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Bio-Growth Stimulants Impact Seed Yield Products and Oil Composition of Chia

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Abstract: Chia (*Salvia hispanica* L.) is a specialty crop capable of providing healthy food and metabolites. The goal of our study was to explore the possibility of expanding seed yield, oil production, and metabolites of chia in response to amino acid, barthenosteriode, and algae extract treatments used as bio-stimulants. The experiment was conducted in the field in a randomized complete block design with three repeats. The treatments were (1) control (spray only with water), (2) amino acids with nutrients (2 mL/L vs. 4 mL/L), (3) brassinolide (5 mL/L vs. 10 mL/L), and algae extract (2 mL/L vs. 4 mL/L). The growth and yield measurements of chia, such as chlorophyll, carotenoids, amino acids, indoles, phenols, macro- and micronutrients, carbohydrates, total oil, and fatty acids were analyzed. The chia plants sprayed with growth stimulant materials showed increases in most studied characteristics, particularly algae extract at 4 mL/L, followed by algae extract at 2 mL/L during the first and second seasons. Meanwhile, amino acids at 4 mL/L led to the third-highest increases in most cases. Conversely, all bio-stimulant treatments decreased total phenols in leaves (mg/100 g f.w.), especially seaweed at 4 mL/L, compared to high levels in the control during both seasons. Control plants showed the lowest levels of the measurements mentioned previously when scored by barthenosteriode at 5 mL/L during the first and second seasons. GLC for fixed oil in chia showed the recognition of four biocomponents. i.e., oleic, linoleic, palmitic, and α-α linolenic acids. The main biocomponent was α-α linolenic acid and reach (49.7 to 57.9%). The application of seaweed at 4 mL/L could be exploited to improve growth, seed crop, fixed oil production, chemicals and bio-constituents, especially the fixed oil composition of chia (*Salvia hispanica* L.) plant.

Keywords: fixed oil; amino acids; barthenosteriode; algae extract; oil productivity; oil fractions; α linolenic contents

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1. Introduction

The chia plant *Salvia hispanica* L., sometimes identified as chia, is a yearly oily plant that is native to southern Mexico and northern Guatemala (family *Lamiaceae*) [1]. The greatest known proportion of plant oil and alpha-α linolenic acid (omega 3) is found in chia [2]. Chia is an oil seed crop that has the potential to be used as a human food. According to Zanqui et al. [3] and Coorey et al. [4], it is commonly growing in Africa and owing to be health advantages. Ayerza and Coates [5] reported that chia has parallel-assembled leaves, a tiny flower (3 to 4 mm in diameter), short corollas, and joined floral components that promote a high self-pollination rate. Chia may grow to a height of 1 m. The seed's oval form and size varies between 1 to 2 mm, and its color ranges from black, grey, and black speckled to white. Chia seeds are rich in mucilage, protein, oil, and fiber. Omega-3 α linolenic acid (ALA) and omega-6 gamma linoleic acid (GLA) make about 60–68% and 20%, respectively, of its 25 to 40% oil contents [6–9]. Chia seeds include protein (15 to 25%), lipids (30 to 33%), carbohydrates (26 to 41%), exceptional nutritive fiber (18 to 30%), ash (4 to 5%), and minerals and vitamins. They also contain a lot of antioxidants [10].

Bio-stimulants, used as risk-free substitutes for hazardous chemical fertilizers, contain auxins, gibberellins, and cytokinins [11]. Bio-stimulants reduce the demand for fertilizer by boosting the assimilation of both micro- and macronutrients [12]. To supplement and/or improve chemical fertilization, foliar applications of bio-stimulants such as amino acids, brassinosteroids, and algal extract have recently been produced. Traditionally, amino acids have been viewed as the developing blocks and constituents of proteins. Numerous amino acids serve as building blocks for other compounds containing nitrogen, such nucleic acids. Through the biosynthesis of auxin, amino acids have a crucial regulatory function in plants, affecting the production of carbohydrates, proteins, a number of enzymes, and gene expression [13,14].

Brassinosteroids (brassinolide, BRs, and steroidal chemicals) are among the naturally occurring bio-stimulants that are conjugated with sugars and fatty acids and are extremely promising and ecologically benign natural substances. Several research studies on the physiological effects of Brassinosteroids on plants have revealed a wide range of plant physiological changes [15–19]. Algae extract has quickly become one of the most popular bio-stimulants on the planet [11,20]. This has been already used as a foliar spray for many crops, including various herbs, vegetables, fruit trees, flowers, and medicinal and aromatic plants, improving the capacity of plants to withstand environmental stress [21–24]. Since it contains useful components with varied levels and functions in an interwoven and integrated way, seaweed extract has a positive impact on plant development [25,26]. This study sought to determine how foliar spraying with growth bio-stimulating substances (amino acids, brassinosteroids, and algal extract) affected chia growing, seed output, chemical bioconstituents, oil efficiency, and fixed oil contents.

2. Material and Methods

2.1. Experimental Layout and Properties

During the 2019–2020 and 2020–2021 seasons, this study was performed on the investigational field and in the labs of the botany and horticulture departments of the Agriculture faculty at Moshtohor, Benha University. The investigational region was situated at an altitude of 45 m above sea height (30.45° N latitude and 31.10° E longitude), and its purpose was to investigate the effects of growth stimulant treatments (such as amino acids, brassinosteroids, and algae extract) on vegetative growth, essential oil contents and oil fractionation of chia seeds.

The chia seeds were obtained from the floriculture farm of the horticulture department of Benha University's Agriculture faculty. On 20 October, in both seasons, chia seeds were planted in plots (1 × 1 m) with two rows of clay loam soil (50 cm. in between). Six weeks later, the plants were clipped to leave only two seedlings per hill in each of the two hills (50 cm apart) that were present in each row.

The methods of Jackson et al. [27] and Black et al. [28] were used to determine the physical and chemical contents of the investigational soil. Table 1 shows the findings of soil analyses including mechanical properties (coarse sand %, fine sand %, silt %, clay %), and textural class during both experiment seasons. In addition, chemical analysis including organic matter %, calcium carbonate %, available nitrogen, available phosphorus, available potassium, pH, and electrical conductivity, was performed on the experimental soil before the experiment was started in each of the two seasons.

Table 1. Mechanical and chemical analysis of the experimental soil.

Parameters	Values		Parameters	Values	
Mechanical Properties			Chemical Analysis		
	(2019–2020)	(2020–2021)		(2019–2020)	(2020–2021)
Coarse sand (%)	9.0	5.8	Organic matter (%)	1.77	1.81
Fine sand (%)	13.7	12.0	Calcium carbonate (%)	0.85	0.99
Silt (%)	22.2	23.2	Available nitrogen (mg Kg^{-1})	0.68	0.74
Clay (%)	55.1	59.1	Available phosphorus (mg kg^{-1})	0.42	0.38
Textural class	Clay loam	Clay loam	Available potassium (mg kg^{-1})	199	210
			pH_{water} (1:1)	7.66	7.59
			Electrical conductivity (dS/m)	0.95	0.77

The experiment used a randomized complete block design (RCBD) with one component and three replicates. The treatments included seven growth stimulant treatments with three duplicate replicates, each with 20 plants, for a total of 60 plants for each treatment.

2.2. Experimental Protocol

2.2.1. Biosafety Growth Bio-Stimulants Treatments

- The untreated group (control spray with tap water).
- Amino acids (2 mL/L and 4 mL/L) as a foliar fertigation; this was a commercial product from the Union for Agriculture Development (UAD) containing 20% free L amino acids, 40% total amino acids, 3% vitamin mix, 3.5% potassium citrate, and some micronutrients such as 1500 ppm Fe, 500 ppm Zn, and 500 ppm Mn.
- Brassinolide at 5 mL/L and 10 mL/L for each as foliar spray were obtained from the Union for Agriculture Development Co., UAP, and Egypt. It structure was (1R,3aS,3bS,6aS,8S,9R,10aR,10bS,12aS)-1-[(1S,2R,3R,4S)-2,3-Dihydroxy-1,4,5-trimethyl hexyl] hexadecahydro-8,9-dihydroxy-10a,12a-dimethyl-6H-benz[c]indeno[5,4-e] oxepin-6-one, (2a,3a,5a,22R,23R,24S)-2,3,22,23-Tetrahydroxy-B-homo-7-oxaergostan-6-one.
- Seaweed extract (created by Orbital Company, 24 Obour Bldgs, Salah Salem, Egypt, Cairo) was used at 2 mL/L and 4 mL/L as a foliar spray. The extract used includes vitamins, enzymes, amino acids, carbohydrates, and plant hormones and some minerals including Fe, Zn, Cu, Mn, and Mo. (i.e., auxins, cytokinins and gibberellins).

The growth stimulants amino acids, brassinolide, and algae extract were administered to plant leaves four times as a foliar spray; the first application was one month after replanting, and the others at 21 day (21 d) periods thereafter. All assessed solutions, including the control, were sprayed over the plant leaves, taking care to uniformly wet the entire leaf surface. Common agricultural procedures (such irrigation, fertilizer, and hand weeding) were performed as required.

2.2.2. Harvesting

The experimental plants were collected on March 15, 120 d after planting in both the first and second seasons when the plants reached maturity, clusters, and seeds.

2.2.3. Experimental Measurements

2.3. Collecting Data and Measurements

The following vegetative and yield measurements were measured at harvest time on 15 March 2020 and 2021: The vegetative stage was cut a centimeter above the soil's surface. Measurements of the characteristics described in the following paragraphs were taken.

2.3.1. Vegetative Parameters at the Start of Flowering

Plant height (cm) was measured as the main stem's height from the soil's exterior to the plant's apex, fresh biomass g/plant, dry biomass g/plant, leaf area per square centimeter, and the number of stems for each plant.

2.3.2. Seeds Yield Parameters

Plants were removed after harvesting for sampling and other measures. Inflorescences (cluster) weights (g/plant), weight per thousand seeds by (g), and seed yield (g/plant) were measured.

