Growth and Yield of Spinach As Affected by Silicon and Fulvic Acid Under Salt Stress

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ABSTRACT: Two pots experiments were conducted at Abu Hummus,El-Beheira Governorate, Egypt, during the successive winter seasons of 2019 and 2020 to investigate the effect of fulvic acid and silicon in elevating the negative impact of salinity on vegetative growth, yield and chemical composition of spinach under different salinity levels. Each experiment includes 20 treatments which were the combinations between four salinity levels (Tap water, 1500, 3000 and 4500 ppm) and soil application treatments of fulvic (1.5 and 3.0 gm / L) , silicon (1.5 and 3.0 mM) and distilled water as control treatment. The experiments were carried out as randomized complete block design (RCBD) in split plot system with three replicates. Whereas, the salinity levels arranged in the main plots while the soil application treatments of fulvic and silicon were randomly located in the sub-plots. Generally, the obtained results indicated, that all tested characters decreased with increasing salinity levels. The reduction rate on any character varied depending on the imposed level of salinity stress. Adding fulvic acid and silicon in all concentrations showed significant effect in improving all studied traits as compared to the control treatment, in both seasons. Application of silicon at 3 mM recorded the highest values of plant height, plant fresh weight, plant dry weight, number of leaves per plant, nitrogen, phosphorus, potassium, protein and total chlorophyll contents and reduced the hazard effect of nitrate and total oxalate comparing to the other treatments in both seasons. The combined treatment of silicon (Si) at the rate of 3 mM and salinity level at zero gave the highest values of the most tested parameters. The outcome of this research recommends the opportunity of adding silicon (Si) or fulvic acid (FA) to enhance spinach plants and minimize the harmful effect of salinity.

***Keywords*:** spinach; salinity; silicon; fulvic acid; stress; growth

INTRODUCTION

Spinach plants (*Spinacia oleracea* L.) belongs to the family *Amaranthacea*. Spinach is originated from south western and central Asia (Avşar, 2011). China is the largest spinach producer followed by United States and Japan (FAOSTAT, 2017). Fresh spinach is rich in many nutrients (protein, Ca ,Mg, Na, P, Fe, vitamins C, B-carotene, vitamins E, and vitamin A). However , spinach leaves also, contains high concentration of oxalates and phytates (Heaney et al., 1988 and McConn and Nakata 2004). Spinach is a moderately salt-tolerant glycophyte in the winter, but sensitive to moderately-sensitive if cultivated in the spring and summer (Ferreira *et al*., 2020). Agriculture sustainability is threated by increased soil salinization, which reduces both the productivity and availability of land for agriculture (Shrivastava and Kumar, 2015). Soil salinity is one of the major abiotic stresses that hinder crop growth and productivity worldwide. It has been reported that approximately 20% of irrigated land worldwide is salt-affected, which represents one-third of food-producing land (Gregory *et al*., 2018). Moreover, the salt-affected areas are increasing at a rate of 10% annually for various reasons, including low precipitation, high surface evaporation, poor cultural practices and irrigation using saline water (Shrivastava and Kumar, 2015). This issue has been further aggravated by the continued trends in global warming and climatic changes. Thus, living with salinity is the only way of sustaining agricultural production in the salt affected soil. So that, it is must to find the best management to alleviate salt hazard (Al-Rawahy *et al*. 2011).

In recent years, exogenous protectants such as osmoprotectants, phytohormones, humic compounds, antioxidants and various elements such as silicon have been found useful to alleviate the salt-induced damages (Khan *et al*., 2017). The development of methods and strategies to ameliorate the deleterious effects of salt stress on plants has received considerable attention (Senaratna *et al.*, 2000). In this respect, application of fulvic acid enhanced transport of minerals, improved plant hormone activity, modified enzyme activities, promoted photosynthesis, solubilization of micro and macro elements, protein synthesis, and reduction of active levels of toxic minerals (Aiken *et al*., 1985; Khang, 2011; Billard *et al*., 2014; Kandil *et al*., 2020). Moreover, the use of silicon can stimulate greater physical performance and better quality because of the positive effects of Si, Ca, Mg, and K absorption (Ferreira *et al*., 2010). Also, silicon mediated decreased uptake and transport of Na^+ and increased uptake and transport of K^+ (Tuna *et al*., 2008; Hashemi *et al*., 2010 and Farshidi *et al*., 2012), from roots to shoots under salt stress. Therefore, the objective of this study was to investigate the role of fulvic acid and silicon in alleviating the negative impacts of salt stress and to evaluate the expected outcomes that may have on its growth and chemical characteristics on spinach plants irrigated with water in different salinity levels.

MATERIALS AND METHODS

Two pots experiments were conducted at Abu Hummus, EL- Beheira Governorate, north Egypt, during the successive winter seasons of 2019 and 2020 to investigate the effect of fulvic acid and silicon in elevating the negative effect of salinity on vegetative growth, yield and chemical composition of spinach (*Spinacia oleracea* L. cv. Balady) under different salinity levels. Soil physical and chemical properties were analyzed at the Agricultural Directorate Lab of Damanhur city, El-Behera Governorate, Egypt. Properties of the selected soil are shown in Table (1).

Table(1): Chemical and physical properties of the experimental soil.

