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Research Article

Bio-stimulants as alternatives to mineral fertilizers: influence on chia (*Salvia hispanica* L.) growth, yield, and fatty acid composition

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Abstract

One of the main components of sustainable agricultural strategies in crop production is substitution of chemical fertilizers with alternatives. This study aimed to examine the effects of partial replacement of mineral fertilizers with bio-stimulants (yeast and/or moringa leaves extract). Field experiment was conducted during two successive seasons 2018/2019 and 2019/2020. The fertilization treatments were distributed in a factorial design. Treatments included 75%, 50% or 0% NPK combined with yeast, moringa extract, or their combination in comparison with control treatment (100% NPK). The partial substitution of mineral fertilizers with yeast and/or moringa extract had significant impacts on growth, yield and quality components. Decreasing NPK level significant decreased growth and yield components of chia plants, except for 75% NPK combined with yeast and/or moringa. In contrast, decreasing NPK level significantly increased the chemical and quality components of chia. Treating with 75% NPK combined with yeast and/or moringa gave the highest micronutrients and fatty acid content. GC analysis showed definition of nine main components, and the major components were linolenic and linoleic acids. Linolenic acid increased with decreasing NPK, and the highest values obtained with bio-stimulant treatments with zero % NPK. The results of this study recommended that partial substitution of 25% of mineral fertilizers with bio-stimulants (yeast and/or moringa) is the best alternative not only for maintaining the growth and yield component, but also improving the quality of chia plants.

Keywords: chia; fatty acids; linolenic acid; moringa extract; NPK; yeast

Introduction

Chia, *Salvia hispanica* L., is an annual herbaceous plant of the family Lamiaceae (Labiatae). It was one of the main foods consumed by numerous Central American civilizations. Just like grain, amaranth, and beans, chia seeds were an important source of nourishment for the Aztecs (Ayerza and Coates, 2004). The chia plant is native to southern Mexico and northern Guatemala. It grows naturally in the tropics and subtropics. Because the chia market is profitable and appealing, it is rapidly expanding from its origins to new growth areas in

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Central Africa, Australia, Europe, and North America (Bochicchio *et al*., 2015). Due to their high quantities of proteins (15-25%), fats (30-33%), carbohydrates (26-41%), dietary fiber (18-30%), and ash (4-5%), chia seeds have been studied and recommended as most recognizable foods based on their nutritional properties and medicinal values. The seed's moisture content ranges from 6% to 8% (Ixtaina *et al*., 2010; Jiménez *et al*., 2013). It is also high in vitamins, minerals, antioxidants, and polyphenols (Ixtaina *et al*., 2008). In addition, it has high content of B vitamins, calcium, phosphorus, and potassium (Muñoz *et al*., 2013). Compared to other natural sources, chia seeds possess the highest concentration of α-linolenic acid (Ш-3), up to 68% (Capitani *et al*., 2012; Guiotto *et al*., 2013).

Chemical fertilizers are critical to the world's food supply because they act as fast food for plants, allowing them to grow faster and more efficiently. While there have been negative consequences associated with the excessive and unbalanced use of these synthetic inputs (Bansal, 2017). In addition, continuous use of traditional chemical fertilizers destabilizes soil ecology, diminishes its fertility, pollutes ground water and has severe consequences on human and animal health (Daneshmandi and Seyyedi, 2019; Tang *et al*., 2019; Ostadi *et al*., 2020). As a result, in order to maximize crop productivity, agrochemical corporations strive to replace chemical fertilizers with bio-stimulants in sustainable agricultural strategies (Sharma *et al*., 2013). It is possible that there is an urgent need to improve agricultural practices in order to balance agricultural production with environmental sustainability. There is also a lot of interest in using microorganisms that help plants grow to make crop production more sustainable (Vessey, 2003). Microorganisms can promote plant growth and health through a variety of mechanisms, including providing plants with biologically fixed nitrogen, phytohormones, volatiles, protection compounds, and enzymes (Ryu *et al*., 2003; Kuklinsky-Sobral *et al*., 2004; Lugtenberg and Kamilova, 2009). Yeast is a low-cost bio-fertilizer that improves not only plant vitamins but also plant energy during the early boom period and rhizophagy's help to plant nutrient uptake (Lonhienne *et al*., 2014). Yeast is eco-friendly, nutritive, and simple to apply, and it has advantages over conventional plant growth regulators and soil conditioners. It would be a good idea to investigate how yeast extract affects reforestation in a semiarid soil area (Xi *et al*., 2019). Yeast extract contains a variety of active ingredients, including low-molecular-weight organic compounds, amino acids, nucleotides, peptides, nitrogen, phosphorus, and trace elements. Furthermore, yeast extract is free of synthetic hormones and toxic ingredients (Zhang *et al*., 2000; Vieira *et al*., 2016).