2.3.3. Chemical Constituents

Using the method outlined by Inskeep and Bloom [29] to determine chlorophyl (Chll.), a, b, and carotenoids in chia leaf were measured using a spectrophotometer and calculated as mg/g fresh weight. Fresh leaves were taken to determine the total free amino acids, according to [30]. A.O.A.C. [30] was used to calculate the overall indoles and overall phenols in new leaves. Dried chia leaves were analyzed for nitrogen, phosphorus, potassium, and total carbohydrates using the assays described by [31–33], and Chaplin and Kennedy [34]. Micronutrients (Fe, Mn, and B) were also assessed in the dried leaves, according to the method of Houk et al. [35].

Fixed Oil Productivity

The fixed oil percentage was determined as a weight/weight ratio using the equation:

$$\text{The fixed oil \%} = \frac{\text{Extracted fixed oil weight}}{\text{Seeds sample weight}} \times 100$$

Fatty Acids Measurement

The methylated fatty acid residues were determined using a G.L.C. technique, as previously described by Stahls [36].

Statistical Analysis

Analyses of variance were performed on the means of all data from the dependent variable (chia plants) in response to the predictor factors in a randomized full block design (ANOVA). Using the statistical software program MSTAT-C® (Version 6.1), Tukey's multiple range test (DMRT) was employed to find out if there were any significant variations among the mean values of the treatments.

3. Results

3.1. Vegetative Growing Parameters

The data presented in Tables 2 and 3 show that all treatment groups, especially seaweeds at 4 mL/L over the course of two seasons, had an impact on all vegetative growth measurements of the chia plant, including plant height, leaf fresh and dry weights/plant, leaf area (cm^2), and number of branches/plants. In addition, seaweeds at 2 mL/L resulted in the second-highest values of the previously indicated criteria. The third-highest value was an amino acid spray at 4 mL/L. When compared to untreated control plants in the first and second seasons, the barthenosteriode treatment at 10 mL/L significantly boosted vegetative growth metrics.

Table 2. Effect of bio-stimulant treatments on chia plant height (cm), fresh weight (g)/plant, and dry weight (g)/plant during the 2019–2020 and 2020–2021 seasons.

Parameters	Plant Height (cm)		Fresh Weight g/Plant		Dry Weight g/Plant	
Treatments	**1st Season**	**2nd Season**	**1st Season**	**1st Season**	**2nd Season**	**1st Season**
Control (tap water)	70.33 ± 0.67 ^{g}	76.43 ± 0.96 ^{f}	78.93 ± 1.58 ^{f}	81.00 ± 0.73 ^{g}	9.07 ± 0.23 ^{d}	11.10 ± 1.68 ^{e}
Amino acid at 2 mL/L	81.77 ± 1.36 ^{f}	85.33 ± 0.74 ^{e}	99.70 ± 0.46 ^{d}	105.50 ± 1.06 ^{d}	12.03 ± 1.69 ^{c}	15.33 ± 0.81 ^{d}
Amino acid at 4 mL/L	84.93 ± 0.67 ^{e}	89.77 ± 1.02 ^{d}	103.90 ± 1.70 ^{c}	110.20 ± 0.95 ^{c}	14.83 ± 0.40 ^{b}	17.93 ± 0.55 ^{c}
Barthenosteriode at 5 mL/L	89.03 ± 1.12 ^{d}	91.13 ± 1.29 ^{d}	89.80 ± 1.06 ^{e}	93.97 ± 1.19 ^{f}	10.00 ± 0.35 ^{cd}	14.03 ± 0.23 ^{d}
Barthenosteriode at 10 mL/L	93.57 ± 0.95 ^{c}	95.97 ± 0.47 ^{c}	92.70 ± 0.53 ^{e}	99.63 ± 0.51 ^{e}	10.60 ± 0.52 ^{cd}	14.67 ± 0.50 ^{d}
Algae extract at 2 mL/L	100.70 ± 0.50 ^{b}	110.4 ± 1.1 ^{b}	115.40 ± 1.15 ^{b}	116.90 ± 1.3 ^{b}	16.95 ± 0.83 ^{b}	23.37 ± 0.68 ^{b}
Algae extract at 4 mL/L	107.60 ± 0.96 ^{a}	113.0 ± 1.12 ^{a}	128.80 ± 1.36 ^{a}	135.2 ± 0.51 ^{a}	21.43 ± 0.83 ^{a}	25.70 ± 0.85 ^{a}

The data represent the means ± standard deviation (SD) of three replications. In the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test (TMRT) at $p \leq 0.05$.

Table 3. Effect of bio-stimulant treatments on chia leaf area/(cm^2) and number of branches/plant during the 2019–2020 and 2020–2021 seasons.

Parameters	Leaf Area (cm^2)		Number of Branches/Plant	
Treatments	**1st Season**	**2nd Season**	**1st Season**	**2nd Season**
Control (tap water)	628.3 ± 2.68 ^{c}	636.6 ± 16.55 ^{c}	13.33 ± 1.58 ^{d}	12.67 ± 1.15 ^{e}
Amino acid at 2 mL/L	909.6 ± 14.50 ^{ab}	908.6 ± 16.62 ^{ab}	17.67 ± 0.58 ^{bc}	19.67 ± 0.58 ^{d}
Amino acid at 4 mL/L	934.1 ± 18.85 ^{ab}	950.8 ± 14.98 ^{ab}	19.00 ± 1.0 ^{b}	23.33 ± 1.15 ^{c}
Barthenosteriode at 5 mL/L	869.0 ± 14.56 ^{ab}	897.9 ± 13.63 ^{ab}	15.67 ± 0.58 ^{cd}	18.33 ± 0.58 ^{d}
Barthenosteriode at 10 mL/L	834.9 ± 18.56 ^{b}	856.6 ± 14.53 ^{b}	16.33 ± 0.58 ^{bc}	19.67 ± 0.58 ^{d}
Algae extract at 2 mL/L	944.7 ± 13.11 ^{ab}	947.2 ± 13.07 ^{ab}	25.00 ± 1.0 ^{a}	26.67 ± 1.15 ^{b}
Algae extract at 4 mL/L	959.4 ± 10.11 ^{a}	972.2 ± 17.23 ^{a}	27.67 ± 0.58 ^{a}	29.67 ± 0.58 ^{a}

The data represent the means ± standard deviation (SD) of three replications. In the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test (TMRT) at $p \leq 0.05$.

3.2. Seed Yield Parameters

Data in Table 4 illustrate that spraying chia plants with seaweeds at 4 mL/L proved to be the most effective treatment for increasing inflorescence weights (g), seeds weights (g/plant), and weight per 1000 seeds (g), when compared to untreated plants in both seasons. Additionally, seaweed at 2 mL/L and amino acid at 4 mL/L led to high increments of the parameters mentioned previously in the two seasons. Meanwhile, the control treatment (untreated group) had the minimum levels of the measurements mentioned in Table 4. Additionally, barthenosteriode at 5 mL/L resulted in an increase in most cases but this did not reach the highest levels.

Table 4. Effect of bio-stimulant treatments on chia inflorescences weights, seed weights and weights per 1000 seeds during the 2019–2020 and 2020–2021 seasons.

Parameters	Inflorescences Weights (g)/Plant		Seeds Weights (g)/Plant		Weight of 1000 Seeds/(g)	
Treatments	**1st Season**	**2nd Season**	**1st Season**	**2nd Season**	**1st Season**	**2nd Season**
Control (tap water)	9.33 ± 0.39 e	9.97 ± 0.54 c	3.63 ± 0.15 e	3.50 ± 0.1 e	1.06 ± 6.5 c	1.10 ± 0.11 c
Amino acid at 2 mL/L	10.87 ± 0.58 de	10.87 ± 0.57 c	5.37 ± 0.32 c	6.43 ± 0.21 c	1.12 ± 0.1 bc	1.15 ± 0.11 bc
Amino acid at 4 mL/L	12.03 ± 0.74 cd	12.83 ± 0.40 b	5.87 ± 0.06 c	7.03 ± 0.29 bc	1.20 ± 0.1 bc	1.19 ± 0.01 bc
Barthenosteriode at 5 mL/L	10.67 ± 0.50 de	11.57 ± 1.0 bc	4.20 ± 0.30 de	5.30 ± 0.20 d	1.15 ± 0.1 bc	1.15 ± 0.01 bc
Barthenosteriode at 10 mL/L	12.93 ± 0.55 c	12.80 ± 0.44 b	4.53 ± 0.35 d	5.43 ± 0.21 d	1.24 ± 0.2 b	1.21 ± 0.02 b
Algae extract at 2 mL/L	15.10 ± 0.56 b	18.90 ± 0.61 a	7.20 ± 0.30 b	7.43 ± 0.32 b	1.40 ± 0.3 a	1.46 ± 0.02 a
Algae extract at 4 mL/L	17.34 ± 0.82 a	20.00 ± 0.34 a	8.57 ± 0.06 a	8.53 ± 0.35 a	1.46 ± 0.3 a	1.50 ± 0.02 a

The data represent the means ± standard deviation (SD) of three replications. In the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test (TMRT) at $p \leq 0.05$.

3.3. Chemical Composition Determinations

In comparison to the control during the two seasons, data from Table 5 and Figures 1–10 show that bio-stimulant treatments statistically increased the leaf Chll. a, b, carotenoids (mg/100 g fresh weight), nitrogen, phosphorus, potassium, total free amino acids (mg/100 g F.W.), total indoles (mg/100 g F.W.), total carbohydrate contents Fe ppm, Mn ppm, and B. Therefore, in the two growth seasons, seaweed at 4 mL/L and amino acid at 4 mL/L provided the greatest results in this regard, respectively. The minimum levels of the parameters mentioned above were recorded in the control. On the other hand, all bio-stimulant treatments decreased leaf total phenols (mg/100 g F.W.), especially seaweed at 4 mL/L, compared to the high values in the control in both growing seasons.

Table 5. Effect of bio-stimulant treatments on chia chlorophyll a, b, and carotenoids during the 2019–2020 and 2020–2021 seasons.