			Chemical properties				
	PH	**EC (dSm^{-1})**	**Organic matter (%)**	**NO3 (ppm)**	**Available N (ppm)**	**Available P (ppm)**	**Available K (ppm)**
2019	7.87	0.46	1.07	22. 75	18.21	14.50	26.42
2020	7.86	0.43	1.06	20.92	18.68	15.13	25.90
			Physical properties				
Season	Sand (%)	Silt (%)	Clay (%)	Texture	Bulk density (g cm^{-3})		
2019	30.08	9.72	60.20	Clay	1.50		
2020	29.9	10.5	59.6	Clay	1.51		

The spinach seeds, cv. Balady, purchased from a local seeds market, were sown in plastic pots (35 cm inner diameter, and 30 cm height), each was filled with 12 kg of soil (Table 1) , and placed in the open field. The seeds were planted on 15^{th} and 10^{th} of November in 2019 and 2020, respectively. Each treatment composed of five replicated pots with four plants in each pot. Each experiment includes 20 treatments which were the combinations between four salinity levels (Tap water, 1500 , 3000 and 4500 ppm) and soil application treatments of fulvic acid (1.5 and 3.0 gm / L) in form potassium fulvate , silicon (1.5 and 3.0 mM) in form potassium silicate and distilled water as the control treatment. The recommended concentrations of soil application treatments were applied as a drench to the spinach plants. The control plants were treated with tap water. Each soil application treatment was applied three times after planting. The first application was conducted in the two specific leaves phase (15 days) after sowing and the others were applied with one week intervals (Smolen and Sady 2012; Fouda, 2016). Harvesting was done after 50 days of planting in both seasons (Barkat *et. al*. 2018).

All experimental pots received identical levels of nitrogen, phosphorus and potassium fertilizers. Ammonium nitrate (33.5% N) at the rate of 60 kg N/fed. was equally divided and side dressed after 21, 28 and 35 days after planting, Calcium super phosphate (15.5 % P_2O_5) at the rate of 150 kg P_2O_5 /fed. was base dressed before planting and potassium sulphate (48 % K_2O) at the rate of 50 kg K_2O /fed. was equally divided and side dressed after 21 and 28 days of planting. All other agricultural practices were adopted whenever they were necessary and as commonly recommended for the commercial production of spinach.

Plant measurements

vegetative growth parameters

Spinach plants were harvested after 50 days and the measurement of vegetative growth parameters was performed immediately. Ten spinach plants from each treatment were randomly taken to measure:

Plant height (cm); it was measured with the help of measuring scale from the surface of the soil to the growing tip of the selected plants and then the average was calculated.

Plant fresh weight (gm); the whole plant sample was weighted and the average weight $plant^{-1}$(gm) was calculated.

Plant dry weight (gm); the collected 10 plants were oven dried at 70 C˚ in a forced air oven till obtaining a constant weight to obtain shoots dry weigh (g $plant^{-1}$) and the dried tissues were ground for further analysis. in a forced-oven at 70 C˚ till the weights became constant., then the dry matter was weighted.

Number of leaves per plant; it was estimated as an average of the selected plants.

Root length (cm) ; it was measured for 10 plants randomly taken, and the average root length (cm) was calculated.

Root fresh weight (gm) ; the whole fresh root for 10 plants was weighted and the average weight (gm) was calculated

Root dry weight (gm) ; the collected fresh root for 10 plants were oven dried at 70 C° in a forced air oven till obtaining a constant weight to obtain roots dry weigh (gm).

Leaf area per plant (cm^2): leaves area / plant was calculated using the weight method as used by Fayed (1997). The leaves from the plant samples (three plants) were cleaned from dust and weighted. then, twenty random disks were taken from the leaves, using a circular puncher and weighted.

$$\text{Leaf area per plant} = \frac{\text{Leaves fresh weight} \times 20 \times \text{area of disk}}{\text{Fresh weight of 20 disk} \times 3}$$

Where 20 = number of random disks
3 = number of plant sample
area of disk = πr^2

Chemical measurements

Total chlorophyll contents; total leaf chlorophyll contents (SPAD index) were measured using spad-502 chlorophyll meter devise (Konica Minolta, Kearney, NE, USA).

Total nitrogen, phosphorus , potassium, sodium and chloride; leaves samples were oven dried at 70ºC till obtaining a constant weight for 48 hours and ground in a mill with stainless steel blades. Wet digestion procedure was performed according to Chapman and Pratt (1978). Nitrogen percentage in leaves was determined by micro kjeldahl method as described by Page *et al.* (1982). Phosphorus percentage was determined calorimetrically as reported by Jackson (1973). Potassium and sodium were determined by atomic absorption Spectrophotometry methods (Bhowmik *et al*. 2012). Chloride was determined according to the method described by Jackson and Brown (1955).

Vitamin C and nitrate contents; vitamin C (mg100 g^{-1}) and nitrate (ppm) were determined according to the method described by Singh (1988).

Total oxalate **;** total oxalate (mg $100g^{-1}$) were determined according to the method described by Mazumdar and Majumder (2003).

Statistical analysis

The experimental design was split plots in a randomized complete block design, whereas the salinity levels arranged in the main plots and the soil application treatments of fulvic and silicon were randomly placed in the sub-plots. All the obtained data were statistically analyzed by CoStat program (Version 6.4, Co Hort, USA, 1998–2008). Least significant difference (LSD) test was applied at 0.05 level of probability to compare means of different treatments according to Williams and Abdi (2010).

RESULTS AND DISCUSSION

The effects of salinity levels, soil applications of fulvic acid , silicon and their interaction on vegetative growth of spinach plants are presented in Tables (2 and 3). Concerning the main effect of salinity levels on plant height **,** plant fresh weight, plant dry weight, number of leaves /plant, leaves area, root length, root fresh weight and root dry weight, results presented in Tables (2 and 3) revealed that all tested parameters decreased by increasing salinity levels. The reduction rate on any character varied depending on the level of imposed salinity stress. The highest values of the given parameters were obtained from the control treatment, while that the rate of 4500 ppm salinity recorded the lowest ones, in both seasons. At salinity of 4500 ppm, the estimated percentage reductions, expressed as plant height, plant fresh weight , plant dry weight, number of leaves , leaves area, root length, root fresh weight and root dry weight of the two seasons, were (26.42 and 31.18 %), (26.59 and 27.59 %), (26.16 and 26.77 %), (17.69 and 16.67%), (20.01 and 29.04 %), (35.98 and 40.32 %), (40.70 and 40.17 %) and (23.07 and 22.79 %) as compared to the control treatment in the first and second season, respectively. The adverse effects of high salinity on plants are related to the following factors: (1) low water potential of soil solution (water stress), (2) nutritional imbalance and disturbing ionic homeostasis (ionic stress), (3) specific ion effect (salt stress), (4) over-production of reactive oxygen species - (oxidative stress) (Parvaiz and Satyawati, 2008; Hasanuzzaman *et al*., 2013).

