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Combined Effect of some Nitrogen and Zinc Sources on some Quality Parameters of Spinach Grown on Salt Affected Soil

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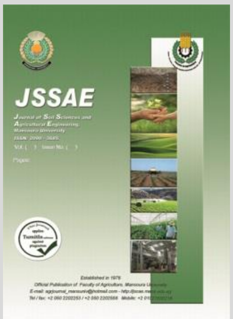

ABSTRACT

Salinity is a major problem affecting vegetable production in Egypt. Thus, the purpose of this investigation was to evaluate the influence of foliar applications of different sources of zinc [(Z_1): Zn-EDTA, (Z_2): $ZnSO_4$ and (Z_3): without Zn (control)] with both $(NH_4)_2 SO_4$ and $Ca(NO_3)_2$ combined together with one level as 6 mixed ratios of $Ca(NO_3)_2$: $(NH_4)_2 SO_4$ [$N_1$100% $Ca(NO_3)_2$: 0.0% $(NH_4)_2 SO_4$, N_2 75% $Ca(NO_3)_2$: 25% $(NH_4)_2 SO_4$, N_3 50% $Ca(NO_3)_2$: 50% $(NH_4)_2 SO_4$, N_4 25% $Ca(NO_3)_2$:75% $(NH_4)_2 SO_4$, N_5 0.0% $Ca(NO_3)_2$: 100% $(NH_4)_2 SO_4$ and N6 control treatment (without nitrogen)] on some quality parameters and chemical composition of spinach plants grown on salt-affected soil during the winter season of 2018. The experiment was designed in a split-plot design with three replicates. As for most determined parameters at both growth stages (30 and 55 days sowing), the findings indicate that the highest values were recorded with N_1 treatment and foliar application of Zn-EDTA (Z_1),while the lowest values were recorded under a combination of N_6 treatment (without N-fertilization) and Z_3 treatment (untreated plants). It can be concluded that; foliar spraying with Zn-EDTA in combination with soil addition of calcium nitrate as a source of N-fertilization is considered the most suitable treatment for overcoming salinity stress in some Egyptian soils and releasing the highest safe yield of spinach plant.

***Keywords*:** Spinach, zinc, salt-affected soil, $Ca(NO_3)_2$, $(NH_4)_2 SO_4$, nutritional elements and quality parameters.

INTRODUCTION

Spinach (*Spinacea oleracea* L.) is an edible flowering plant in the family of Chenopodiaceae. It is containing relatively high amounts of protein, mineral salts (mainly iron and calcium), as well as provitamin A and vitamins C, B_1, and B_2. It is a vegetable, which is easily assimilated by humans and is recommended for child nutrition (El-Sirafy *et al.* 2015).Salinity is a major problem affecting crop production all over the world: 20% of cultivated soil in the world, and 33% of irrigated soil, are salt-affected and degraded. It is a major factor limiting crop productivity in the arid and semi-arid areas of the world. It has a negative influence on soil physical and chemical properties (Herpin *et al*. 2007). Zinc plays an essential role as enzymes metal component (superoxide dismutase, alcohol dehydrogenase, carbonic anhydrase and RNA polymerase) or as a functional, the structural, or regulatory cofactor of a large number of enzymes (El-Gizawy and Mehasen, 2009). Traditional agriculture practices employ Zn sulfate ($ZnSO_4$) or EDTA-Zn chelate for application to leaves and ground. Zn absorption through the leaves seems to be determined by the source of the micro-nutrients (García-López *et al*. 2019).The effects of different sources of nitrogen on growth and the accumulation of nitrate on vegetables have attracted significant attention in recent years. In several studies, urea and ammonium nitrate are the most used N fertilizers.While there are very few studies that compare $(NH_4)_2SO_4$, Ca $(NO_3)_2$ and other nitrogen sources. Also, the development of more efficient foliar Zn fertilizers is limited by a lack of knowledge regarding the distribution, mobility, and speciation of Zn in leaves once it is taken up by the plant. Thus, the purpose of this investigation is to study the effect of foliar applications of different sources of zinc [Zn-EDTA(14%Zn) , $ZnSO_4$ (21.3% Zn) and without Zn (control)] with using both $(NH_4)_2 SO_4$ and $Ca(NO_3)_2$ which combined together with one level as ratio between $Ca(NO_3)_2$: $(NH_4)_2 SO_4$) [100%: 0.0%, 75%:25%, 50% :50%, 25%:75%,100% :0.0%, and control treatment (without nitrogen)] on accumulation of nitrate and nitrite and chemical composition of spinach plants grown on salt-affected soil.