Parameters	Chlorophyll a (mg/g Fresh Weight)		Chlorophyll b (mg/g Fresh Weight)		Carotenoids (mg/100 g Fresh Weight)	
Treatments	**1st Season**	**2nd Season**	**1st Season**	**2nd Season**	**1st Season**	**2nd Season**
Control (tap water)	0.540 ± 0.02 d	0.546 ± 0.04 c	0.260 ± 0.09 e	0.263 ± 0.02 d	0.350 ± 0.01 b	0.346 ± 0.01 b
Amino acid at 2 mL/L	0.653 ± 0.01 c	0.667 ± 0.01 b	0.327 ± 0.02 d	0.337 ± 0.02 c	0.447 ± 0.01 a	0.443 ± 0.01 a
Amino acid at 4 mL/L	0.673 ± 0.01 bc	0.670 ± 0.01 b	0.333 ± 0.02 cd	0.333 ± 0.01 c	0.423 ± 0.03 ab	0.427 ± 0.01 ab
Barthenosteriode at 5 mL/L	0.677 ± 0.01 b	0.677 ± 002 b	0.333 ± 0.02 cd	0.340 ± 0.01 c	0.437 ± 0.01 ab	0.443 ± 0.01 a
Barthenosteriode at 10 mL/L	0.690 ± 0.01 b	0.690 ± 0.01 b	0.347 ± 0.05 c	0.353 ± 0.04 bc	0.430 ± 0.01 ab	0.430 ± 0.03 ab
Algae extract at 2 mL/L	0.783 ± 0.02 a	0.780 ± 0.02 a	0.370 ± 0.03 b	0.370 ± 0.01 b	0.440 ± 0.02 ab	0.433 ± 0.01 ab
Algae extract at 4 mL/L	0.793 ± 0.02 a	0.797 ± 0.01 a	0.407 ± 0.02 a	0.400 ± 0.02 a	0.487 ± 0.02 a	0.500 ± 0.02 a

The data represent the means ± standard deviation (SD) of three replications. In the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test (TMRT) at $p \leq 0.05$.

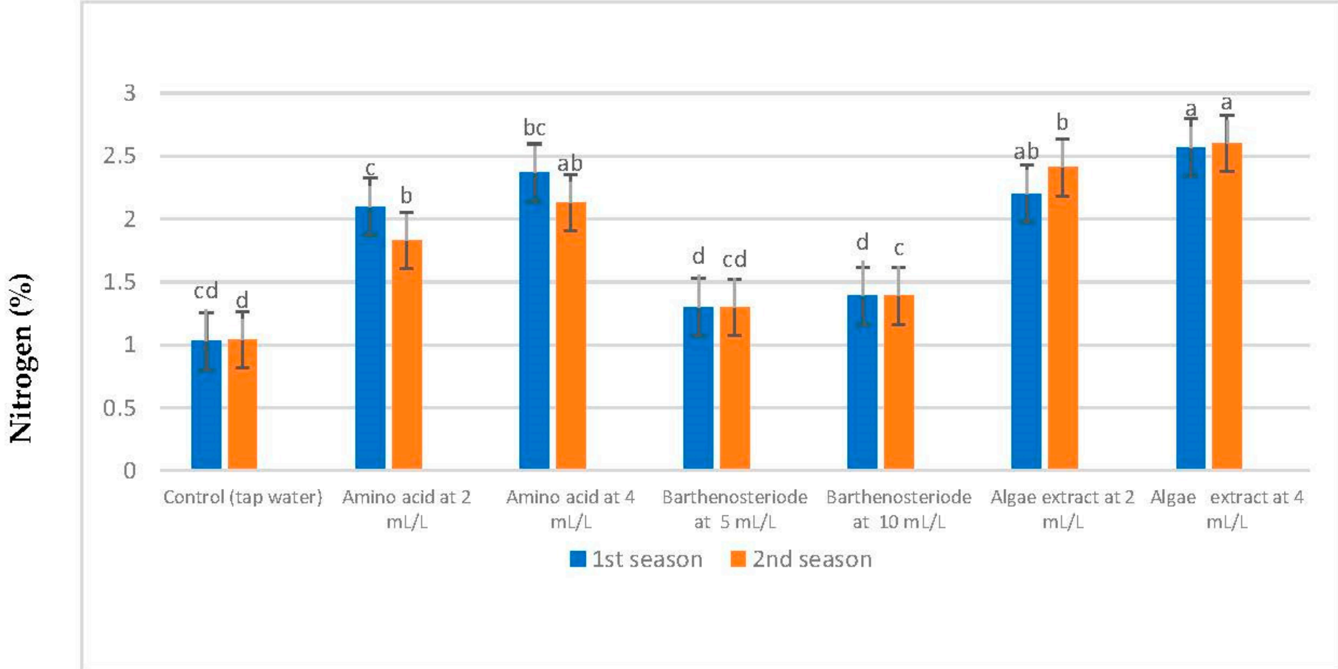

Figure 1. Effect of various bio-stimulant materials on nitrogen (%) of chia *(Salvia hispanica* L.) plants during the 2019–2020 and 2020–2021 seasons. For the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test (TMRT) at $p \leq 0.05$.

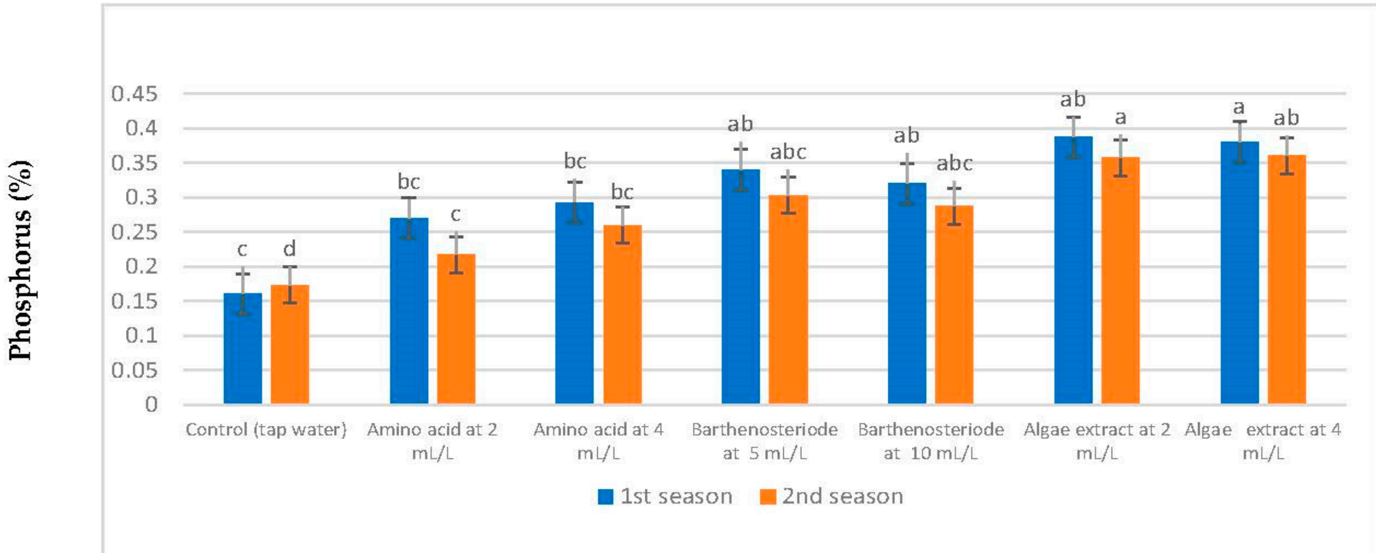

Figure 2. Effect of various bio-stimulant treatments on phosphorus (%) of chia *(Salvia hispanica* L.) plants during the 2019–2020 and 2020–2021 seasons. For the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test (TMRT) at $p \leq 0.05$.

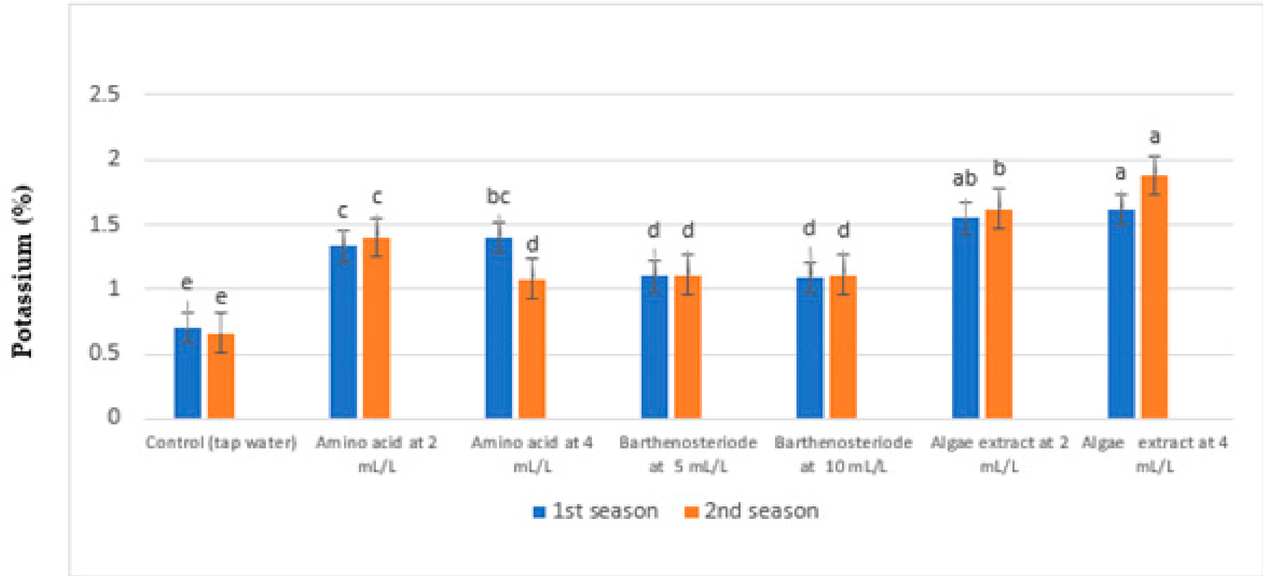

Figure 3. Effect of various bio-stimulant treatments on potassium (%) of chia *(Salvia hispanica* L.) plants during the 2019–2020 and 2020–2021 seasons. For the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test.