These results were in harmony with those reported by Mohammad *et al.(*1998) on tomato, Shereen *et al.* (2005) on radish , Gama *et al.(*2007) on common bean, Céccoli *et al.*(2011) and Siddikee *et al.*(2011) on sweet pepper, Brengi (2019) on cucumber, Ors and Suarez (2017) , Seven and Sağlam (2020), Fayed, *et al*. (2021) and Kim *et al*., (2021) on spanish who reported, generally, that vegetative growth parameters, decreased with increasing salinity rates.

Regarding the main effect of the ameliorative treatments (fulvic acid and silicon) on the plant height , plant fresh weight and plant dry weight, number of leaves , leaves area, root length, root fresh weight and root dry weight of spinach plants, results presented in Tables (2 and 3) exhibited that adding FA and Si in all concentrations showed significant effect in improving all the studied traits as compared with the control treatment, in both seasons. For instance, application of silicon at the highest level (i.e. 3 mM) recorded, generally, the highest values of plant height, plant fresh weight , plant dry weight , number of leaves, root length, root fresh weight and root dry weight compared to the other treatments, in both seasons . However, leaves area reached its maximum when plants were treated with FA at the rate of 1.5 gm /l in both seasons. The particular treatment of Si at the rate of 3 mM the resulted estimated percentages increase in plant height, plant fresh weight , plant dry weight, root length, root fresh weight and root dry weight of 34.84 and 35.59 %), (25.19 and 27.34 %) , (26.19 and 28.65), (37.04 and 33.99 %) , (23.48 and 14.36 %) and (45.25 and 34.77%) comparing to the control treatment in the first and second season, respectively. The positive effects of Si could plays different roles in plant growth and development, improve plant resistance to diseases and pests, increase photosynthesis, regulate respiration and increase the tolerance of the plant to elements toxicity (Zargar *et al.,* 2019). Moreover, Si fertilizer application can alleviate the adverse effects of various abiotic (e.g., drought, salt and metal toxicity) and biotic (pests and plant diseases) stresses on plants (Ma *et al*. 2004). Silicon seems to affect acquisition of other essential nutrients such as nitrogen, phosphorus and calcium and other micronutrients as well (Liang *et al.,* 2003 and Farshidi *et al*., 2012), thereby improving the growth of plants and the generally tolerance against salt stress

Table (2): Plant height, plant fresh weight , plant dry weight , Number of leaves and Leaves area of spinach plants as affected by salinity and soil application of both fulvic acid and silicon in both seasons of 2019and 2020.

Treatments		Plantheight(cm)		Plantfreshweight(gm)		Plantdryweight(gm)		Numberofleaves		Leavesarea(cm^2)	
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Salinity levels (ppm)	Tap water	34.63A*	35.40A	71.39A	70.60A	7.40A	7.32A	7.04A*	7.60A	605.98A	610.26
	1500	31.33B	31.37B	66.07B	65.10B	6.75B	6.67B	6.41B	6.70B	543.13B	539.72
	3000	29.05C	27.99C	60.85C	58.85C	6.30C	6.11C	6.03C	6.32B	518.44C	500.68
	4500	25.48D	24.36D	52.40D	51.12D	5.47D	5.36D	5.79C	6.33B	448.33D	433.04
Protection treatments	control	24.79D	24.17D	52.89C	51.11C	5.43C	5.25C	5.17B	5.11B	444.20D	431.58C
	Fulvicacid1.5g mperlitter	30.07C	30.22C	64.12B	63.15B	6.57B	6.50B	6.50A	6.83A	569.23A	566.39A
	Fulvicacid3gm perlitter	30.87B	30.39C	65.04AB	64.07AB	6.74B	6.64AB	6.58A	7.25A	545.10B	537.17B
	Silicon1.5mM	31.46B	31.36B	65.12AB	63.68B	6.81A	6.69A	6.50A	7.33A	536.23C	529.45B
	Silicon3mM	33.43A	32.77A	66.22A	65.08A	6.85A	6.76A	6.83A	7.17A	550.07B	540.04B
Tapwater	control	31.95de	33.45c	63.27e	64.05cd	6.57e	6.62d	6.53bcd	6.67bc	536.32fg	555.68de
	Fulvicacid1.5g mperlitter	33.65bc	35.17b	72.08b	71.90a	7.25bc	7.24bc	7.33ab	7.67ab	624.38ab	637.86a
	Fulvicacid3gm perlitter	34.73b	35.43b	73.23ab	71.90a	7.56ab	7.42ab	7.00abc	7.67ab	618.42bc	619.11abc
	Silicon1.5mM	35.19b	35.37b	73.50ab	71.80a	7.79a	7.62a	6.67abcd	8.00a	617.45bc	612.00bc
	Silicon3mM	37.63a	37.60a	74.87a	73.33a	7.86a	7.71a	7.67a	8.00a	633.31a	626.67ab
1500	control	24.37j	24.33k	57.25f	57.19f	5.58f	5.61e	5.38ef	5.51d	455.81j	455.34hi
	Fulvicacid1.5g mperlitter	31.64def	31.93cd	66.93d	64.83c	6.81de	6.60d	6.67abcd	7.00abc	604.79c	598.48c
	Fulvicacid3gm perlitter	32.57cd	31.67de	68.09cd	67.13b	7.10cd	7.00c	6.67abcd	7.33ab	547.32ef	537.31efg
	Silicon1.5mM	33.07cd	33.37c	68.51cd	68.23b	7.11cd	7.11bc	6.67abcd	6.67bc	546.40f	547.80def
	Silicon3mM	35.03b	35.53b	69.57c	68.10b	7.16c	7.02c	6.67abcd	7.00abc	561.31de	559.69d
3000	control	22.21k	20.83l	50.61h	45.97h	5.13g	4.67f	4.82fg	4.27e	423.90k	391.40j
	Fulvicacid1.5g mperlitter	29.42gh	28.83gh	62.85e	62.17de	6.55e	6.51d	6.33bcde	6.67bc	562.46d	554.04de
	Fulvicacid3gm perlitter	30.29fg	29.30fg	63.02e	61.23e	6.57e	6.38d	6.67abcd	6.67bc	542.34f	525.87fg
	Silicon1.5mM	30.75efg	30.23efg	63.48e	62.00de	6.60e	6.50d	6.00cde	7.33ab	524.82g	514.40g
	Silicon3mM	32.58cd	30.73def	64.30e	62.90cde	6.63e	6.51d	6.33bcde	6.67bc	538.69fg	517.67g
4500	control	20.63l	18.05m	40.45i	37.23i	4.44h	4.10g	3.97g	4.00e	360.79l	323.92k
	Fulvicacid1.5g mperlitter	25.57ij	24.93jk	54.60g	53.70g	5.69f	5.63e	5.67def	6.00cd	485.30h	475.18h
	Fulvicacid3gm perlitter	25.90ij	25.17jk	55.83fg	56.00f	5.71f	5.76e	6.00cde	7.33ab	472.33hi	466.40hi
	Silicon1.5mM	26.83i	26.47ij	55.00fg	52.67g	5.73f	5.53e	6.67abcd	7.33ab	456.25j	443.58i
	Silicon3mM	28.47h	27.20hi	56.13fg	56.00f	5.76f	5.78e	6.67abcd	7.00abc	466.99ij	456.14hi