Plant growth regulators have been widely used in crop production for decades, whether through soil application or foliar spraying. Plant stimulants play a key role not only in plant growth and nutrition, but also in abiotic and biotic stress tolerance (du Jardin, 2015). The term “bio-stimulant is relatively new, and its application in the scientific community is yet unclear. du Jardin (2012) proposed a broad definition: "Plant bio-stimulants are substances or materials that, with the exception of nutrients and pesticides, have the capacity to modify physiological processes in plants in a way that provides potential benefits to growth, development, or stress response when applied to plants, seeds, or growing substrates in specific formulations”. Several studies have reviewed the role of bio-stimulants in relation to promoting growth and nutrient availability (du Jardin, 2012; du Jardin, 2015; Colla and Rouphael, 2015). In addition, many studies have investigated the effect of bio-stimulants like protein hydrolysates, seaweed extracts, silicon, chitosan, humic acid, and fulvic acid (Van Oosten *et al*., 2017). For example, moringa leaves extract favorably boost seeds germination, plant growth and yield, nutrient usage efficiency, crop quality features, as well as tolerance to environmental stresses (Abdel-Rahman and Abdel-Kader, 2020; Zulfiqar *et al*., 2020; Hassanein *et al*., 2021).

The main objective of this study was to examine the effect of partial substitution of mineral fertilizers (N-P-K) with bio-stimulants (yeast and/or moringa extract) on the growth, yield, and fatty acid composition of chia (*Salvia hispanica* L.) plants.

Materials and Methods

Plant material and growth conditions

During the two consecutive seasons of 2018/2019 and 2019/2020, a field experiment was carried out at the Agricultural Experimental Farm of the Faculty of Agriculture and Natural Resources, Aswan University, Aswan, Egypt. Chia (*Salvia hispanica* L.) seeds were obtained from the National Research Centre in Cairo, Egypt. Permissions were obtained from the administration of the National Research Centre to collect seeds, and from the administration of the Faculty of Agriculture and Natural Resources at Aswan University to conduct the experiment. National Research Centre was identified the plant material which used in this study. The voucher specimen of this material has not been deposited in a publicly available herbarium. This study was conducted in according with relevant institutional and national guidelines and regulations.

The experiment was set up in a randomized complete block factorial design with three replicates. The plot was 3×1.5 m long and had two rows. Chia seeds were directly planted at 30 cm in row and 70 cm between rows at the beginning of November for both seasons. Soil samples were collected from a depth of 30 cm below the used soil surface, and physical and chemical properties were analyzed using the methods described by Jackson (1973) and Black *et al*. (1982) as shown in Table (1).

Ten different treatments have been compared as follows: T1, 100% NPK suggested dose as control (375 kg ha^{-1}, ammonia nitrate 33.5%, 250 kg ha^{-1}, superphosphate 19.5% and 125 kg ha^{-1} potassium sulphate 49.5%), T2: 75% NPK plus yeast extract, T3: 75% NPK plus moringa leaves extract, T4: 75% NPK plus yeast and moringa leaves extract, T5: 50% NPK plus yeast extract, T6: 50% NPK plus moringa leaves extract, T7: 50% NPK plus yeast and moringa leaves extract, T8: Yeast extract only, T9: moringa leaves extract only, and T10: yeast and moringa leaves extract. Active dry yeast extract *Saccharomyces cerevisiae* (home yeast) was used (6 g L^{-1}) with level of 50 ml per plant. Moringa leaves extract was prepared by mixing 100 g of fresh leaves with one liter of distilled water. For both seasons, the treatments were applied to the soil three times, starting 45 days after planting with a consistent two weeks interval between applications. Drip irrigation and all recommended agricultural practices were followed.

