

## ROLE OF SOME MICRONUTRIENTS IN IMPROVING PRODUCTIVITY AND QUALITY OF SEEDS IN CHIA PLANTS (*SALVIA HISPANICA* L.) IN SANDY SOIL

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**ABSTRACT:** Chia plant (*Salvia hispanica* L.) is a new crop introduced to the Egyptian agriculture system to enrich it with new species of medicinal plants that have healthy and beneficial ingredients. The purpose of this work was to study the possibility of improvement growth, seed yield, fixed oil production and chemical constituents of chia plants in response to various micronutrients under sandy soil conditions. The field experiment was conducted in the Experimental Farm of EL-Quassassin, Horticulture Research Station, Ismailia Governorate, Egypt. The treatments were (1) control (spray only with tap water), (2) boron (B) at 50 ppm, (3) boron (B) at 100 ppm, (4) iron (Fe) at 100 ppm, (5) iron (Fe) at 200 ppm, (6) zinc (Zn) at 100 ppm, (7) zinc (Zn) at 200 ppm, (8) combination of B (50 ppm) + Fe (100 ppm) + Zn (100 ppm) and (9) combination of B (100 ppm) + Fe (200 ppm) + Zn (200 ppm). In general, the results showed that the growth, seed yield, fixed oil yield, and chemical measurements of chia plant, such as fixed oil composition (fatty acids), photosynthetic pigments content, macro- and micronutrients, total carbohydrates, protein content, total phenols and antioxidant activity of the oil were improved by foliar application of micronutrients solely or in combination compared to control in both seasons. Combination of boron (50 ppm) + iron (100 ppm) + zinc (100 ppm), followed by a combination of B (100 ppm) + Fe (200 ppm) + Zn (200 ppm) led to the highest increases with no significant difference between them in most cases. GC for fixed oil of chia seeds revealed the estimation of seven components *i.e.*, lauric, myristic, palmitic, oleic, linoleic, linolenic and arachidic acids. The main component was linolenic acid (55.34 to 61.43%). The highest percentage of linolenic acid achieved with the combination treatment of B (50 ppm) + Fe (100 ppm) + Zn (100 ppm). While, combination of B (100 ppm) + Fe (200 ppm) + Zn (200 ppm) had significant increases in total phenols and oil antioxidant activity over the control. The improvement of growth, seed yield, oil production and their quality of *Salvia hispanica* L. by the foliar spraying of these micronutrients may be useful for growing and productivity of plants in Egyptian sandy soil conditions.

**Keywords:** *Salvia hispanica* L., chia, boron, iron, zinc, seed yield, oil production, chemical composition.

### INTRODUCTION

Chia (*Salvia hispanica* L.) is an annual oil seed crop, that belongs to the Lamiaceae

family which is native to Mexico and the north of Guatemala (Ullah *et al.*, 2016 and da Silva *et al.*, 2017). Today, chia plants are cultivated in a wide range of African

countries. Seeds of chia plants consider one of the important sources of unsaturated fatty acids mainly  $\alpha$ -linolenic acid, hence chia plants are rich in omega-3 fatty acids. Chia seeds are rich in essential amino acids, proteins, several minerals, vitamins, carbohydrates, as well as high dietary fibers content. They also contain bioactive compounds of high antioxidant activity (Ixtaina *et al.*, 2008; Punia and Dhull, 2019 and Kulczyński *et al.*, 2019). Chia seeds contain 39% oil (mass of dry seed). Its oil contains linolenic acid ranging 50-57% and linoleic acid (17-26%) (Ciau-Solis *et al.*, 2014).

Due to the aforementioned composition of chia seeds and its oil, it considers future super nutrient for food, medical and pharmaceutical sectors. Thus, it has anti-ageing, anti-carcinogenic characteristics, protect against cardiovascular diseases (Ullah *et al.*, 2016). Also, it is associated with the reduction of inflammation and cholesterol (Ali *et al.*, 2012), control of diabetes, hypertension, used as anti-blood clotting, antidepressant, laxative, analgesic, immune improver and antimicrobial activity (Kobus-Cisowska *et al.*, 2019). Furthermore, the plant repels insects, pests, and disease, so is thus considered a good candidate for organic production (Ullah *et al.*, 2016). It must be known that chia seeds are free from gluten and as such may be consumed by celiac patients (Munoz *et al.*, 2013). Therefore, the European Parliament and Council of Europe approved it as a “novel food”.

Micronutrients are important for increasing productivity of crops (Senthilkumar, 2018). Despite micronutrients being added in very small amounts (Shukla *et al.*, 2018), poor growth and yield of plants were observed in the absence of them, and their adequate supplies enhanced plants' productivity and quality (Kobraee *et al.*, 2013). Foliar spraying of micronutrients is cheap, available, more effective strategy, reduces environmental pollution (Aziz *et al.*, 2021) and promote

root growth, hence it increases uptake of nutrients by crops (Bhatt *et al.*, 2020).

Micronutrients as boron, iron and zinc are essential for completion of the plant's life cycle (Ali *et al.*, 2020), have important functions in maintain the stability and biosynthesis of proteins, cellular structures, contribute to the activities of molecules and enzymes, carbohydrate and metabolism, increases a plant's resistance to environmental stresses, chlorophyll maintenance, electron transport and anti-oxidative systems (Arigony *et al.*, 2013 and Das *et al.*, 2016). They may also have an impact on respiration, cell division, photosynthesis, plant maturity, and the growth of meristematic tissues. (Zeidan *et al.*, 2006). Moreover, micronutrients help in reproduction, seed formation and oil synthesis in the seeds (Kumar *et al.*, 2014). Hence, foliar application of micronutrients is essential for optimum crop nutrition and development (Prusty *et al.*, 2022).

Boron (B) is a micronutrient required for growth of plants, involved in pollen grain germination, pollen tube elongation and seeds yield (Zheng *et al.*, 2019).

Iron (Fe) is required as a cofactor for a variety of enzymes that catalyze many biochemical reactions. Hence, iron is necessary for a variety of plant development processes, including chlorophyll creation, chloroplast development as well as it required in various steps in the biosynthetic pathways (Boghdady, 2017).

