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Nitrate Accumulation and Oxalate Formation in Spinach Plants (*Spinacia oleracea,* L.) as Affected by Nitrogen Fertilization levels and Iron Foliar Application.

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ABSTRACT

Two field experiments were organized at the Tag Elez Station Experimental Farm; Governorate of Dakahlya, during the two successive winter seasons of 2014-2015 and 2015-2016 using spinach plant (*Spinacia oleracea, L.* cv. "Balady"*).* Spinach seeds were sown on 2nd and 6th of December, seasons 2014 and 2015, respectively. Ten treatments were arranged in split plot design with 3 replicates to evaluate the combination effect of five levels of N-fertilizer {50, 75, 100, 125 and 150 %} from the recommended dose (RD) by the Ministry of Agric. and Soil Recl. (MASR) in the form of $NH_4 NO_3$ (33.5% N) as soil application in the presence and absence of Fe as foliar way $FeSo_4$ (20%Fe) was used as a source of Fe at the rate of 300 ppm Fe. The obtained results indicated that: * A nitrogen fertilizer application at the rates of this survey significantly increased the average values of dry weigh, chlorophyll contents, N, P, K, Fe and VC in the leaves of spinach plant tell the rate of 100% RD. increasing the rate of N-fertilizer from 100% to 150% RD significantly decreased the mean values of all the aforementioned traits during both seasons of the experimentation. At any level of N-fertilization; foliar applied of Fe has been recorded a pronounced high effects on the average values of all parameters under the current study. * Increasing the rate of N-fertilization from 50 to 150%RD sharply and significantly increased the average value of nitrate and nitrite accumulation in spinach leaves, while such effect significantly decreased the activity of nitrate reductase enzyme in spinach plant. Moreover, foliar application of Fe in combination with any rate of N-fertilizer has been corrected this trend. * A favorable effect on oxalate formation in spinach leaves due to an addition of iron in foliar way combined with any level of N-fertilization as compared to the plants treated with the same levels of nitrogen in single form.

Keywords: $NH_4 NO_3$, nitrite, nitrate, Oxalate, Iron, spinach.

INTRODUCTION

Spinach (*Spinacia oleracea L.*) is one of the vegetables whose leaves and stems are utilized processed or fresh. This plant produces large measures of fresh leafy mass in a short time of vegetation. Spinach is a restorative and appetizing plant that contains vitamins, antioxidants (flavonoids and ascorbic acid) and nutrients. It has been likewise recognized as one of the vegetables having innately high nitrate fixation and its petioles have a few fold higher concentration than its leaf blades. High concentration of nitrate and arrangement of oxalate are found in leafy vegetables for all intents and purposes under application of nitrogen treatment (Dehkharghanian *et al*., 2010).

Nitrogen is important for plant growth, it is a constituent of all proteins and nucleic acids, some of the plant growth regulators, and in many of the vitamins and hence of call protoplasm. As a component of these and many other compounds, nitrogen is involved in most of the biochemical reactions that compose life. An addition of huge amount of nitrogen to leafy vegetables such as cabbage, lettuce radish and spinach to maintain bright green color of the foliage and maximum yield, can lead to the accumulation of excessive levels of NO_3-N in the plant. In Egypt, farmers consume a large amount of N-fertilizers aiming to increase the yield without any care of the bad residual effect on the quality of crops Abd-Allah, (2001).

Nitrate is essential to life but a nuisance and possibly a hazard. Nitrate can be changed into nitrite by microbes requiring oxygen. This can happen in the soil, in water and in our bodies. When that happen in our bodies we have to consider two problems methaemoglobinaemia or the blue syndrome and stomach cancer. No_3^- ions in the soil usually complexed with the production of some pesticides degradation like amino compounds to produce nitrosamine compounds were carthenogenic to human and animals. The critical level recommended by FAO (2000) amounted 3.6 mg day^{-1} for (a 60-kg person) for nitrite.

Another factor governing the NO_3-N accumulation in the vegetables is the status of micronutrients in plant especially Fe. In the case of insufficient concentration of Fe in plant, the application of this element will decrease NO_3-N accumulation. This effect may be due to the role played by iron on NO_3 and NO_2 reductases activity. In this respect, Crawford and Campbell, (1990) reported that NO_3 was first transported into the cell and then reduced to NH_4 by the consecutive action of two enzymes NO_3- reductase and NO_2 reductases. Both enzymes were metalloflavoprotein the enzyme system includes a reduced pyridine nucleotide (NADPH or NADH) as electron donor, flavin adenine dinucleotide (FAD) as a prosthetic group and an iron-containing hydrochlorine (siroheme) for NO_2-reductase.