MATERIALS AND METHODS

A pot experiment was carried out at the Experimental Farm of Faculty of Agric. El- Mansoura Univ. during the winter season of 2018 to investigate the response of spinach (*Spinacia oleracea* L.) grown on salt-affected soil to foliar applications of different sources of zinc with using different types of N-fertilizers as a soil addition. Eighteen treatments were in a split-plot design with three replicates, which were the simple possible combination between two forms of zinc fertilization in a foliar way as main plots and six ratios of [$(NH_4)_2 SO_4$ and $Ca(NO_3)_2$] as soil addition as subplots. Main plots were zinc treatments which included three forms *i.e.* (Z_1): Zinc was foliar applied as Zn -EDTA (14%Zn) at rate of 100 mgL^{-1}, (Z_2): Zinc was foliar applied as Zn SO_4 (21.3% Zn)

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at rate of 100 mgL^{-1} and (Z_3): Without zinc (control treatment).While, subplots were N-fertilization treatments. $Ca(NO_3)_2$ and $(NH_4)_2SO_4$) were combined with one level 60 kg N fed^{-1} as a ratio between $Ca(NO_3)_2$: $(NH_4)_2SO_4$) as follows; (N_1):100% calcium nitrate : 0.0% ammonium sulfate, (N_2): 75% calcium nitrate:25% ammonium sulfate, (N_3) 50% calcium nitrate : 50% ammonium sulfate,(N_4)25% calcium nitrate :75% ammonium sulfate, (N_5):0.0% calcium nitrate : 100% ammonium sulfate and (N_6): without nitrogen (control treatment). Fifty-four plastic pots (30 cm diameter and 40 cm height) were used in the experimental season. Each pot was filled by air-dry soils equal to 10 kg oven-dry soil taken from Kalapshoo Village, Belqas District, Dakahlia Governorate, and analyzed according to Dewis and Feritas (1970) as shown in Table (1).

Table 1. Some physical and chemical characteristics of the experiment soil.

Particle size distribution (%)				**Textural class**		**EC, dSm^{-1}***	**pH ****	**$CaCO_3$**	**O.M**	**F.C**	**SP**
C.sand sand	F. sand	Silt	Clay	Clay				(%)			
4.99	10.41	35.1	49.5			5.30	8.20	3.85	1.35	45.0	90.0
Soluble cations (meq L^{-1})				Soluble anions (meq L^{-1})				Available element, mg kg^{-1}			
Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}	Zn	N	P	K
6.50	12.70	20.10	13.70	-	2.75	22.55	27.70	0.85	42.6	4.14	229.2

*** Soil Electrical Conductivity (EC) and soluble ions were determined in soil paste extract. ** Soil pH was determined in soil paste.**

Planting date was on 12th of February during the experimental season. Twenty seeds of spinach c.v DASH were planted in each pot and were thinned to the most suitable ten uniform plants per pot after 14 days from planting (3true leaves). The watering was every 5-7 days to reach the soil moisture to 70% of field capacity by weight. The P and K fertilizers were added to the soil of pots cultivated with spinach plants as recommended by the Ministry of Agriculture and Soil Reclamation (MASR). Phosphorus fertilizer was added before planting to the soil, while potassium fertilizer was added in one dose after 23 days from planting. As for nitrogen fertilization, [$(NH_4)_2SO_4$ and $Ca(NO_3)_2$] combined with one level 60 kg N/fed as mentioned above ratios between $Ca(NO_3)_2$: $(NH_4)_2SO_4$) as recommended by the MASR for leafy vegetables, the doses were divided into two equal doses, the first application was done after 15 days from planting and the second 15 days later. Zinc fertilizers in both studied forms of (EDTA) Ethylene Diamine Tetra Acetic Acid (14%Zn) and zinc sulfate ($ZnSO_4$ 21.3%Zn) were prepared then sprayed three times (after 20, 30 and 45 days of sowing).Spinach plants were harvested on April 8, 2018 (after 55 days from planting).

At two different stages (30 and 55 days after sowing of spinach plant); representative samples were randomly taken from each experimental pot to determine the Zn, Ca, N, P and K contents in spinach leaves and some quality parameters of the fresh plants, such as total phenolic, NO_3-N and NO_2-N contents and vitamin C. The global standard methods which used to measure the concentrations of Zn, Fe, Ca, N, P and K in shoots of spinach plant at the both investigated stages (at 30 and 55 days from sowing) were shown in Table 2.

Table 2. Global standard methods of plant chemical analysis.

Parameters	**Methods**	**References**
Nutrients content		
Digested for Zn, Ca, Fe, N, P and K analysis	Mixed $HClO_4 + H_2SO_4$ method.	Cotteni *et al.* 1982
Total Zn ,Ca and Fe	Atomic Absorption Spectrophotometer	Chapman and Pratt, 1978
Total N	Micro-kjeldahl method	Jones *et al.* 1991
Total P	spectrophotometrically	Peters *et al.* 2003
Total K	Flame photometer	Peters *et al.* 2003
Quality parameters Vitamin C	Titrimetric estimation with 2, 6 dicholoro phenol dye solution.	Mazumdar and Majumder, 2003
Total phenolic	Spectrophotometrically using the modified Folin-Ciocaltue colorimetric method.	Eberhardt *et al.* 2000
NO_3-N	Extracted by 2% acetic acid using of N-1 naphthyle ethylene diamine dihydrochlorid as an indicator (spectrophotometrically)	Singh, 1988

Statistical analysis:

Data were statistically analyzed according to Gomez and Gomez (1984) using CoStat (Version 6.303, CoHort, USA, 1998–2004).

RESULTS AND DISCUSSION

1- Plant Nutritional Status.

N, P and K contents.

