

RESPONSE OF THE SEED YIELD OF CHIA PLANT TO NPK FOLIAR FERTILIZATION AND BIOFERTILIZER MIXTURE

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ABSTRACT

The cultivation of *Salvia hispanica*, commonly known as chia, is a recent addition to Egyptian agriculture aimed at diversifying the cultivation system with new medicinal and aromatic plant species or varieties. Chia is an annual herbaceous plant belonging to the mint family (*Lamiaceae*) and originates from southern Mexico and Northern Guatemala. Chia seeds show potential as a rich source of antioxidants due to their omega-3 content and the presence of polyphenols, chlorogenic acid, caffeic acids, myricetin, quercetin, and kaempferol. This investigation aimed to assess the impact of foliar fertilization with NPK and/or a combination of biofertilizer (*Azotobacter chroococcum* + *Bacillus megaterium* + *Bacillus subtilis*) on the seed yield of the chia (*S. hispanica*) plant. The findings indicated a significant increase in the seed yield of the chia plant with various treatments of NPK and/or a combination of biofertilizer.

Keywords: Chia seeds, *Salvia hispanica*, biofertilizer, NPK, foliar fertilization.

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INTRODUCTION

Chia (*Salvia hispanica*) plant is a newly introduced crop to cultivation under the Egyptian environment. It aims to enrich the region with new species or varieties of medicinal and aromatic plants. *Salvia hispanica*, commonly known as chia (Ali *et al.*, 2012), is an annual herbaceous plant belonging to the mint family (*Lamiaceae*) and is native to southern Mexico and northern Guatemala (Yeboah *et al.*, 2014; Silva *et al.*, 2016). Due to its ability to grow in arid environments, it has been highly recommended as an alternative crop for the field crop industry (Peiretti and Gai 2009). The crop cycle duration in most cases ranges from 140 to 180 days (Ayerza and Coates 1996; De Kartzow 2013).

Chia seeds were mechanically harvested under low-input conditions, with an average yield of around 600 kg/ha, but it can reach up to 1200 kg/ha. In high-input conditions, including irrigation and fertilization, experimental trials in Argentina have demonstrated yields as high as 2500 kg/ha (Coates 2011). Chia is also considered an interesting forage crop in Greece (Bilalis *et al.*, 2016) and is well-suited for Mediterranean and desert climates, as observed in Chile (Baginsky *et al.*, 2016; Cortes *et al.*, 2017). The European Commission has approved the use of chia seeds in bread products, with a limit of not more than 5%. Currently, chia seeds and its oil are utilized in various applications such as breakfast items, cookie snacks, fruit juices, cakes, and yogurt (Borneo *et al.*, 2010).

Chia has the potential to play a role in reducing the risk of chronic degenerative diseases (Rosa *et al.*, 2017). The lipid content in chia seeds varies from 25% to 40%, with 60% of the total lipids consisting of alpha-linolenic acid (ALA, omega-3) and 20% composed of linoleic acid (omega-6) (Bresson *et al.*, 2009; Coorey *et al.*, 2012; Salas *et al.*, 2014). Chia seeds are a promising source of antioxidants due to their content of omega-3 and the presence of polyphenols, chlorogenic acids, caffeic acids, myricetin, quercetin and kaempferol (Reyes *et al.*, 2008; Ixtaina *et al.*, 2011 a,b). Chia seeds and oil also represent a rich source of compounds which have

beneficial effects on human health such as vitamin B (**Bushway *et al.*, 1984**). One of the most promising features of chia seeds is the 5–6 % content of fiber, which can be used as dietary fiber (**Ayerza and Coates 2001; Reyes *et al.*, 2008**). Chia seeds contain all the essential amino acids, so they have a better protein quality than cereals and the other oily seeds (**Nitrayová *et al.*, 2014**).

Nitrogen, phosphorus and potassium are macronutrients that play essential roles in various plant processes. Nitrogen is the main yield-limiting mineral nutrient, participating in numerous physiological and biochemical plant processes. It serves as a structural component of amino acids, nucleic acids, enzymes, proteins, chlorophyll, and cell walls. Phosphorus is also a highly required macronutrient, playing vital roles in energy transfer, cell membranes, nucleic acids phospholipids, and co-enzymes. Potassium increases plant resistance to diseases and prevents excessive water loss. Overall, NPK fertilizers provide plants with macro-elements necessary for both growth and yield (**Wiedenhoeft 2006, Fageria 2009; Ezz El-Din and Hendawy 2010**).