Oxalate in vegetables is one of toxin and anti-nutrient. Free oxalic acid and soluble oxalate able to combine with Ca^{++} and other mineral resulting in other foods deficiencies of Ca, Fe, Mg and Cu. Uptake of oxalate-accumulating vegetables, such as spinach, can led to kidney stone formation due to actuating a huge increment in urinary oxalate excretion. Furthermore, abundance oxalate ingested in human bodies can cause afunctional hypocalcemia with tetany in intense cases and acute poisoning of oxalate. A deadly dose of oxalic acid arranged from 2 to 30 g was conducted for people depending on variety of factors and 2 grams of oxalate was the minimum lethal dose for human adult (Nakata, 2003; Bohn *et al*., 2004 and Massey, 2007).

Therefore, the objective of this study are to investigate nitrate accumulation and oxalate formation in spinach plants (*Spinacia oleracea*, L.) as affected by nitrogen fertilization levels and foliar iron application.

MATERIALS AND METHODS

Two field experiences were conducted at Tag Elez Station Experimental farm; Governorate of Dakahlya, during the two successive winter season of 2014-2015 and

2015-2016 using spinach plant (*Spinacia Oleracea,* cv. "Balady"). Spinach seeds were sown on 2nd and 6th of December, 2014 and 2015 seasons, respectively. Soil sample was air dried and analyzed to determine some physical and chemical properties as shown in Table 1.

Table 1. Some physical and chemical properties of the experimental soil during both seasons of 2014-2015 and 2015-2016

Soil Properties		Season 2014-2015	Season 2015-2016
Mechanical analysis %	Coarse Sand	3.75	4.53
	Fine Sand	30.04	29.36
	Silt	39.12	39.82
	Clay	27.09	26.29
	Texture class	Sandy clay loam	Sandy clay loam
Organic matter %		1.83	1.75
$CaCO_3$ %		4.51	4.39
pH * (1: 2.5)		8.12	8.14
E.C dS m^{-1}		9.85	9.55
SP %		53.5	52.1
Soluble cation meq/100g soil	Ca^{+2}	2.05	2.01
	Mg^{+2}	1.43	1.44
	Na^{+}	6.22	6.24
	K^{+}	0.39	0.29
Soluble anions meq/100g soil	Co_3^{-2}	---	---
	HCo_3^{-}	2.25	2.03
	Cl^{-}	5.93	6.03
	So_4^{-2}	1.91	1.92
Available ppm	N	48.6	41.8
	P	3.75	2.98
	K	184	179
	Fe **	3.41	3.34
	Mo **	0.169	0.156

*** Soil suspension 1:2.5 (soil: water)**
**** extracted by DTPA**

Experimental design: Ten treatments were organized in a split plot design or two factorials with 3 replicates, to evaluate the combination effect of five levels of N-fertilizer (50, 75, 100, 125 and 150 %) from the recommended dose (RD) by the Ministry of Agric. and Soil Recl. (MASR) in the form of $NH_4 NO_3$ (33.5% N) as a soil application in the presence and absence of Fe as a foliar way, $FeSo_4$ (20%Fe) was used as a source of Fe at the rate of 300 ppm Fe.

N doses were divided into two equal parts; the first addition was done after 21 days from sowing and the second one 15 days later. Foliar application of Fe was done at the same time of nitrogen fertilizer addition.

All cultivation processes were carried out according to the recommendation of the Egyptian Ministry of Agriculture.

Cultivation: Six meters square (2x3m) of plots were built-up. Five rows in each plot. Spinach seeds cv. Balady were sown on 2nd and 6th of December, 2014 and 2015 seasons, respectively; in hills; 15 cm apart on both sides of rows. After 21 days from planting; the plants were thinned at three plants per hill.

Experimental procedures: At harvesting stage (60 days after sowing of spinach, samples of twenty plants were taken at random from each experimental plot. plant parameters expressed in; Fresh weight (g $Plant^{-1}$), dry weight (g $Plant^{-1}$) and chlorophyll contents (mg g^{-1}). Fresh weight of plant was determined as the average weight per plant in grams and then, NO_3-N, NO_2-N content, the activity NO_3-reductase enzyme, vitamin C, total oxalate, soluble oxalate and insoluble oxalate were determined. Plant sample were oven dried and then; chemical analysis of plant expressed in N, P and K % as well as Fe ppm were estimated.