Means of N, P and K percentages in spinach leaves at different growth stages (30 and 55 days after planting) as affected by different Zn-sources, different N-sources and their interactions during the season of the experimentation are shown in Table 3. Regarding the effect of zinc foliar spraying on N, P and K percentages in spinach leaves, data in Table (3) indicate that using both Zn-EDTA and $ZnSO_4$ led to a significant increase in the values of N and K (%) compared with untreated plant (without zinc), while the value of P (%) in spinach leaves were significantly decreased under both studied Zn-forms compared with untreated plant (without zinc). On the other words; the highest values of N and K (%) in leaves of spinach plants were recorded at Z_1treatment (Zn-EDTA) followed by Z_2 ($ZnSO_4$) and lastly Z_3 treatment (control). This is due to the zinc element may be stimulating N and K uptake. Also, translocation of Zn away from the treated leaf to other plant parts under EDTA form was faster than that $ZnSO_4$

form, thus the spinach plants treated with Zn-EDTA gave results better than $ZnSO_4$. Similar results were also concluded by Tian *et al.* (2015).

On the contrary, the highest values of P (%) in spinach leaves were realized at Z_3 treatment (control) followed by Z_2 treatment ($ZnSO_4$) and lastly Z_1treatment (Zn-EDTA). These results may be attributed to the antagonism between zinc and phosphorus (El-Agrodi *et al.* 2017). These results are supported by the findings of Amin and Ghaly (1984) and Bouain *et al.* (2014) who stated that Zn-translocation into faba bean leaves decreased by P-application especially when Zn was applied as foliage. P-translocation showed the same general trend as affected by Zn-treatments. They concluded that there is a mutual interaction between phosphorus and zinc, which affected the translocation of both nutrients whenever either element exceeded some threshold value.

Table 3. Nitrogen, phosphorus and potassium (%) of spinach plant at different growth stages (30 and 55 days from sowing) as affected by different sources of zinc, different types of N-fertilizers and their interactions.

Treatments		N%		P%		K%	
		30 days	55 days	30 days	55 days	30 days	55 days
Zn fertilization							
Z_1		2.09	2.86	0.283	0.436	3.13	3.83
Z_2		1.85	2.55	0.287	0.441	2.92	3.45
Z_3		1.64	2.22	0.316	0.482	2.59	3.17
LSD $_{at\ 5\%}$		0.02	0.01	0.005	0.014	0.12	0.01
Nitrogen forms							
N_1		2.46	3.39	0.362	0.555	3.44	4.20
N_2		2.19	2.94	0.335	0.505	3.17	3.89
N_3		1.89	2.58	0.306	0.469	3.13	3.58
$N_4)_2$		1.70	2.33	0.277	0.427	2.76	3.37
N_5		1.52	2.10	0.254	0.393	2.50	3.05
N_6		1.40	1.91	0.237	0.368	2.30	2.82
LSD $_{at\ 5\%}$		0.02	0.01	0.003	0.013	0.13	0.01
Interaction							
Z_1 (Zn-EDTA)	N_1	2.74	3.72	0.349	0.536	3.73	4.56
	N_2	2.44	3.26	0.323	0.498	3.43	4.20
	N_3	2.12	2.90	0.293	0.451	3.23	3.95
	$N_4)_2$	1.92	2.63	0.265	0.407	3.01	3.71
	N_5	1.71	2.42	0.242	0.374	2.81	3.43
	N_6	1.60	2.23	0.224	0.348	2.55	3.15
Z_2 (Zn SO_4)	N_1	2.41	3.39	0.354	0.542	3.42	4.15
	N_2	2.18	2.94	0.326	0.502	3.13	3.85
	N_3	1.90	2.59	0.297	0.456	3.51	3.53
	$N_4)_2$	1.70	2.33	0.268	0.413	2.76	3.34
	N_5	1.53	2.11	0.245	0.380	2.47	3.00
	N_6	1.41	1.92	0.232	0.355	2.27	2.80
Z_3(Without Zn)	N_1	2.23	3.08	0.383	0.586	3.17	3.90
	N_2	1.94	2.63	0.356	0.516	2.94	3.63
	N_3	1.65	2.26	0.327	0.502	2.64	3.25
	$N_4)_2$	1.49	2.02	0.297	0.460	2.52	3.05
	N_5	1.32	1.78	0.275	0.425	2.22	2.71
	N_6	1.20	1.56	0.255	0.401	2.08	2.51
LSD $_{at\ 5\%}$		0.04	0.03	0.006	0.021	0.23	0.02

Z_1:At the rate of 100 mgL^{-1} Zn using [Zn -EDTA 6%Zn]; Z_2:At the rate of 100 mgL^{-1}Zn using [Zn SO_4 21.3% Zn]; Z_3:The control treatment (without zinc); N_1:(100% calcium nitrate : 0.0% ammonium sulfate); N_2: (75% calcium nitrate : 25% ammonium sulfate); N_3: (50% calcium nitrate : 50% ammonium sulfate); N_4:(25% calcium nitrate : 75% ammonium sulfate) N_5:(0.0% calcium nitrate : 100% ammonium sulfate) and N_6:The control treatment (without nitrogen).