The application of N, P, and K fertilizers at different growth stages has been shown to significantly impact root growth and the concentration of bioactive compounds in *Salvia miltiorrhiza* (**Lu *et al.*, 2013**). In a study on chia, **Jeena *et al.*, (2018)** found that plants treated with (90:60:75 kg NPK/ha) produced a significantly higher yield (676.58 kg/ha). Biofertilizers are eco-friendly inputs and are less damaging to the environment than chemical fertilizers use (**Gentili and Jumpponen, 2006**). Biofertilizers are substances that contain living microorganisms. When applied to seeds, plant surfaces, or soil, they colonize the rhizosphere or the interior of the plant, promoting growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. The microorganisms in biofertilizers, as highlighted by (**Kapoor *et al.*, 2015**), play a crucial role in restoring the soil's natural nutrient cycle and building soil organic matter. They are extremely

advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying organic nutrients through microorganisms and their products. Importantly, biofertilizers do not contain any harmful chemicals to the living soil (Vessey 2003).

The addition of a mixture of biofertilizers (*Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Bacillus megaterium*) along with chemical fertilizer resulted in increased measured traits compared to the application of either biofertilizer or chemical fertilizer alone in fennel plants (Mahfouz and Sharaf-Eldin, 2007). Al-Fraihat *et al.* (2011) reported that *Majorana hortensis* L., plant recorded the maximum values of herb fresh and dry yield when biofertilizers (Nitraboein and Halex-2 at a rate of 988 g/ha) were used. Also, El Sebai *et al.* (2019), in a study on flax plants, found that the highest oil percentage (27.29%) was obtained through the interaction between biofertilizer and chemical fertilizer. As chia is a new crop for Egyptian cultivation, there is a lack of information on its optimal agronomic management practices, particularly in terms of fertilization management. This study was carried out to evaluate the effect of foliar fertilization of NPK and/or mixture of biofertilizer (*Azotobacter chroococcum* + *Bacillus megaterium* + *Bacillus subtilis*) on the seeds yield of chia (*S. hispanica*) plant.

MATERIALS AND METHODS

Study location

This study was carried out on a private farm at the Hawareya location, Beheira Governorate (Northwest of the Nile Delta), Egypt, during the two successive seasons (2016/2017 and 2017/2018).

Physico-chemical characteristics

The physical and chemical characteristics of the experimental soil and water irrigation were determined before cultivation according to **Jackson (1973)** and the results are presented in **Table (1 and 2)**. The physical and chemical analyses of the soil used for this experiment revealed that the soil is clay loam soil in texture.

Table (1): Some physical and chemical properties of the experimental soil sites during 2016/2017 and 2017/2018 seasons.

Physical Properties	First Season	Second Season
Clay (%)	48.60	48.60
Silt (%)	29.40	29.56
Sand (%)	22.00	21.84
Soil Texture	Clay Loam	Clay Loam
Organic Matter (%)	1.88	1.94
Chemical Properties		
pH (1 soil:2.5 water suspension)	7.77	7.80
EC(ds/m ³ at 25°C)	1.80	1.82
(meq/L):		
Ca ⁺²	9.40	11.20
Mg ⁺²	5.20	7.10
Na ⁺¹	10.10	10.30
K ⁺¹	0.91	1.15
HCO ₃ ⁻	5.46	7.93
Cl ⁻	8.51	9.14
Available macronutrients (ppm)		
N	145	155
P	46	40
K	310	290
Available micronutrients (ppm)		
Fe	18.30	18.20
Zn	1.10	1.12
Mn	7.56	7.52
Cu	13.55	13.18

The analysis was carried out at the National Research Centre (NRC), Egypt.

Table(2):Chemical composition of water used for irrigation of soil during 2016/2017 and 2017/2018 seasons.