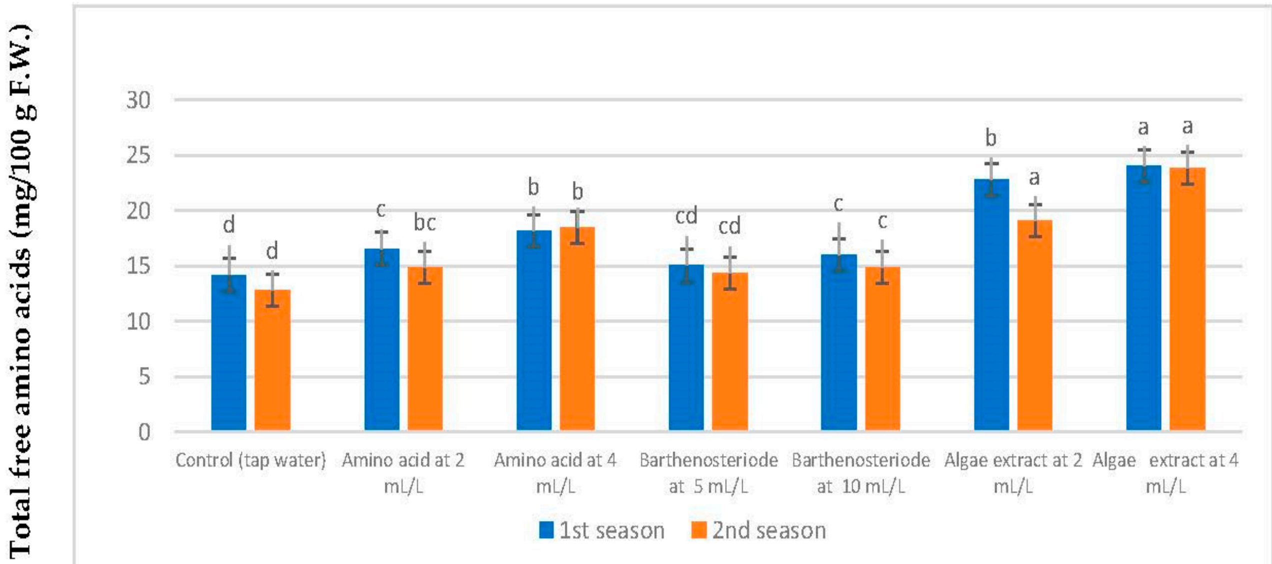

Figure 4. Effect of various bio-stimulant treatments on chia (*Salvia hispanica* L.) plant total free amino acids (mg/100 g F.W.) during the 2019–2020 and 2020–2021 seasons. For the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test.

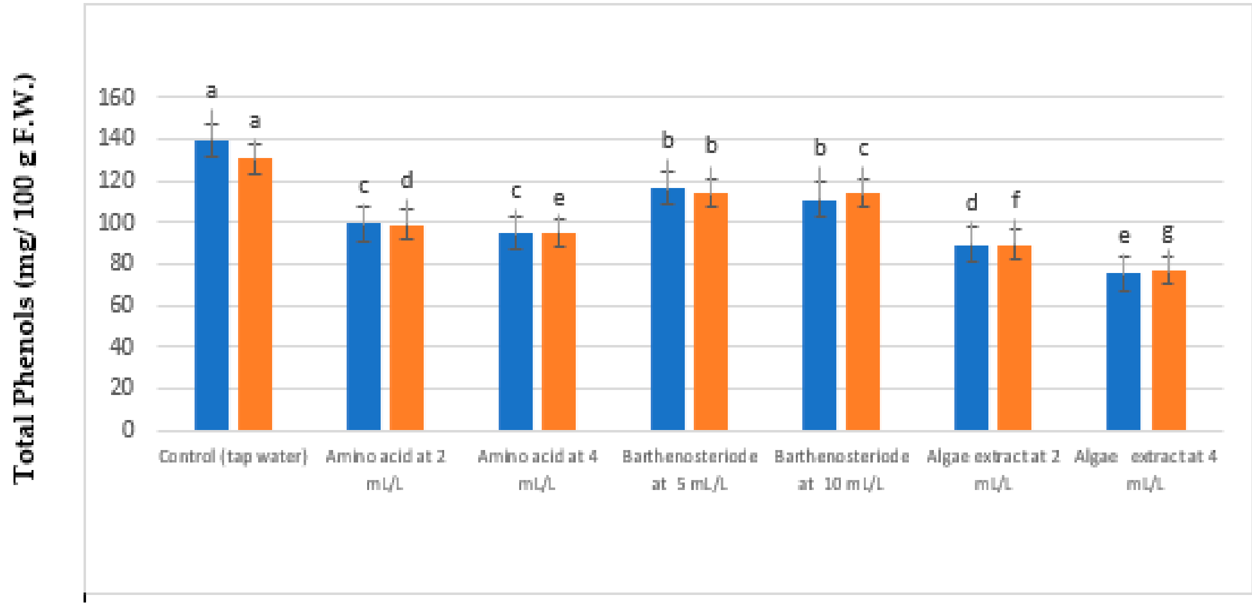

Figure 5. Effect of various bio-stimulant treatments on chia (*Salvia hispanica* L.) plant total phenols (mg/100 g F.W.) during the 2019–2020 and 2020–2021 seasons. For the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test.

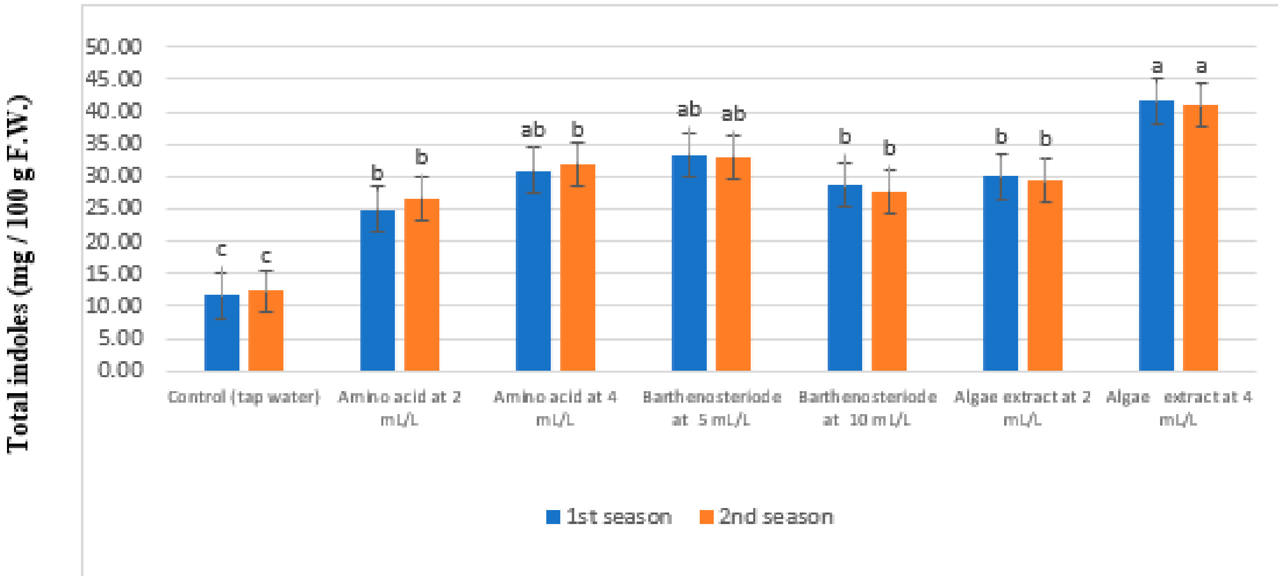

Figure 6. Effect of various bio-stimulant treatments on chia (*Salvia hispanica* L.) plant total indoles (mg/100 g F.W.) during the 2019–2020 and 2020–2021 seasons. For the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test.

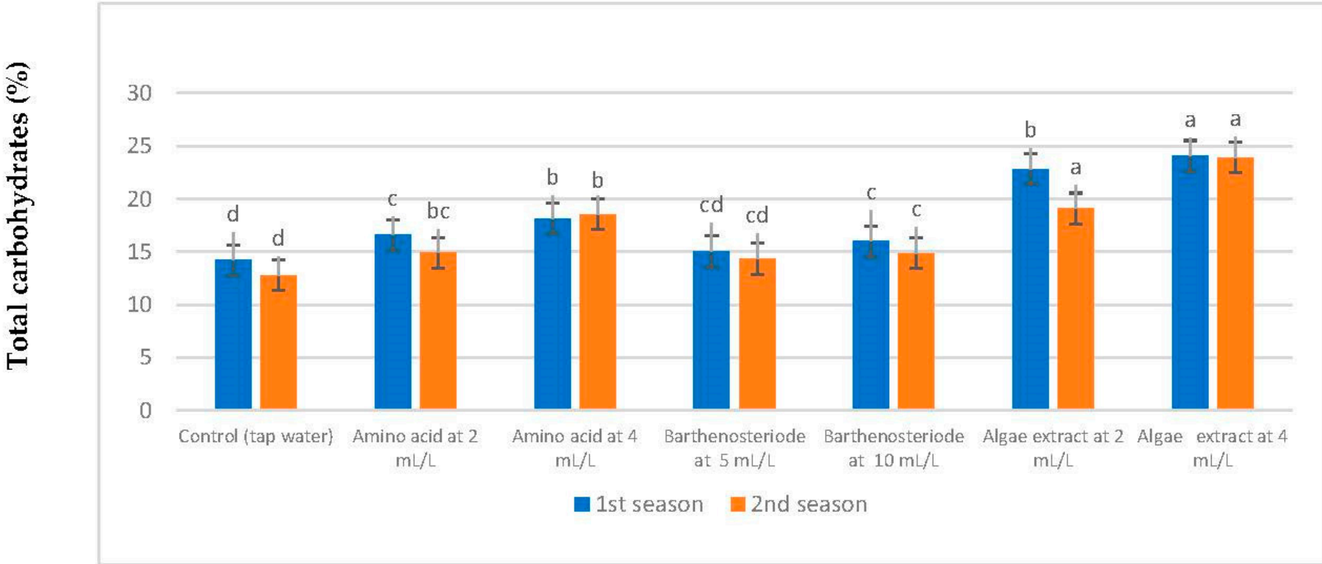

Figure 7. Effect of various bio-stimulants treatment on total carbohydrates (%) of chia (*Salvia hispanica* L.) plants during the 2019–2020 and 2020–2021 seasons. For the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test.

