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Effect of Saline Water Stress in the Presence of Silicon Foliar Application on Growth, Productivity and Chemical Constituents of Chia (*Salvia hispanica* L.) under Egyptian Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. Author WMAM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ASMY, YFYM and MAEGE managed the analyses of the study. Author MAAEW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The pot experiment was carried out during two successive seasons (2016/2017 & 2017/2018) to study the effect of five levels of salinity and three levels of silicon (Si), as a foliar-spray application as well as their combinations on growth, productivity and chemical constituents of chia (*Salvia hispanica* L.) plants. Results showed that there was a negative relationship between vegetative growth measurements i.e., plant height, fresh weight and dry weight, flowering growth and yield parameters i.e., main inflorescence height, main inflorescence weight, inflorescences weight, seeds weights, weight of 1000 seeds and calculated seeds yield /m^2 and root growth measurements i.e. root length, root weight and root diameter values and salinity treatments in both seasons. Hence, as the concentrations of salinity increased, the values of these parameters decreased to reach the maximum decreasing at the high concentration (4.69 dS m^{-1}). Therefore, the combination treatment

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between 0.68 dS m^{-1} salinity concentration and 2000 ppm silicon scored the highest values of these parameters, in the 1st and 2nd seasons. Meanwhile, the maximum values of N, P and K contents were recorded by the combination treatment between 0.68 dS m^{-1} salinity concentration and 2000 ppm silicon in both seasons. Whereas, the highest values of free proline, sodium and chloride content were gained by the high concentration of salinity 4.96 dS m^{-1} especially those received silicon at 0 ppm in both seasons. Conclusively, the highest growth, productivity and chemical constituents of chia (*Salvia hispanica* L.) plant, it is preferable to grow the plants under saline water irrigation concentration at (0.68 dS m^{-1}) and spray with silicon at 2000 ppm.

Keywords: Chia; Salvia hispanica; saline water; salinity; silicon.

1. INTRODUCTION

Salvia hispanica L. belongs to family Lamiaceae as an annual plant, and its center of origin is between Mexico and Guatemala [1,2].

The seed contains from 25% to 40% oil with 60% of its comprising α-linolenic acid and 20% of linoleic acid. Chia (*Salvia hispanica* L.) can grow up to 60 – 180 cm tall and has opposite arranged leaves. Chia flowers are small flower (3-4 mm) with small corollas and fused flower parts that contribute to a high self-pollination rate, presents a subangular ramified stem, leaves with different degrees of pubescence, blue or white flowers, and very small indescent dry fruits commonly called seeds, which have varied colors from black, grey, and black spotted to white, the shape is oval with size ranging from 1 to 2 mm [3-8].

Chia seed is composed of protein (15–25%), fats (30– 33%), carbohydrates (26–41%), high dietary fiber (18–30%), ash (4-5%), minerals, vitamins, and dry matter (90–93%). It also contains a high amount of antioxidants [2]. Another key feature of chia seed is that it does not contain gluten [9]. Its oil provides the richest plant alpha linolenic fatty acid known [10,11]. Chia a source of plant ω-3 fatty acids and nutraceuticals, since research is targeting new and functional foods worldwide [12].

Salinity is considered the most serious environmental stresses which affect the physiology and biochemistry of plants and significantly decreases the yield. Salinity is one of the world's most large environmental problems [13,14].

Many studies have proved the harm effect of water salinity on most different plants, Valifard [15]. On Salvia macrosiphon, Chrysargyris et al. [16] on lavender (*Lavandula angustifolia* Mill.) plants, Grzeszczuk et al. [17] on *Salvia coccinea* Sun et al. [18] on *Tagetes s*p.

Silicon is the second most abundant element in soils, the mineral substrate for most of the world's plant life. Silicon as a mineral constituent of plants, it is not counted among the elements defined as essential. Silicon plays a large role in their growth, mineral nutrition, mechanical strength, and resistance to fungal diseases, herbivory and adverse chemical conditions of the medium [19].

Benefits of silicon to minimize and reduce the adverse effects of salinity were established by Badawy et al. [20] on zinnia, Dawood [21] on oregano plant (*Origanum syriacum* L.); Radkowski et al. [22] on meadows, Ashour [23] on *Cupressus macrocarpa* 'Goldcrest Wilma, Chrysargyris et al. [16] on lavender and Menesy et al. [24] on *Pimpinella anisum.*

Of the great importance of chia plant and its recent entry into Egyptian agriculture. It was necessary to estimate its productivity under Egyptian conditions. This investigation will study the effect of salinity on the growth and productivity of chia and silicon additives to reduce its harmful effects.

2. MATERIALS AND METHODS

A pot experiment was done during the two consecutive winter seasons of 2016/2017 and 2017/2018 at a Privet Farm in Toukh, El-Qaloubia Governorate, Egypt (geographical latitude 30°18'54.7" N 31°07' 29.2"E). The seeds of chia were sown in the seedling trays on the 4th October of each season, then the seedlings were transplanted 25th October and 27th October in the first and second season respectively, in plastic pots 25 cm diameter. Each pot was filled with 4.6 kg washed sand and 0.9 kg wet compost at 5:1 (w/w). After one week of transplanting the plants were thinned to one plant per pot. The pots of each treatment were organized in three replicate, each replicate contains, 5 pots, every treatment contains 15 plants. The sandy soil in the texture and the chemical properties of compost are shown in Table 1.

The plants were irrigated after one month of transplanting. The plastic pots were irrigated with 450 ml. Soil moisture was kept at 75% of field capacity by watering to a constant weight every 2 days during the growing seasons. Five levels of saline water irrigation were used in irrigation water as follows: ECw 0.68, 2.34, 3.13, 3.91, and 4.69 dS m^{-1}, the first level of water salinity (0.68 dS m^{-1}) was obtained from a natural well in the farm. Meanwhile, the other levels (2.34, 3.13, 3.91 and 4.69 dS m^{-1}) were made up by adding sodium chloride (NaCl) with purity 95% obtained from El Gomhoureya Co. For Pharmaceuticals Company, the salt was dissolved in the water of well (0.68 dS m^{-1}) to produced different levels of water salinity. The same well water (0.68 dS m^{-1} as a control) was used to wash the residual effect of soil salinity every 3 doses of saline water irrigation. Water analyses of well water (control) and the highest salinity level of irrigation water used in the present investigation are shown in Tables 2 and 3.