*Means having the same letter (s) within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability.

Table (3): Root length, root fresh weight and root dry weight of spinach plants as affected by salinity and soil application of both fulvic acid and silicon during in both seasons of 2019and 2020.

Treatments		Rootlength(cm)		Rootfreshweight(gm)		Rootdryweight(gm)	
		2019	2020	2019	2020	2019	2020
Salinity levels (ppm)	Tapwater	11.44A*	10.39A	9.97A	10.63A	1.20A	1.27A
	1500	8.53B	9.21B	8.46B	9.07B	1.05B	1.13B
	3000	7.81C	7.79C	6.58C	7.17C	0.96C	1.04C
	4500	7.32C	6.20D	5.91D	6.36D	0.92D	0.98D
Protection treatments	control	7.05D	6.72C	6.78C	7.61C	0.77C	0.87C
	Fulvicacid1.5gmperlitter	8.97BC	8.89AB	7.98AB	8.79A	1.05B	1.15B
	Fulvicacid3gmperlitter	8.81C	8.63AB	7.68B	8.22B	1.10AB	1.17A
	Silicon1.5mM	9.38AB	8.74B	7.86B	8.22B	1.12A	1.17A
	Silicon3mM	9.67A	9.00A	8.37A	8.70AB	1.12A	1.17A
Tapwater	control	9.35d	9.36de	9.20bcd	10.25bc	0.99gh	1.10d
	Fulvicacid1.5gmperlitter	11.50bc	10.57b	9.67bc	10.27bc	1.23a	1.30a
	Fulvicacid3gmperlitter	11.00c	10.30bc	9.67bc	10.22bc	1.26a	1.32a
	Silicon1.5mM	12.33ab	10.53b	10.00b	10.62b	1.25a	1.30a
	Silicon3mM	13.00a	11.20a	11.33a	11.81a	1.25a	1.31a
1500	control	7.25g	7.80g	7.42e	8.42ef	0.83i	0.95f
	Fulvicacid1.5gmperlitter	8.77de	9.83cd	8.99cd	9.85bcd	1.06cdef	1.16bc
	Fulvicacid3gmperlitter	8.57def	9.53de	8.43d	8.93de	1.11bc	1.17b
	Silicon1.5mM	8.83de	9.60de	8.60d	8.92de	1.13b	1.18b
	Silicon3mM	9.23d	9.27e	8.87cd	9.21cde	1.13b	1.18b
3000	control	6.18h	5.33j	5.51hi	5.64ij	0.73j	0.74g
	Fulvicacid1.5gmperlitter	8.03efg	8.53f	7.00ef	8.15efg	0.95h	1.10d
	Fulvicacid3gmperlitter	7.97efg	8.30fg	6.62efg	7.44fgh	1.01fg	1.13cd
	Silicon1.5mM	8.40def	8.47f	6.78efg	7.15ghi	1.07cde	1.12d
	Silicon3mM	8.47def	8.30fg	7.00ef	7.49fgh	1.07cde	1.12d
4500	control	5.44h	4.37k	4.97i	6.12j	0.54k	0.67h
	Fulvicacid1.5gmperlitter	7.57fg	6.63i	6.28fgh	6.91hi	0.96h	1.05e
	Fulvicacid3gmperlitter	7.70fg	6.40i	6.00gh	6.31ij	1.01fg	1.06e
	Silicon1.5mM	7.93efg	6.37i	6.05gh	6.18ij	1.04defg	1.06e
	Silicon3mM	7.97efg	7.23h	6.27fgh	6.28ij	1.05def	1.05e

*Means having the same letter (s) within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability.