Soil methods of analysis: Mechanical analysis of the used soil was determined following the international pipette method Piper, (1950).

Calcium carbonate, organic matter, available N, P, K, in soil were determined using the methods adopted by Dewis and Fertais, (1970); Jackson, (1967); Bremner and Mulvany, (1982); Olsen and Sommers, (1982) and Black, (1965), respectively.

EC and pH: were measured according to the method of US Salinity Lab (1954) and Jackson (1967). Iron in the soil was extracted using DTPA and determined by an Atomic Absorption Spectrophotometer as described by Chapman and pratt (1961).

Plant analysis: The oven dry materials of plant samples were ground and wet digested as described by Gotteni *et al*., (1982). The total N, P, K and Fe were determined using the techniques described by Jones *et al*., (1991); Peters *et al*., (2003); Black, (1965) and Chapman and Pratt, (1961), respectively.

Nitrate and nitrite were measured by using a rapid method of Singh (1988). Nitrate reductase enzyme activity described by Hageman and reed, (1980).

Chlorophyll content, Vitamin C, Total oxalate and soluble oxalate contents were determined according to the method of Sadasivam and Manickam, (1996); Mazumdar and Majumder, (2003) and Zhang *et al*., (2005), respectively.

Statistical analysis: The statistical analysis of the data collected was carried out according to the method described by (Gomez and Gomez 1984) using LSD to compare the means of treatment values.

RESULTS AND DISCUSSION

Results

Data illustrated in Table 2, the effect of N levels as soil fertilization and Fe as foliar application on fresh, dry weight (g $plant^{-1}$) and the average values of chlorophyll (a, b & total mg g^{-1}) for spinach plant at marketing stage.

Concerning the N-fertilization levels studied affected in single form; data in Table 2 indicated that; the average values of all the parameters studied (except for fresh weight g $plan^{-1}$) were significantly increased when the fertilization rate N increased by 100% N. Increase the rate of N-fertilization from 100% to 125 or 150% N significantly decreased the mean values of all the aform mentioned traits. The same trend realized in the 2nd season of the experiment. In this connect the mean values of fresh weight g $plan^{-1}$ were significantly increased as the rate of N-fertilization was increased from 50 to 125% from the recommended dose.

The effect of Fe as foliar way on the plant parameters under the presented study are shown in the same Table. It can be observed that; the best plant parameters were obtained by spinach plant treated with Fe at any level of N-fertilization as compared to the untreated plants. On the other hand; the same trend of N-fertilization

levels in the absence of Fe foliar spraying was reflected on the average values of all the previously mentioned traits in the presence of Fe foliar applied. This trend was the same during both seasons of 2014-2015 and 2015-2016.

Table 2. Effect of nitrogen fertilization levels and Fe as foliar way on fresh, dry weight and the average values of chlorophyll of spinach plant during 2014-2015 and 2015-2016 seasons.

Treatments		Fresh Weight (g plant^{-1})		Dry Weight (g plant^{-1})		Chlorophyll a mg g^{-1} FW		Chlorophyll b mg g^{-1} FW		Total chlorophyll mg g^{1} FW	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
50%	0	36.43	46.17	5.04	6.54	0.511	0.568	0.322	0.408	0.833	0.976
	Fe	59.83	69.33	7.98	9.80	0.603	0.668	0.405	0.497	1.008	1.164
75%	0	38.43	52.20	5.65	7.42	0.528	0.594	0.435	0.431	0.963	1.025
	Fe	63.07	74.27	8.57	10.45	0.622	0.684	0.414	0.511	1.036	1.196
100%	0	40.40	54.63	5.91	7.70	0.569	0.605	0.423	0.441	0.992	1.046
	Fe	65.40	76.17	8.87	10.69	0.635	0.694	0.426	0.521	1.062	1.215
125%	0	42.73	56.00	5.33	7.13	0.516	0.583	0.327	0.423	0.843	1.006
	Fe	66.60	71.97	8.27	10.18	0.613	0.676	0.411	0.504	1.025	1.180
150%	0	44.47	57.87	4.75	6.83	0.507	0.577	0.315	0.414	0.822	0.991
	Fe	69.27	67.27	7.72	9.54	0.593	0.658	0.398	0.487	0.991	1.145
LSD at 5%		1.25	1.12	0.12	0.08	0.024	0.006	0.008	0.007	0.027	0.009

Data in Table 3 revealed the effect of nitrogen fertilization and iron foliar application on N, P, K and Fe contents in the dry matter of leaves at the stage of marketing. Also, VC mg 100g^{-1} FW.