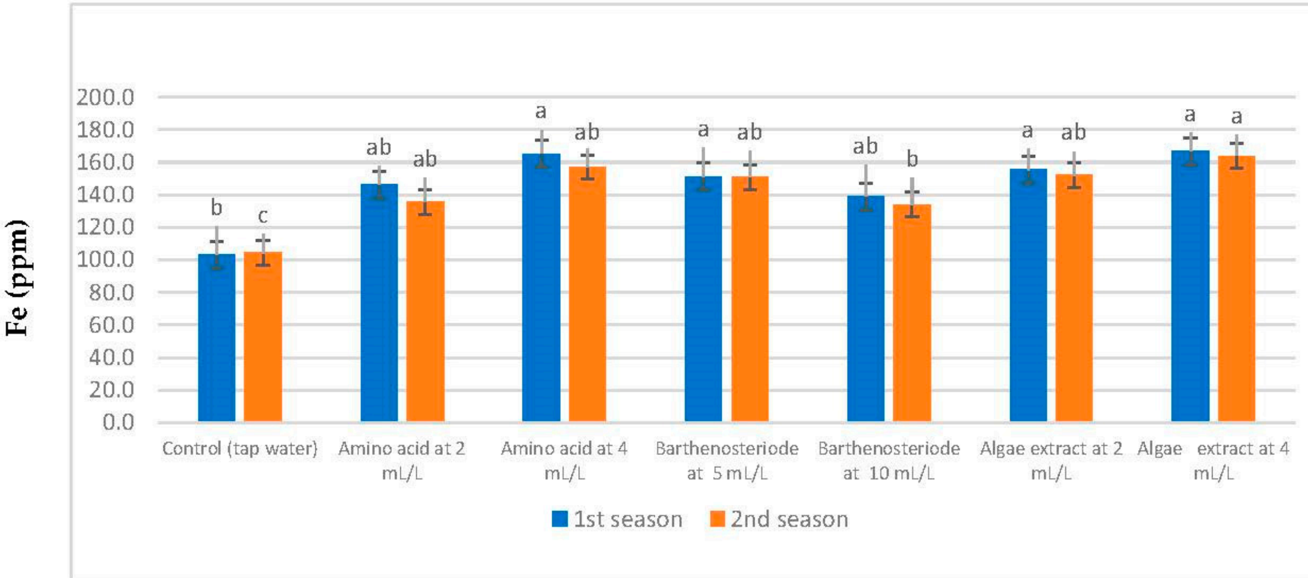

Figure 8. Effect of various bio-stimulant treatments on Fe (ppm) of chia (*Salvia hispanica* L.) plants during the 2019–2020 and 2020–2021 seasons. For the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test.

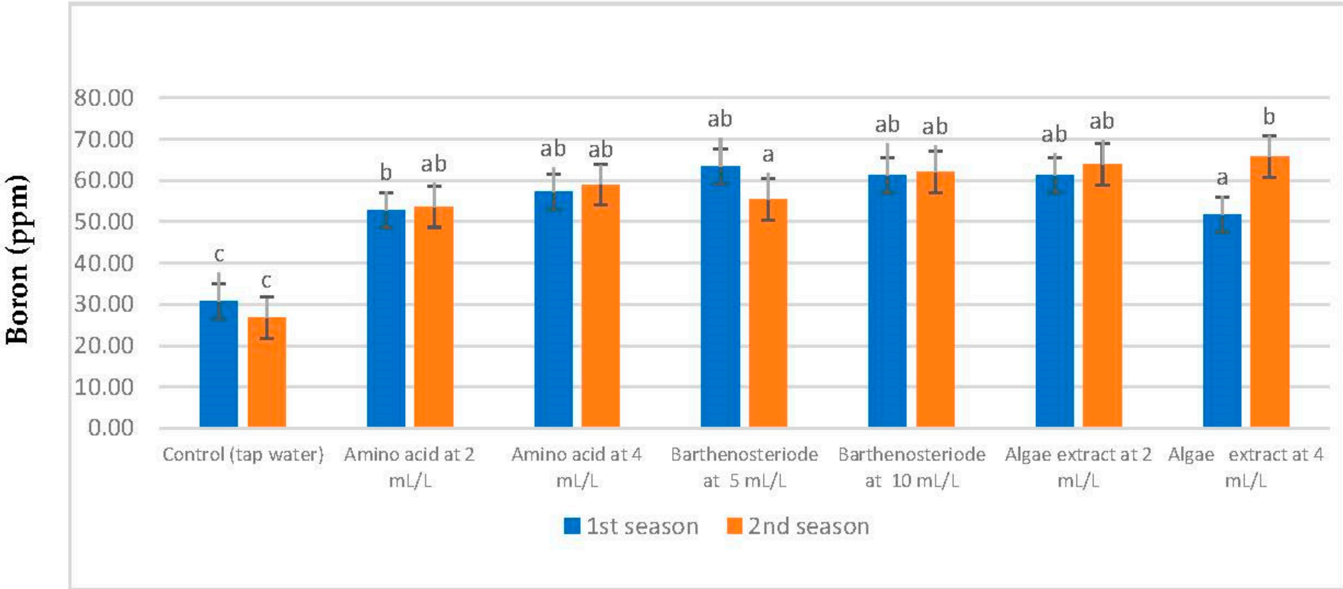

Figure 9. Effect of various bio-stimulants treatments on boron (ppm) of chia (*Salvia hispanica* L.) plants during the 2019–2020 and 2020–2021 seasons. For the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test.

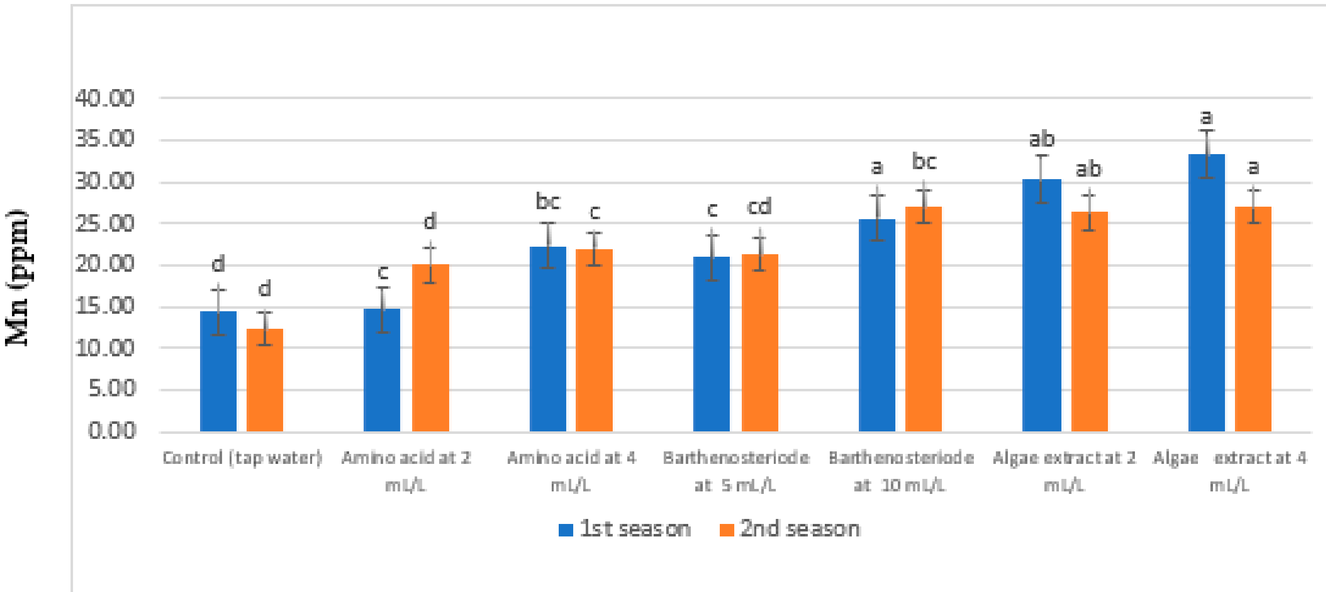

Figure 10. Effect of various bios-timulant treatments on Mn (ppm) of chia (*Salvia hispanica* L.) plant during the 2019–2020 and 2020–2021 seasons. For the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test.

3.4. Fixed Oil

According to the data in Figure 11, utilizing all bio-stimulant treatments had a greater impact on the fixed oil percentage of chia seeds than the control in both seasons During the first and second seasons, the seaweed gave the highest fixed oil percentage at 4 mL/L, followed (in descending order) by seaweed at 2 mL/L. Furthermore, amino acid at 4 mL/L yielded the third-highest results in this regard. On the other hand, the control yielded the lowest levels of this factor during both seasons.

