

RESPONSE OF SPINACH PLANTS TO COBALT SUPPLEMENT UNDER DIFFERENT NITROGEN RATES

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Abstract

Two field experiments were carried out to study the effect of cobalt on spinach leaves yield quantity and quality under different nitrogen rates. Experiments were conducted at Research and Production Station, National Research Centre, El-Nubaria Site, Beheara Governorate, Delta Egypt under drip irrigation system during two the successive seasons of 2016 and 2017. Spinach growth, yield, chlorophyll content, mineral composition as well as chemical constituents significantly decreased as nitrogen level in plant media decreased. Cobalt has a significant promotive effect on spinach growth, yield nutritional status and chemical constituents with all nitrogen rates compared with specially with 100% and 75% nitrogen compared with control (100% N without cobalt). As nitrogen rates in plant media decreased, Nitrate, Nitrite, Calcium Oxalate in spinach leaves significantly decreased. Cobalt resulted adverse noticeable effect on the content of Nitrate, Nitrite and calcium oxalate in spinach leaves compared with 100% nitrogen fertilizer alone. Cobalt at 9 ppm save 25% from nitrogen fertilizer compared with control (100% nitrogen fertilizer alone).

Keywords: Cobalt - Spinach - Yield quantity and quality.

Introduction

Spinach is a member of the chenopodiaceae (*Goosefoot family*). Spinach (*Spinacia oleracea*) is a low growing fleshly leaved annual that forms a heavy rosette of broad. Spinach is one of the major leafy vegetables, which is widely cultivated in spring, autumn, and winter seasons. Spinach is a good source of vitamins A, B₁, B₂, and C as well as minerals such as calcium, iron and magnesium (Kawazu *et al.*, 2003).

Spinach vegetable is among the oddest vegetable crops in the world and success stories of its cultivation are attributed to the plant genetic physiological fixibility that allows for adaption to wide range of environments. The nutritional value of this vegetable is highly rated (Khandaker *et al.*, 2008).

Nitrogen (N) is one of the most important nutrients limiting plant growth (Mengel and Kirkby, 1987) which is evidenced by its high demand for vegetable production. Although the application of N fertilizer can improve the yield of leafy vegetables. Its oversupply may cause economic loss and environmental degradation as well as a reduction in food quality (Zhang *et al.*, 2014). In addition, the over use of N could also increase nitrate concentration in spinach which may be noxious to both animals and humans (Citak and Sonmez, 2010).

Nitrogen has many functions in plant life being responsible for the biosynthesis of enzymes, nucleoproteins, amino acids, proteins, sugars, polypeptides, chlorophylls and encoouragecell division (Marschner, 1995). In this respect, mineral nitrogen fertilizer enhanced plant growth (El-desuki *et al.*, 2010), leaf chlorophyll content, yield and its components (Danesh *et al.*, 2012).

According to UK-Food-Safety-Directorate (1996), Nitrite (NO_2^-) inhibit oxygen transport by blood, leading to methaemoglobin formation, and producing a medical condition known as "hemoglobinemia", to which infants an at greater risk than adults because the lower acidity in their stomach. Reduction of NO_3^- in the intestinal trace may occur by bacterial activating NO_2^- (the nitrite ions) to react readily with hemoglobin.

Cobalt is considered as a beneficial element for higher plant despite absence of evidence of its direct role in plant metabolism.

Cobalt is an essential element for the synthesis of vitamin B_{12} which is required for human and animal nutrition (Young, 1983). Unlike other heavy metals, cobalt is saver for human consumption and up to 8 ppm can be consumed on a daily basis without health hazard (Smith, 1991). Laila Helmy and Nadia Gad (2002) found that cobalt significantly increased both growth and yield parameters, minerals composition as well as total soluble solids and vitamin "C" as L. ascorbic acid in parsley leaves. The main aroma constituent of parsley leaves, 1,3,8-p-methatriene which forms about (76%) of leaves essential oil, showed about (10%) increase over that of control with 50 mg/kg soil.

Nadia Gad and Zaghloul (2007) reported that the addition of 10.0 and 12.5 ppm cobalt for some contaminated soils (Helwan and El-Gabal El-Asfar) were the best treatments to have healthy lettuce plant and significantly decrease the content of heavy metals such as Pd, Cd, Ni and Fe content. Cobalt application as an amendment may help in minimizing the hazard of heavy metals in these soils.

Eman Aziz *et al.* (2013) stated that all cobalt doses gave a significant positive effect on sweet basil growth, herb yield and essential oil compared with control. Cobalt at 15 ppm gave the greatest values of fresh and dry herb yield along with essential oil components.