Regarding the effect of nitrogen fertilization, the data show that the application of nitrogen at (50, 75, 100, 125 and 150%) from the recommended dose by (MASR) resulted in significant increase in the N% value in spinach leaves, while such effect for P, K, Fe and VC content were realized at the rate of 100%N in single form, the lowest mean values were associated with N-fertilization at the rate of 50%. This trend was happened during both seasons of the experimentation.

A superiority effect on the mean values of (N, P, K, Fe and VC content) was obtained due to an application of Fe in foliar way which recorded the highest mean values in the both seasons. The lowest mean values were recorded for the control treatment during both seasons of the experiment.

Table 3. Effect of nitrogen fertilization levels and Fe as foliar way on (N, P, K, Fe and VC content) of spinach plant during 2014-2015 and 2015-2016 seasons.

Treatments		N%		P%		K%		Fe ppm		VC (mg 100g^{-1} FW)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
50%	0	2.22	2.32	0.404	0.354	3.21	3.07	40.98	40.27	35.63	40.53
	Fe	2.89	2.32	0.472	0.433	3.63	3.95	69.12	65.37	47.95	51.64
75%	0	2.26	2.60	0.416	0.376	3.30	3.31	43.87	43.87	38.09	43.51
	Fe	2.96	2.50	0.482	0.451	3.71	4.03	73.80	68.87	50.53	53.67
100%	0	2.33	2.68	0.427	0.384	3.38	3.38	45.99	47.07	36.98	44.26
	Fe	2.98	3.18	0.489	0.460	3.75	4.11	75.95	70.33	49.20	54.94
125%	0	2.38	2.71	0.417	0.369	3.28	3.25	42.03	42.10	34.25	42.63
	Fe	3.06	3.43	0.476	0.443	3.65	4.12	71.15	67.17	46.51	52.65
150%	0	2.45	2.91	0.397	0.362	3.21	3.14	39.04	38.53	32.98	41.37
	Fe	3.12	3.55	0.467	0.430	3.59	3.88	67.24	63.73	45.15	50.75
LSD at 5%		0.20	0.30	0.006	0.009	0.06	0.21	0.36	0.92	0.13	0.19

Data shown in Table 4, the effect of N fertilization levels and foliar Fe spraying on nitrate and nitrite accumulation as well as nitrate reductase (NR) activity in spinach leaves at the commercialization stage.

The data obtained in Table 4 indicated that, the average values of NO_3-N and NO_2-N mg kg^{-1} in spinach leaves were significantly increased as the level of nitrogen fertilization was increased, while the nitrate reductase enzyme activity was significantly decreased as the level of N-fertilization was increased. On other words, the highest values of nitrate and nitrite accumulation were associated with the plants treated with N-fertilizer at 150% of RD, while such effect has been recorded the lowest level of nitrate reductase activity.

Regarding the effect of Fe as foliar application data in the same Table showed that; in the first and second seasons the highest values of nitrate were recorded for the untreated plants, while the lowest values were realized with the plants treated with Fe. Furthermore, N.R activity was also, influenced by the application of Fe was applied by foliar way; the lowest level of N.R enzyme activity was realized for the untreated plants.

Table 4. Effect of nitrogen fertilization levels and Fe as foliar way on NO_3-N, NO_2-N (mg kg^{-1}) and N.R.A. of spinach leaves during 2014-2015 and 2015-2016 seasons.

Treatments		NO3-N mg kg^{-1}		NO2-N mg kg^{-1}		NR. Activity	
		1st	2nd	1st	2nd	1st	2nd
50%	0	624	594	5.10	4.87	0.091	0.079
	Fe	562	543	4.63	4.47	0.125	0.109
75%	0	640	606	5.15	4.97	0.080	0.075
	Fe	575	551	4.72	4.58	0.123	0.103
100%	0	652	615	5.27	5.06	0.074	0.068
	Fe	587	563	4.80	4.56	0.113	0.098
125%	0	660	628	5.36	5.14	0.068	0.063
	Fe	598	572	4.90	4.67	0.107	0.091
150%	0	670	446	5.45	5.32	0.061	0.057
	Fe	611	583	4.98	4.76	0.099	0.087
LSD at 5%		6.50	21.61	0.06	0.21	0.005	0.96

The mean values of soluble, insoluble and total oxalate (mg $100g^{-1}$ F.W) found in spinach plant as influenced by single application of N levels and foliar application of iron are presented in Table 5.