Regarding the interaction effect between salinity levels and the ameliorative treatments (fulvic acid and silicon) on plant height **,** plant fresh weight , plant dry weight, number of leaves , leaves area, root length, root fresh weight and root dry weight of spinach plants, whereas results presented in Tables (2 and 3) showed significant interactions between both variables. The combined treatment between zero salinity and silicon at 3 mM accomplished , generally, the highest values of aforementioned characters, in both seasons compared to other treatments.

Percentages of nitrogen, phosphor, potassium ,sodium and chloride in leaves

Regarding the main effect of salinity levels on the percentages of nitrogen, phosphor potassium, sodium and chloride in plant leaves, result presented in Table (4) revealed that nitrogen, phosphor , potassium decreased as salinity levels increased. However sodium and chloride percentages increased as salinity levels increased. The highest values of nitrogen, phosphor and potassium were obtained from control treatment, while that of 4500 ppm salinity gave the lowest ones, in both seasons. At salinity of 4500 ppm, the estimated percent reductions, for nitrogen, phosphor and potassium, were (22.63 and 30.61%), (11.11 and 22.80 %) and (29.90 and 26.58 %) in the first and second seasons, respectively and relative to the control treatment. Also, The highest values of sodium and chloride percentages were obtained from salinity at 4500 ppm treatment, while that of control treatment gave the lowest ones, in both seasons.

The nutritional disorders may result from the effect of salinity on nutrient availability, competitive uptake, transport, or distribution within the plant. Numerous reports indicated that salinity reduces nutrient uptake and accumulation of nutrients into the plants (Rogers et al. 2003; Hu and Schmidhalter 2005). A number of laboratory and greenhouse studies have shown that salinity can reduce N, P and K accumulation in plants (Feigin *et al*., 1991; Pessarakli, 1991; Al-Rawahy *et al.,* 1992). This is not surprising since an increase in Cl uptake and accumulation is often accompanied by a decrease in shoot-NO_3 concentration. Examples of such an effect have been found in cucumber (Martinez and Cerda, 1989), eggplant (Savvas and Lenz, 1996), melon (Feigin et al., 1987), and tomato (Kafkafi et al., 1982; Feigin et al., 1987; Martinez and CerdaÂ, 1989). In addition, Salinity stress decreases the uptake and concentration of P in plant tissues. Thus, plants exhibit reduced and stunted growth, dark green coloration of the leaves, production of slender stems, and death of older leaves (Taiz and Zeiger, 2006). Under saline-sodic or sodic conditions, high levels of external Na^+ not only interfere with K^+ acquisition by the roots, but also may disrupt the integrity of root membranes and alter their selectivity. The selectivity of the root system for K^+ over Na^+ must be sufficient to meet the levels of K^+ required for metabolic processes, for the regulation of ion transport, and for osmotic adjustment (Martinez and Cerda (1989).

Concerning the main effect of the soil application treatments (fulvic acid and silicon) on the percentages of nitrogen, phosphor, potassium, sodium and chloride results presented in Tables (4) showed that application of fulvic acid and silicon exhibited significant effect on the percentages of nitrogen, phosphorus and potassium as compared with the control treatment in both seasons. However, the differences between the two concentrations of either fulvic acid (1.5 and 3.0 g/l) or silicon (1.5 and 3.0 mM) were not significant. Whereas, soil application of fulvic acid and silicon differed in their effect on the contents of Na. The highest mean value of sodium was obtained with fulvic acid at 3 gm/l, where the lowest value was achieved with silicon at 3mM, in both seasons. So, fulvic acid activated the absorption of sodium, while silicon reduced it. Silicon at the rate of 3.0 mM reduced chloride ,compared to the other treatments, in both seasons. At silicon concentration of 3.0 mM, the estimated percent increase in nitrogen, phosphor and potassium, were (7.48 and 7.37%), (15.21 and 8.33 %) and (8.44 and 17.10 %) in the first and second seasons, respectively relative to the control treatment. Meanwhile, for silicon at 3.0 mM, the estimated percent decrease in sodium and chloride were (23.23 and 22.08%) and (20.87and 20.53 %) in the first and second seasons, respectively in relative to the control treatment.

The positive effect of silicon could be mediated decrease in the uptake and transport of Na^+ and increased uptake and transport of K^+ (Tuna *et al*., 2008; Hashemi *et al*., 2010 and Farshidi *et al.,* 2012), from roots to shoots under salt stress. Silicon seems to affect acquisition of other essential nutrients such as nitrogen, phosphorus and calcium and other micronutrients as well (Liang *et al.,* 2003 and Farshidi *et al*., 2012), thereby improving the growth of plants and generally the tolerance against salt stress. Moreover, P concentration and total P contents were increased by adding silicon under saline conditions. The possible causes for this may be associated with both Si-stimulated root activity showed by root dehydrogenase activity and Si-improved P bioavailability in soils due to the chemical competition between $H_2PO_4^-$ and silicate ($H_3SiO_4^-$) anions for the sorption sites. (Liang *et al*. ,1999) .

Pertaining the interaction effect between salinity levels and protection treatments (fulvic acid and silicon) on the percentages of nitrogen, phosphor, potassium, sodium and chloride in spinach leaves, results offered in Table (4) indicated significant differences among the interactions between both variables. The combined treatment between zero salinity and silicon at the rate of 3 mM achieved , generally, the highest values of N, P and K percentages in both seasons compared to other treatments, except nitrogen in the second season and potassium in both seasons. However, the combination between 4500 ppm salinity level and fulvic acid at 3 gm/L achieved the highest values of sodium **,** in both seasons. Moreover, the combination between 4500 ppm salinity level and control treatment reached, generally, the highest values of chloride **,** in both seasons.