Season	pH	EC mmohos /cm	Soluble ions (meq / L)						SAR	
			Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	HCO ₃ ⁻	Cl ⁻		SO ₄ ⁻
First Season	6.9	0.81	3	2.1	2.74	0.23	1.6	1.8	4.7	2
Second Season	7.3	0.89	3.2	1.9	2.86	0.28	1.8	1.66	4.5	

SAR: sodium adsorption ratio.

-The analysis was carried out at the National Research Centre (NRC), Egypt.

Experimental design

The experiment was laid out in a split-plot design within a randomized complete blocks design, comprising 20 treatments with three replicates for each treatment. The ten NPK treatments control, 1.5, 3, and 4.5 g/l of NPK and biofertilizer. Treatments were applied as 2 and 2 sprays. {1 (control), 2 (1.5 g/l one portion), 3 (3 g/l one portion), 4 (4.5 g/l one portion), 5 (1.5 g/l two portions), 6 (3 g/l two portions), 7 (4.5 g/l two portions), 8 (1.5 g/l three portions), 9 (3 g/l three portions), 10 (4.5 g/l three portions)} were randomly distributed in the main plots; whereas, the two biofertilizer foliar applications, namely (Nil and Bio) were randomly distributed in the sub-plots. The experiment repeated three times.



Fig.1: Chia (*Salvia hispanica*) plant.

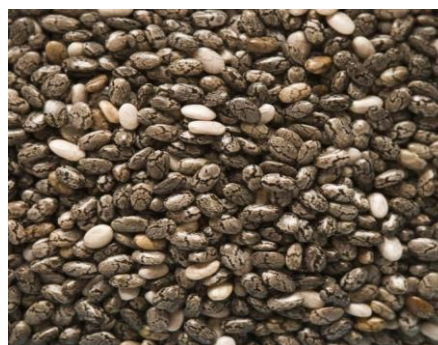


Fig. 2: Chia seeds.

Plant source and description

The seeds of chia (*Salvia hispanica*) were imported from Original Hanoju Deutschland UG Company, Germany. *Salvia hispanica*, commonly known as chia, is an annual herbaceous plant belonging to the mint family (*Lamiaceae*) and is native to southern Mexico and northern Guatemala. The crop cycle duration in most cases ranges from 140 to 180 days. Chia seeds were mechanically harvested under low-input conditions, with an average yield of around 600 kg/ha. The lipid content in chia seeds varies from 25% to 40%, with 60% of the total lipids consisting of alpha-linolenic acid (ALA, omega-3) and 20% composed of linoleic acid (omega-6). Chia seeds are a promising source of antioxidants due to their content of omega-3 and the presence of polyphenols, chlorogenic acids, caffeic acids, myricetin, quercetin and kaempferol.

Fertilizers

All treatments were received 15 m³/fed of cattle manure + 300 kg/fed super phosphate (15.5% P₂O₅) during preparation and hoeing the soil while ammonium nitrate (33.5% N) at the rate of 200 Kg/fed was added after 21 days from sowing. Chia seeds were directly sown in hills at distance of 30 cm between hills and intro-row spacing of 60 cm on 15th of October in both seasons (2016-2017 and 2017-2018), after 5 weeks of planting, some plots were sprayed with Nitrophoska foliar fertilizer from Shoura Chemical Company, Egypt with different levels that added at different portions at different dates of application for the same level of fertilizer. Three different amounts of foliar fertilizer (1.5, 3 and 4.5 g/l) were used. Each dose of foliar fertilizer was applied either once (at 50 days from sowing) or was divided into two equal portions (added at 35 and 50 days after sown) or was divided into three equal portions (added at 35, 50 and 65 days after sown). The chemical composition of the used foliar fertilizer is presented in **Table (3)**. After 45 days from sowing, some plots were sprayed with a mixture of biofertilizers introduced from National

Research Centre (NRC), Egypt consists of (*Azotobacter chroococcum* 10 g/l+ *Bacillus megaterium* var. phosphaticum 10 g/l+ *Bacillus subtilis* 10 g/l) were grown according to (Abd El- Malek and Ishac 1968; Dobereiner *et al.*, 1976), on two portions and the second portions was applied after 75 days from sowing date. The mixed cultures of bacterial species containing 1×10^6 colony forming unit's ml⁻¹, were used for plant inoculation.