Table 1. Physical and chemical analysis of studied soil

Soil constituents	Value
Sand	13.66
Silt	46.19
Clay	40.15
Texture grade	silt clay
EC (m.mhos/cm)	0.60
Organic matters %	1.30
Available N %	0.08
Available P% (ppm)	8.00
Available K% (mg/100 g.soil)	0.62

Vegetative growth parameters

At the end of March, various plant growth parameters were recorded including plant height (cm), spike number per plant, spike height (cm), number of lateral branches per plant, and spike fresh and dry weight/plant. Additionally, leaf area (mm^2), seeds yield per plant, and 100 seeds weight were measured. Leaf area was measured using a portable leaf area meter AM350 (Eijkelkamp company, EM Giesbeek, The Netherlands).

The relative leaf greenness

The SPAD chlorophyll meter was used to measure the relative leaf greenness (level of total chlorophyll content) in chia leaves using SPAD chlorophyll meter (SPAD-502plus, Konica Minolta, INC., Osaka, Japan). Chia SPAD values were measured on the middle of the leaf blade.

Nitrogen, phosphorus, and potassium analysis

Nutrient elements were extracted from dried plant samples of chia with known weights (0.2 g). The nitrogen (N) percentage was calculated using the semi-micro Kjeldahl technique (Black *et al*., 1965). The phosphate percentage was calculated by using vanadate-molybdate-yellow method (Chapman and Parattm, 1961). A flame photometer calibrated with standard solution was used to quantify potassium percentage (Chapman and Parattm, 1961).

Total fatty acids content and composition

The fatty acid content of oil was measured using AOAC method 996.01 (Satchithanandam *et al*., 2001). The composition of total fatty acids (saturated and unsaturated fatty acids) was measured. The fatty acid composition was determined by converting of oil to fatty acid methyl esters prepared by adding 1.0 mL of n-hexane to 15 mg of oil followed by 1.0 mL of sodium methoxide (0.4 mol). The mixtures were stirred for 30 seconds then let 15 minutes to settle. The upper phase containing the FAMEs was recovered and analyzed by gas chromatography (GC-FID). FAMEs were analyzed on a Perkin Elmer (model 8700), fitted with a non-bonded biscyanopropyle siloxane stationary phase, polar capillary column SP-2340 (60 m-0.25 mm), 0.2 mm film thickness and a flame ionization detector. Nitrogen (oxygen-free) was used as a carrier gas at a flow rate of 3.5 mL/min. Other conditions were as follows: initial oven temperature, 130 °C; ramp rate, 4 °C/min; final temperature, 220 °C; injector temperature, 260 °C; detector temperature, 270 °C; temperature hold, 2 minutes before the run and 17 minutes after the run. A sample volume of 1.0 mL was injected. FAMEs were identified by comparing their relative and absolute retention times to those authentic standards of FAMEs. All of the quantifications were done by a built-in data handling program provided by the manufacturer of the gas chromatograph (Perkin Elmer). The fatty acid composition was reported as a relative percentage of the total peak area.

Statistical analysis

The obtained data were statistically analyzed using the "F" Test (Snedecor and Cochran, 1989), means were compared using a least significant difference (L.S.D.) test as described in Gomez and Gomez (1984). Statistical analysis was performed using Statistix 8.1 program.

Results

Analysis of variance (ANOVA) showed that partial substitution of mineral fertilization (NPK) with bio-stimulants (yeast, moringa extract or their combination) had significantly affected vegetative and yield parameters. In the first season, plant height significantly decreased when the recommended chemical NPK dose was reduced to 50% and combined with bio-stimulants (Table 2). Moringa leaves extract produced significantly better results compared to yeast and the combined treatment (Table 2). The number of lateral branches per plant was dramatically decreased in all treatments as compared to the control (100% NPK) in both seasons. The lowest values were recorded when yeast and moringa extract were used alone (Table 2). On the other hand, the highest leaf area was observed in plants treated with 75% NPK with moringa extract. There were significant decreases in leaf area begin at 50% NPK, and the smallest leaves were shown in plants treated with only yeast (Table 2). The number of spikes significantly decreased in both seasons in plants treated with 50 %t NPK plus bio-stimulants or bio-stimulants alone. Moringa extract was much better than yeast alone and its combination with moringa in the first season, while combined treatment of moringa extract and yeast was much better in the second season (Table 2). The tallest spike has been shown in plants treated with 75% NPK combined with yeast, and the spike height decreased under other treatment compared to control. Moringa extract gave much better results than yeast and their combination.

Table 2. Plant height, number of branched, leaf area, number of spike and spike height of chia plants as affected by partial substitution of mineral fertilizers (NPK) with bio-stimulants (yeast, moringa, or combination) in the 1st and 2nd seasons (2018/2019 & 2019/2020).