As shown in Table 5 there were significant increases in soluble and total oxalate mg $100g^{-1}$ F.W recorded with ammonium nitrate at 100% of recommended dose, while the highest values of insoluble oxalate mg $100g^{-1}$ F.W were recorded of 50% of nitrogen fertilizer in both seasons.

Table 5. Effect of nitrogen fertilization levels and Fe as foliar way on total, soluble and insoluble oxalate mg $100g^{-1}$ F.W of spinach leaves during 2014-2015 and 2015-2016 seasons.

Treatments		Soluble. oxalate mg$100g^{-1}$		Insoluble. Oxalate mg$100g^{-1}$		Total. Oxalate mg$100g^{-1}$	
		1st	2nd	1st	2nd	1st	2nd
50%	0	560	572	293	311	854	884
	Fe	656	632	282	288	938	919
75%	0	583	580	287	299	870	879
	Fe	678	653	275	272	953	926
100%	0	589	595	290	306	879	901
	Fe	685	660	276	273	962	934
125%	0	574	588	289	297	863	885
	Fe	665	646	280	286	945	932
150%	0	554	575	292	303	846	877
	Fe	649	639	283	279	932	918
LSD at 5%		7.44	6.74	4.69	5.73	7.59	9.60

Data at the same Table clear that, the effect of foliar application of iron were significantly affected data of the previously mentioned traits of spinach leaves due to the foliar application of iron comparing with the control treatment. In the same way, the adding of Fe decreased significantly the average values of insoluble oxalate mg $100g^{-1}$ while increased soluble and total oxalate over the control. Data clearly showed that; spinach plants tended to accumulate more soluble and total oxalate as a result of foliar applied of Fe than that obtained from the untreated plants. On the contrary of this trend; a significant decrees was happened in insoluble oxalate which, treated with Fe compared with the untreated plants. The same trend was realized during the both seasons of the experiment.

Discussion

Results mentioned previously can be discussed as follow:

Results of the previously mentioned traits indicated that; an application of N-fertilization at the rates of this investigation significantly increased the average values of dry weigh, chlorophyll contents, N, P, K, Fe and VC in the leaves of spinach plant tell 100% of RD. increasing the rate of N-fertilizer from 100% to 150% of RD significantly decreased the mean values of all the aform mentioned traits during both seasons of the experimentation. The increases in these parameters in spinach leaves due to raising the nitrogen doses tell the rate of 100% may be attributed to the beneficial effect of nitrogen on stimulating the meristematic activity producing more tissues and organs since nitrogen is a constituent of proteins, nucleic acid and many important substances of plant cell. It can be noticed that in spite of the high significant increase in these parameters tell 100% of RD more increasing N-fertilizer tell the rate of 150%RD significantly increased the average values of fresh weight of spinach leaves only, which mean that the increase was in moisture percent in leaves not in the dry matter.

These results in accordance with the findings of Shaheen *et al*., (2012); El-Mergawi *et al*., (2014); Zhang *et al*., (2014); Abdelraouf, (2016); Awaad *et al*., (2016) and Mahlangu *et al*., (2016).

Results also indicated that at any rates of nitrogen fertilization; foliar applied of iron has been recorded a pronounced high effects on the average values of all previous parameters under study. The ideal part of iron on invigorating vegetative development of spinach plant might be alluded to the pretended by Fe on plant bioactivities, for example, enzymatic framework in charge of biosynthesis of amino acids, protein, chlorophyll and in addition impartment of the supplement status.

Such results were reported by El-Talawy, (1998); Abd-Allah, (2001); Salem, (2009); El-Ghamry, (2010); Abu Zinada *et al*., (2011) and EL-Aila *et al.,* (2015).

The results of this investigation have proved that, increasing the rate of N-fertilization from 50 to 150% of RD sharply and significantly increased the average value of nitrate and nitrite accumulation in spinach leaves, while such effect significantly decreased the activity of nitrate reductase enzyme in spinach plant. Moreover, foliar application of Fe in combination with any rate of N-fertilizer has been corrected this trend. These results may be explained on basis of; under heavy nitrogen application spinach plants may absorb great quantity of nitrogen than its assimilation capacity. The different between N-absorption and assimilation may be great and the unutilized nitrogen well be stored as nitrate and nitrites in plant tissues. In respect to the effect of Fe spraying on decreasing the content of nitrate and nitrite in spinach plant, such finding could be explained on the basis of the beneficial and stimulation effect of iron on the activity of nitrate and nitrite enzymes.