Silicon was added as potassium silicate (K_2SiO_3) which contain 25% silicon oxide (SiO_2) and 10% potassium oixide (k_2O) in liquid form (obtained from Abo Ghaneima Company for Fertilizer and Chemical Industries, Egypt), three levels of silicon Si 1, Si 2 and Si 3 i.e. 0, 1000 and 2000 ppm were used as a foliar-spraying in early morning after one week from transplanting and were contained every two weeks until the end of the experiment, The plants were harvested on 25th and 27th February in the first and second season, respectively. The vegetative parts were cut about 1 cm above the soil surface, all other agricultural practices were performed when needed.

The layout of the experiment was a completely randomized design with two factors. The first factor was silicon as a foliar application S1 0, S2 1000 and S3 2000 ppm and the second factor was salinity concentrations of irrigation water (0.68, 2.34, 3.13, 3.91 and 4.69 dS m^{-1}). The experiment included 15 treatments with three replicate, each replicate consists of 5 plants i.e. 15 plants in each treatment.

2.1 Data Collected

2.1.1 Vegetative characteristics at beginning of flowering

Plant height (cm), Fresh weight $plant^{-1}$ (g), dry weight $plant^{-1}$ (g), No. branches, root weight $plant^{-1}$ (g), root length $plant^{-1}$ (cm), root diameter $plant^{-1}$ (mm).

2.1.2 Yield evaluation

After harvesting time plants were removed to station for sampling and other measurements the following measurements were taken: number of branches = (number of inflorescences) $plant^{-1}$, main inflorescence length $plant^{-1}$ (cm), main inflorescence weight $plant^{-1}$ (g), Inflorescences weight $plant^{-1}$ (g), Seeds weight $plant^{-1}$ (g), weight of 1000 seeds (g), seeds weight/m^2 (g) and calculated seeds yield fed^{-1} (kg).

2.1.3 Chemical constituents

Chemical analyses were determined for chemical constituents as follows: N, P, K, Na and Cl^- (%) in dry leaves, while free proline content was determined in fresh herb.

Nitrogen content (%) was determined by modified micro Kjeldahle method as described by A. O. A. C. [26]. Phosphorus was colorimetrically determined using the method described by Murphy and Riley [27]. As for potassium, sodium and calcium it was estimated using flame photometry according to Cottenie et al. [28]. Chloride (Cl^-) content in the dry leaves as a percentage was assessed according to the method described by Higinbothon et al. [29]. A portion of extract (10 ml) was taken for titration with 0.02 mol silver nitrates using potassium chromate as an indicator [30]. Free proline concentration was measured colorimetrically in the extract of fresh leaf material according to Bates et al. [31].

The means of all obtained data from the studied factors were subjected to analyses of variance (ANOVA) as factorial experiments in a complete randomized block. The differences between the mean values of various treatments were compared by using the least significant differences (L. S. D.) at 0.05%, as given by Snedecor and Cochran [32] using MSTAT-C statistical software package.

Table 1. Chemical analyses of compost

pH	E. C. ($dS\ m^{-1}$)	Soluble cations ($mmolc\ L^{-1}$)				Soluble anions ($mmolc\ L^{-1}$)			Ash (%)	O. M. (%)	N (%)	P (%)	K (%)
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	$SO4^{--}$					
8.10	0.92	0.75	3.75	3.71	0.86	0.74	1.15	7.5	9.0	65.0	2.0	1.5	1.0

Compost was analyzed in the Sekem Co. Laboratories

Table 2. Water analyses of the fresh water (Control)

pH	E.C. ($dS\ m^{-1}$)	Soluble cations ($mmolc\ L^{-1}$)				Soluble anions ($mmolc\ L^{-1}$)			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	SO_4^{--}	Cl^{-}
7.4	1.04	4.28	3.93	1.76	0.23	0.00	7.10	0.81	2.29

Analyzed in the Desert Research Center laboratories according to Rainwater and Thatcher (1960) [25]

Table 3. Water analyses of the high salinity water (4.69 $dS\ m^{-1}$)

pH	E.C. ($dS\ m^{-1}$)	Soluble cations ($mmolc\ L^{-1}$)				Soluble anions ($mmolc\ L^{-1}$)			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	SO_4^{--}	Cl^{-}
7.6	4.69	3.99	5.04	41.74	0.23	0.00	7.60	3.52	35.89

Analyzed in the Desert Research Center laboratories according to Rainwater and Thatcher (1960) [25]

3. RESULTS AND DISCUSSION

3.1 Plant Height (cm), Fresh Weight (g), Dry Weight (g) and No. of Branches Plant^{-1}

Data presented in Tables 4 and 5 illustrated that all tested concentrations of salinity decreased all vegetative growth measurements i.e., plant height, fresh weight, dry weight and No. of branches plant^{-1} of chia (*Salvia hispanica* L.) plants of both seasons. However, the tallest plant and the heaviest fresh weight, dry weight and No. of branches plant^{-1} were gained by 0.68 ds / m^{-1} (control) in the first and second seasons. In this concern, the second values of these parameters were recorded by 2.34 dS m^{-1} salinity concentration in both seasons. Concerning the effect of the studied silicon treatments, it was interested to note that there was a positive relationship between vegetative growth measurement bvalues and silicon treatments in both seasons. Hence, as the concentrations of silicon increased, the values of these parameters increased to reach the maximum increase at the high concentration (2000 ppm). Therefore, 2000 ppm silicon -sprayed plants scored the highest values of these parameters of chia plants in both seasons. As for the interaction effect between salinity treatments and silicon concentration, it was clear that all resulted combinations treatments succeeded in decreasing the values of vegetative growth measurements as compared to the combination of 0.68 dS m^{-1} (control) and 2000 ppm silicon in the two seasons.