Regarding the effect of studied N-treatments (N_1, N_2, N_3, N_4, N_5 and N_6) on aforementioned traits, obtained results in Table (3) indicate that all studied N-sources (N_1, N_2, N_3, N_4 and N_5) significantly increased N, P and K percentages as compared to N_6 treatment (untreated plants), where the application of Ca $(NO_3)_2$ ratio at rate of (100% calcium nitrate : 0% ammonium sulfate) (N_1) was superior treatment regarding N, P and K percentages in spinach leaves compared with other sources and untreated plant (control).Also, the lowest values of the aforementioned traits were obtained at control treatment(N_6),where the treatments sequence from top to less was the $N_1 > N_2 > N_3 > N_4 > N_5 > N_6$ (control), such effect was the same at both studied growth stages (30 and 55 days sowing) during the season of the experimentation. The significant increase in N, P and K percentages in spinach leaves due to applied N-sources in different ratios compared to control confirm the deficiency of nitrogen in the investigated soil (42.6 mg kg^{-1}) as well as weakness of root absorption due to the high salinity of the investigated soil (5.3 dSm^{-1}). Spinach plant can grow under moderate soil fertility circumstances but production is enhanced with application of nitrogen fertilizer especially from calcium source such as calcium nitrate. The results of this indicate that, the soil applications of nitrogen at 60 kg $Nfed^{-1}$ as calcium nitrate fertilizer significantly increased the N, P and K contents in spinach leaves compared to control at both stages of growth. These results are in harmony with the findings of El-Sirafy *et al.* (2015) and Shormin and Kibria, (2018).

Concerning the interaction effect between the treatments under study , data in Table (3) reveal that the values of N, P and K percentages in spinach leaves at the both growth stages (30 and 55 days sowing) were significantly affected due to the addition of all investigated treatments. At the both growth stages (30 and 55 days sowing), the highest values of N and K (%) in spinach leaves were recorded with N_1 treatment ((100% Ca $(NO_3)_2$: 0.0% $(NH_4)_2SO_4$)) and foliar application of Zn- EDTA (Z_1) .While the lowest values of N and K (%) in spinach leaves were recorded under combination between N_6 treatment (without N-fertilization) and Z_3 treatment (untreated plants). Also, under all the zinc treatments, the application of $Ca(NO_3)_2$ ratio at rate of (100% calcium nitrate : 0% ammonium sulfate) (N_1) gave higher N and K (%) in spinach leaves. On the other hand, the folair sprying of Zn-EDTA (Z_1) gave higher N and K (%) in spinach leaves under all of N treatments. On the contrary; because of the antagonism between zinc and phosphorus, the value of P (%) under any studied N-treatment significantly decreased at the both studied Zn-forms(Zn-EDTA and $ZnSO_4$) compared with the untreated plant (without zinc). Generally, under all the zinc treatments, the highest value of P (%) in spinach leaves were recorded with N_1 treatment ((100% $Ca(NO_3)_2$: 0.0% $(NH_4)_2SO_4$)),while the lowest value were recorded with N_6 treatment (without N-fertilization),but the values of P (%) under Z_3 treatment (without zinc) is greater than those under the both Z_2 ($ZnSO_4$) and Z_1(Zn-EDTA) treatments due to antagonism between zinc and phosphorus. Moreover, the values of P (%) under Z_2treatment were higher than those obtained for the Z_1 at any studied N-treatment. The present results agree with those obtained by (El-Sirafy *et al.* 2015; El-Agrodi *et al.* 2017; Doolette *et al.* 2018 and García-López *et al.* 2019).

Ca, Fe and Zn contents:

Calcium, iron and zinc contents in spinach leaves are considered to be the most important chemical characters of spinach plant especially the iron content. Data of Table (4) show the effect of different Zn- sources, different N-sources and their interactions on Ca%, Fe and Zn (mg kg^{-1}) in spinach leaves at two different growth stages (30 and 55 days sowing) during the season of the experimentation.

The different sources of Zn nutrient [Zn-EDTA, $ZnSO_4$, and control (without Zn)] pronouncedly affected the values of Ca%, Fe and Zn (mg kg^{-1}) in spinach leaves at two different growth stages (30 and 55 days sowing) during the season of the experimentation. The values of all aforementioned traits of spinach plants treated with the two different Zn- sources (Zn-EDTA and $ZnSO_4$) were significantly increased compared with the untreated plant (without zinc) but the resulting increase with Zn-EDTA is greater than $ZnSO_4$, where the highest values were realized when the plants treated with Zn-EDTA followed by $ZnSO_4$ and lastly control treatment (without Zn). The present results agree with those obtained by Doolette *et al.* (2018) and García-López *et al.* (2019). The statistical analysis illustrated that the differences in the values of Ca%, Fe and Zn (mg kg^{-1}) in spinach leaves under different nitrogen sources were statically significant. It was evident from the results that, the maximum values of Ca%, Fe and Zn (mg kg^{-1}) in spinach leaves were obtained with applying nitrogen from $Ca(NO_3)_2$ ratio at rate of (100% $Ca(NO_3)_2$:0% $(NH_4)_2SO_4$) followed by (75% $Ca(NO_3)_2$: 25% $(NH_4)_2SO_4$),while the minimum values of Ca%, Fe and Zn(mg kg^{-1}) in spinach leaves were found with control treatment (without nitrogen).The finding of the present study is in concurrence with Chohura and Kolota, (2011) and El-Sirafy *et al.* (2015).