These results are confirmed with those obtained by Hanafy *et al.,* (1997); El-Talawy, (1998); Abd-Allah, (2001); Gulser, (2005); Hammad *et al.,* (2007); Salem, (2009); El-Ghamry, (2010); Commission Regulation, (2011); Vattani *et al.,* (2012); Zdravkovic *et al.,* (2012); Qiu *et al*., (2014) and Sakara, (2016).

Finally, a favorable effect on oxalate formation in spinach leaves due to an addition of iron in foliar way combined with any level of N-fertilization as compared to the plants treated with the same levels of nitrogen in single form. Such effect may be due to the role played by Fe on activating the metabolism thought it's important in enzymatic system in the cell of spinach plant.

These results in agreement with those obtained by Tei *et al*., (2006); Stagnari *et al.,* (2007); Zhang *et al*., (2009): Musa *et al*. (2011); Lin *et al.,* (2014); Svein *et al,* (2015) and Sakara, (2016).

CONCLUSION

Under the same conditions of this investigation it can be recommended that, the highest safe yield of spinach plant and the best quality parameters were realized for the plants treated with N-fertilization at the rate of 100% from the recommended dose by the ministry of agriculture and soil reclamation as soil

addition in combination with Iron at the rate of 300 ppm in foliar way.

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تأثير مستويات التسميد النيتروجيني والرش الورقي بالحديد علي تراكم النترات وتكوين الاكسالات في نبات السبانخ.

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تم تنفيذ تجربةحقليه في محطة بحوث تاج العز – محافظة الدقهلية خلال موسمي النمو الشتويين المتتاليين ٢٠١٤ , ٢٠١٥ بإستخدام نبات السبانخ (صنف بلدي) لدراسة ما يلي: • تاثير مستويات من التسميد النيتروجيني(٥٠, ٧٥ , ١٠٠ , ١٢٥ , ١٥٠ ٪) كنسبه من الموصي بة وايضا تاثير الرش الورقى بالحديد على النمو وتراكم النترات وتكوين الاكسالات فى السبانخ. اشتملت التجربه على ١٠ معاملات في تصميم قطع منشقه مره واحده او عاملين في ٣ مكررات باستخدام ٥ مستويات من نترات الامونيوم ٣٣.٥٪N (٥٠, ٧٥ , ١٠٠ , ١٢٥ , ١٥٠ ٪) كنسبه من الموصي بة بمعرفة وزارة الزراعة واستصلاح الاراضي كإضافة ارضية في وجود او عدم وجود الحديد بمستوي ٣٠٠ جزء / مليون (سلفات الحديد ٢٠ ٪ Fe) بالرش الورقي. ويمكن تلخيص النتائج المتحصل عليها على النحو التالى : • الاضافة الارضية لمستويات التسميد النتروجيني موضوع الدراسة ادت الي زيادة معنوية في الوزن الجاف , محتوي الكلوروفيل , النتروجين , الفسفور , البوتاسيوم , الحديد وكذلك فيتامين c حتي مستوي ١٠٠ ٪ من الموصي بة , زيادة مستوي النتروجين من ١٠٠- ١٥٠ ٪ ادي الي حدوث نقص معنوى في قيم كلا من الصفات السابقة . الاضافة الورقية للحديد عن اي مستوي من مستويات اضافة النتروجين ادي الي حدوث زيادة ملحوظة في قيم جميع الصفات المدروسة واتضح ذلك خلال موسمي النمو . • زيادة مستوي اضافة السماد النتروجيني من ٥٠ الي ١٥٠ ٪ من الموصي بة ادي الي حدوث زيادة معنوية في تراكم النترات والنيتريت في اوراق السبانخ . – في حين ادت نفس المعاملات الي حدوث نقص معنوي في نشاط انزيم النيترات ريدكتاز . –كما ادي الرش الورقي بالحديد عند اي مستوي من مستويات التسميد النيتروجيني الي تصحيح هذا الاتجاة . • الرش الورقي بالحديد عند اي مستوي من مستويات التسميد النيتروجيني ادي الي حدوث تأثير مشجع علي اتجاة تكوين الاكسالات في اوراق السبانخ مقارنة بالاضافة الارضية لمستويات النتروجين علي صورة فردية. الاستنتاج :تحت نفس ظروف هذة الدراسه نوصى باستخدام التسميد الارضى بالاسمده النيتروجينيه المعدنيه عند مستوى 100% من الموصى به فى وجود الرش الورقى بسلفات الحديد لتحقيق محصول امن من السبانخ .