Treatments	Plant height (cm)		Number of branches/plant		Leaf area (mm^2)		Number of spike/plant		Spike height (cm)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
T1	51.1	69.5	43.6	43.7	1808.1	1513.2	32.5	35.5	15.8	19.4
T2	48.4	67.1	34.8	35.3	2072.7	1687.7	31.2	29.3	12.5	18.6
T3	49.7	78.6	26.9	34.3	1743.3	1739.5	24.0	32.3	13.1	23.7
T4	48.4	71.8	29.4	43.0	1820.9	1614.7	27.5	33.6	13.0	19.7
T5	44.0	67.2	25.7	32.1	1676.9	1470.7	24.6	25.8	17.6	18.9
T6	46.3	67.2	16.6	27.9	1747.4	1574.7	23.9	30.3	13.2	18.2
T7	45.5	71.6	25.8	33.9	1339.5	1478.1	25.0	30.5	13.2	19.5
T8	38.9	55.5	15.9	15.2	1302.8	1344.8	17.0	10.1	10.4	13.4
T9	35.0	53.3	14.4	16.9	1160.9	1131.7	9.2	14.5	8.6	12.8
T10	37.9	49.0	24.3	21.9	1244.1	1074.7	8.8	15.9	9.3	12.7
LSD 0.05	2.8	2.4	3.9	3.0	123.1	132.2	2.2	2.9	2.0	2.4
F value	37.7	129.6	47.4	94.3	53.5	24.3	121.5	134.2	2.4	19.1
P	***	***	***	***	***	***	***	***	***	***

Treatments: T1 (100% NPK), T2 (75% NPK+ yeast), T3 (75%NPK+ moringa), T4 (75% NPK+ yeast+ moringa), T5 (50% NPK+ yeast), T6 (50%NPK+ moringa), T7 (50% NPK+ yeast+ moringa), T8 (yeast only), T9 (moringa only), and T10 (yeast+ moringa). *** represented the significance of differences at probability of 0.05%.

Spike fresh and dry weights decreased as NPK levels decreased, the lowest values were recorded in both seasons under zero NPK (Table 3). The combination of yeast and moringa extract resulted in a significantly higher seed yield than either yeast or moringa extract alone (Table 3). In both seasons, seed yields per plant decreased with decreasing NPK levels. Treatment with 75% NPK combined with yeast and moringa extract gave higher yield of seeds compared to combined yeast or moringa. In contrast, the lowering NPK levels in both seasons had no effect on the weight of 100 seeds. The best value of 100 seeds weight has been shown under treatment with 100% NPK with moringa extract.

Table 3. Spike fresh and dry weight, seed yield and 100 seeds weight of chia plants as affected by partial substitution of mineral fertilizers (NPK) with bio-stimulants (yeast, moringa, or combination) in the 1st and 2nd seasons (2018/2019 & 2019/2020)

Treatments	Spike fresh weight/plant (g)		Spike dry weight/plant (g)		Seed yield/plant (g)		100 seeds weight (g)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
T1	24.8	16.1	20.9	14.3	9.45	7.46	0.13	0.15
T2	10.9	10.2	8.6	9.6	3.83	4.63	0.13	0.17
T3	10.6	12.3	10.0	11.6	4.48	6.08	0.13	0.15
T4	13.2	13.3	11.4	12.8	6.12	6.01	0.13	0.16
T5	10.8	8.7	8.5	7.4	3.57	3.63	0.13	0.15
T6	9.6	11.1	9.0	10.2	4.24	5.07	0.13	0.15
T7	13.0	11.8	10.4	11.1	4.19	5.34	0.12	0.15
T8	6.7	3.0	4.9	2.3	1.89	1.15	0.12	0.13
T9	6.1	2.9	3.9	2.2	1.40	1.23	0.13	0.13
T10	10.8	2.4	9.4	1.7	3.73	0.86	0.12	0.14
LSD 0.05	1.2	2.5	1.5	1.4	1.24	1.04	0.01	0.02
F value	149.4	191.1	83.6	93.5	28.5	44.1	1.06	5.16
P	***	***	***	***	***	***	ns	***

Treatments: T1 (100% NPK), T2 (75% NPK+ yeast), T3 (75%NPK+ moringa), T4 (75% NPK+ yeast+ moringa), T5 (50% NPK+ yeast), T6 (50%NPK+ moringa), T7 (50% NPK+ yeast+ moringa), T8 (yeast only), T9 (moringa only), and T10 (yeast+ moringa). *** represented the significance of differences at probability of 0.05%, and ns referred to non-significant.