Nutritional elements concentrations, *i.e,* calcium, iron and zinc in spinach plant as affected by the interaction between all treatments under study are tabulated in Table (4). The highest values of Ca%, Fe and Zn (mg kg^{-1}) in spinach leaves were recorded at (Z_1× N_1) treatment, while the lowest values were recorded at (Z_6× N_6) treatment. Also, the application of $Ca(NO_3)_2$ ratio at rate of (100%:0%) (N_1) gave higher values of Ca%, Fe and Zn (mg kg^{-1}) in spinach leaves at any studied Zn treatment but the resulting increase under Zn-EDTA is greater than $ZnSO_4$ than control. For example, the values of Ca%, Fe and Zn (mg kg^{-1}) in spinach leaves at the both growth stages under (Z_1× N_1) treatment were greater than (Z_2×N_1) treatment as well as the values under (Z_2× N_1) treatment were greater than (Z_3×N_1)treatment. Generally, as mentioned earlier, Zn enhances cation-exchange capacity of the roots, which in turn enhances absorption of essential nutrients under salinity stress. Also, the nitrogen fertilizers have appositive effect on plant content from essential nutrient. Thus, calcium, iron and zinc contents in spinach leaves is enhanced with combination between foliar spraying of Zn element especially from Zn-EDTA and soil application of nitrogen fertilizer especially from calcium source such as calcium nitrate. Very close results were found by Abdelraouf (2016) who reported that increasing N fertilization rates generally increased the spinach content of N, P, K, and Fe as well as did not affect the contents of S, Ca, Mg, and Zn. In our study, the increase in Ca and Zn contents were due to Ca and Zn applications. The present results agree with those obtained by (El-Sirafy *et al.* 2015; Doolette *et al.* 2018 and García-López *et al.* 2019).

Table 4. Calcium (%), iron and zinc (mg kg^{-1}) of spinach plant at different growth stages (30 and 55 days from sowing) as affected by different sources of zinc, different types of N-fertilizers and their interactions.

Treatments		Ca (%)		Fe (mg kg^{-1} DM)		Zn (mg kg^{-1} DM)	
		30 days	55 days	30 days	55 days	30 days	55 days
Zn fertilization							
Z_1		1.99	2.81	18.46	26.26	23.99	37.08
Z_2		1.74	2.53	18.19	26.00	20.90	34.63
Z_3		1.56	2.06	17.03	24.38	6.28	10.82
LSD at 5%		0.02	0.14	0.02	0.05	0.12	0.01
Nitrogen forms							
N_1		3.35	4.41	18.88	26.96	21.37	34.04
N_2		2.72	3.62	18.61	26.59	19.86	31.14
N_3		1.72	2.51	18.32	26.09	17.97	28.46
$N_4)_2$		1.36	1.90	17.74	25.35	16.38	25.63
N_5		0.86	1.40	17.22	24.59	15.46	23.74
N_6		0.55	0.96	16.58	23.69	11.31	22.06
LSD at 5%		0.03	0.13	0.03	0.03	0.13	0.03
Interaction							
Z_1 (Zn-EDTA)	N_1	3.59	5.02	19.15	27.30	29.09	44.90
	N_2	2.94	4.13	18.84	26.91	26.97	41.70
	N_3	1.96	2.75	18.84	26.40	24.75	38.30
	$N_4)_2$	1.60	2.28	18.23	26.01	22.55	34.87
	N_5	1.10	1.56	17.94	25.62	20.95	32.37
	N_6	0.75	1.11	17.74	25.31	19.63	30.36
Z_2 (Zn SO_4)	N_1	3.33	4.54	18.92	27.04	27.33	42.28
	N_2	2.69	3.74	18.62	26.62	25.35	39.14
	N_3	1.71	2.49	18.29	26.13	22.98	35.70
	$N_4)_2$	1.34	1.96	17.95	25.72	20.95	32.39
	N_5	0.84	1.46	17.73	25.33	19.26	29.99
	N_6	0.50	0.99	17.61	25.14	9.55	28.27
Z_3(Without Zn)	N_1	3.14	3.66	18.57	26.53	7.71	14.94
	N_2	2.53	2.99	18.37	26.24	7.26	12.58
	N_3	1.51	2.29	17.85	25.75	6.17	11.38
	$N_4)_2$	1.15	1.47	17.03	24.33	5.65	9.63
	N_5	0.64	1.17	15.99	22.82	6.18	8.85
	N_6	0.40	0.78	14.40	20.63	4.74	7.54
LSD at 5%		0.05	0.23	0.05	0.08	0.23	0.06

Z_1:At the rate of 100 mgL^{-1} Zn using [Zn -EDTA 6%Zn]; Z_2:At the rate of 100 mgL^{-1}Zn using [Zn SO_4 21.3% Zn]; Z_3:The control treatment (without zinc); N_1:(100% calcium nitrate : 0.0% ammonium sulfate); N_2: (75% calcium nitrate : 25% ammonium sulfate); N_3: (50% calcium nitrate : 50% ammonium sulfate); N_4:(25% calcium nitrate : 75% ammonium sulfate) N_5:(0.0% calcium nitrate : 100% ammonium sulfate) and N_6:The control treatment (without nitrogen).