Zinc (Zn), is another necessary micronutrient for enhancing plant growth and productivity. It acts as an enzyme activator in some reactions, e.g., carbonic anhydrase, hexose kinase and alcohol dehydrogenase. Zinc is needed for biosynthesis of the indole-3-acetic acid, auxin, help in cell division process and protein metabolism (Verma, 2007), involved in photosynthesis and CO<sub>2</sub> sequestration, and biosynthesis of chlorophylls, (Singh *et al.*, 2018).

Though many researches were mainly concerned with several plants, there is limited information available on the impact of micronutrients on the growth, productivity and chemical constituents of chia plant. Additionally, the impact of boron, iron and zinc foliar application on chia plants is still unknown, especially under sandy soil conditions. Therefore, the objectives of this research were to (1) evaluate the response of chia plant to boron, iron and zinc foliar application treatments in terms of growth, crop yield and chemical composition, and (2) determine the appropriate levels of iron, zinc and boron for the plant production under sandy soil conditions.

## **MATERIALS AND METHODS**

### **The experimental site:**

The current study was conducted during two successive seasons of 2020/2021 and 2021/2022 at the Experimental Farm of EL-Quassassin, Horticulture Research Station, Ismailia Governorate, Egypt (30° 34' N Latitude, 31° 56' E Longitude) to examine the influence of some micronutrients as boron, iron, zinc and their combination on plant growth, yield and chemical composition of chia plant. Chia plant grown under sandy soil conditions. Drip irrigation system was used. The physical and chemical analyses of the soil are shown in Table (1).

### **Plant materials and procedures:**

The seeds of chia plants (*Salvia hispanica* L.) were obtained from Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt. The seeds were sown in hills at distances of 30 cm between hills and 60 cm between rows on October 19<sup>th</sup> in both seasons. After 15 days from sowing, seedlings were thinned to be one plant/hill.

### **Chemical fertilizers (NPK):**

Chemical fertilizers (NPK) were added according to Fouad *et al.* (2018) at the recommended rate. All amounts of calcium superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>) at the rate of

350 kg fed<sup>-1</sup> was added during soil preparation. While, ammonium nitrate (33.5% N) at the rate of 200 kg fed<sup>-1</sup> and potassium sulphate (48% K<sub>2</sub>O) at rate of 75 kg fed<sup>-1</sup> were applied in two equal doses, after 30 and 60 days from sowing.

The layout of the experiment was a randomized complete blocks design with nine treatments and three replicates.

### **The treatments were as follows:**

1. Control (sprayed with tap water only).
2. Boron (B1) at 50 ppm.
3. Boron (B2) at 100 ppm.
4. Iron (Fe1) at 100 ppm.
5. Iron (Fe2) at 200 ppm.
6. Zinc (Zn1) at 100 ppm.
7. Zinc (Zn2) at 200 ppm.
8. Boron at 50 ppm (B1) + iron at 100 ppm (Fe1) + zinc at 100 ppm (Zn1).
9. Boron at 100 ppm (B2) + iron at 200 ppm (Fe2) + zinc at 200 ppm (Zn2).

Boron, iron and zinc were applied as boric acid, ferrous sulphate and zinc sulphate respectively. Foliar application of micronutrients solely and their combinations were applied three times. The first one at 30 days after sowing and was repeated twice after 21 days intervals.

### **Harvesting:**

The plants were harvested on 28<sup>th</sup> of March and 1<sup>st</sup> April in the first and second seasons, respectively.

### **Data recorded:**

#### **1. Vegetative growth parameters:**

Plant height (cm), number of main branches, herb fresh weight (g) and herb dry weight (g).

#### **2. Yield parameters:**

Number of inflorescences plant<sup>-1</sup>, main inflorescence length plant<sup>-1</sup>, seed yield (g) plant<sup>-1</sup>, seed index [weight of 1000 seeds (g)] and seed yield (kg) fed<sup>-1</sup>.

**Table 1. Physical and chemical properties of the experimental soil.**

Properties	Value	Properties	Value
Clay	4.8	E.C (mmohs/cm) (1 soil: 5 water)	2.83
Silt	16.7	pH	7.94
Sand	78.5	Saturation (%)	33
Texture	Sandy loam	Soluble cations and anions (mq/l)	
<b>Plant available nutrients contents in the soil (mg kg<sup>-1</sup>soil)</b>		CO <sub>3</sub> <sup>-</sup>	0
N	59	HCO <sub>3</sub> <sup>-</sup>	1.5
P	2.18	Cl <sup>-</sup>	20.3
K	27.2	SO <sub>4</sub> <sup>-</sup>	3.2
Fe	1.72	Ca <sup>++</sup>	7.2
Zn	0.41	Mg <sup>++</sup>	3.4
Mn	1.72	Na <sup>+</sup>	14.1
B	0.57	K <sup>-</sup>	0.3

### 3. Fixed oil production:

The fixed oil was extracted from the seeds using n-hexane as a solvent in a Soxhlet system HT apparatus according to the methods of (AOAC, 1970). Fixed oil percentage (%), content (g plant<sup>-1</sup>) and yield (kg fed<sup>-1</sup>) were calculated.

### 4. Fatty acid profile:

Fatty acid profile of chia seeds oil was determined through two steps:

- Preparation of fatty acid methyl esters (FAMES) as described by (ISO 5509, 2000).
- Fatty acids composition was determined using gas chromatography (GC HP 6890) as described by Coates (2011).

### 5. Photosynthetic pigments content (mg g<sup>-1</sup> fresh weight):

Chlorophyll a, chlorophyll b and carotenoid contents were determined in the fresh leaves, using the method described by Saric *et al.* (1976).

### 6. Macro-elements [N, P and K (%)] and micro-elements [Fe, Zn and B (ppm)]:

The samples of seeds were ground for chemical elements determination as follows, nitrogen (N) was determined using the modified micro-Kjeldahl method (Helrich, 1990). Phosphorus (P) was analyzed calorimetrically using the chlorostannous molybdophosphoric acid blue color method

in sulphuric acid (Jackson, 1973). As well as, potassium (K) concentrations was analyzed using the flame photometer apparatus (CORNING M 410, Germany).