In general, the highest values of plant height, fresh weight, dry weight and No. of branches plant^{-1} of chia (*Salvia hispanica* L.) plants were recorded by the combination of 0.68 ds m^{-1} (control) and 2000 ppm silicon, followed descendingly by 0.68 dS m^{-1} and 1000 ppm silicon as compared with the other ones in most cases of both seasons. Additionally, the combination of 2.34 dS m^{-1} and 2000 ppm silicon ranked the third values in this respect. On the contrary, the lowest values of these parameters were scored by high salinity concentration 4.69 dS m^{-1} and received no silicon treatments (0 ppm) in both seasons. The remained treatments occupied an intermediate position between the above mentioned treatments in both seasons.

3.2 Flowering Growth and Yield Parameters: Number of Inflore-scences/Plant, Main Inflorescence Height (cm), Main Inflorescence Weight (g), Inflorescences Weight (g)/ Plant, Seeds Weights/Plant, the Weight of 1000 Seeds and Calculated Seeds Yield/m^2 (g)

Data presented in Tables 6 and 7 declare that all tested concentrations of salinity decreased flowering growth and yield parameters i.e., number of inflorescences, main inflorescence height, main inflorescence weight, inflorescences weight, seeds weights, the weight of 1000 seeds and calculated seeds yield/m^2 of chia (*Salvia hispanica* L.) plants of both seasons.

However, the highest values of these parameters were gained by 0.68 dS m^{-1} (control) salinity concentration followed in descendingly by 2.34 dS m^{-1} in the first and second seasons. Concerning the effect of the studied silicon treatments, it was interested to note that there was a positive relationship between all flowering growth and yield parameters values and silicon treatments in both seasons. Hence, as the concentrations of silicon increased, the values of these parameters increased to reach the maximum increase at the high concentration (2000 ppm).

Therefore, 2000 ppm silicon -sprayed plants scored the highest values of these parameters of chia plants in both seasons. As for the interaction effect between salinity treatments and silicon concentration, it was clear that all resulted combinations treatments succeeded in decreasing the values of these parameters as compared of the combination of 0.68 dS m^{-1} (control) and 2000 ppm silicon in the two seasons.

Generally, the highest values of these parameters of chia (*Salvia hispanica* L.) plants were recorded by the combination of 0.68 dS m^{-1} (control) and 2000 ppm silicon, followed descendingly by 0.68 dS m^{-1} and 1000 ppm silicon or 2.34 dS m^{-1} and 2000 ppm silicon as compared with the other ones of both seasons. On reverse, the lowest values of these parameters were scored by high salinity concentration 4.69 dS m^{-1} and received no silicon treatments (0 ppm) in both seasons. The remained treatments occupied an intermediate position between the abovementioned treatments in both seasons.

3.3 Root Growth Measurements: Root Length (cm), Root Weight (g) and Root Diameter

Data in Tables 8 and 9 indicate that there was negative relationships between root growth measurements i.e. root length, root weight and root diameter values and salinity treatments in both seasons.

Hence, as the concentrations of salinity increased, the values of these parameters decreased to reach the maximum decreasing at the high concentration (4.69 dS m^{-1}). Therefore, 0.68 dS m^{-1} (control) salinity concentration scored the highest values of these parameters of chia plants in both seasons. As for the effect of silicon treatments, it was observed that all applied silicon concentrations statistically increased root length, root weight and root diameter/plant, especially the high concentration in both seasons.

Concerning the interaction effect between salinity treatments and silicon concentrations, data in Table 6 reveal that the combination treatment between 0.68 dS m^{-1} salinity concentration and 2000 ppm silicon scored the highest values of these parameters, followed in descendingly by the combination treatment between 0.68 dS m^{-1} salinity concentration and 1000 ppm silicon in most cases in the first and the second seasons. On the contrary, the lowest value of these parameters was produced by 4.69 dS m^{-1} and 0 ppm silicon treatments as compared with the other treatments in the two seasons.

3.4 Seeds Weights Plant^{-1}, Seeds Weights/m^2 and Weight of 1000 Seeds

Data presented in Tables 7 and 10 it could be concluded that all applied silicon concentrations statistically increased the weight of the seeds plant^{-1}, seeds weights/^{2} and weight of 1000 seeds, especially the high concentration (2000 ppm) in both seasons.

On the other hand, the saline water irrigation treatments induced decreased the seeds weights plant^{-1}, seeds weights/m^2 and weight of 1000 seeds of chia as compared with control 0.68 dS m^{-1} in both seasons. However, the heaviest seeds weight plant^{-1} seeds weights / m^2 and weight of 1000 seeds were obtained from the 0.68 dS m^{-1} saline water irrigation concentration in the two seasons. Also, 2.34 dS m^{-1} produced the second highest values of these parameters in both seasons.

Moreover, the combination treatment between silicon at 2000 ppm and 0.68 dS m^{-1} saline water irrigation concentration scored the highest values of the abovementioned parameters in both seasons. Also, the combined treatment between 1000 ppm silicon and 0.68 dS m^{-1} saline water irrigation concentration or 2000 ppm silicon and 2.34 dS m^{-1} saline water irrigation concentration resulted in high increments of these parameters in both seasons.

Table 4. Effect silicon concentrations, saline water irrigation treatments and their combination on plant height (cm) and fresh weight (g) of chia (*Salvia hispanica* L.) plant during 2016/2017 and 2017/2018 seasons