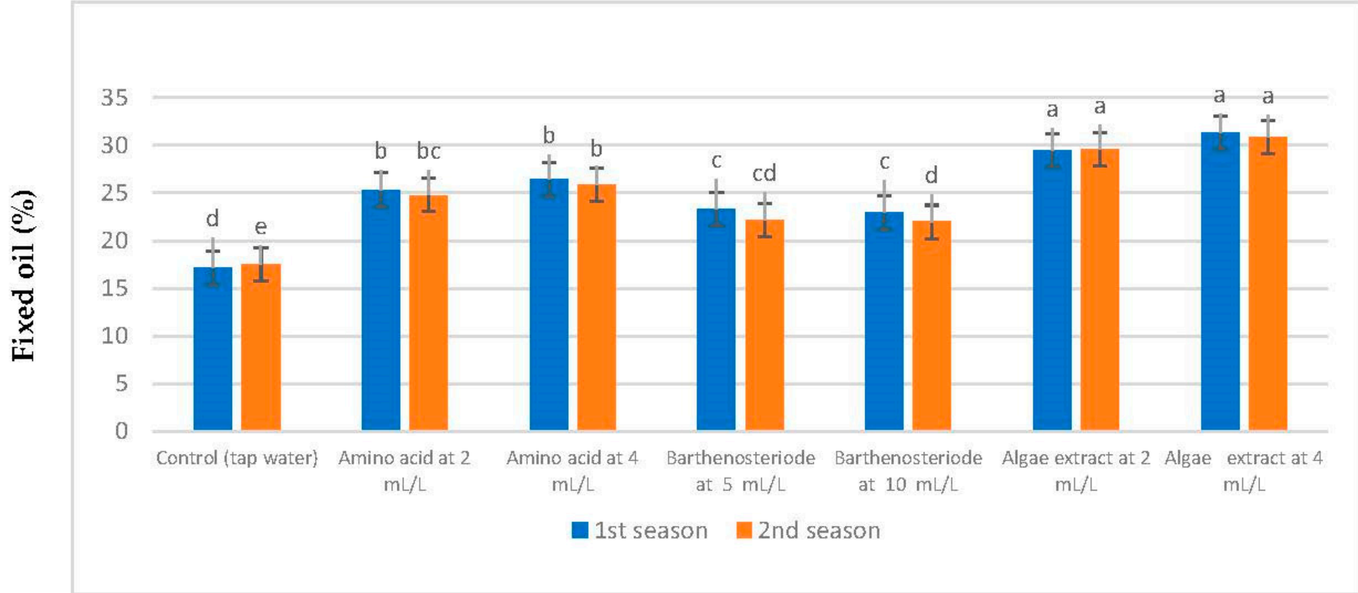

Figure 11. Effect of various bio-stimulants treatments on fixed oil (%) of chia (*Salvia hispanica* L.) plant during the 2019–2020 and 2020–2021 seasons. For the same column, means followed by the same letter are not statistically different, according to the Tukey multiple range test.

3.5. Oil Fractionation

Data described in Table 6 and Figures 12–18 show the effects of bio-stimulant treatments on chemical properties of the fixed oil contents of chia seeds. The fixed oil contents of chia seed contain four major fatty acids. The main biocomponent was α-α linolenic acid (49.72 to 57.89%) and other major components included linoleic acid (20.93 to 23.32%), oleic acid (12.09 to 16.32%) and palmitic acid (8.99 to 10.95%). Algae extract at 4 mL/L registered the greatest levels of α-α linolenic acid at 57.89%, followed (in descending order) by algae extract at 2 mL/L (56.46%) and amino acid 4 mL/L (55.18%), when compared to the minimum values of barthenosteriode at 5 mL/L as (49.72%). Meanwhile, barthenosteriode at 5 mL/L gave the greatest values of linoleic acid (23.32%). Furthermore, all treatments mentioned previously registered decreases in the percentage of oleic acid, from 14.70% for the control to 12.09% for algae extract at 4 mL/L, with the exception of barthenosteriode at 5 mL/L at 16.32%. From the above-mentioned results and the data indicated in the tables and figures, it can be concluded that α linolenic acid is the major content of different treatments and, in addition, algae extract was a superior treatment for linolic acid during the second experimental season.

Table 6. Effect of different treatments on the fractions of fixed oil contents of chia (*Salvia hispanica* L.) plants during the second season (2020–2021).

Peak No.	Component Name	Area%						
		Control (Tap Water)	Amino Acid at 2 mL/L	Amino Acid 4 mL/L	Barthenosteriode at 5 mL/L	Barthenosteriode at 10 mL/L	Algae at 2 mL/L	Algae at 4 mL/L
1	Palmitic acid	9.52	10.65	10.41	10.63	10.35	8.99	9.10
2	Oleic acid	14.70	13.49	13.15	16.32	13.47	12.84	12.09
3	Linoleic acid	23.12	21.81	21.25	23.32	21.30	21.72	20.93
4	α-α linolenic acid	52.65	54.05	55.18	49.72	54.88	56.46	57.89
	Solvent	-	-	-	-	-		-

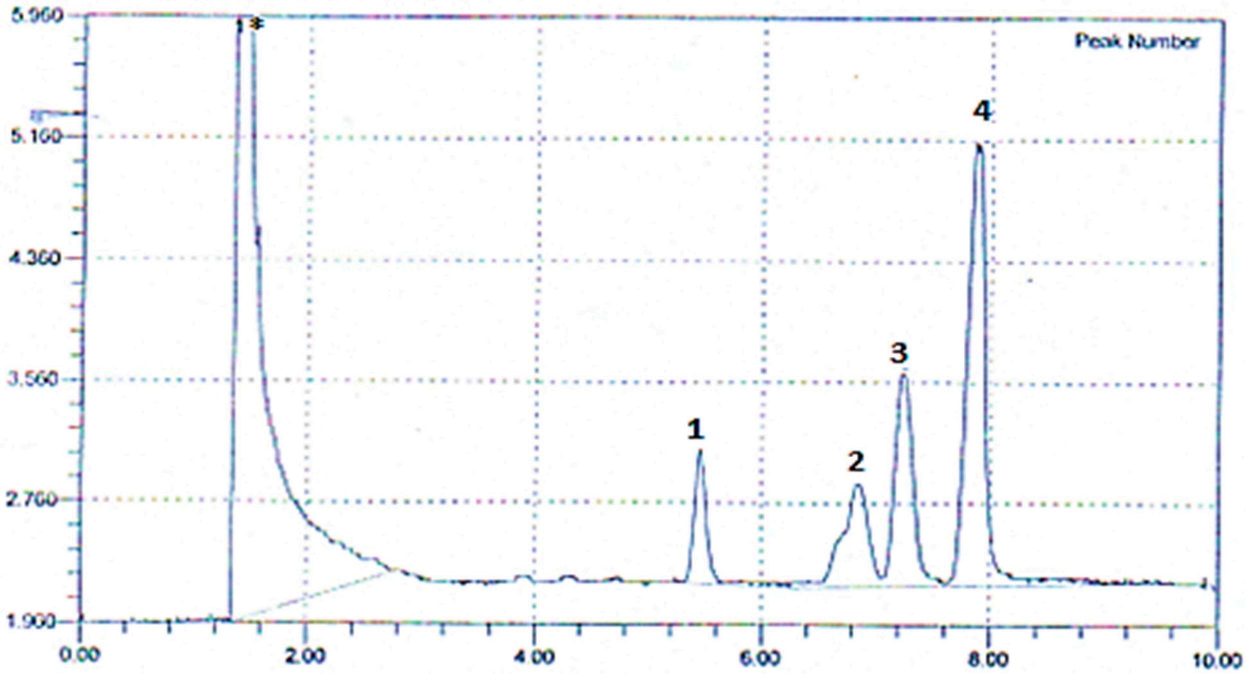

Figure 12. Untreated (control) GLC. (* is the solvent, 1 is the palmitic acid, 2 is oleic acid, 3 is a Linoleic acid and 4 is the α-α linolenic acid) of chia (*Salvia hispanica* L.) fixed oil content as impacted by various bio-stimulating materials.

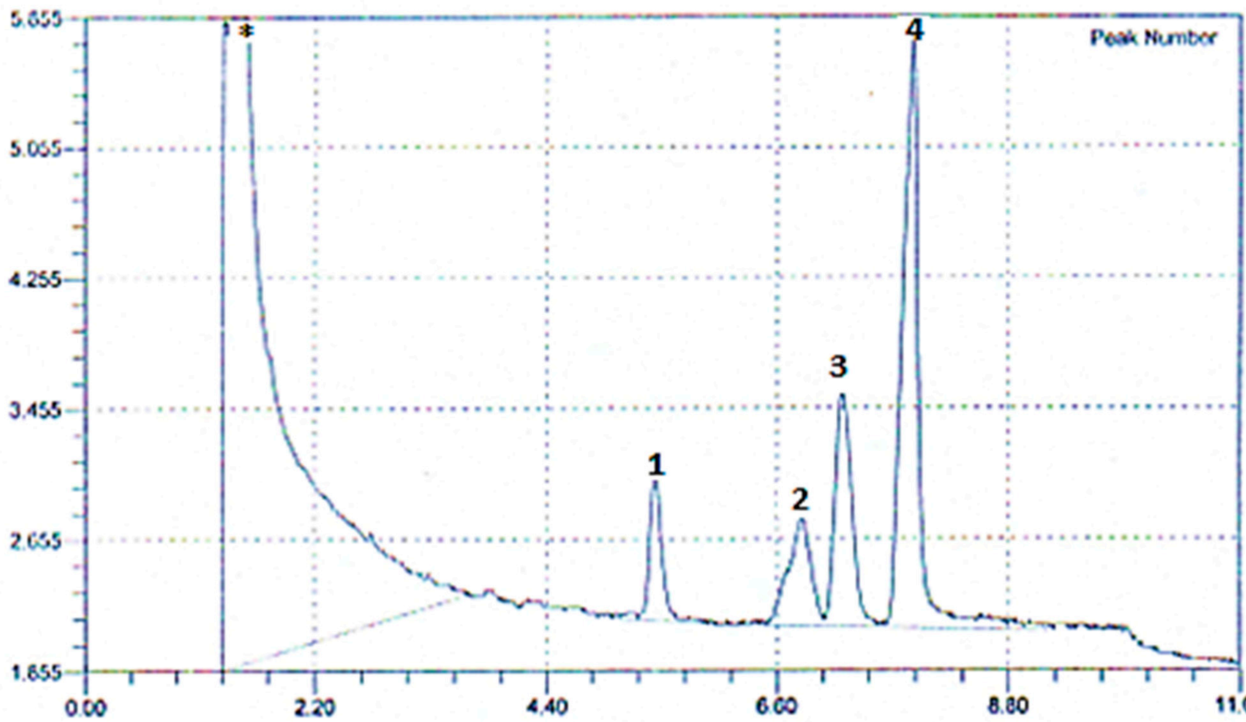

Figure 13. Amino acid at 2 mL/L GLC. (* is the solvent, 1 is the palmitic acid, 2 is oleic acid, 3 is a Linoleic acid and 4 is the α-α linolenic acid) of chia (*Salvia hispanica* L.) fixed oil content as impacted by various bio-stimulating materials.