Table (3): The chemical composition of the used foliar fertilizer (Nitrophoska).

Macronutrients (%)					Micronutrients (ppm)						
N	P	K	Mg	S	Mn	Fe	Cu	Zn	B	Mo	
20	19	19	0.5	0.3	1000	1000	400	380	130	30	

Data of the present study were statically analyzed as a split plot system in randomized complete blocks design using the General Linear Models procedure of the Costat program (version 6.4; CoHort Company, Birmingham, UK, 1998 –2008) by (Cardinali and Nason 2013). Mean comparisons were assumed by Duncan's multiple-range test (DMR) (Duncan 1955 ; Snedecor and Cochran 1967)

RESULTS AND DISCUSSION

The results of chia seeds weight (g/plant and kg/fed) as affected by foliar fertilization doses in the first and the second seasons are shown in Table (4).

Effect of NPK fertilizer

NPK foliar fertilizer application resulted in significant increase in seeds weight in both seasons.

Table (4): Effect of NPK foliar fertilization and biofertilizer (Bio) on seed production of *Salvia hispanica* plants during the two seasons (2016/2017 (S1) and 2017/2018 (S2)).

Treatments		Seeds weight (g/plant)		Seeds weight (kg/fed)	
NPK	Bio	S1	S2	S1	S2
*1	-	6.16 f	7.50 e	143.73 f	174.99 e
2	-	8.89 c	9.44 cd	207.43 c	220.26 cd
3	-	9.71 b	10.17 c	226.10 c	237.30 c
4	-	8.84 c	9.19 d	206.26 c	214.43 d
5	-	7.80 e	7.58 e	182.00 e	176.86 e
6	-	11.37 a	16.22 a	265.30 a	378.46 a
7	-	8.69 cd	7.86 e	202.76 cd	183.40 e
8	-	8.88 c	7.92 e	207.20 c	184.80 e
9	-	10.35 b	11.49 b	241.50 b	268.10 b
10	-	8.02 de	7.25 e	187.13 de	169.16 e
	Nil	7.87 b	7.50 b	177.63 b	175.09 b
	Bio	9.93 a	11.42 a	227.79 a	266.48 a
1	Nil	5.72 g	6.00 h	133.46 g	140.00 h
	Bio	6.61 fg	9.00 c-f	154.23 fg	210.00 c-f
2	Nil	7.77 ef	8.11 e-g	181.30ef	189.23 e-g
	Bio	10.01bc	10.77 ab	233.56 bc	251.30 ab
3	Nil	8.08 de	9.44 c-e	188.53 de	220.26 c-e
	Bio	11.33 ab	10.91 ab	264.02 ab	254.56 ab
4	Nil	8.47 c-e	8.89 c-f	197.63 c-e	207.43 c-f
	Bio	9.20 b-d	9.50 cd	214.66 b-d	221.66 cd
5	Nil	7.36 ef	6.83 gh	171.73 ef	159.36 gh
	Bio	8.25 de	8.33 d-f	192.50 de	194.36 d-f
6	Nil	10.33 bc	8.22 d-f	241.02 bc	191.80 d-f
	Bio	12.88 a	24.22 a	300.53 a	565.13 a
7	Nil	7.46 ef	5.50 h	174.06 ef	128.33 h
	Bio	9.92 b	10.22 c	231.46 b	238.46 c
8	Nil	8.07 de	5.83 h	188.30 de	136.03 h
	Bio	9.69 bc	10.00 c	226.10 bc	233.33 c
9	Nil	7.96 ef	9.44 c-e	185.73 ef	220.26 c-e
	Bio	12.74 a	13.54 b	297.26 a	315.93 b
10	Nil	7.43 ef	6.78 gh	173.36 ef	158.20 gh
	Bio	8.62 b-e	7.72 fg	201.13 b-e	180.13 fg

Means with the same letters in each column indicate no significant difference between treatments at the 5% level of probability. *1(control), 2 (1.5 g/l one portion),3 (3 g/l one portion),4 (4.5 g/l one portion), 5 (1.5 g/l two portions), 6 (3 g/l two portions),7 (4.5 g/l two portions), 8 (1.5 g/l three portions), 9 (3 g/l three portions),10 (4.5 g/l three portions).