As for microelements, the concentrations of B, Fe and Zn were analyzed by an atomic absorption spectrophotometer with air-acetylene and fuel (PyeUnicam, model SP-1900, US) according to (AOAC, 1980).

### 7. Protein content:

The percentage of raw protein in the seeds was obtained by multiplying the percentage of N by a factor of 6.25 (AOAC, 2000).

### 8. Total carbohydrates (% of dry matter):

Total carbohydrates (% of dry matter) were determined in the seeds using the method described by Herbert *et al.* (1971) by a Milton Roy colorimeter (model Spectronic 21 D) at 490 nm, and the glucose standard curve was used to determine the total carbohydrates.

### 9. Total phenolic content:

Total phenolic content was determined by two processing:

- Extraction of polyphenols as described in (Peschel *et al.*, 2007).
- Determination of total phenolic content (mg GAE/100 g DW) based on the method of Gutfinger (1981).

## 10. Antioxidant activity of the fixed oil:

The antioxidant activity was measured in terms of radical-scavenging ability, using the stable radical DPPH (2,20-Diphenyl-1-picrylhydrazyl) according to Brand-Williams *et al.* (1995).

The inhibition percentage of the DPPH radical was calculated according to the formula:

$$I \% = [(AB - AS)/AB] \times 100$$

Where I =DPPH inhibition %,

AB = absorbance of control sample (t=0 h),

and AS = absorbance of a tested sample at the end of the reaction (t=1 h).

Each assay was carried out in triplicate.

### Statistical analysis:

Data recorded on vegetative growth, flowering, seed yield, as well as oil production and chemical composition, were subjected to analysis of variance (ANOVA), and the means were compared using the "Least Significant Difference (L.S.D.)" test at the 5% level, as described by (Snedecor and Cochran, 1980).

## RESULTS AND DISCUSSION

### Effect of micronutrients and their combinations on:

#### 1. Vegetative growth parameters:

Data in Tables (2 and 3) showed that, all concentrations of micronutrients and their combinations significantly increased the studied vegetative growth parameters (plant height, number of branches, fresh and dry weight/plant) as compared to the control in both seasons, especially combination of B1 + Fe1 + Zn1 treatment which increase plant height (cm), herb fresh and dry weight (g) per plant by (26.51 and 26.50%), (57.13 and 53.94%) and (62.36 and 53.66%) in the 1<sup>st</sup> and 2<sup>nd</sup> seasons, respectively, relative to the control followed by the combination of Fe2 + Zn2 + B2 treatment with no significant differences between these two treatments. Regarding to number of branches, the

combination of Fe2 + Zn2 + B2 treatment have the highest value with no significant difference with treatments B1, Zn2 and combination of B1 + Fe1 + Zn1 in the first season and treatments B1, Fe1 and combination of B1 + Fe1 + Zn1 in the second season. Our results are in agreement with the finding of Khater and Abd-Allah (2017) on sweet basil, Mumivand *et al.* (2021) on *Satureja khuzistanica* Jamzad and Pandey *et al.* (2022) on tomato. They found that, spraying the plants with micronutrients separately or mixed increased the vegetative growth parameters of the studied plants.

The advantages of boron may be to its role in cell wall ingredients formation which contains several enzymes that involved in cell expansion and division. Accordingly, it stimulates division and elongation of the cells especially in meristematic tissues; hence it increases plant growth (Cohen and Lepper, 1977 and Zheng *et al.*, 2019). Where, the marked effect of iron may be attributed to its role in enhancement chlorophyll synthesis and activate enzymes in the respiration process, so it is reflected in vegetative growth (Al-Sahhaf, 1989 and Shareef, 2020). Zinc accelerates cell division through involved it in the metabolism of RNA and ribosomal content in plant cells, stimulate carbohydrates, proteins and DNA formation, help in the synthesis of tryptophan, control auxin metabolism, consider as a precursor of IAA, which acts as a growth promoting substance (Amberger, 1974 and Chandler, 1982). So, increased vegetative growth parameters of plants as a result of spraying with mixture of microelements might be due to their effects on cell division and elongation (Malakouti and Tehrani, 1999).

#### 2. Yield parameters and its component:

##### a. Number and length of the inflorescences:

It could be noticed that spraying by different concentrations of the three micronutrients and their combinations showed a significant increase in the number and length of the inflorescences compared to

**Table 2. Effect of some micronutrients and their combinations on plant height (cm) and number of branches of chia plant during 2020/2021 and 2021/2022 seasons.**

Treatments	Plant height (cm)		Number of branches plant <sup>-1</sup>	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Control (tap water)	73.20	74.20	12.00	12.33
B1	85.00	85.28	15.00	16.33
B2	87.50	89.00	13.00	14.34
Fe1	79.20	79.98	14.00	16.00
Fe2	84.00	83.00	13.00	14.67
Zn1	85.30	86.10	14.34	14.67
Zn2	86.00	87.00	15.00	15.67
Fe1+Zn1+B1	92.61	93.87	15.33	16.00
Fe2+Zn2+B2	92.01	93.26	15.67	16.67
LSD at 5%	2.14	1.78	0.82	0.83

\* B1: boron at 50 ppm; B2: boron at 100 ppm; Fe1: iron at 100 ppm; Fe2: iron at 200 ppm; Zn1: zinc at 100 ppm and Zn2: zinc at 200 ppm.