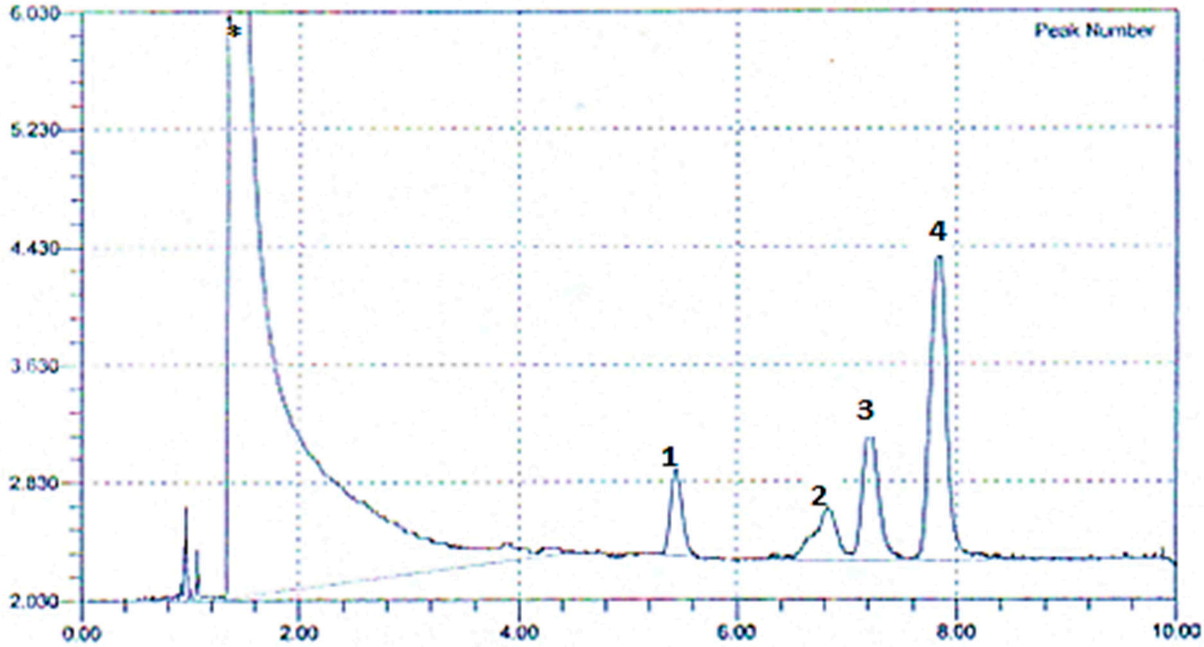

Figure 14. Amino acid at 4 mL/L GLC. (* is the solvent, 1 is the palmitic acid, 2 is oleic acid, 3 is a Linoleic acid and 4 is the α-α linolenic acid) of chia (*Salvia hispanica* L.) fixed oil content as impacted by various bio-stimulating materials.

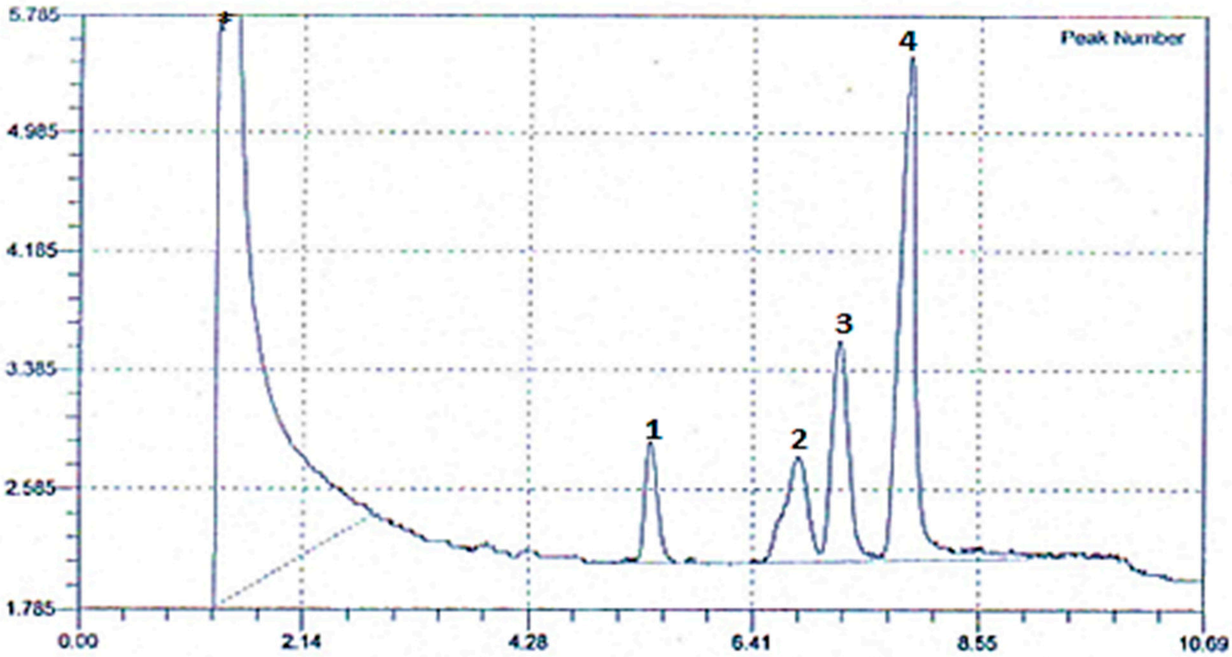

Figure 15. Barthenosteriode at 5 mL/L GLC. (* is the solvent, 1 is the palmitic acid, 2 is oleic acid, 3 is a Linoleic acid and 4 is the α-α linolenic acid) of chia (*Salvia hispanica* L.) fixed oil content as impacted by various bio-stimulating materials.

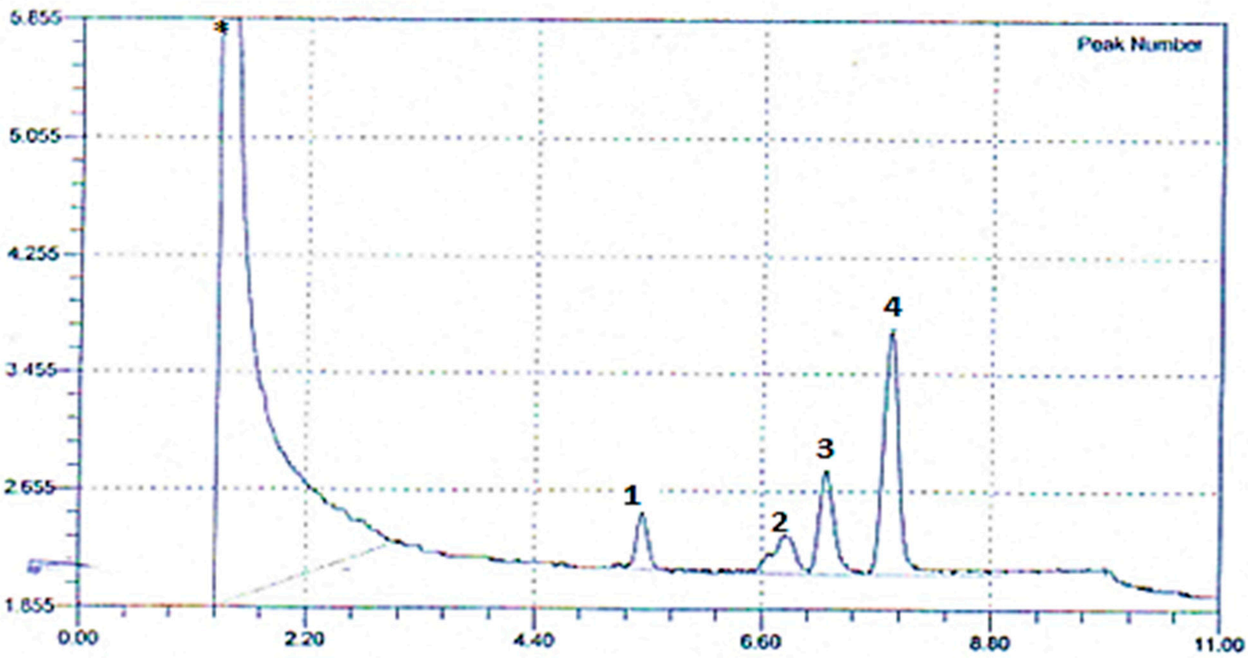

Figure 16. Barthenosteriode at 10 mL/L GLC. (* is the solvent, 1 is the palmitic acid, 2 is oleic acid, 3 is a Linoleic acid and 4 is the α-α linolenic acid) of chia (*Salvia hispanica* L.) fixed oil content as impacted by various bio-stimulating materials.

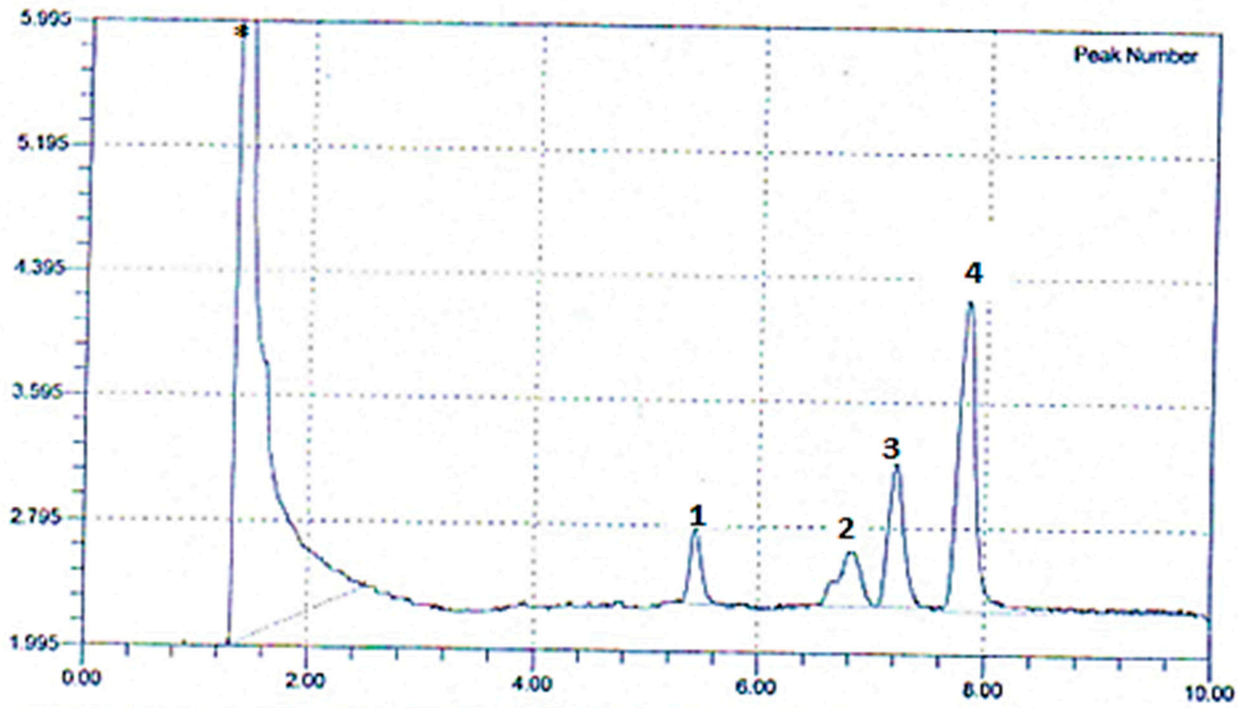

Figure 17. Algae at 2 mL/L GLC (* is the solvent, 1 is the palmitic acid, 2 is oleic acid, 3 is a Linoleic acid and 4 is the α-α linolenic acid) of chia (*Salvia hispanica* L.) fixed oil content as impacted by various bio-stimulating materials.