	Plant height (cm)								Fresh weight (g)							
	First season			Mean	Second season			Mean	First season			Mean	Second season			Mean
S.W. I. Silicon	**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**	
0.68 dS m^{-1}	87.97	97.65	111.69	99.10	85.74	106.10	112.80	101.6	33.67	40.83	43.33	39.28	32.50	36.37	45.90	38.26
2.34 dS m^{-1}	86.56	94.76	96.44	92.59	84.12	94.78	96.22	91.70	26.67	25.87	37.67	30.07	29.67	32.77	39.33	33.92
3.13 dS m^{-1}	81.02	83.17	90.33	84.84	80.28	86.75	90.33	85.79	16.93	23.30	28.33	22.86	25.20	28.00	30.73	27.98
3.91 dS m^{-1}	78.48	79.44	73.50	77.14	77.76	80.33	78.37	78.82	10.67	20.87	23.83	18.46	13.67	25.77	23.17	20.87
4.69 dS m^{-1}	53.44	69.15	72.36	64.98	67.81	73.83	70.33	70.66	8.83	10.20	12.47	10.50	10.53	11.23	11.83	11.20
Mean	77.49	84.83	88.86		79.14	88.36	89.62		19.35	24.21	29.13		22.31	26.83	30.19	
L.S.D $_{at\ 0.05\ \%}$																
Silicon		4.52				3.78					1.68				1.44	
S. W. I.		5.83				4.89					2.17				1.86	
Silicon * S. W. I.		10.09				8.46					3.76				3.23	

S 1= 0 ppm silicon, S 2 = 1000 ppm silicon, S 3 =2000 ppm silicon and S. W. I = saline water irrigation

Table 5. Effect of silicon concentrations, saline water irrigation treatments and their combination on dry weight (g) and No. branches (number) of chia (*Salvia hispanica* L.) plant during 2016/2017 and 2017/2018 seasons

	Dry weight (g)								No. branches (number)							
	First season			Mean	Second season			Mean	First season			Mean	Second season			Mean
S. W. I. Silicon	**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**	
0.68 dS m^{-1}	3.87	5.48	6.58	5.31	3.73	4.94	6.89	5.19	22.11	25.45	31.67	26.41	19.67	22.33	33.22	25.07
2.34 dS m^{-1}	3.13	3.53	5.68	4.12	3.41	4.44	5.90	4.58	14.33	18.33	21.45	18.04	15.67	18.33	23.00	19.00
3.13 dS m^{-1}	1.95	3.13	4.28	3.12	2.91	3.81	4.60	3.77	13.00	15.00	17.67	15.22	13.22	14.57	16.34	14.71
3.91 dS m^{-1}	1.18	3.07	3.47	2.57	1.56	3.49	4.35	3.13	7.45	11.67	12.33	10.48	9.22	12.11	14.11	11.81
4.69 dS m^{-1}	1.08	1.47	1.94	1.50	1.21	1.52	1.78	1.50	4.34	6.34	8.33	6.34	6.56	7.00	7.89	7.15
Mean	2.24	3.34	4.39		2.56	3.64	4.71		12.25	15.36	18.29		12.87	14.87	18.91	
L.S.D $_{at\ 0.05\ \%}$																
Silicon	0.23				0.20				1.76				3.78			
S. W. I.	0.29				0.26				2.27				4.88			
Silicon * S. W. I.	0.51				0.45				3.93				8.45			

S 1= 0 ppm silicon, S 2 = 1000 ppm silicon and S 3 = 2000 ppm silicon

Table 6. Effect of silicon concentrations, saline water irrigation treatments and their combination on main inflorescence height (cm) and main inflorescence weight (g) of chia (*Salvia hispanica* L.) plant during 2016/2017 and 2017/2018 seasons

	Main inflorescence height (cm)								Main inflorescence weight (g)							
	First season			Mean	Second season			Mean	First season			Mean	Second season			Mean
S. W. I. Silicon	**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**	
0.68 dS m^{-1}	17.05	19.50	24.17	20.24	17.20	18.02	22.59	19.27	1.48	2.18	2.48	2.05	1.71	2.59	2.67	2.32
2.34 dS m^{-1}	14.19	17.43	19.93	17.19	14.69	15.95	18.39	16.34	1.32	1.44	1.52	1.43	1.29	1.63	1.82	1.58
3.13 dS m^{-1}	11.07	14.87	15.93	13.96	13.08	15.37	16.00	14.82	0.88	1.37	1.41	1.22	0.96	1.55	1.72	1.41
3.91 dS m^{-1}	10.50	11.23	12.25	11.33	10.06	10.82	11.50	10.79	0.66	0.86	0.90	0.81	0.65	0.91	0.98	0.85
4.69 dS m^{-1}	5.28	7.91	8.90	7.36	7.15	8.12	9.00	8.09	0.39	0.58	0.80	0.59	0.43	0.74	0.74	0.64
Mean	11.62	14.19	16.24		12.44	13.66	15.49		0.89	1.29	1.42		1.01	1.48	1.59	
L.S.D $_{at\ 0.05\ \%}$																
Silicon	1.38				1.75				0.20				0.23			
S. W. I.	1.78				2.26				0.26				0.29			
Silicon * S. W. I.	3.08				3.9				0.45				0.51			

S 1= 0 ppm silicon, S 2 = 1000 ppm silicon, S 3 =2000 ppm silicon and S. W. I = saline water irrigation

Table 7. Effect of silicon concentrations, saline water irrigation treatments and their combination on main inflorescences weight (g) and weight of 1000 seeds (g) of chia (*Salvia hispanica* L.) plant during 2016/2017 and 2017/2018 seasons

	Inflorescences weight/ plant (g)								Weight of 1000 seeds (g)							
	First season			Mean	Second season			Mean	First season			Mean	Second season			Mean
S. W. I. Silicon	**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**	
0.68 dS m^{-1}	6.93	7.63	12.73	9.10	7.49	8.92	13.87	10.09	1.33	1.21	1.27	1.21	1.39	1.42	1.86	1.56
2.34 dS m^{-1}	4.11	6.17	10.12	6.80	4.67	7.08	9.50	7.08	0.94	1.11	1.15	1.06	1.22	1.26	1.31	1.26
3.13 dS m^{-1}	3.40	4.50	8.00	5.30	3.72	5.04	7.90	5.55	0.91	1.02	1.11	1.01	0.93	1.20	1.23	1.12
3.91 dS m^{-1}	2.57	3.11	5.00	3.56	2.66	3.70	5.30	3.89	0.77	0.98	1.02	0.93	0.87	1.01	1.19	1.02
4.69 dS m^{-1}	1.12	1.91	3.12	2.05	1.39	2.50	2.95	2.28	0.25	0.52	0.67	0.48	0.58	0.65	0.75	0.66
Mean	3.63	4.66	7.79		3.99	5.45	7.90		0.80	0.97	1.05		1.00	1.11	1.27	
L.S.D $_{at\ 0.05\ \%}$																
Silicon	0.85				1.02				0.11				0.09			
S. W. I.	1.10				1.30				0.14				0.11			
Silicon * S. W. I.	1.90				2.25				0.24				0.20			