**Table 3. Effect of some micronutrients and their combination on herb fresh and dry weight (g) of chia plant during 2020/2021 and 2021/2022 seasons.**

Treatments	Herb fresh weight (g) plant <sup>-1</sup>		Herb dry weight (g) plant <sup>-1</sup>	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Control (tap water)	73.28	76.65	15.12	16.53
B1	79.17	85.17	17.70	19.71
B2	92.99	96.37	19.87	20.57
Fe1	77.15	85.24	17.03	19.54
Fe2	93.90	100.20	20.04	21.71
Zn1	81.86	93.87	19.37	22.22
Zn2	96.31	103.54	21.04	22.62
Fe1+Zn1+B1	115.15	118.00	24.55	25.40
Fe2+Zn2+B2	112.83	115.25	24.55	25.37
LSD at 5%	2.33	3.13	1.80	2.09

\* B1: boron at 50 ppm; B2: boron at 100 ppm; Fe1: iron at 100 ppm; Fe2: iron at 200 ppm; Zn1: zinc at 100 ppm and Zn2: zinc at 200 ppm.

the control in the two seasons (Table, 4). The best results were obtained in plants treated with combination of B1 + Fe1 + Zn1 and there is no significant difference between this treatment and the treatments B2, Zn1, Zn2 and combination of B2 + Fe2 + Zn2 in the first season while Zn2 and the combination of B2 + Fe2 + Zn2 in the second season. Regarding the length of the inflorescences, the same treatment (B1 + Fe1 + Zn1) scored the highest values with no significant difference with treatments Fe2

and combination of B2 + Fe2 + Zn2 in the first season and treatments B2, Fe2 and combination of B2 + Fe2 + Zn2 in the second season. Our results match those reported by Abd El-Aziz and Balbaa (2007) on blue sage, Alamer *et al.* (2020) on marigold, Mumivand *et al.* (2021) on *Satureja khuzistanica* and Kumar *et al.* (2022) on chamomile. They concluded the beneficial effects of boron, iron and zinc on flower production of different plants and hence increase the inflorescences length and

**Table 4. Effect of some micronutrients and their combination on number of Inflorescences and length of inflorescences (cm) of chia plant during 2020/2021 and 2021/2022 seasons.**

Treatments	Number of inflorescences plant <sup>-1</sup>		Length of inflorescences (cm) plant <sup>-1</sup>	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Control (tap water)	20.00	20.67	12.50	12.50
B1	26.00	26.00	14.67	15.67
B2	29.00	30.00	14.00	16.00
Fe1	27.00	28.33	13.50	13.80
Fe2	24.00	24.33	15.50	16.50
Zn1	28.00	30.00	14.50	15.80
Zn2	29.33	30.45	14.00	14.00
Fe1+Zn1+B1	30.00	32.33	16.00	17.00
Fe2+Zn2+B2	29.00	32.00	15.67	16.67
LSD at 5%	2.28	2.32	1.01	1.04

\* B1: boron at 50 ppm; B2: boron at 100 ppm; Fe1: iron at 100 ppm; Fe2: iron at 200 ppm; Zn1: zinc at 100 ppm and Zn2: zinc at 200 ppm.

number. Zinc acts in flower bud formation, synthesis of tryptophan, the movement of carbohydrates to the bud development site or to the bud itself, while boron is an essential element for flower reproduction and pollen tube elongation (Davarpanah *et al.*, 2016). Beside this, iron increases cell division, plays as a catalyst role in respiration process and several chemical reactions that are directly responsible for enhanced growth and development (Khosa *et al.* 2011).

**b. Seed yield characteristics:**

Different concentrations of micronutrients and their combinations significantly increased all studied yield attributes in both seasons (Table, 5). Spraying with combination of B1 + Fe1 + Zn1 was the most effective and improved the weight of 1000 seeds (g), seeds yield (g) per plant and seed yield (kg) per fed by (13.79 and 13.60%), (59.73 and 67.46%) and (59.73 and 74.09%) as compared to the control in the first and second seasons, respectively. Regarding to seed index (weight of 1000 seeds; g) character, there is no significant difference between the combination treatments of B1 + Fe1 + Zn1 and the combination of B2 + Fe2 + Zn2. Our results are parallel with those obtained by Mohamed and Ghatas (2020) on chia plant, Adib *et al.*

(2020) on cumin, Khuong *et al.* (2022) on sesame and Venugopalan *et al.* (2022) on lentil. Seed yield is a complex trait as it is the product of various individual yield components as number of branches, number and length of inflorescences per plant which are closely correlated with seeds yield per plant and seeds yield per fed. (Yassin, 1973). These results may be attributed to the direct function of boron on flowering and seed formation (Padasalagi *et al.*, 2019). Also, the foliar application strategy of iron and zinc lead to availability of nutrients might have helped in increasing the translocation of photosynthetic to sink leading to improvement in pollen viability, flowering and seed production yield as reported by Marschner, (1995) and Choudhary *et al.* (2015). Thus, spraying micronutrients on leaves resulted in higher growth and yield parameters (Loomis and Durst, 1992).

**c. Fixed oil percentage and its yield (g plant<sup>-1</sup> and kg fed<sup>-1</sup>):**

It could be noticed that the application of micronutrients solely or in combinations showed a significant increase in fixed oil percentage and yield over untreated plants in both seasons (Table, 6). The foliar spray combination of B1 + Fe1 + Zn1 treatment lead to the best results with a significant

**Table 5. Effect of some micronutrients and their combinations on seed index [weight of 1000 seeds (g)], seeds weight (g) plant<sup>-1</sup> and seed weight yield (kg) fed<sup>-1</sup> of chia plant during 2020/2021 and 2021/2022 seasons.**

Treatments	Seed index weight of 1000 seeds (g)		Seeds weight (g) plant <sup>-1</sup>		Seed weight yield (kg) fed <sup>-1</sup>	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Control (tap water)	1.45	1.47	3.03	3.35	70.70	78.17
B1	1.53	1.52	3.83	4.31	90.30	100.57
B2	1.56	1.58	4.15	4.83	96.83	112.70
Fe1	1.56	1.58	3.62	3.54	84.47	82.60
Fe2	1.5	1.52	3.59	3.74	83.77	87.27
Zn1	1.5	1.51	4.01	4.03	93.57	94.03
Zn2	1.6	1.62	4.11	4.25	95.90	99.17
Fe1+Zn1+B1	1.65	1.67	4.84	5.61	112.93	130.90
Fe2+Zn2+B2	1.65	1.65	4.62	5.13	107.80	119.70
LSD at 5%	0.04	0.03	0.15	0.13	3.45	3.02

\* B1: boron at 50 ppm; B2: boron at 100 ppm; Fe1: iron at 100 ppm; Fe2: iron at 200 ppm; Zn1: zinc at 100 ppm and Zn2: zinc at 200 ppm.