Nadia Gad *et al.* (2014) showed that cobalt significantly increased Rosemary growth, herb yield, mineral composition along with essential oil content and its components compared with control. Cobalt at 10 ppm gave the highest values. Cobalt improved growth, herb yield quantity and quality of Rosemary herb. Nadia Gad *et al.* (2016) stated that all studied cobalt levels (3,6,9 and 12 ppm) has a promotive effect on cabbage growth and yield parameters, nutritional

status and chemical constituents compared with control. Cobalt at 6 ppm gave the highest figures.

This work aimed to study the effect of cobalt concentration on spinach growth, and leaves yield, leaves quantity and quality under different nitrogen doses.

Materials and Methods

Two field experiments were conducted in the Research and Production Station, National Research Centre, El-Noubaria Site, Behiera Governorate, Delta Egypt. These experiment were carried out to study the effect of cobalt levels on vegetative growth, leaves yield quantity and quality of spinach under different nitrogen levels.

Soil Analysis

Physical and chemical properties of Nubaria Soil were determined and particle size distributions along with soil moisture were determined as described by Blackmore (1972). Soil pH, EC, cations and anions, organic matter, CaCO₃, total nitrogen and available P, K, Fe, Mn, Zn, Cu were run according to Black *et al.* (1982). Determination of soluble, available and total cobalt was determined according to method described by Cottenie *et al.* (1982). Some physical and chemical properties of Nubaria soil are shown in Table (1).

Table 1 : Some physical and chemical properties of Nubaria soil

Physical properties													
Particle size distribution %								Soil moisture constant %					
Sand Silt Clay 70.8 25.6 3.6		y 5	Soil t Sand	il texture Saturation ndy loam 32.0		FieldWiltinCapacityPoin19.26.1		ng A	vailable Water 13.1				
Chemical properties													
Soluble ca							ions (meq ⁻¹ L) Soluble anions (meq ⁻¹			L)			
pН	EC	CaCO ₃ OM		DM	Ca ⁺⁺	Mg ⁺⁺	K ⁺	Na⁺	HCO ₃	CO3	Cľ	$SO_4^=$	
1:2.5	$(dS m^{-1})$	% %		%									
8.49	1.74	3.4 0.20		0.20	0.8	0.5	1.6	1.80	0.3	-	1.9	0.5	
Cobalt Total							Available Available micronutrients				ts		
(ppm) (mg 100							⁻¹ soil) (ppm)						
Soluble	Soluble Available Total]	N	Р	K	[Fe	Mn	Zn	Cu		
0.35	4.88	4.88 9.88		2	5.1	13.3 4.49		19	4.46	2.71	4.52	5.2	

FC (Field capacity), WP (Welting point), AW (Available water).

Experimental works

Preliminary pot experiment was conducted at Wirehouse of National Research Centre to evaluate the effect of cobalt concentration which resulted highest growth and yield of spinach plant. At 14^{th} of November 2015, seeds of spinach (*Spinacia olesea L.*) were sown in plastic pots of 10 kg capacity. Pots were filled with sandy loam soil taken from Noubaria farm. Ten seeds of spinach were sown and thinned to five plants per pot. After three weeks from sowing, seedlings were irrigated once with cobalt concentrations: (0.0, 3, 6, 9, 12 and 15 ppm) as cobalt sulphate form. All required agriculture managements for plants growth and production were carried out as recommended by Ministry of Agriculture. After 70 days from sowing date, spinach growth and yield parameters were recorded.

Two field experiments were carried out to evaluate the effect of cobalt on spinach growth and production under different levels of nitrogen. The experiments were conducted at Research and Production Station, National Research Centre, El-Noubaria Site, Beheira Governorate, Delta Egypt, under drip irrigation system during 2016 and 2017 successive seasons. Each experiment consists of 8 treatments. Each treatment was represented by 3 plot area 5x3 meter. phosphorous as super phosphate at rate 200 kg/fed and potassium fertilizers as potassium sulphate 50kg/fed were added during soil preparation.

Seeds of spinach (*Spinacia oleracea L.*) were sown on 14 and 17 November during 2016 and 2017 seasons.

Ammonium sulphate NH₄SO₄ (20.5%N) at the rate 250 kg/fed was basic amount (100 N) as control. The levels of Ammonium sulphate treatments were calculated to be corresponding to 75, 50 and 25% of the control. According to the preliminary experiment results, cobalt at 9 ppm resulted the greatest growth and yield values. After three weeks from sowing seedlings were irrigated once with cobalt at 9 ppm.

A number of 8 treatments were concluded:-

- 1- NH₄ SO₄ 100% N recommended dose as control
- 2- NH₄ SO₄ 75%N
- 3- NH₄ SO₄ 50% N.
- 4- NH₄ SO₄ 25% N.
- 5- NH₄ SO₄ 100% N + cobalt at 9 ppm.
- 6- NH₄ SO₄ 75% N + cobalt at 9 ppm.
- 7- NH₄ SO₄ 50% N + cobalt at 9 ppm.
- 8- NH₄ SO₄ 25% N + cobalt at 9 ppm.