2- Quality Parameters of Spinach Plant.

Nitrate and nitrite.

Nitrate and nitrite concentration in spinach leaves as influenced by zinc addition, N-forms and their interactions are present in Table (5),where obtained data show the individual effect of the different sources of Zn nutrient [Zn-EDTA, $ZnSO_4$, and control (without Zn)] on nitrate and nitrite concentration in spinach leaves .It indicated that; the values of NO_3-N and NO_2-N (mg kg^{-1}) in spinach leaves significantly declined due to an addition of Zn in foliar way as compared to the untreated planted. Also, the foliar spraying of Zn-EDTA gave nitrate and nitrate accumulation in spinach leaves less than $ZnSO_4$ (Z_2 treatment).

The present results partly agree with those obtained by Gheshlaghi *et al.* (2014) who evaluated the influence of foliar zinc and harvest times on the decline of both nitrate accumulation and nitrate reductase activity, where their treatments were two zinc rates (7.0 and 50.0 ppm), two harvest times (29 and 46 days from sowing) and two plants of lettuce and spinach. The results confirmed that with increasing zinc concentration, NO_3-N concentration in shoots of lettuce in both times markedly decreased and NO_3-N concentration in roots declined during the second harvest, whereas no reduction was reported in roots and shoots of spinach plants. Nitrate reductase activity was significantly increased in both plants with application of high rates of zinc. Increased nitrate accumulation was found over time in both investigated plants.

Table 5. NO_3-N and NO_2-N (mg kg^{-1}) of spinach plant as affected by different sources of zinc, different types of N-fertilizers and their interactions.

Treatments		NO_3-N mg kg^{-1}	NO_2-N mg kg^{-1}
Zn fertilization			
Z_1		263.19	2.93
Z_2		269.28	3.20
Z_3		276.71	4.64
LSD at 5%		1.15	0.02
Nitrogen forms			
N_1		217.12	2.87
N_2		287.59	3.42
N_3		302.51	3.98
$N_4)_2$		322.80	4.44
N_5		339.56	4.31
N_6		148.77	2.51
LSD at 5%		0.87	0.02
Interaction			
Z_1 (Zn-EDTA)	N_1	211.37	2.10
	N_2	281.76	2.61
	N_3	296.65	3.16
	$N_4)_2$	316.20	3.81
	N_5	331.55	4.03
	N_6	141.62	1.84
Z_2 (Zn SO_4)	N_1	217.42	2.61
	N_2	286.52	3.14
	N_3	301.55	3.61
	$N_4)_2$	322.57	4.39
	N_5	338.47	3.07
	N_6	149.12	2.37
Z_3(Without Zn)	N_1	222.57	3.89
	N_2	294.50	4.51
	N_3	309.33	5.16
	$N_4)_2$	329.63	5.14
	N_5	348.66	5.82
	N_6	155.56	3.31
LSD at 5%		1.52	0.04

Z_1:At the rate of 100 mgL^{-1} Zn using [Zn -EDTA 6%Zn]; Z_2:At the rate of 100 mgL^{-1}Zn using [Zn SO_4 21.3% Zn]; Z_3:The control treatment (without zinc); N_1:(100% calcium nitrate : 0.0% ammonium sulfate); N_2: (75% calcium nitrate : 25% ammonium sulfate); N_3: (50% calcium nitrate : 50% ammonium sulfate); N_4:(25% calcium nitrate : 75% ammonium sulfate) N_5:(0.0% calcium nitrate : 100% ammonium sulfate) and N_6:The control treatment (without nitrogen).

The results of this study show that nitrate metabolism in plants influenced by the plant species, harvest time, activity of nitrate reductase enzyme and plant nutrients like zinc. With respect to the effect of N-forms it is evident that; the lowest values of nitrate and nitrite accumulation were recorded for the untreated planted (without N-fertilization) followed by the planted treated with N-fertilizer in form of calcium nitrate (100%).Also, N-fertilizer in form of ammonium sulfate (either alone or mixed with calcium nitrate) caused raising nitrate and nitrite accumulation compared with the planted treated with N-fertilizer in form of calcium nitrate (100%).

The effect of ammonium sulfate in the remarkably raising nitrate and nitrite accumulation in the spinach plant is due to its high content of nitrogen (21%N in ammonium form) which turns in to nitrate form under Egyptian conditions (nitrification process) according to the following formula.