The leaf greenness significantly decreased under zero NPK level treatment in the first season. Significant increases have been shown under 75% NPK combined with moringa extract. Yeast alone showed much greenness than moringa extract and combined of both of them (Table 4). Nitrogen content increased under treatment with 75% NPK combined with moringa extract, and the content decreased when NPK level decreases. Combined moringa extract and yeast gave higher nitrogen content compared to moringa extract or yeast alone. Phosphorus content also increased when plants treated with 75% NPK combined with bio-stimulants, especially yeast, and significant decreased has been observed only in plants treated with moringa or yeast alone. Potassium content also increased under combined 75% NPK with bio-stimulants compared to control, and the lowest value has been shown under moringa extract treatment alone (Table 4). In contrast, total crude fat content increased with decreasing NPK level, and the highest values have been shown under treatment with yeast or combined of yeast and moringa extract with zero NPK (Table 4).

Table 4. Relative leaf greenness, nitrogen (N), phosphorus (P), potassium (K), and crude fat content of chia plants as affected by partial substitution of mineral fertilizers (NPK) with bio-stimulants (yeast, moringa, or combination) in the 1st and 2nd seasons (2018/2019 & 2019/2020)

Treatments	Leaf greenness		N %		P %		K %		Crude fat	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
T1	30.2	17.8	3.57	3.74	0.33	0.58	2.72	2.26	31.3	29.1
T2	32.4	20.1	3.99	2.60	0.70	0.61	4.12	2.23	28.4	29.0
T3	28.6	18.9	2.21	3.05	0.83	0.59	3.83	2.23	31.3	30.6
T4	32.4	18.7	4.06	3.68	0.77	0.59	4.31	2.66	31.5	30.6
T5	27.4	15.6	2.35	2.92	0.64	0.58	3.13	1.97	32.2	30.5
T6	28.0	15.9	1.72	4.39	0.75	0.59	3.55	2.07	32.8	29.9
T7	28.3	16.5	2.24	2.98	0.59	0.59	4.00	1.91	32.6	31.5
T8	20.2	10.1	2.00	3.11	0.25	0.59	3.00	1.99	32.2	32.1
T9	25.7	13.8	1.40	3.40	0.19	0.58	3.50	1.75	35.1	33.4
T10	20.1	10.0	3.47	4.06	0.43	0.58	3.96	1.89	34.6	32.9
LSD 0.05	2.88	1.96	0.34	0.26	0.06	0.019	0.01	0.03	1.12	0.52
F value	19.6	28.2	70.9	39.7	119.9	1.34	149.3	945.4	24.3	74.0
P	***	***	***	***	***	ns	***	***	***	***

Treatments: T1 (100% NPK), T2 (75% NPK+ yeast), T3 (75%NPK+ moringa), T4 (75% NPK+ yeast+ moringa), T5 (50% NPK+ yeast), T6 (50%NPK+moringa), T7 (50% NPK+ yeast+ moringa), T8 (yeast only), T9 (moringa only), and T10 (yeast+ moringa). *** represented the significance of differences at probability of 0.05%, and ns referred to non-significant.

In the chromatographic analysis of fatty acid, nine components have been defined in the first season 2018/2019 and seven components have been defined in the second season 2019/2020. The major component in fatty acid included linolenic acid and linoleic acid, which made up about 82% of the fatty acids in both seasons (Table 5). The main component was linolenic acid which consisted about 59-62% in the first season and 61-63.5% in the second season, while linoleic acid consisted about 19-21% of fatty acids in both season. Treatment with combined yeast and moringa extract, even alone or combined with 75% or 50% recommended dose of NPK, had significant effect on fatty acid quality in term of linolenic acid percentage.