S 1= 0 ppm silicon, S 2 = 1000 ppm silicon, S 3 =2000 ppm silicon and S. W. I = saline water irrigation

Table 8. Effect of silicon concentrations, saline water irrigation treatments and their combination on root length (cm) and root weight/plant (g) of chia (*Salvia hispanica* L.) plant during 2016/2017 and 2017/2018 seasons

	Root length (cm)								Root weight (g)							
	First season			Mean	Second season			Mean	First season			Mean	Second season			Mean
S. W. I. Silicon	**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**	
0.68 dS m^{-1}	24.07	32.80	34.54	30.47	19.37	30.83	33.95	28.05	23.30	26.08	27.60	25.66	19.55	28.75	34.87	27.72
2.34 dS m^{-1}	17.70	31.77	33.33	27.60	17.80	30.25	31.00	26.35	15.63	18.78	25.77	20.06	13.62	16.60	23.25	17.82
3.13 dS m^{-1}	17.10	17.50	18.79	17.80	16.57	18.18	20.21	18.32	8.99	11.29	14.50	11.59	7.51	9.85	12.68	10.01
3.91 dS m^{-1}	8.50	10.57	11.24	10.10	9.67	10.26	12.14	10.69	3.09	5.43	6.15	4.89	2.28	4.23	6.42	4.31
4.69 dS m^{-1}	3.93	6.00	6.50	5.48	5.68	8.88	9.30	7.95	1.17	4.12	4.54	3.28	1.58	3.29	3.95	2.94
Mean	14.26	19.73	20.88		13.82	19.68	21.32		10.44	13.14	15.71		8.91	12.55	16.23	
L.S.D $_{at\ 0.05\ \%}$																
Silicon	1.80				1.49				1.11				1.43			
S. W. I.	2.33				1.93				1.43				1.85			
Silicon * S. W. I.	4.03				3.34				2.48				3.20			

S 1= 0 ppm silicon, S 2 = 1000 ppm silicon, S 3 =2000 ppm silicon and S. W. I = saline water irrigation

Table 9. Effect of silicon concentrations, saline water irrigation treatments and their combination on root diameter (mm) of chia (*Salvia hispanica* L.) plant during 2016/2017 and 2017/2018 seasons

	Root diameter (mm)							
	First season			Mean	Second season			Mean
S. W. I. Silicon	**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**	
0.68 dS m^{-1}	11.33	11.50	12.00	11.61	10.25	10.97	11.50	10.91
2.34 dS m^{-1}	9.00	10.00	10.50	9.83	8.12	9.25	10.10	9.15
3.13 dS m^{-1}	6.88	8.00	8.65	7.84	7.00	7.22	8.03	7.42
3.91 dS m^{-1}	5.00	6.12	7.00	6.04	6.10	6.87	7.25	6.74
4.69 dS m^{-1}	4.16	4.00	4.85	4.34	3.00	4.50	5.12	4.21
Mean	7..27	7.92	8.60		6.89	7.76	8.40	
L.S.D $_{at\ 0.05\ \%}$								
Silicon	0.39				0.32			
S. W. I.	0.51				0.41			
Silicon * S. W. I.	0.88				0.71			

S 1= 0 ppm silicon, S 2 = 1000 ppm silicon, S 3 =2000 ppm silicon and S. W. I = saline water irrigation

Table 10. Effect of silicon concentrations, saline water irrigation treatments and their combination on seeds weight/plant (g) and seeds weight/ m^2 (g) of chia (*Salvia hispanica* L.) plant during 2016/2017 and 2017/2018 seasons

	Seeds weight/ plant (g)								Seeds weight/ m^2 (g)							
	First season			Mean	Second season			Mean	First season			Mean	Second season			Mean
S. W. I. Silicon	**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**	
0.68 dS m^{-1}	3.02	3.82	5.37	4.07	3.22	3.57	6.02	4.27	48.37	61.07	85.97	65.14	51.49	57.16	96.34	68.33
2.34 dS m^{-1}	1.33	2.05	2.97	2.12	1.55	2.76	3.92	2.74	21.23	32.85	47.47	33.85	24.85	44.18	62.67	43.90
3.13 dS m^{-1}	1.08	1.60	2.60	1.76	1.49	1.77	2.22	1.82	17.23	25.60	41.60	27.14	23.80	28.27	35.52	29.20
3.91 dS m^{-1}	0.81	0.90	1.27	0.99	0.77	0.98	1.33	1.03	13.01	14.40	20.32	15.91	12.30	15.75	21.24	16.43
4.69 dS m^{-1}	0.20	0.33	0.42	0.31	0.19	0.25	0.52	0.32	3.15	5.23	6.67	5.01	3.00	3.95	8.25	5.07
Mean	1.29	1.74	2.53		1.44	1.87	2.80		20.60	27.83	40.41		23.09	29.86	44.80	
L.S.D $_{at\ 0.05\ \%}$																
Silicon	0.51				0.75				8.15				12.01			
S. W. I.	0.66				0.97				10.52				15.51			
Silicon * S. W. I.	1.14				1.68				18.22				26.86			

S 1= 0 ppm silicon, S 2 = 1000 ppm silicon, S 3 =2000 ppm silicon and S. W. I = saline, water irrigation

The lowest value of these parameters was produced by 0 ppm silicon treatments and 4.69 dS m^{-1} saline water irrigation concentration as compared with the other treatments in both seasons.

The aforementioned results of silicon (Si) on vegetative growth are in agreement with Soundararajan et al. [33] on *Salvia splendens*, Manivannan et al. [34] on *Zinnia elegans*, Dawood [21] on oregano plant (*Origanum syriacum* L.), the highest values in vegetative growth parameters under study were obtained when 2000 ppm Si was applied, Chrysargyris et al. [16] illustrated that Zn and Si application, had lesser effects on the content of growth and development of lavender, even though altered salinity induced changing, Menesy et al. [24] on *Pimpinella anisum* found that, silica nanoparticles enhanced the seeds yield fed.$^{-1}$, number of umbels and root diameter plant^{-1}.