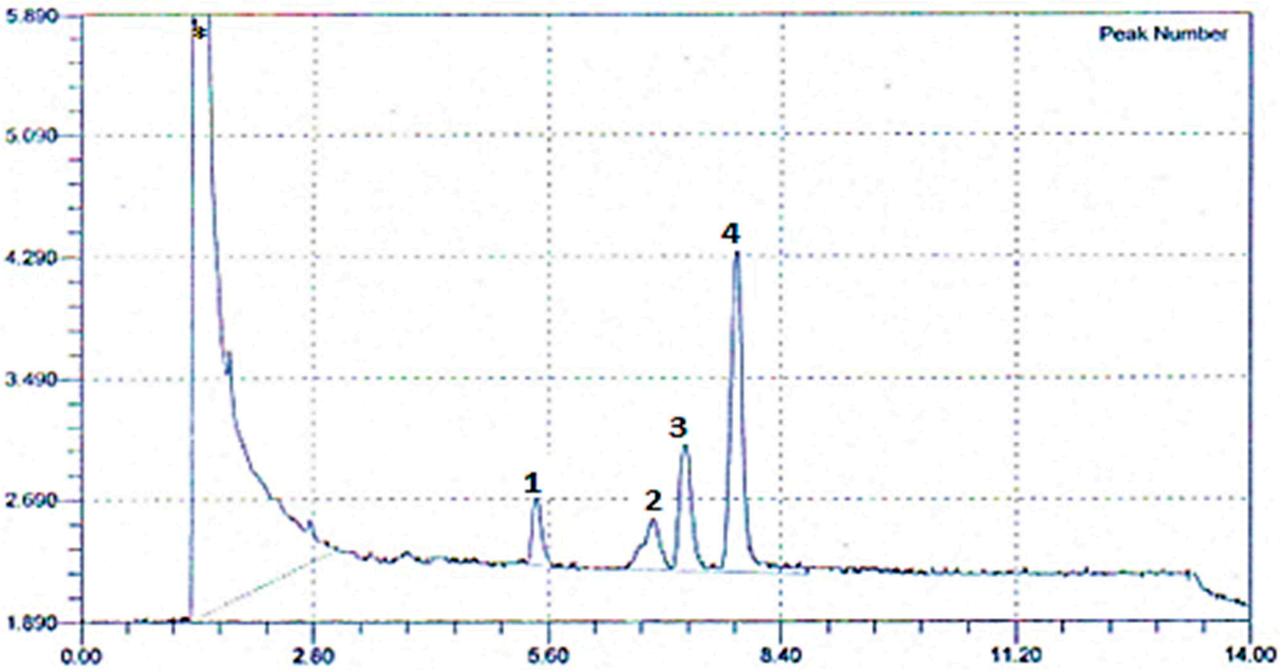

Figure 18. Algae at 4 mL/L G.L.C. (* is the solvent, 1 is the palmitic acid, 2 is oleic acid, 3 is a Linoleic acid and 4 is the α-α linolenic acid) of chia (*Salvia hispanica* L.) fixed oil content as impacted by various treatments.

4. Discussions

Bio growth stimulants materials were successful in accelerating plant growth relative to the control because they enhanced chia plant growing, seed yield, chemical composition, oil output, and fixed oil components relative to untreated plants in all growth-stimulant treatments.

For all bio-stimulant treated groups, especially seaweeds at 4 mL/L over the course of two seasons, there was an impact on all vegetative growth measurements of the chia plant, including plant height, leaf fresh and dry weights/plant, leaf area (cm^2) and number of branches/plants.

One of the most well known bio-stimulants on the globe is algae extract [11,20]. Algae extract has been used as a foliar spray for many crops, including different herbs, vegetables, fruit trees, flowers, medicinal and aromatic plants, and more. It includes key and mineral elements, amino acids, vitamins, cytokinins, auxins, and also abscisic acid as growth promoters [20,21], and increases plant tolerance to environmental stress [22–24]. The mechanism of algal extract's favorable influence on plant development occurs because it includes various helpful components in varying amounts and operates in an interlocking and integrated manner [25,26].

Proteins, amines, purines, pyrimidines, alkaloids, vitamins, enzymes, terpenoids, and other organic compounds are all synthesized from amino acids. Additionally, amino acids help plants deal with any immediate or incidental biotic or abiotic harm by acting as originators for minor components and hormones [37].

The effect of brassinolide on growth may be the metabolism of nucleic acids and proteins, as well as improvement of cell growth, differentiation, amplification, division, and change of membrane potential [38–41].

Applying seaweeds at 4 mL/L to chia plants proved to be the most effective treatment for increasing inflorescence weights (g), seeds weights (g/plant), and weight per 1000 seeds (g), when compared to untreated plants in both seasons. Also, bio-stimulant treatments statistically increased the leaf Chll. a, b, carotenoids (mg/100 g fresh weight), nitrogen, phosphorus, potassium, total free amino acids (mg/100 g F.W.), total indoles (mg/100 g F.W.), total carbohydrate contents Fe ppm, Mn ppm, and B. Therefore, in the two growth seasons, seaweed at 4 mL/L and amino acid at 4 mL/L provided the greatest results in this regard, respectively.

Several of the previously mentioned studies on growth stimulants materials, including Mohamed et al., [42] working on basil (*Ocimum basilicum*, L.) and Mohamed and Ghatas [43] on Dutch fennel plant (*Foeniculum vulgare Mill.* spp. *vulgare*), examined the effects of several growth bio-stimulants (amino acids, brassinolide, and seaweed extract), as well as arbuscular mycorrhizal fungi. They reported that adding arbuscular mycorrhizal fungus (AMF) to seaweed extract at a dose of 2 mL/L improved the growth characteristics on *Artemisia annua* L. Ghatas et al. [44] showed that the use of seaweed extract significantly improved all growing parameters and artemisinin content. The primary contents of chia oil have been determined by several research studies, including those by Ayerza and Coates [45], who claimed that palmitic, stearic, -α linolenic, linoleic, and oleic acids were the essential constituents. Segura-Campos et al. [46] and Silva et al. [47], measured the fatty acids of chia seeds prepared with different types of solvents and found that -α linolenic was combined with linoleic, oleic, palmitic, and stearic acids. Other researchers have found that chia oil includes α linolenic, linoleic, oleic, palmitic, and stearic acids [45]. According to Moghith et al. [48]. The primary components were α linolenic acid, linoleic acid, oleic acid, and palmitic acid. Mohamed [49] reported that -α linolenic acid was the primary component of chia (37.28 to 39.72 percent). A linolenic, linoleic, oleic, and palmitic acids were the main components. Also, Plant height, number of branches, fresh and dry weights, photosynthetic pigments (chlorophyll a, b, and carotenoids), macronutrients (N, P, K), and total carbohydrates were significantly increased by using seaweed extract at a rate of 2 mL/L or amino acids at a rate of 1 g/L at the two cuts in both seasons during which these results were recorded by [49,50].

Among the commercial bio-stimulants, most species of seaweed extract contain a range of biologically active chemicals, including growth regulators, vitamins, and minerals. Seaweed is rich in phytohormones such as auxins and cytokinins, as well as alginates, amino acids, and trace levels of macro and microelements [51]. The formulation of bio-stimulants derived from natural seaweed extract is distinguished by a high concentration of free amino acids (aliphatic, aromatic, acidic, and basic amino acids) produced by enzymatic hydrolysis [40]. Seaweed also includes organic nitrogen, boron, magnesium, iron, zinc, manganese, molybdenum, and many micro elements in, addition to the after mentioned compounds [50].

All bio-stimulant treatments had a greater impact on the fixed oil percentage of chia seeds than the control in both seasons; during the first and second seasons, the seaweed gave the highest fixed oil percentage at 4 mL/L, followed (in descending order) by seaweed at 2 mL/L. The fixed oil contents of chia seed contained four major fatty acids. The main biocomponent was α-α linolenic acid (49.72 to 57.89%) and other major components included linoleic acid (20.93 to 23.32%), oleic acid (12.09 to 16.32%), and palmitic acid (8.99 to 10.95%).

5. Conclusions

According to our findings, algal extract at a concentration of 4 mL/L may be utilized to improve the chia plant's growth, seed yield, chemical composition, fixed oil output, and fixed oil bio-constituents. Additionally, it improved the contents using key oil constituents and increased GLC for fixed oil in chia showed the recognition of four bio-components, i.e., oleic, linoleic, palmitic, and α-α linolenic acids. The main bio-component was α-α linolenic acid. In addition, the application of seaweed extract increased oil contents, which could increase the medicinal revenue of the plants. The application of seaweed at 4 mL/L can be exploited for refining growth, seed crop, fixed oil production, chemicals and bio-constituents, especially fixed oil composition of chia (*Salvia hispanica* L.) plant.

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