Table 5. Fatty acids composition of chia plants as affected by partial substitution of mineral fertilizers (NPK) with bio-stimulants (yeast, moringa, or combination) in the 1st and 2nd seasons (2018/2019 & 2019/2020)

Composition	Treatments									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
	1st season 2018/2019									
Laueic acid	---	---	---	0.05	----	0.05	0.05	---	---	---
Palmitic acid	7.28	7.34	7.18	8.06	7.53	7.68	7.51	7.04	7.13	7.35
Heptadecanoic acid	0.18	0.22	0.17	0.17	0.17	0.20	0.19	0.27	0.40	0.28
Stearic acid	3.63	3.67	3.76	3.84	3.72	3.97	3.92	3.69	3.92	3.84
Oleic acid	6.27	6.77	6.57	6.34	6.47	6.94	6.47	7.01	7.00	6.22
Elaidic acid	0.77	1.00	0.80	0.78	0.88	0.75	0.84	0.77	0.82	0.84
Linoleic acid	19.32	20.15	19.82	20.57	19.56	21.03	21.09	19.03	20.74	19.18
Linolenic acid	61.54	60.59	61.36	59.42	61.12	58.63	59.18	61.66	59.99	61.97
Arachidic acid	0.30	0.27	0.33	0.26	0.35	0.34	0.32	0.30	----	0.31
Total	99.29	100.0	99.99	99.49	99.8	99.59	99.57	99.77	100.0	99.99
2nd season 2019/2020										
Palmitic acid	7.66	7.34	7.27	7.11	7.09	7.19	7.15	7.26	6.99	7.12
Stearic acid	3.29	4.06	3.37	3.26	3.36	3.18	3.38	3.31	3.35	3.08
Oleic acid	6.37	5.70	6.18	5.93	6.13	5.99	6.04	6.51	6.50	6.23
Elaidic acid	0.88	0.86	0.83	0.87	0.86	0.87	0.82	0.86	0.79	0.86
Linoleic acid	19.21	19.49	19.54	18.99	20.32	19.49	19.06	21.00	20.46	20.52
Linolenic acid	62.60	62.55	62.82	63.61	61.99	63.04	63.56	61.07	61.66	61.96
Arachidic acid	---	---	---	0.24	0.25	0.25	---	---	0.26	0.24
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Treatments: T1 (100% NPK), T2 (75% NPK+ yeast), T3 (75%NPK+moringa), T4 (75% NPK+ yeast+ moringa), T5 (50% NPK+ yeast), T6 (50%NPK+moringa), T7 (50% NPK+ yeast+ moringa), T8 (yeast only), T9 (moringa only), and T10 (yeast+ moringa).

Discussion

The production and quality of crops are strongly influenced by growing conditions, such as fertilization. The role of nutrition in the growth and development of all agricultural plants is important. Nutrients can successfully boost active substances such as oil output and quality in medicinal plants that generate secondary metabolites (Aziz *et al*., 2010; Jabbari *et al*., 2011; Sharafzadeh *et al*., 2011; Zheljazkov *et al*., 2011). The importance of the mineral micronutrients N, P, and K in enhancing the plant's vegetative development features has been exhaustively described in order to analyze their principal roles in plant growth, development, and quality enhancement. Nitrogen is a major element of amino acids, enzymes, and energy-transmitting molecules such as chlorophyll, ADP, and ATP (Bidwell, 1974). Phosphorus is also essential for cell division, glucose metabolism, photosynthesis, and biological oxidation (Lambers *et al*., 2010). Potassium's synergistic is interacted with carbohydrates as an osmotic agent (Mengel and Kirkby, 1987). In sustainable horticulture production systems, natural plant bio-stimulants are essential. Dry yeast extract for example is a bio-substance having stimulate, nutritional and protective characteristics. Yeast is a source of several plant growth compounds, including cytokinins, vitamin B, and microelements (P, K, S, Na, Ca, and Mg). In addition to protein, carbohydrates, DNA, and lipids, it contains auxins, cytokinins, and other endogenous plant hormones (Mahdi *et al*., 2010). Khalil and Ismail (2010) were found that yeast may be useful in increasing nutrient availability for crop production. Yeast extract has been found to improve the growth, productivity, and quality of several plants (Ahmed *et al*., 2011; Mahmoud *et al*., 2023). Yeasts have been proposed as an improved source of phyto-hormones (particularly cytokinins), nutrients, enzymes, amino acids, and minerals (Barnett *et al*., 1990). Furthermore, it has been shown to stimulate cell division and development, protein and nucleic acid synthesis, and chlorophyll production (Castelfranco and Beale, 1983). It is a bio-substance containing a variety of nutritional nutrients and semi-effective growth regulators such as auxins, gibberellins, and cytokinins. In contrast, prior research has demonstrated that moringa leaf extracts improve seed germination, plant development, yield, nutrient utilization efficiency, crop quality, and resistance to abiotic stresses (Abdel-Rahman and Abdel-Kader, 2020; Zulfiqar *et al*., 2020; Hassanein *et al*., 2021). Moringa extracts are a suitable alternative supply of inorganic fertilizers because to their high amount of micro and macro mineral nutrients, protein, and vital amino acids, which help to complement the nutritional needs of plants (Yasmeen *et al*., 2014). Latif and Mohamed (2016) attributed growth and yield responses when treated with moringa to the presence of growth-promoting hormones in moringa, as analyses showed that moringa extract contains antioxidants, gibberellins, IAA and ABA (Rady and Mohamed, 2015; Azam *et al*., 2020).