Major effects of added Si on plant leaves included increased rigidity of the mature leaves which had a rougher texture and were held more horizontally. Also, they were darker green, and senescence was delayed. The mature high-Si leaves acquired characteristics of leaves grown in higher light intensity, i.e. they had shorter petioles and an increased fresh weight per unit area, dry weight per unit area and chlorophyll content [35].

As for the growth regulators, it has been shown that Si application leads to enhancement of GA_1 and is a precursor of GA_{20} which results in increased growth parameters via cell enlargement required for intermodal elongation [36]. It's known, gibberellins since quite a long time ago are known to affect cell enlargement and division which leads to internode elongation in stems and increases stem height [37].

Si enhanced salt tolerance is believed to be associated with a decrease in sodium concentration and an increase in potassium concentration as much in roots and shoots in plant by an active process associated with ATP-driven H^+ pump in the plasma membrane [38].

It could be suggested that Si application might achieve its favorable effect to counteract the detrimental effects of salinity when the plants would show obvious stunting via increasing cell wall elasticity during extension growth [39].

Si increased induction of antioxidant enzymes and their obvious protective role of membranes also caused increasing tolerance of barley plant to damages [40]. Si plays an important role in moderating damage to chloroplasts and their metabolism in saline environments [41].

The abovementioned results of salinity on vegetative growth are nearly similar to those obtained by Ali and Hassan [42] on chamomile plant, [43] showed that, salinity treatments of, 100 mM NaCl did not affect the growth of *Salvia miltiorrhiza* in a morphological sense but significantly inhibit the accumulation of dry matter.

Mohamadiyeh et al. [44] on (*Mentha spicata* L.) showed that the highest fresh and dry weights of leaf, plant height and number of lateral branches were observed in the control treatment. Menezes et al. [45] on basil (*Ocimum basilicum* L.) demonstrated that, the plant height, stem diameter, number of leaves, dry mass and inorganic solutes in different organs, absolute membrane integrity and relative water content were evaluated, Valifard [15] on *Salvia macrosiphon,* results showed that, salinity stress affects plant growth by changing plants, both fresh and dry weights, Chrysargyris et al. [16] on lavender plants, Grzeszczuk et al. [17] on *Salvia coccinea* (Lamiaceae)., Sun et al. [18] on marigolds (*Tagetes* sp.) Plants were irrigated weekly with nutrient solution at an electrical conductivity (EC) of 1.2 dS m^{-1} (control) or saline solutions at an EC of 3.0 or 6.0 dS m^{-1}. Marigold plants began to show foliar salt damage (leaf burn and necrosis) at 6 weeks after the initiation of treatment and Parvez et al. [46] on two quinoa genotypes.

Salinity stress reduces plant growth and productivity by affecting morphological, anatomical, biochemical and physiological characteristics, processes and functions. Disturbed water and nutritional balance of plants may cause reduced crop yields affected by salinity. Reduction in plant height and other growth parameters are the most distinct and obvious effect of salt stress since inhibition of growth is probably the most general response of plants to stress [47,48].

The mechanisms by which salinity affects plant growth were reported and summed up by Meiri and Shahavet [49] who attributed the effect of salinity to the four causes as following:

1. Salinity raises salts within plant cells may which results in turgor reduction and growth retardation.
2. Salinity affects root and stomata resistance to water flow through manipulating the balance between root and shoot hormones changes considerably.
3. Salinity changes the structure of the chloroplasts and mitochondria and such changes may interfere with normal metabolism and growth.
4. Salinity increases respiration and reduces photosynthesis products available for growth.

Salinity stress affected the values of all the examined parameters, both morphological and physiological, and caused the inhibition of plant growth, the degradation of photosynthetic capacity and stomatal behavior, a decrease in the photosynthetic pigments contents and relative water content, an increase in the Malondialdehyde (MDA) content and relative electrolytic conductivity, and the accumulation of Na^+ and Cl^- content. The presence of relatively high concentrations of organic osmolytes, the activation of antioxidant enzymes, and the ionic transport capacity from the root to shoots may represent a constitutive mechanism of defense against stress Zhou et al. [50].

The inhibitory effect of saline water irrigation on vegetative parameters of chia acquired here could be explained by the fact that increasing salt concentrations in water irrigation results always in a decrease in partial molar-free energy as an extra amount of energy needed to be generated and is required for absorption of water and minerals. Often, plants must use up more energy to absorb a particular amount of water instead of using it on the growth and accumulation of assimilates inside plants [51-54].

3.5 Chemical Composition Determinations: N, P and K Contents in Dry Leaves (%)

Data in Tables 11 and 12 declare that, sprayed the plant with silicon concentration significantly increased N, P and K contents (%) when compared to the untreated (0 ppm silicon), particularly the highest concentration 2000 ppm silicon in both seasons.

On the other hand, all saline water irrigation treatments significantly decreased N, P and K contents (%) in the dry leaves of chia (*Salvia hispanica* L.) when compared to control (0.68 dS m^{-1}) in both seasons. However, the highest values of these parameters scored by 0.68 dS m^{-1}, followed 2.34 dS m^{-1} in the two seasons.

Moreover, data in Tables 11 and 12 show that combination treatment between silicon at 2000 ppm and 0.68 dS m^{-1} saline water irrigation concentration recorded the highest values of these parameters in the first and the second seasons. Furthermore, the combined treatment between 1000 ppm silicon and 0.68 dS m^{-1} saline water irrigation concentration or 2000 ppm silicon and 2.34 dS m^{-1} saline water irrigation concentration recorded highly increases of these parameters in both seasons. The lowest value of these parameters was produced by 0 ppm silicon treatments and 4.69 dS m^{-1} saline water irrigation concentration as compared with the other treatments in the two seasons.

