

To explain the positive effect of Ch on fruit number and yield, it is clear to our knowledge that this stimulating effect may be due to the role of Ch in increasing nitrogen metabolism enzyme activities [57] and increasing water and essential nutrients uptake [44].

4.3. Fruit's Physical Parameters

Considerable attention to the results of fruit's physical parameters found that the foliar application of AA, Ch, and MO in high concentrations significantly obtained the highest values of fruit weight, diameter, length, and size compared with control and the other concentrations.

The positive results with regard to fruit weight, diameter, length, and size may be due to the essential role of AA in many metabolic and physiological processes in addition to its role in cell division regulation [8]. These results are in accordance with those reported by El-Tohamy et al., 2008 [9] on eggplant and Johkan et al., 2008 [58] on sweet pepper.

In regard to the favorable results of Ch on fruit's physical parameters, it is clear that the foliar spray with Ch at the concentration of 200 mg/L caused higher fruit weight, diameter, length, and size than the lower concentrations. These results may be attributed to the role of Ch in water and essential nutrient uptake encouragement by increasing cells' osmotic pressure and, furthermore, increasing antioxidant enzyme activity and retarding free radicals accumulation [44]. Also, Ch improves carbohydrates and protein translocation; these results are in harmony with the findings of Aly et al., 2019 [16] and Sultana et al., 2017 [15].

The positive effect of MO on fruit physical parameters was confirmed by Elzaawely et al., 2017 [59], Matthew, 2016 [60], and Culver et al., 2012 [48] on snap bean, pepper, and tomato.

4.4. Fruit's Chemical and Biochemical Parameters

The results of the fruit's chemical and biochemical characteristics reflected that the foliar application of AA, Ch, and MO at the concentrations of 300, 200, and 1000 mg/L, respectively, showed the highest dry matter, AA, total phenols, and total flavonoids content compared to the lower concentrations and control. These results may be due to the role of AA in photosynthetic machinery activation as a result of the stimulatory effects of the used plant growth biostimulator on the photosynthetic process [61]. Therefore, it could be expected that applied AA increased phenolic concentration as a result of the increase in carbohydrate and vitamin C synthesis, which elevates induced resistance in plants; these obtained data are in agreement with Elwan et al., 2007 [62].

With respect to the effect of chitosan foliar application on fruit dry matter and AA content, it is revealed that these favorable results may be due to the fact that Ch can enhance the photosynthesis process, which is strongly correlated with the synthesis of sugars, polysaccharides, and vitamins, in addition to dry matter [42].

The positive results of the effect of chitosan foliar spray on total phenols may be attributed to encouraging plant phenolic substances biosynthesis by chitosan [63]. Moreover,

chitosan application may encourage phenolic substance accumulation that is reflected in the polyphenolic substance content of fruits [64].

This study found that foliar applications of AA, Ch, and MO significantly boosted antioxidant activity in eggplants, with AA at 300 mg/L showing the highest scavenging activity in both DPPH and ABTS assays. MO showed a dose-dependent response, with antioxidant activity increasing up to 1000 mg/L, while Ch increased antioxidant content within a narrower optimal range. Untreated plants had the lowest antioxidant activity, confirming that biostimulant treatments effectively enhance plant resistance to oxidative stress. These results suggest that AA, Ch, and MO can be valuable for boosting crop resilience and quality in sustainable agriculture [65].

In this concern, all biostimulants used in this study (AA, Ch, and MO) can increase the eggplant plants' response to environmental stress by raising induced resistance by increasing antioxidant enzyme activity, phenols, vitamins, chlorophyll, and carbohydrates content in addition to essential elements uptake, and this study demonstrates how the applied biostimulants mitigated these stresses and significantly improved growth, yield, and quality, highlighting their potential for broader agricultural use.

5. Conclusions

During the 2021–2022 and 2022–2023 seasons, this study assessed the effectiveness of foliar treatments of ascorbic acid (AA), chitosan (Ch), and moringa oil (MO) as biostimulants to improve eggplant growth, yield, and fruit quality in loamy sand soil. Higher dosages of biostimulants were found to significantly increase plant height, chlorophyll content, and fruit yield. The application of AA at 300 mg/L yielded the highest plant height (78.14 cm in 2021–2022 and 76.15 cm in 2022–2023), chlorophyll content (63.93 and 63.01, respectively), and fruit yield (15.67 and 15.33 fruits per plant, equating to 37.73 and 36.46 tons per feddan). Both Ch at 200 mg/L and MO at 1000 mg/L also demonstrated significant positive effects, particularly on fruit weight, diameter, and size.

Additionally, higher concentrations of these biostimulants improved fruit biochemical quality, including increased dry matter, ascorbic acid, total phenols, and flavonoids with higher concentrations, with AA at 300 mg/L giving the best results. Antioxidant activity, measured through DPPH and ABTS assays, was significantly elevated with AA, Ch, and MO treatments. Principal component analysis revealed that 93.8% of the data variability was explained by two components, confirming strong correlations between biostimulant treatments and improvements in plant growth, yield, and quality traits.

In conclusion, the application of these biostimulants offers a practical and sustainable solution to enhancing crop productivity, improving fruit quality, and promoting environmental resilience. By reducing the reliance on synthetic growth enhancers and chemical inputs, AA, Ch, and MO applications represent a sustainable approach to agricultural productivity, promoting environmental resilience and supporting safer, chemical-free farming practices.

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