Foliar application of NPK at 3g/l divided into two portions, showed the best seed weight of plants (11.37 g/plant and 265.30 kg/fed) for 1st season and (16.22 g/plant and 378.46 kg/fed) for 2nd season. It is clear from data in Table (4) that control plants gave the mean value of (6.16 g/plant and 143.73 kg/fed) for 1st season and (7.50 g/plant and 174.99 kg/fed) for 2nd season. The increment in seeds weight (g/plant and kg/fed) as a result of the application of NPK 3g/l (2 portions) treatment reached to (116.27%) than the control for 2nd season, while in the first season it reached (84.58%). These results are in harmony with those reported by **Yeboah *et al.*, (2014)** on chia plants. Also, **Hashemi and Mojaddam, (2015)** showed that triple super phosphate increased the seed yield of sesame. **Jeena *et al.*, (2018)** found that fertilization with 90:60:75 kg NPK/ha gave the best result of seed yield (623.60 kg/ha). They added that the variation in yield was associated with the variation in plant population and number of spikes produced, as well as difference in the amount of available nutrients in the rhizosphere of plant system. **Abumere *et al.*, (2019)**, in a study on sunflower (*Helianthus annuus* L.) reported that the maximum mean value of seed yield was 1.21 ton/ha by using 30 kg N/ha.

Parhizkar *et al.*, (2012) reported that the highest grain yield of flax has been achieved with 120 kg phosphorus /ha. As is well known, phosphorus is a necessary nutrient that contributes to the storage and transfer of chemical energy in plants, accelerates growth, and promotes increased flowering and early maturity (**Hashemi and Mojaddam, 2015**). The increase in the seed yield could be attributed to the effect of phosphorus on growth and development. It might be due to the noticeable increases in growth criteria of plant, which gave a chance to the plant to carry more flowers, pods and hence more seeds. Moreover, marked increases in the photosynthetic pigments content, which could lead to an increase in photosynthesis, resulting in greater transfer of photo-assimilates to the seeds and leading to an increase in the weight of grains, as well as due to transfer of more assimilates into the grains (**Sadiq *et al.*, 2017**).

Effect of biofertilizer

The results of seed weight in chia plants (g/plant and kg/fed) as affected by biofertilizer in the first and the second seasons are shown in **Table (4)**. Biofertilizer application resulted in significant increase in seeds weight in both seasons as shown in **Table (4)**. The mean increment values in seed weight (g/plant and kg/fed) were (26.18%) for 1st season and (52.27%) 2nd season, compared to the control plants. These results were in agreement with those reported by **Yadav and Khurana (2000)** on fennel plants, as they reported that seed treatment with *Azotobacter* improved umbels/plant, seeds/umbel and total seed yield. **Gad (2001)** found a significant increase in fruit weight/plant as a result of using biofertilizers on *Foeniculum vulgare* and *Anethum graveolens*.

Kandeel et al., (2004) recorded a significant increase in fruits weight/plant due to using biofertilizers (Biogene, Nitrobenzene and Serialene) on *Anethum graveolens* and *Foeniculum vulgare*. **El-Gendy et al., (2012)** studied the effect of different levels of cattle manure and biofertilizers (phosphorein and/or nitrobenzene) as well as their interactions on the growth, sepals and seeds yield on roselle plants and they found that the interactions treatments between cattle manure (30 m³/fed.). The highest values of sepals yield were obtained when combined with biofertilizers alone or in a mixture.

Effect of interaction between NPK and biofertilizer

The results of chia seed weight (g/plant and kg/fed) as affected by foliar fertilization and/or biofertilizer in the first and the second seasons are shown in **Table (4)**. Seeds weight (g/plant and kg/fed) responded significantly to interaction between NPK and biofertilizer in both seasons as shown in **Table (4)**.