**Table 6. Effect of some micronutrients and their combinations on fixed oil percentage fixed oil yield (g) plant<sup>-1</sup> and fixed oil yield (kg) fed<sup>-1</sup> in seeds of chia plant during 2020/2021 and 2021/2022 seasons.**

Treatments	Fixed oil (%)		Fixed oil yield (g plant <sup>-1</sup> )		Fixed oil yield (kg fed <sup>-1</sup> )	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Control (tap water)	20.65	21.29	0.63	0.71	14.59	16.61
B1	27.52	29.59	1.10	1.19	25.72	27.78
B2	28.77	31.61	1.18	1.34	27.55	31.30
Fe1	22.56	23.26	0.87	1.00	20.35	23.35
Fe2	25.59	26.38	1.06	1.27	24.74	29.67
Zn1	26.15	28.73	0.95	1.02	22.07	23.69
Zn2	28.02	30.80	1.01	1.15	23.45	26.83
Fe1+Zn1+B1	31.20	34.29	1.51	1.92	35.17	44.83
Fe2+Zn2+B2	30.50	33.51	1.41	1.72	32.82	40.06
LSD at 5%	1.03	1.14	0.07	0.08	1.67	2.27

\* B1: boron at 50 ppm; B2: boron at 100 ppm; Fe1: iron at 100 ppm; Fe2: iron at 200 ppm; Zn1: zinc at 100 ppm and Zn2: zinc at 200 ppm.

increase in the fixed oil percentage by (51.09 and 61.06%), fixed oil yield (g) per plant by (139 and 170%) and fixed oil yield (kg) per fed by (141 and 169%) compared to the control in both seasons, respectively. In addition, there was no significant difference between the two combination treatments of B1 + Fe1 + Zn1 and B2 + Fe2 + Zn2. Increased oil yield could be attributable to an increase in seed yield and oil percentage as a

result of the positive effects of boron, iron and Zinc application. Many researchers found that spraying of micronutrients significantly increases the oil content and yield of several oil-producing plants, for example fenugreek (Boghdady, 2017) and black cumin (Rezaei-Chiyaneh *et al.*, 2018). This might be due favorable effects of boron on pollination, seed formation, protein synthesis, carbohydrates and lipid

metabolism, which lead to increase seed fertility and increasing full seeds, hence oil content increase (Brighenti and Castro, 2008). In addition, zinc and iron are beneficial in oil biosynthesis (Ebrahimian *et al.*, 2010). Our results agreed with those obtained by Yasin and Abdelsalam (2022) who found that, foliar spray of mixture zinc and iron resulted in improvement oil yield in sesame.

### **3. Chemical composition:**

#### **a. Fixed oil fractionation:**

Data presented in the Table (7) showed the positive effects of micronutrients and their combination treatments on chemical properties of the fixed oil contents of chia seeds in the second season. The fixed oil composed of 7 well known fatty acids which it was rich with unsaturated fatty acids, chiefly linolenic (55.34 to 61.43%), linoleic acid (14.48 to 18.22%) and oleic fatty acids (11.29 to 13.94%). The Combination of B1 + Fe1 + Zn1 registered the highest levels of linolenic acid at 61.43%, followed (in descending order) by the combination of B2 + Fe2 + Zn2 (60.22%) and B2 (59.25%), when compared to the control (55.34%). Furthermore, combination of B1 + Fe1 + Zn1, combination of B2 + Fe2 + Zn2 and B2 treatments had the highest total unsaturated fatty acids content and lowest total saturated fatty acids content as compared to the control. Generally, the highest content of saturated fatty acids especially palmitic acid was obtained with the control treatment. From the aforementioned results and the data cleared in Table (7), it can be concluded that linolenic acid is the major component of chia seed oil as observed in all treatments. In addition, combination of B1 + Fe1 + Zn1 was a superior treatment for improving the quality of the oil during the second experimental season. In general, foliar spray of micronutrients improved oil quality of chia plants. Our results are in line with Boghdady (2017) on fenugreek and Rezaei-Chiyaneh *et al.* (2018) on black cumin. According to Ebrahimian *et al.* (2010) on sunflower pointed out that, zinc foliar spray

significantly increased linolenic, palmitoleic, oleic and myristic acid content. In addition, iron increased total unsaturated fatty acids. Also, boron resulted in an increase of palmitic, stearic and oleic acids (Bahaa El-Din, 2015).

#### **b. Chlorophyll a, chlorophyll b and carotenoids (mg g<sup>-1</sup> F.W.):**

Data in Table (8) described the positive effect of micronutrients foliar application on photosynthetic pigments. In general, the combination of B1 + Fe1 + Zn1 treatment has the highest values in improving photosynthetic pigments, the recorded increments in chlorophyll a (mg g<sup>-1</sup>) and carotenoids (mg g<sup>-1</sup>) were by (26.58 and 27.39%) and (23.32 and 23.14%) over the control in 1<sup>st</sup> and 2<sup>nd</sup> seasons, respectively followed by the treatment of B2 + Fe2 + Zn2 with no significant differences between the two treatments. Concerning chlorophyll b (mg g<sup>-1</sup>), there is a significant differences between treatments (combination of B1 + Fe1 + Zn1, Fe2 and Zn2). This may be owing to the role of boron, iron and zinc in chlorophyll synthesis. Boron lead to significant improvements in plant chlorophyll content and leaf photosynthesis rates (Nasef *et al.*, 2006). While, iron is crucial for chlorophyll biosynthesis, energy transmission, acts as co-enzymatic for activation various enzymes responsible for the anabolism of chlorophyll and redox enzymes that participate in the electron chain in the respiration process, so it participates in chlorophyll synthesis and increases the accumulation of Phytoferritin in the chloroplast and lead to chloroplast development (Focus, 2003, Gill and Tuteja, 2010 and Shareef, 2020). In addition, zinc have a considerable role in various dehydrogenases, CO<sub>2</sub> assimilation (Said-Al Ahl and Omer, 2009 and Khalifa *et al.*, 2011.) and carbonic anhydrase activation which involved in the control of stomata (Hu *et al.*, 2010). These results agreed with those reported by Khuong *et al.* (2022) on sesame, Alamer *et al.* (2020) on *Tagetes erecta* L. and Venugopalan *et al.* (2022) on lentil.