In this study, fertilization treatments showed significant impacts on the growth and yield parameters; plant height, number of branches, leaf area, number of spike, spike height, spike fresh and dry weights, seeds weight and 100 seeds weight (Tables 1 and 2). In general, all growth parameters decreased when amount of mineral fertilizer (NPK) decreased, and the lowest values were shown under zero NPK treatment. The effect of partial substitution of NPK with bio-stimulants on growth and yield components was evidently good, especially when 25% of NPK was replaced with a combination of yeast and moringa extract. This is consistent with previous study demonstrating the significance of bio-stimulant in compensating for the decrease of chemical fertilizers on rosemary (Singh *et al*., 2005), basil (Hassan *et al*., 2015), and chia (Salman *et al*., 2019).

Analysis of variance also revealed that partial substitution of mineral fertilizer with bio-stimulants had a substantial impact on the chemical and qualitative characteristics of chia plants (Table 4). Relative chlorophyll content (relative leaf greenness) dramatically rose when 25% of mineral fertilization N-P-K replaced with bio-stimulants, notably moringa extract. Similarly, the nitrogen, phosphorus, and potassium levels increased by 75% NPK combined with bio-stimulants. These results are due to richness of yeast and moringa with micronutrients and hormone-like contents (Barnett *et al*., 1990; Khalil and Ismail, 2010; Mahdi *et al*., 2010; Abdel-Rahman and Abdel-Kader, 2020; Zulfiqar *et al*., 2020; Hassanein *et al*., 2021).

On the contrary, the quality of chia plants as measured by total crude fat content and fatty acid composition increased significantly with lowering NPK level, with the greatest value demonstrated under bio-stimulant treatment with 0% NPK. Partial replacement of NPK with bio-stimulant significantly enhanced total fat content (Table 4). Plants treated with bio-stimulant with minimal NPK had the best results when treated with linolenic acid, a major component of fatty acids. Linolenic acid increased significantly with decreasing mineral fertilizer. These results suggested that mineral fertilization had negative effect on the quality of chia oil and its fatty acid composition. In contrast, the bio-stimulants, even yeast or moringa, have positive impact on the quality component and this may due to organic components of yeast and moringa which involved in the pathway of fatty acid synthesis. Improving the quality of medicinal plants as a result of bio-stimulants treatment have been documented in various studies (Ahmed *et al*., 2011; Jiménez *et al*., 2013; Abdel-Rahman and Abdel-Kader, 2020; Zulfiqar *et al*., 2020; Hassanein *et al*., 2021).

Conclusions

It is concluded that partial substitution of mineral fertilizers with bio-stimulants had significantly impacts on growth, yield and quality components. The growth and yield component significantly decreased with decreasing NPK level, except for 75% NPK combined with bio-stimulants. In contrast, the chemical and quality component increased with decreasing mineral fertilizer. Treatment with 75% NPK combined with bio-stimulants gave the highest micronutrients and fatty acid content. The major component of fatty acid, linolenic acid, increased with decreasing NPK, and the highest values obtained with bio-stimulants treatment with zero % NPK.

Authors' Contributions

Conceptualization, S.M.E., S.F.H., S.S., and W.S.S.; methodology, S.M.E., S.F.H., S.S., and W.S.S.; software, S.M.E., and A.M.A.; validation, S.F.H. and W.S.S.; formal analysis, S.M.E. and W.S.S.; investigation, S.M.E. and S.S.; resources, S.F.H. and S.S.; data curation, S.M.E., A.M.A., and W.S.S.; writing—original draft preparation, S.M.E. and W.S.S.; writing—review and editing, A.M.A., and W.S.S.; visualization, S.F.H.; supervision, S.F.H., S.S., and W.S.S.; project administration, A.M.A., and W.S.S.; funding acquisition, S.M.E. A.M.A., and W.S.S. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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