The combined treatment of NPK 3g/l (2 portions) + biofertilizer gave the maximum mean values (12.88 g/plant and 300.53 kg/fed) for 1st season and (24.22 g/plant and 565.13 kg/fed) for 2nd season. Such increase in seed weight as a result of NPK 3g/l (2

portions) + biofertilizer treatment reached (122.73%) for 1st season and (303.67%) for 2nd season than the control plants. **Abd-El-Raaof et al., (2013)** on *Nigella sativa* L. revealed that fertilization with mineral NPK exhibited the best vegetative growth parameters and the highest number of capsule/plant, seed yield /plant and /fed. Also, **Jafari et al., (2015)** found significant effect of chemical fertilizer and biofertilizer application on all measured traits on *Salvia officinalis*. Also, our results were in line with those reported by **Coates (2011)**, on *Salvia hispanica* L. In low input conditions, the average seed yield of chia was around 600 kg/ha, but it could reach up to 1200 kg/ha. Conversely, under high input conditions with irrigation and fertilization, seed yield as high as 2500 kg/ha have been demonstrated in experimental trials in Argentina. **Buriro et al., (2015)**, in a study on sunflower, showed that the best mean value of seed yield was (2017.74 kg/ ha) by using 6 ton/ha poultry manure + 90:45:45 kg NPK /ha. **Jeena et al., (2018)** on chia (*Salvia hispanica* L.) found that plants treated by (90:60:75 kg NPK /ha) produced significantly the highest yield (676.58 kg/ha). **El Sebai et al., (2019)** on flax plant found that, the maximum seed yield was (771.25 kg/fed) by using the interaction between Phosphate Solubilizing Bacteria (PSB) and Single Super Phosphate (SSP) 120 Kg/ha.

The findings of our investigation revealed a notable impact of chemical fertilizers on the measured traits related to both vegetative and yield growth. Macronutrients such as nitrogen, phosphorus, and potassium played essential roles in numerous plant processes. Nitrogen, identified as the primary yield-limiting mineral nutrient, actively participated in various physiological and biochemical plant processes, serving as a structural component of amino acids, nucleic acids, enzymes, proteins, chlorophyll, and the cell wall. Phosphorus, being a highly essential macronutrient, plays crucial roles in energy transfer, cell membranes, nucleic acids, and various other key compounds. Additionally, potassium has been reported to participate in rapid cell division (**Belorkar et al., 1992, Wiedenhoft 2006, Tiessen 2008, Fageria 2009 and Ezz El-Din and Hendawy 2010**).

CONCLUSION

Based on the results, it can be concluded that the optimal treatment for achieving the highest seed yield of *S. hispanica* is the application of NPK at a concentration of 3 g/l (applied in two portions) along with biofertilizer under the environmental conditions of Behira Governorate and similar regions.

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الملخص العربي

إستجابة المحصول البذري لنبات الشيا للتسميد الورقي NPK ومخلوط السماد الحيوي

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أجريت التجربة الحقلية الخاصة بهذه الدراسة في موسمين متتاليين (2016-2017، 2017-2018) على نبات الشيا كأحد المحاصيل الحديثة التي تم إدخالها مؤخراً في الزراعة المصرية وهو من النباتات الطبية الهامة المنتشرة بالخارج وذلك لإثراء الزراعة المصرية بأنصاف جديدة من النباتات الطبية والعطرية، تعد بذور الشيا مصدراً واعداً لمضادات الأكسدة نظراً لمحتواها العالي من أوميغا 3 ، البوليفينول ، حمض الكلوروجينيك ، الكافيين ، الميريستين ، الكيرسيتين والكامفيرول. وكان الهدف من التجربة هو دراسة إستجابة المحصول البذري لنبات الشيا للتسميد الورقي بإستخدام سماد NPK المركب وكذلك مزيج من الأسمدة الحيوية والتداخل بينهما. أظهرت النتائج أن المعاملات المختلفة لسماد NPK و/أو خليط الأسمدة الحيوية أدت إلى زيادة معنوية في إنتاجية بذور نبات الشيا. من النتائج المذكورة ومناقشتها ومن الناحية الإقتصادية، يوصي بتسميد نبات الشيا (*Salvia hispanica*) بالرش الورقي 3جم/ لتر NPK (تضاف على مرتين) + سماد حيوي لتحقيق أفضل إنتاجية من محصول البذور وذلك تحت الظروف المشابهة لمحافظة البحيرة- مصر.

الكلمات المفتاحية: بذور الشيا، سالفيا هيسبانيكا، الأسمدة الحيوية، سماد مركب NPK، التسميد الورقي.