**Table 7. Effect of micronutrients treatments and their combinations on fatty acids of chia (*Salvia hispanica* L.) oil during the second season (2021/2022).**

Treatments	Saturated fatty acids (%)				Components Unsaturated fatty acids (%)					Total fatty acids (%)	Unknown
	Lauric acid	Myristic acid	Palmitic acid	Total	Oleic acid	Linoleic acid	Linolenic acid	Arachidic acid	Total		
Control (tap water)	0.45	1.44	6.23	8.12	11.29	14.48	55.34	3.19	84.30	92.42	7.58
B1	0.34	1.01	3.71	5.06	12.87	17.33	59.13	2.16	91.49	96.55	3.45
B2	0.21	1.36	2.52	4.09	13.22	17.69	59.25	2.37	92.53	96.62	3.38
Fe1	1.24	1.83	4.38	7.45	11.74	15.56	56.41	2.68	86.39	93.84	6.16
Fe2	1.62	2.11	4.12	7.85	11.98	15.82	56.77	3.19	87.76	95.61	4.39
Zn1	0.56	0.95	4.26	5.77	12.41	16.64	57.23	2.73	89.01	94.78	5.22
Zn2	0.73	1.14	4.03	5.90	12.63	17.41	57.90	1.55	89.49	95.39	4.61
Fe1+Zn1+B1	0.56	0.81	2.47	3.84	13.76	18.22	61.43	2.00	95.41	99.25	0.75
Fe2+Zn2+B2	0.65	0.87	3.25	4.77	13.94	17.73	60.22	1.21	93.10	97.87	2.13

\* B1: boron at 50 ppm; B2: boron at 100 ppm; Fe1: iron at 100 ppm; Fe2: iron at 200 ppm; Zn1: zinc at 100 ppm and Zn2: zinc at 200 ppm.

**Table 8. Effect of some micronutrients and their combinations on chlorophyll a ( $\text{mg g}^{-1}$ ), chlorophyll b ( $\text{mg g}^{-1}$ ) and carotenoids ( $\text{mg g}^{-1}$ ) of chia plant during 2020/2021 and 2021/2022 seasons.**

Treatments	Chlorophyll a ( $\text{mg g}^{-1}$ fresh weight)		Chlorophyll b ( $\text{mg g}^{-1}$ fresh weight)		Carotenoids ( $\text{mg g}^{-1}$ fresh weight)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Control (tap water)	0.662	0.690	0.382	0.398	0.253	0.255
B1	0.752	0.782	0.349	0.369	0.248	0.255
B2	0.742	0.781	0.330	0.361	0.265	0.270
Fe1	0.811	0.861	0.422	0.448	0.299	0.307
Fe2	0.813	0.853	0.436	0.464	0.296	0.297
Zn1	0.811	0.843	0.408	0.438	0.261	0.270
Zn2	0.811	0.830	0.433	0.443	0.300	0.305
Fe1+Zn1+B1	0.838	0.900	0.451	0.481	0.321	0.324
Fe2+Zn2+B2	0.830	0.879	0.421	0.430	0.312	0.314
LSD at 5%	0.024	0.023	0.025	0.027	0.016	0.016

\* B1: boron at 50 ppm; B2: boron at 100 ppm; Fe1: iron at 100 ppm; Fe2: iron at 200 ppm; Zn1: zinc at 100 ppm and Zn2: zinc at 200 ppm.

Also, Alijani *et al.* (2022) pointed the positive effect of B + Fe + Zn on chlorophyll content in soybean.

### c. Macronutrients content (%):

Micronutrients at different concentrations and their combinations had a significant effect on nitrogen, phosphorus and potassium contents (%) except those

treatments of Fe1 (100 ppm) and zn1 (100 ppm) on nitrogen content in both seasons and Fe1 (100 ppm) on potassium content in the 1<sup>st</sup> season, they had no significant differences with the control (Table, 9). Spraying the combination of B1 + Fe1 + Zn1 was the most effective treatment and lead to a significant increase in nitrogen content by (77.97 and 78.64%), phosphorus content by

**Table 9. Effect of some micronutrients and their combinations on nitrogen, phosphorus and potassium contents (%) in seeds of chia plant during 2020/2021 and 2021/2022 seasons.**

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Control (tap water)	2.86	3.09	0.48	0.43	1.39	1.59
B1	3.84	4.27	0.59	0.57	1.65	1.74
B2	4.37	4.80	0.66	0.64	1.74	1.79
Fe1	3.29	3.52	0.55	0.50	1.44	1.64
Fe2	3.79	4.02	0.61	0.56	1.58	1.71
Zn1	3.36	3.59	0.57	0.53	1.55	1.69
Zn2	4.01	4.34	0.64	0.61	1.63	1.75
Fe1+Zn1+B1	5.09	5.52	0.74	0.69	1.86	1.83
Fe2+Zn2+B2	4.65	5.08	0.70	0.67	1.84	1.80
LSD at 5%	0.52	0.50	0.05	0.04	0.07	0.03

\* B1: boron at 50 ppm; B2: boron at 100 ppm; Fe1: iron at 100 ppm; Fe2: iron at 200 ppm; Zn1: zinc at 100 ppm and Zn2: zinc at 200 ppm.