It is vital to note that silicon reduced the risk effect of either sodium or chloride because it plays different roles in plant growth and development, improve plant resistance to diseases and pests, increase photosynthesis, regulate respiration and increase the tolerance of the plant to elements toxicity (Deshmukh *et al.,* 2017). Roshdy and Brengi (2016) found that silicon treatment resulted in significant decrease in leaves Na and Cl but increased K/Na ratio in snap bean leaves under salt stress condition.

Table (4): Percentages of nitrogen (N), protein, and phosphor (P) in leaves of spinach plants as affected by salinity and soil application of both fulvic acid and silicon in both seasons of 2019and 2020.

Treatments		N(%)		P(%)		K(%)	
		2019	2020	2019	2020	2019	2020
Salinity levels (ppm)	Tap water	3.49A*	3.56A	0.54A	0.57A	3.88A	3.95A
	1500	3.23B	3.18B	0.52B	0.53B	3.39B	3.61B
	3000	2.92C	2.74C	0.49C	0.47C	3.17C	3.28C
	4500	2.70D	2.47D	0.48D	0.44D	2.72D	2.90D
Protection treatments	control	2.94C	2.85C	0.46C	0.48C	3.08B	3.04B
	Fulvicacid1	3.06B	2.99B	0.51B	0.51B	3.33A	3.53A
	Fulvicacid2	3.13A	3.01AB	0.52AB	0.52AB	3.35A	3.51A
	Silicon1	3.12A	3.02AB	0.52AB	0.51B	3.36A	3.54A
	Silicon2	3.16A	3.06A	0.53A	0.52A	3.34A	3.56A
Interaction							
Tap water	control	3.51ab	3.56a	0.53cd	0.55cd	3.84a	3.92a
	Fulvicacid1.5gmperlitter	3.39bc	3.61a	0.54bc	0.58ab	3.93a	3.99a
	Fulvicacid3gmperlitter	3.53a	3.53a	0.55ab	0.60a	3.88a	3.96a
	Silicon1.5mM	3.49ab	3.58a	0.54bc	0.56bc	3.88a	3.94a
	Silicon3mM	3.53a	3.54a	0.56a	0.57b	3.87a	3.94a
1500	control	3.08f	3.00c	0.50efg	0.53d	2.97f	3.00d
	Fulvicacid1.5gmperlitter	3.23e	3.20b	0.52de	0.53d	3.42c	3.72b
	Fulvicacid3gmperlitter	3.21e	3.17b	0.52de	0.54d	3.51bc	3.75b
	Silicon1.5mM	3.25de	3.23b	0.53cd	0.53d	3.52b	3.79b
	Silicon3mM	3.36cd	3.28b	0.54bc	0.53d	3.52b	3.81b
3000	control	2.73h	2.56f	0.43h	0.44h	2.82g	2.67e
	Fulvicacid1.5gmperlitter	2.90g	2.71de	0.50fg	0.47fg	3.26de	3.46c
	Fulvicacid3gmperlitter	3.00fg	2.80d	0.51efg	0.48f	3.26de	3.39c
	Silicon1.5mM	2.98fg	2.78d	0.51efg	0.48f	3.31d	3.44c
	Silicon3mM	2.97fg	2.84d	0.50efg	0.50e	3.21e	3.46c
4500	control	2.46i	2.28h	0.39i	0.41i	2.69h	2.56f
	Fulvicacid1.5gmperlitter	2.72h	2.42g	0.49g	0.44h	2.71h	2.97d
	Fulvicacid3gmperlitter	2.77h	2.54fg	0.50efg	0.45gh	2.75gh	2.96d
	Silicon1.5mM	2.76h	2.50fg	0.50efg	0.45gh	2.71h	2.99d
	Silicon3mM	2.78h	2.60ef	0.50efg	0.47fg	2.75gh	3.03d

* Means having the same letter (s) within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability.

Total chlorophyll, protein, ascorbic acid, nitrate and total oxalate contents

Regarding the main effect of salinity levels on the total chlorophyll, protein, ascorbic acid, nitrate and total oxalate contents , data presented in Table (5) revealed that the total chlorophyll, protein and nitrate decreased by salinity levels increased, whereas, ascorbic acid and total oxalate increased with increasing salinity levels , in the two seasons. The reduction rate on total chlorophyll ,protein and nitrate varied depending on the level of imposed salinity stress. The highest values of total chlorophyll ,protein and nitrate content were obtained from the control treatment, while that 4500 ppm of salinity gave the lowest ones, in both seasons. However, the highest values of ascorbic acid and total oxalate were attained from 4500 ppm salinity, although that zero salinity reached the maximum values, in both seasons. At salinity of 4500 ppm, the estimated percent reductions, in total chlorophyll ,protein and nitrate were (13.72 and 14.37%), (22.71 and 30.68 %) and (30.75 and 30.24 %), in the first and second seasons, respectively relative to the control treatment .However, at salinity of 4500 ppm, the estimated percent increase in ascorbic acid and total oxalate were (10.11 and 9.53%) and (45.53 and 40.17%) in the first and second seasons, respectively relative to the control treatment . The present results are in agreement with those of Parida *et al. (*2005) who stated that salt stress has been shown to change the photosynthesis, osmoregulation, mineral ion contents, and chlorophyll content of spinach treated with 0–200 mmol L^{-1} NaCl and that salt stress showed toxic effects on plants and lead to metabolic changes, like loss of chloroplast activity and decreased photosynthetic rate. Also, the same conclusion were obtained by Khan *et al.*(2013) and Berengi (2019) in cucumber. The decrease in chlorophyll content under stress is a commonly reported phenomenon, and in various studies, this may be due to different reasons, one of them is related to membrane deterioration (Mane *et al.*, 2010). Also, with increasing salinity levels, total chlorophyll in pepper leaves significantly decreased, this reduction may be related to enhanced activity of the chlorophyll-degrading enzyme, chlorophyllase, as suggested by Mishra and Sharma, (1994) who indicated that increasing saline increased oxidation of chlorophyll leading to its decreased concentration. Moreover, other investigators indicated that during water stress brought about by salt stress, generation of reactive oxygen species (ROS) are thought to play important roles in inhibiting photosynthesis and H_2O_2 and OH- are responsible for injurious effect of salt stress on chlorophyll content and chloroplast ultra-structure (Yamane *et al*., 2004). Also, in spinach, Seven and Sağlam(2020) found that chlorophyll and total protein content in spinach leaves were reduced as salinity increased. Furthermore, increased salt content also interfered with protein synthesis and influences the structural component of chlorophyll (Jalee *et al*., 2008).Vaidyanathan *et al.* (2003) reported that the non-enzymatic antioxidants such as ascorbic acid, glutathione, α-tocopherol, and flavonoids, showed an accumulation in root tissues in rice plants subjected to salt stress.