NH_4^+→ Hydroxylamine→ Nitroxyl→Nitrohydroxylamine → Nitrate →Nitrite

Especially under salt affected soil which need big quantities of irrigation water to leach the salt (suitable oxidation condition) such as our investigated soil. While calcium nitrate contains15.5% N in nitrate form. Thus, because of the fast change of nitrogen from ammonium to nitrate under Egyptian conditions, most of added NH_4^+ infiltrate to soil solution in NO_3^- form, therefore the plants absorb large quantities of N in NO_3^- form. Due to nitrification process, the plant absorbs most of its nitrogen requirements in NO_3^- form which turn into NH_4^+ inside plants. Nitrates in excess of plant requirements remain in NO_3^- form. Thus, in the case of using ammonium sulfate in fertilization compared with calcium nitrate, the nitrite accumulation is more in plants. Generally, dependence on our results, the ammonium sulfate usage in fertilization led to nitrate accumulation more than calcium nitrate. The finding of the present study is in concurrence with Chohura and Kolota, (2011) and El-Sirafy *et al.* (2015) who found that the highest mean values of nitrate and nitrite accumulation were recorded for the plants treated with N-fertilization in the form of ammonium nitrate without an addition of Ca by foliar way, while the lowest values of such traits were realized with foliar applied of calcium on spinach plant. On the contrary to our results Zeka *et al.* (2014) reported that the application of Ca $(NO_3)_2$ resulted in a higher content of nitrate compared to NH_4NO_3, and CO $(NH_2)_2$. Statically analysis of the data in Table (5) revealed the values of NO_3-N and NO_2-N (mg kg^{-1}) in spinach leaves as affected by combination between the various treatments under investigation. In this respect, the lowest values of all quality parameters mentioned were recorded with control treatment (Z_3× N_6). On the contrary of this trend, the highest values of nitrate and nitrite accumulation (mg kg^{-1}) were recorded with the treatments of [Z_3 (without zinc) × N_5 (0.0% $Ca(NO_3)_2$: 100% $(NH_4)_2SO_4$)]. Under all zinc treatments, the lowest values of all quality parameters mentioned were recorded with the untreated planted (without N-fertilization) followed by the planted treated with N-fertilizer in form of calcium nitrate (100%), while the highest values were recorded with the planted treated with N-fertilizer in form of ammonium sulfate (100%).The present results agree with those obtained by (El-Sirafy *et al.* 2015; Doolette *et al*. 2018 and García-López *et al*. 2019). On the contrary to our results, Bowman and Paul (1992) did not observe any differences in plants treated with urea, ammonium and nitrate nitrogen.

Total phenol and vitamin C (mg/100g F.W).

Data presented in Table (6) indicate the effect of different Zn-sources, different N-sources as well as its interactions on quality parameters, *i.e.* total phenol and vitamin C (mg/100g F.W) of spinach plant at harvest stage (55 after sowing) during the season of the experimentation.

There was a positive effect of zinc application with different forms (Zn-EDTA and $ZnSO_4$) recorded on quality parameters, *i.e.* total phenol and vitamin C of spinach plant. Data presented in Table 6 revealed that foliar spraying of Zn-EDTA (Z_1treatment) significantly and sharply increased the values of the aforementioned traits than those obtained for the spinach plant treated with $ZnSO_4$ (Z_2 treatment). On the other hand, the spinach plant treated with Zn-EDTA (Z_1 treatment) or $ZnSO_4$ (Z_2 treatment) had the highest values of the aforementioned traits comparing with the Z_3 treatment (control). Treatments sequence from top to less was the Zn-EDTA > $ZnSO_4$> control (without Zn). The present results agree with those obtained by Doolette *et al.* (2018) and García-López *et al*. (2019). Similar results was found by Sarkar *et al.* (2017) who revealed that the application of zinc at 15 kg/ha through soil and 1.5 g/ litre (in $ZnSO_4$ form) markedly influenced total phenol and vitamin C as compared with the control.

Table 6. Total phenol and vitamin C (mg/100g F.W) of spinach plant as affected by different sources of zinc, different types of N-fertilizers and their interactions.

Treatments		T. phenol (mg/100g D.W)	Vitamin C (mg/100g F.W)
Zn fertilization			
Z_1		922.44	54.70
Z_2		902.42	51.26
Z_3		866.78	46.73
LSD at 5%		12.55	0.04
Nitrogen forms			
N_1		979.14	59.06
N_2		930.14	56.20
N_3		912.09	52.75
$N_4)_2$		881.89	49.29
N_5		867.53	45.42
N_6		814.07	42.66
LSD at 5%		13.01	0.03
Interaction			
Z_1 (Zn-EDTA)	N_1	1004.07	62.44
	N_2	957.63	59.64
	N_3	937.67	56.35
	N_4	909.63	53.07
	N_5	885.00	49.55
	N_6	840.67	47.13
Z_2 (Zn SO_4)	N_1	986.30	59.07
	N_2	935.90	56.64
	N_3	919.80	53.94
	$N_4)_2$	887.70	50.03
	N_5	861.60	45.64
	N_6	823.20	42.22
Z_3(Without Zn)	N_1	942.30	55.68
	N_2	896.90	52.31
	N_3	878.80	47.95
	$N_4)_2$	848.34	44.76
	N_5	856.00	41.08
	N_6	778.33	38.63
LSD at 5%		22.52	0.05

Z_1:At the rate of 100 mgL^{-1} Zn using [Zn -EDTA 6%Zn]; Z_2:At the rate of 100 mgL^{-1}Zn using [Zn SO_4 21.3% Zn]; Z_3:The control treatment (without zinc); N_1:(100% calcium nitrate : 0.0% ammonium sulfate); N_2: (75% calcium nitrate : 25% ammonium sulfate); N_3: (50% calcium nitrate : 50% ammonium sulfate); N_4:(25% calcium nitrate : 75% ammonium sulfate) N_5:(0.0% calcium nitrate : 100% ammonium sulfate) and N_6:The control treatment (without nitrogen).