(54.17 and 60.47%) and potassium content by (33.81 and 15.09%) compared with the control in the 1<sup>st</sup> and 2<sup>nd</sup> seasons, respectively followed by the combination treatment of B2 + Fe2 + Zn2 with no significant difference between these two treatments. The same results were observed by Khalifa *et al.* (2011) that foliar application of boron and zinc significantly increased N, P and K concentrations in iris. Also, Khuong *et al.* (2022) on sesame seeds. According to Kumar *et al.* (2019) plants require micronutrients as boron for translocation of phosphorus and metabolism of nitrogen as it enhances nitrate levels. Spraying of micronutrients in the field increased N<sub>2</sub> fixation which lead to increase grain N contents in chickpea and lentils (Yanni, 1992). Boron, iron and zinc improvement the growth processes and seed yield resulted in improvement in nutrient concentrations.

**d. Micronutrient content (ppm):**

The results from Table (10) showed a significant influence of the micronutrients and their combinations of foliar application on boron, iron and zinc contents in the seeds of chia in both seasons except treatments of Fe1 (100 ppm) on boron content and zn1 (100 ppm) on iron content, these two

treatments had no significant differences when compared with the control. The application of the combination treatment of B1 + Fe1 + Zn1 significantly promoted the boron content by (81.88 and 76.29%), iron content by (36.97 and 39.23%) and zinc content by (72.58 and 85.25%), compared to the control in the 1<sup>st</sup> and 2<sup>nd</sup> seasons, respectively followed by the combination treatment of B2 + Fe2 + Zn2 with no significant difference between these two treatments. Foliar spray of micronutrients significantly affected micronutrient accumulation. The increase in boron, iron and zinc concentrations with the combined foliar application of micronutrients might be related with the enhanced bioavailability of these nutrients and synergistic effect between them. According to Ahmed *et al.* (2011) B have a positive correlation on Fe and Zn. Our results were supported by the findings of Ravi *et al.* (2008) on safflower and Venugopalan *et al.* (2022) on lentil.

**e. Total carbohydrate and protein content (%):**

The results shown in Table (11) revealed that, spraying micronutrients solely or in combinations significantly increased total carbohydrate content (%) and protein content (%). Where, the best results for total

**Table 10. Effect of some micronutrients and their combinations on boron, iron and zinc contents (ppm) in seeds of chia plant during 2020/2021 and 2021/2022 seasons.**

Treatments	Boron (ppm)		Iron (ppm)		Zinc (ppm)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Control (tap water)	18.71	20.04	5.22	5.48	2.59	2.17
B1	27.19	28.79	5.80	6.20	3.46	3.04
B2	30.63	32.33	6.30	6.63	4.01	3.69
Fe1	19.64	21.34	5.87	6.19	3.11	3.28
Fe2	23.70	25.40	6.15	6.58	3.43	3.44
Zn1	21.60	23.30	5.36	5.65	3.70	2.69
Zn2	24.21	25.91	5.90	6.30	3.36	3.01
Fe1+Zn1+B1	34.03	35.33	7.15	7.63	4.47	4.02
Fe2+Zn2+B2	31.80	33.60	6.77	7.22	4.21	3.79
LSD at 5%	2.53	2.65	0.40	0.34	0.20	0.17

\* B1: boron at 50 ppm; B2: boron at 100 ppm; Fe1: iron at 100 ppm; Fe2: iron at 200 ppm; Zn1: zinc at 100 ppm and Zn2: zinc at 200 ppm.

**Table 11. Effect of some micronutrients and their combinations on total carbohydrate content and protein content of chia plant during 2020/2021 and 2021/2022 seasons.**

Treatments	Total carbohydrate content (%)		Protein content (%)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Control (tap water)	13.67	13.53	17.88	19.31
B1	15.71	15.18	24.00	26.69
B2	16.43	16.22	27.33	30.02
Fe1	14.80	14.94	20.54	21.98
Fe2	17.56	17.75	23.69	25.13
Zn1	13.42	13.82	20.98	22.42
Zn2	14.98	15.43	25.06	27.13
Fe1+Zn1+B1	19.77	20.36	31.81	34.50
Fe2+Zn2+B2	18.04	18.58	29.06	31.75
LSD at 5%	1.05	1.31	1.68	1.11

\* B1: boron at 50 ppm; B2: boron at 100 ppm; Fe1: iron at 100 ppm; Fe2: iron at 200 ppm; Zn1: zinc at 100 ppm and Zn2: zinc at 200 ppm.

carbohydrate content (%) and protein content (%) were obtained in plants treated with the combinations treatment of Fe1 + Zn1 + B1 as a foliar application improved them by (44.62 and 50.48%) and (77.91 and 78.66%) in comparison to the control in 1<sup>st</sup> and 2<sup>nd</sup> seasons, respectively. The increment of total carbohydrates (%) may be indirectly attributed to the effect of micronutrients on increasing the photosynthesis as a result of the increasing pigment content in the leaves. Obviously, any factor causes increase in

plant pigments rising carbohydrate content. Also, boron act as protector to sugars from polymerization and hence they are more available for translocation. These results are in agreement with the findings of Öztürk *et al.* (2010) on canola and Hemantaranjan and Trivedi (2015) on soybean. Increasing in protein content may be due to the role of micronutrients in nitrogen metabolism. Where, boron caused a synergistic effect on nitrogen uptake, enhanced nitrogen metabolism, amino acids which is directly

linked with accumulation of amino acids, RNA, and synthesis of protein hence, it increases the protein content while the role of zinc in RNA metabolism and ribosomal content in plant cells, leading to the stimulation of carbohydrates metabolism and proteins synthesis. Similar results were reported by Rajae and Ziaei (2009) on corn, Farokhi *et al.* (2014) on sunflower and Amirani and Kasraei (2015) on mungbean who pointed that iron, boron and zinc lonely or a mixture increase protein content in studied plants.