Concerning nitrate contents, our results were in agreement with those obtained by Bian *et al*.(2020) who reported that chloride showed an opposite trend to nitrate as it is well-known that salinity can reduce nitrate accumulation in leafy vegetables due to antagonism between nitrate and chloride for the same root anion channel. A linear decrease in nitrate concentration has been reported in romaine lettuce baby-leaf grown in high salinity solution (Scuderi *et al*., 2011 ; Barbieri *et al*., 2011 ; Bonasia *et al*., 2017)). The increasing in EC resulted in a reduction in nitrate concentration along with a Cl^- rise in soilless-grown wild rocket (Bonasia et al., 2017). Moreover, it is known that chloride ions inhibit the activity of the enzymes involved in the N metabolism and consequently N assimilation (Barber et al., 1989; Debouba et al., 2006, 2007). Oxalic acid in lettuce leaves increased by increasing NaCl treatments (Tarakcioglu and Inal ,2002).

Regarding, the main effect of the soil application of fulvic acid and silicon on the total chlorophyll, protein, ascorbic acid, nitrate and total oxalate contents , results presented in Tables (5) demonstrated that soil application of fulvic acid and silicon revealed significant effect on total chlorophyll and protein, in both seasons, compared to control treatment. However, ascorbic acid contents reached the maximum values when plants received control treatment, in both seasons. Nevertheless, the differences between the two levels of both fulvic acid and silicon in total chlorophyll and protein percentage, generally, were not significant, in both seasons. Nitrate contents reached its maximum when plants were treated with fulvic acid followed by silicon at low concentration. Also, the differences between the high concentration of silicon (3 mM) and the control treatment were not significant, in both seasons. The highest values of total oxalate contents were obtained from control treatment, followed by the fulvic acid treatments, while that of silicon treatments recorded the lowest ones, in both seasons. So, silicon reduce the hazard effect of oxalate.

These results were in agreement with those of Lobato *et al.* (2009) who, documented that silicon encouraged a progressive increase in total chlorophyll in (*Capsicum annuum* L.) under water stress compared to control. Also, Li *et al.* (2015) indicated that chlorophyll contents were increased as a results of adding Si application to tomato seedlings under salt stress. Moreover, in spinach plants, exogenous application of Si increased chlorophyll concentration under salinity stress (Eraslan *et al*., 2008). Application of fulvic acid enhanced transport of minerals, improved plant hormone activity, modified enzyme activities, promoted photosynthesis, solubilization of micro and macro elements, protein synthesis, and reduction of active levels of toxic minerals (Aiken *et al*., 1985; Khang, 2011; Billard *et al.,* 2014; Kandil *et al.,* 2020).

Regarding, the interaction effect between salinity levels and protection treatments (fulvic acid and silicon) on total chlorophyll, protein, ascorbic acid, nitrate and total oxalate contents of spinach plants, results in Table (5) indicated that the combined treatment between salinity level at 4500 ppm and control gave , generally, the lowest chlorophyll and protein contents. The combination between 4500 ppm salinity level and fulvic acid at 3 gm/L achieved the highest values of ascorbic acid contents **,** in both seasons. Moreover, the combination between zero salinity and fulvic acid at 1.5 gm/l achieved the highest values of nitrate in both seasons compared to other treatments. However, the combined treatment between salinity level of 4500 ppm and control treatment attained , generally, the highest values of total oxalate contents, compared to other treatments.

Table (5) Total chlorophyll, protein, ascorbic acid and total oxalate contents of spinach plants as affected by salinity and soil application of both fulvic acid and silicon in both seasons of 2019and 2020.