3.6 Free Proline Content in Fresh Leaves (µmole/g f.w.)

Data in Table 12 observed that all applied silicon treatments statistically decreased proline content, especially the highest concentration 2000 ppm silicon, followed in ascendingly by 1000 ppm silicon in both seasons with non-significant differences between them in the first season only.

As for the effect of saline water irrigation, there was a positive relationship between proline content in fresh leaves values and saline water irrigation treatments in both seasons. Hence, as the concentrations of saline water irrigation increased, the values of proline content increased to reach the maximum increase at the high concentration (4.69 dS m^{-1}). Therefore, 4.69 dS m^{-1} saline water irrigation concentration scored the highest values of this parameter of the chia plant in both seasons.

With regard to the interaction effect between silicon concentrations and saline water irrigation treatments, data in Table 12 reveal that all the combination treatment between silicon concentrations and saline water irrigation treatments succeeded in increasing proline content as compared with the combined treatment of the highest concentration of silicon at 2000 ppm and saline water irrigation treatments at 0.68 dS m^{-1} (control) in both seasons, particularly the combination of 0 ppm silicon and 4.69 dS m^{-1} saline water irrigation treatments.

Additionally, the combination of 1000 ppm and silicon 4.69 dS m^{-1} saline water irrigation treatments ranked the second value of this parameter in this respect. On the contrary, the lowest value of these parameters was produced by 2000 ppm silicon treatments with 0.68 dS m^{-1} saline water irrigation treatments as compared with the other treatments in the two seasons.

3.7 Sodium and Chloride Content in Dry Leaves (%)

Data in Table 13 illustrated that all applied concentrations of silicon statistically decreased sodium and chloride content (%) in dry leaves, especially the low concentration 2000 ppm silicon followed in descending by 1000 ppm silicon in both seasons.

As for the effect of saline water irrigation treatments, it was observed that there was a positive relationship between sodium and chloride content (%) in dry leaves values and saline water irrigation treatments in both seasons. Hence, as the concentrations of saline water irrigation increased, the values of sodium and chloride content increased to reach the maximum increase at the high concentration (4.69 dS m^{-1}). However, 4.69 dS m^{-1} saline water irrigation concentration scored the highest values of this parameter in both seasons.

Concerning the interaction effect between silicon concentrations and saline water irrigation treatments, data in Table 13 reveal that all the combination treatment between saline water irrigation treatments and silicon concentrations succeeded in increasing sodium and chloride content as compared with the combined treatment of silicon at 2000 ppm and 0.68 dS m^{-1} (control) in both seasons, particularly the combination of 4.69 dS m^{-1} and 0 ppm silicon.

Additionally, the combination of 1000 ppm silicon and 4.69 dS m^{-1} ranked the second values of this parameter in this respect. On the contrary, the lowest value of these parameters was produced by 2000 ppm silicon treatments and 0.68 dS m^{-1} saline water irrigation treatments as compared with the other treatments in the two seasons.

In the present study, added Si significantly increased N, P and K elements contents and at the same time reduced Na^+, Cl^- and free proline contents in the leaves of chia plant. Similar results recorded by Asgharipour and Mosapour [55] on fennel plants, Ali and Hassan [56] on roselle plants, Dawood [21] on (*Origanum syriacum* L.) showed that Si application increased N, P and K, while at the same time it decreased Na^+ and Cl^- contents., Shekari et al*.,* [57] on dill (*Anethum graveolens*), found that supplementary Si or Se decreased Na^+ concentration and increased K^+ concentration in roots and shoots and Ashour [23] on *Cupressus macrocarpa* 'Goldcrest Wilma' and Ghalati et al. [58] on guava seedlings found that NaCl caused an increase in catalase, polyphenol oxidase, carotenoids, proline and a decrease in peroxidase, chlorophyll (a, b, and total).

This enhancement in N, P and K content results can be interpreted as a consequence of improved root growth of oregano plants, by Si application which enhanced root structures as shown earlier here and as reported before by Vlamis and Williams [59] Si salt tolerance enhancement is believed to be associated with a decrease in Na concentration and an increase in K concentration [39].

In the present study, Si application under saline water irrigation stress led to decreased free proline concentration inside fresh leaves of chia plant. The results of this study are similar with previous studies have reported that proline level is lowered by addition of silicon in different salt-stressed plant species such as grapevine Mauad et al. [60] on rice plants, Saleh et al. [61] on wheat, Delavar et al. [62] on maize [63]. Applying Si to plants in these conditions of stress decreased proline concentration, which could be due to the reaction between proline and Si [64].

Results showed that saline water irrigation caused a decrease in N, P and K contents and at the same time increased Na and Cl contents in dry leaves of chia plant. The results in the same line with Olfa et al. [65] on marjoram (*Origanum majorana*), Abdel-Rahman et al. [66] on sweet basil cultivars, Mehrizi et al. [67] on rosemary, Gengmao et al. [43] on *Salvia miltiorrhiza*, showed that Na^+ content significantly increased with increasing salinity but the K^+ and Ca^+ contents were reversed, indicating that a high level of external Na^+ resulted in a decrease in both K^+ and Ca^+ concentrations., Xu et al. ([68] found that Na^+ accumulation increased significantly in all plant parts of mint (*Mentha canadensis* L.)., Dawood [21] reported the decrease in N, P and K elements contents and increased in both elements of Na^+ and chloride Cl^- contents in oregano plants and

Table 11. Effect of silicon concentrations, saline water irrigation treatments and their combination on N (%) and P (%) in the dry leaves of chia (*Salvia hispanica* L.) plant during 2016/2017 and 2017/2018 seasons