**f. Total phenolic content in the seeds and oil antioxidant activity:**

Data in Table (12) cleared a positive effect in total phenolic content and oil antioxidant activity by foliar application of different micronutrients and their combinations. Spraying plants with combination of B2 + Fe2 + Zn2 was the most effective treatment and resulted in improvement total phenolic content by (45.74 and 45.68 %) and antioxidant activity of oil by (33.49 and 33.46%) over the control in the two seasons, respectively followed by the combination of B1 + Fe1 + Zn1 and B2 treatments. This effect may be related to the role of boron in the metabolism of phenolic compounds (Wojcik and Wojcik, 2006)

while Zn acts as an activation factor in antioxidant enzymes, involved in carbon assimilation, scavenging of free radicals by protect the membrane proteins and lipids, effect on photosynthetic carbon metabolism and translocation in relation to secondary metabolite biosynthesis and its role in the carbon allocation to produce phenolic compounds in shikimic acid and acetate pathways (Misra *et al.*, 2005). Plants that own high phenol content exhibit high antioxidant activity (Mumivand *et al.*, 2017). The results were in harmony with Mumivand *et al.* (2021) on *Satureja khuzistanica* Jamzad and Meriño-Gergichevich *et al.* (2020) on blueberry who reported a positive effect of boron and zinc on the total phenol content and antioxidant activity in the studied plants. So, micronutrients act as an antioxidant promoter.

**CONCLUSION**

According to our findings, foliar application of the combination of boron (50 ppm) + iron (100 ppm) + zinc (100 ppm) could be a promising way to improve growth and yield of chia plant (*Salvia hispanica* L.) as it improved seed yield, oil yield, chemical composition, fixed oil production. In addition, it improved the quality of the oil as it increased the concentration of unsaturated

**Table 12. Effect of some micronutrients and their combinations on total phenolic content in seeds and antioxidant activity of the oil in seeds of chia plant during 2020/2021 and 2021/2022 seasons.**

Treatments	Total phenolic content (mg GAE/100 g DW)		Oil antioxidant activity (%)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Control (tap water)	33.49	39.05	53.29	57.02
B1	38.02	44.32	59.06	63.18
B2	42.21	49.18	63.37	67.80
Fe1	35.41	42.46	55.46	59.36
Fe2	37.17	45.74	57.73	61.78
Zn1	36.44	43.26	57.17	61.20
Zn2	39.25	47.32	60.22	64.42
Fe1+Zn1+B1	44.42	51.81	68.46	73.25
Fe2+Zn2+B2	48.81	56.89	71.14	76.10
LSD at 5%	1.02	1.36	1.99	2.02

\* B1: boron at 50 ppm; B2: boron at 100 ppm; Fe1: iron at 100 ppm; Fe2: iron at 200 ppm; Zn1: zinc at 100 ppm and Zn2: zinc at 200 ppm.

fatty acids especially linolenic acid, the main component. The application of a combination of boron (100 ppm) + iron (200 ppm) + zinc (200 ppm) significantly improved total phenols and oil antioxidant activity.

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### دور بعض العناصر الصغرى في تحسين إنتاجية وجودة البذور في نباتات التشاي (*Salvia hispanica* L.) في الأراضي الرملية

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نبات التشاي من النباتات التي لها فوائد غذائية وطبية وهي حديثة الزراعة في مصر. الهدف من إجراء هذه الدراسة هو إمكانية تحسين النمو ومحصول البذور والمحتوى الكيماوي وإنتاجية الزيت والخواص الكيميائية للزيت الثابت لنبات التشاي بواسطة الرش الورقي بالعناصر الصغرى تحت ظروف الأرض الرملية. أجريت التجربة الحقلية في مزرعة التجارب بمحطة بحوث البساتين بالقصاصين، محافظة الاسماعيلية. وكانت المعاملات كالتالي: الكنترول (ماء الصنبور)، الرش بالبورون ٥٠ و ١٠٠ جزء في المليون، الرش بالحديد ١٠٠ و ٢٠٠ جزء في المليون، الرش بالزنك ١٠٠ و ٢٠٠ جزء في المليون، الرش بالبورون والحديد والزنك ٥٠ و ١٠٠ و ١٠٠ جزء في المليون بالترتيب، الرش بالبورون والحديد والزنك ١٠٠ و ٢٠٠ و ٢٠٠ جزء في المليون بالترتيب. أعطت النباتات التي تم رشها بالعناصر الصغرى سواء منفردة أو مجتمعة زيادة معنوية لكل الصفات التي تم دراستها وهي كالتالي: النمو الخضري، محصول البذرة، نسبة الزيت الثابت ومحصول الزيت، مكونات الزيت الثابت (الأحماض الدهنية)، كلوروفيل أ، ب والكاروتينويدات، نسبة العناصر الكبرى والصغرى، الكربوهيدرات الكلية، محتوى البروتين، محتوى الفينولات الكلية والنشاط المضاد للأكسدة للزيت خاصة معاملة الرش بالبورون والحديد والزنك ٥٠ و ١٠٠ و ١٠٠ جزء في المليون بالترتيب معاً تليها معاملة الرش بالبورون والحديد والزنك ١٠٠ و ٢٠٠ و ٢٠٠ جزء في المليون بالترتيب معاً. والجدير بالذكر أنه لا يوجد إختلاف معنوي بين هاتين المعاملتين في كثير من الصفات. أظهرت البيانات الخاصة بالتحليل الكروموتجرافي لمكونات الزيت الثابت لنبات التشاي أنه يتكون من ٧ أحماض دهنية وكانت المكونات الكبرى للزيت الثابت هي حمض اللوريك وحمض الميريستيك وحمض البالميستيك وحمض الأوليك وحمض اللينوليك وحمض اللينولينك وحمض الاراشيستيك. وكان المكون الرئيسي للزيت الثابت هو حمض اللينولينك (٥٥,٣٤ - ٦١,٤٣٪) أوضحت النتائج أن أعلى نسبة من حمض اللينولينك كانت مع معاملة الرش بالبورون والحديد والزنك ٥٠ و ١٠٠ و ١٠٠ جزء في المليون بالترتيب سوياً. بينما كانت أعلى نسبة محتوى الفينولات الكلية في البذور والنشاط المضاد للأكسدة للزيت كانت مع معاملة الرش بالبورون والحديد والزنك ١٠٠ و ٢٠٠ و ٢٠٠ جزء في المليون بالترتيب سوياً. ولهذا فإن استخدام الرش الورقي بالعناصر الصغرى كالبورون والحديد والزنك أدى إلى تحسين إنتاجية بذور نبات التشاي ومكوناتها تحت ظروف الأراضي الرملية.