Treatments		Chlorophyll(SPADUnit)		Protein(%)		Ascorbicacid(vitaminc)(mg/100gm)		totaloxalate	
		2019	2020	2019	2020	2019	2020	2019	2020
Salinity levels (ppm)	Tapwater	39.57A*	39.94A	21.80A	22.26A	49.38D	45.11D	577.15D	574.97D
	1500	37.28B	37.27B	20.16B	19.85B	52.27C	46.50C	766.07C	641.71C
	3000	36.697C	35.6C	18.22C	17.10C	53.59B	47.94B	807.92B	721.02B
	4500	34.14D	34.2D	16.85D	15.43D	54.37A	49.41A	839.94A	805.92A
Protection treatments	control	35.88B	34.59C	18.40C	17.82C	53.08A	50.15A	744.91A	734.37A
	Fulvicacid1	36.88A	36.92B	19.11B	18.66B	52.88AB	48.72B	761.00A	710.851B
	Fulvicacid2	37.14A	37.34AB	19.54A	18.80AB	52.89AB	47.33C	764.78A	671.609C
	Silicon1	37.29A	37.25AB	19.50A	18.89AB	51.97BC	46.70C	750.34B	659.16D
	Silicon2	37.41A	37.67A	19.74A	19.14A	51.20C	43.32D	717.49C	653.55D
Interaction									
Topwater	control	39.17ab	39.34ab	21.91ab	22.24a	47.795g	41.36j	574.03ef	576.25hi
	Fulvicacid1	39.5a	40a	21.20bc	22.57a	49.65fg	47.88cdef	576.73ef	565.62i
	Fulvicacid2	40a	40.67a	22.03a	22.03a	49.53fg	44.59ghi	628.71e	590.82hi
	Silicon1	39.5a	40a	21.79ab	22.35a	49.62fg	45.19fghi	563.66ef	589.491hi
	Silicon2	39.67a	39.67ab	22.05a	22.11a	50.28f	46.52efgh	542.61f	552.68i
1500	control	36de	35.34fgh	19.25f	18.77c	51.71ccdef	49.36bcd	731.34d	647.47fg
	Fulvicacid1	37.13cd	37.34cde	20.17e	20.02b	50.96ef	42.64ij	799.06abcd	633.37fg
	Fulvicacid2	37.49bcd	37.67cd	20.06e	19.83b	53.56abcd	45.97fgh	789.69abcd	657.191ef
	Silicon1	37.47bcd	37.34cde	20.31de	20.18b	52.55bcde	46.59defgh	749.701cd	655.72ef
	Silicon2	38.29abc	38.67bc	21.01cd	20.47b	52.58abcde	47.96cdef	760.57bcd	614.78gh
3000	control	35.16ef	35.34fgh	17.09h	15.98f	51.28def	50.89ab	808.27abc	727.49cd
	Fulvicacid1	36.87cd	37.34cde	18.10g	16.93de	53.15abcde	43.96hi	821.49abc	711.65cd
	Fulvicacid2	37.24cd	37.67cd	18.77fg	17.48d	53.91abc	47.39cdefg	801.24abcd	738.42c
	Silicon1	37.197cd	37.34cde	18.61fg	17.40d	54.49ab	48.03cdef	813.24abc	736.77c
	Silicon2	37.01cd	38.67bc	18.55fg	17.73d	54.69ab	49.44bc	795.36abcd	690.76de
4500	control	32.34g	30.67j	15.35i	14.28h	54.02ab	48.86bcde	912.51a	822.27a
	Fulvicacid1	34f	34.34hi	16.97h	15.12g	54.14ab	45.32fghi	846.69a	790.72ab
	Fulvicacid2	34.67ef	34.67gh	17.30h	15.87fg	54.97a	52.46a	830.17ab	820.47a
	Silicon1	35ef	35.34fgh	17.27h	15.64fg	54.43ab	49.51bc	814.34abc	818.63a
	Silicon2	34.67ef	36efg	17.37	16.26ef	54.799ab	50.97ab	841.099a	777.51b

* Means having the same letter (s) within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability.

The present study provided an evidence for the possibility of using silicon or fulvic acid (especially silicon) to enhance spinach plants and minimize the harmful effect of salinity.

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الملخص العربي

النمو والمحصول لنبات السبانخ متأثرا بالسيلكون وحمض الفولفيك تحت إجهاد الملوحة

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أجريت تجربتان أصص في مزرعة خاصة بمنطقة أبوحمص محافظة البحيرة, مصر خلال الموسم الشتوي لعامي 2019 , 2020 بهدف معرفة تأثير كلا من حمض الفولفيك والسيلكون في تقليل الأثر الضار للملوحة على نمو ومحصول و التركيب الكيماوي للسبانخ تحت مستويات ملوحة مختلفة . اشتملت كل تجربة على 20 معاملة وهي عبارة عن 4 معاملات ملوحة (ماء الصنبور 1500 , 3000 , 4500 جزء في المليون) مع 5 معاملات إضافة أرضية لحمض الفولفيك (1,5 و 3جم/لتر) والسليكون (1,5 و 3مللي مول) بالإضافة إلى معاملة الكنترول.

كان التصميم الإحصائي عبارة عن تجارب قطع منشقة مرة واحدة في تصميم القطاعات العشوائية الكاملة في 3 مكررات حيث كانت مستويات الملوحة في القطع الرئيسية بينما الإضافة الأرضية لكلا من حمض الفولفيك والسيلكون في القطع تحت الرئيسية . أوضحت النتائج المتحصل عليها بصفة عامة أن كل المقاييس المختبرة نقصت بزيادة الملوحة وأن معدل النقص مرتبط بتركيز مستويات الأملاح . أظهرت الدراسة أيضًا أن إضافة حمض الفولفيك والسليكون أعطت نتائج معنوية في تحسن كل المقاييس المختبرة مقارنة بالكنترول . أوضحت الدراسة أيضًا أن إضافة السليكون عند مستوى 3 مللي مول أعطى بصفة عامة أعلى القيم لكلا من ارتفاع النبات, الوزن الطازج للنبات, الوزن الجاف للنبات , عدد الأوراق , محتوى الأوراق من عناصر النيتروجين والفوسفور والبوتاسيوم والبروتين والكلوروفيل الكلي وأيضًا قلل الأثر الضار لمحتوى النباتات من النترات و الاوكسالات الكلية , مقارنة بباقي المعاملات في موسمي الزراعة , وأظهرت الدراسة أن أفضل معاملة تداخلية هي إضافة السليكون عند مستوى 3 مللي مول مع ملوحة صفر, أعطت أفضل النتائج لمعظم الصفات المختبرة , توصي هذه الدراسة أن إضافة حمض الفولفيك والسيلكون يحسن من صفات نحو نباتات السباخ ويقلل الأثر الضار للملوحة .

الكلمات المفتاحية سبانخ : ملوحة : سيلكون : فولفيك اسيد : الإجهاد : النمو.