	N (%)								P (%)							
	First season			Mean	Second season			Mean	First season			Mean	Second season			Mean
S. W. I. Silicon	**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**	
0.68 dS m^{-1}	2.51	2.63	2.81	2.65	2.65	2.77	2.92	2.78	0.277	0.290	0.313	0.293	0.253	0.280	0.303	0.279
2.34 dS m^{-1}	2.36	2.41	2.62	2.46	2.22	2.52	2.70	2.48	0.233	0.260	0.277	0.257	0.203	0.223	0.260	0.229
3.13 dS m^{-1}	2.21	2.29	2.44	2.31	2.09	2.36	2.65	2.37	0.207	0.227	0.247	0.227	0.190	0.213	0.247	0.217
3.91 dS m^{-1}	1.92	2.11	2.24	2.09	1.87	2.04	2.38	2.10	0.190	0.207	0.227	0.208	0.180	0.193	0.210	0.194
4.69 dS m^{-1}	1.81	1.97	2.17	1.99	1.79	1.88	2.02	1.90	0.1401	0.167	0.180	0.162	0.170	0.180	0.190	0.180
Mean	2.16	2.28	2.45		2.12	2.32	2.54		0.209	0.230	0.248		0.199	0.218	0.242	
L.S.D $_{\text{at 0.05 \%}}$																
Silicon	0.20				0.22				0.001				0.001			
S. W. I.	0.25				0.28				0.001				0.001			
Silicon * S. W. I.	0.44				0.49				0.002				0.002			

S 1= 0 ppm silicon, S 2 = 1000 ppm silicon, S 3 =2000 ppm silicon and S. W. I = saline water irrigation

Table 12. Effect of silicon concentrations, saline water irrigation treatments and their combination on k (%) and proline amino acid (µmole/g fresh) in the dry leaves of chia (*Salvia hispanica* L.) plant during 2016/2017 and 2017/2018 seasons

	K (%)								Proline amino acid (µmole/g fresh)							
	First season			Mean	Second season			Mean	First season			Mean	Second season			Mean
S. W. I. Silicon	**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**	
0.68 dS m^{-1}	1.67	1.81	1.61	1.70	1.55	1.91	1.94	1.80	0.83	0.82	0.80	0.82	0.92	0.91	0.88	0.91
2.34 dS m^{-1}	1.29	1.46	1.56	1.44	1.38	1.66	1.62	1.56	2.03	1.85	1.72	1.87	1.93	1.72	1.69	1.78
3.13 dS m^{-1}	0.94	1.65	1.46	1.35	1.24	1.52	1.47	1.41	3.44	3.21	3.15	3.27	3.26	3.09	3.39	3.25
3.91 dS m^{-1}	0.91	1.61	1.35	1.29	0.98	1.33	1.15	1.16	4.10	3.80	3.63	3.85	3.63	3.47	3.17	3.42
4.69 dS m^{-1}	0.78	1.06	1.29	1.04	0.76	1.04	1.13	0.97	4.80	4.13	3.85	4.26	4.66	4.42	3.64	4.24
Mean	1.12	1.52	1.45		1.18	1.49	1.46		3.04	2.76	2.63		2.88	2.72	2.56	
L.S.D $_{\text{at 0.05 \%}}$																
Silicon	0.20				0.20				0.71				0.23			
S. W. I.	0.26				0.25				0.91				0.30			
Silicon * S. W. I.	0.44				0.44				1.58				0.52			

S 1= 0 ppm silicon, S 2 = 1000 ppm silicon, S 3 = 2000 ppm silicon and S. W. I = saline water irrigation

Table 13. Effect of silicon, saline water irrigation and their combination on sodium (%) and chloride (%) in the dry leaves of chia (*Salvia hispanica* L.) plant during 2016/2017 and 2017/2018 seasons

	Na (%)								Cl (%)							
	First season			Mean	Second season			Mean	First season			Mean	Second season			Mean
S. W. I. Silicon	**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**		**Si 1**	**Si 2**	**Si 3**	
0.68 dS m^{-1}	0.17	0.14	0.12	0.15	0.13	0.12	0.11	0.12	0.29	0.26	0.22	0.26	0.39	0.26	0.23	0.29
2.34 dS m^{-1}	0.28	0.16	0.13	0.19	0.18	0.16	0.12	0.16	0.76	0.52	0.54	0.61	0.80	0.62	0.58	0.67
3.13 dS m^{-1}	0.33	0.18	0.13	0.22	0.22	0.19	0.15	0.19	0.81	0.61	0.56	0.66	0.87	0.67	0.65	0.73
3.91 dS m^{-1}	0.42	0.21	0.15	0.26	0.27	0.23	0.16	0.22	0.91	0.67	0.66	0.75	1.10	0.82	0.78	0.90
4.69 dS m^{-1}	0.45	0.41	0.18	0.35	0.33	0.31	0.23	0.29	1.54	1.30	1.22	1.35	1.59	1.44	1.40	1.48
Mean	0.33	0.22	0.14		0.23	0.20	0.17		0.86	0.67	0.64		0.95	0.76	0.73	
L.S.D $_{at\ 0.05\ \%}$																
Silicon	0.03				0.02				0.04				0.06			
S. W. I.	0.04				0.03				0.05				0.08			
Silicon * S. W. I.	0.07				0.05				0.09				0.14			

S 1= 0 ppm silicon, S 2 = 1000 ppm silicon, S 3 =2000 ppm silicon and S. W. I = saline water irrigation

Garcia-Caparros et al. [69] on lavender (*Lavandula multifida*) stated that, Na^+ and Cl^- were increased in high salinity.

Results showed that saline water irrigation caused an increase in free proline amino acid content in fresh leaves of chia plants. In higher plants, proline, a nontoxic and protective osmolyte under osmotic stress, is frequently involved in osmotic protection and is reportedly associated with salt tolerance [70]. Proline is one of the important compatible solutes that accumulate under stress conditions and has been considered to play a substantive role in osmotic adjustment [71]. Proline and sucrose are the two most commonly known solutes that accumulate under saline conditions [48].

Proline could accumulate in the cytoplasm without having any detrimental effect on cytosolic enzyme activities [72], i.e. activities related to the aqueous component in the cytoplasm of cells. Also, the primary role of proline may not be sole as an osmolyte, but it also helps the cells to overcome oxidative stress in salt-stressed plants [73].

4. CONCLUSION

The highest growth, productivity, chemical constituents and chemical composition of seed oil of chia (*Salvia hispanica* L.) plant, it is preferable to grow the plants under saline water irrigation concentration at (0.68 dS m^{-1}) and spray with silicon at 2000 ppm.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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