The statistical analysis of the data presented in Table (6) show the individual effect of studied N-sources (N_1, N_2, N_3, N4, $N_5$and N_6) on spinach plant quality parameters such as total phenol and vitamin C at harvest stage (55 after sowing). It could be observed that the values of all aforementioned traits were significantly affected due to the soil application of all different studied N-sources, where the highest values were recorded with the application of $Ca(NO_3)_2$ ratio at rate of (100%:0%) (N_1) following with N_2 treatment (75% $Ca(NO_3)_2$: 25% $(NH_4)_2SO_4$) following with N_3 treatment (50% $Ca(NO_3)_2$: 50% $(NH_4)_2SO_4$) following with N_4 treatment (25% $Ca(NO_3)_2$: 75% $(NH_4)_2SO_4$) following with N_5 treatment (0.0% $Ca(NO_3)_2$: 100% $(NH_4)_2SO_4$) and lately N_6 treatment (control).The finding of the present study is in concurrence with Chohura and Kolota, (2011) and El-Sirafy *et al.* (2015).The interaction influence between the treatments under study are presented in Table (6). It could be observed that the values of total phenol and vitamin C at harvest stage (55 after sowing) were significantly affected due to the addition of all investigated treatments The highest values were recorded with N_1 treatment ((100% $Ca(NO_3)_2$: 0.0% $(NH_4)_2SO_4$)) and foliar application of Zn- EDTA (Z_1), while the lowest values were recorded under combination between N_6 treatment (without N-fertilization) and Z_3 treatment (untreated plants).Data in the same Table also reveal that under all the zinc treatments, the application of $Ca(NO_3)_2$ ratio at rate of (100% calcium nitrate : 0% ammonium sulfate) (N_1) gave higher total phenol and vitamin C at harvest stage(55 after sowing). On the other hand, the folair sprying of Zn-EDTA (Z_1) gave higher total phenol and vitamin C at harvest stage (55 after sowing) under all the nitrogen treatments. According to our results, we found that the significant reduction in the values of total phenol and vitamin C of spinach plant at control treatment ($Z_3\times N_6$) resulting from soil salinity can be enhanced by the combination of zinc and calcium nutrients. These results are supported by the findings of El-Sirafy *et al.* (2015); Doolette *et al.* (2018) and García-López *et al*. (2019).

CONCLUSION

According to the obtained results in this investigation it can concluded that; foliar spraying with Zn-EDTA in combination with soil addition of calcium nitrate as a source of N-fertilization is considered the most suitable treatment for overcoming salinity stress in some of Egypt soils and releasing the highest safe yield of spinach plant.

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التأثير المشترك لبعض مصادر النيتروجين والزنك على بعض مقاييس الجودة للسبانخ النامي علي أرض متأثرة بالأملاح

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ملوحة الأراضي تعتبر مشكلة رئيسية تواجه إنتاج الخضر في مصر لذلك كان الغرض من هذه الدراسة هو تقييم الرش الورقي لعنصر الزنك من مصادر مختلفة [زنك مخلبيZ_1, كبريتات زنكZ_2, كنترولZ_1] مع الإضافة الأرضية للسماد النيتروجيني وهو عبارة عن خليط من نترات الكالسيوم وكبريتات الأمونيوم بنسب مختلفة [(100%نترات كالسيوم : 0% كبريتات أمونيوم)N_1, (75%نترات كالسيوم : 25% كبريتات أمونيوم)N_2 , (50%نترات كالسيوم : 50% كبريتات أمونيوم)N_3 , (25%نترات كالسيوم : 75% كبريتات أمونيوم)N_4 , (0%نترات كالسيوم : 100% كبريتات أمونيوم)N_5 بالإضافة الي الكنترول N_6]علي مدلولات الجودة والمحتوي الكيماوي لنبات السبانخ وكذلك تراكم النترات في نباتات السبانخ النامية في تربة متأثرة بالأملاح خلال الموسم الشتوي 2018. كان التصميم التجريبي عبارة عن تصميم قطع منشقة مع تكرار كل معاملة ثلاث مرات. أشارت النتائج في مرحلتي النمو المختلفة (30 و55 يوم من الزراعة)، أن أعلي القيم لمعظم العناصر الغذائية في نباتات السبانخ وكذلك مدلولات الجودة تم الحصول عليها عند الجمع بين المعاملتين زنك مخلبيZ_1 و (100%نترات كالسيوم: 0% كبريتات أمونيوم) N_1. بينما أقل القيم تم الحصول عليها عند الجمع بين المعاملتين الكنترول (بدون زنك وبدون تسميد نيتروجيني (Z_3 N_3). يمكن أن نستنتج أن الرش الورقي للزنك المخلبي Zn-EDTA مع الإضافة الأرضية لسماد نترات الكالسيوم تعتبر الطرقة الأنسب للتغلب على الإجهاد الملحي في بعض الأراضي المصرية وكذلك الحصول على أعلى محصول آمن من نبات السبانخ.