All required agriculture managements for plants growth and production were carried out as recommended by Ministry of Agriculture. The experimental design was a Factorial experimental in complete randmized block design with three replication for each treatment.

Measurement of vegetative growth and yield

After 70 days from sowing the growth parameters of spinach such as plant hight, leaves fresh and dry weights were recorded according to FAO (1980).

Measurement of NO₃-N and NO₂-N:-

The concentration to both NO_3 -N and NO_2 -N in spinach leaves were determined according to Cottenie (1982).

Measurement of calciumoxalate content

Calcium oxalate content in spinach fresh leaves was determined according to Human Nutrition Information Service USDA (1984).

Measurement of total chlorophyll content

Total chlorophyll content in spinach fresh leaves, using chlorophyll meter spad 501 according to Wood *et al.* (1992).

Measurement of Chemical Constituents:

In spinach leaves, total proteins, total carbohydrates, vitamin "A" and Vitamin "C" as L-Ascorbic acid were determined according to A.O.A.C (1995).

Measurement of Nutritional Status:-

Macronutrient (N,P and K) and Micronutrients (Fe, Mn, Zn and Cu) along with cobalt were determined in spinach leaves according to Cottenie *et al.* (1982).

Statistical Analysis

All data were subject to statistical analysis according to procedure by SAS (1996) computer program and means were compared by method according to Snedecor and Cochran (1982).

Results and Discussion

Growth and yield characteristics

(i) Effect of nitrogen

Data in Table (2) indicate that all growth and yield parameters such as plant height, leaves number per plant, leaf area index, leaves fresh weight per plant, leaves dry weight plant and fresh yield (ton/fed) recorded the highest values with control 100% NRR followed by 75% followed by 50% while 25% gave the lowest ones. These results are in harmony with those obtained by Marschner (1995) who stated that the simulative effect of nitrogen on different plant growth parameters may owe much to that nitrogen is an essential element for building up protoplasm, amino acids and protein which promote cell division. Also, nitrogen plays vital role in several biochemical processes related to plant growth. Confirm these results Erman *et al.* (2009) who found that the application of nitrogen to pea plants significantly enhanced vegetative growth parameters.

(ii) Effect of cobalt

Data in Table (2) also show that cobalt significantly increased all growth and yield parameters under different nitrogen levels compared with untreated plants. It clear that cobalt enhance the studied growth and yield parameters of spinach such as plant hight, leaves number per plant, leaf area, leaves fresh and dry weights along with fresh leaves yield in both two seasons especially with 100% and 75% nitrogen compared with the control (100% nitrogen alone). With 50% nitrogen, all growth parameters were reduced. While 25% nitrogen gave the lowest values. These observations are consistent with previous reports obtained by Nadia Gad (2006b) who found that cobalt being with positive effect due to several induced effects in hormonal synthesis and metabolic activity, while it`s reduce the activity of some enzymes such as peroxides and catalase in tomato plants and hence increasing the anabolism rather than catabolism. Confirm these results Abdul Jaleel *et al.* (2009a and b) who pointed that cobalt addition in soil increased all growth parameters along with yield parameters such as seedling vigor, number and weight of pods and seeds yield per plant in green gram (*Vigna rodiate*) and maize (*Zec maiz* L.) plants.

Cobalt at 9 ppm save 25% from nitrogen fertilizer compared with control (100% nitrogen alone).

The contents of NO₃-N, NO₂,-N and Oxalate:-

(i) Effect of nitrogen:-

Table (3) show the contents of NO_3^-N , NO_2^-N , Oxalate and calcium as affecting by nitrogen levels. Both NO_3^-N and NO_2^--N significantly decreased as nitrogen levels in plant media decreased. The content of NO_3-N in Spinach leaves ranged between 1051-1464 mg/kg⁻¹ fresh weight. These results are agree with those obtained by Gianquinto *et al.* (1992) who stated that the safety limits of nitrate adopted by some countries ranged between 2000-2500 ppm in spinach fresh leaves of and lettuce. Data in Table (3) also show, the content of NO_2-N in spinach fresh leaves ranged between 1.89-762 mg/kg⁻¹ fresh weight.

As nitrogen level decrease in plant media, the content of NO_2^-N significantly was decreased. These results are good agreement with those obtained by Craddock (1983) who found that, with the fatal dose for humans being 20 mg No₂,-N/kg of body weight daily. Data in Table (3) also indicate that the harvesting time was more effective on nitrate formation in leaves. The content of NO₃-N and NO₂,-N in spinach leaves which harvested at 7- pm lower than those which harvested at 10-am with all nitrogen levels. This may explained on the basis of results reported by UK-Food-Safety-Directorate (1996) who showed that the harvesting time was more effective on nitrate formation in spinach leaves. Nitrate and Nitrite concentration in excess of the proposed limits depending on the harvesting time.

Data in Table (3) also reveal, the content of calcium oxalate significantly decreased as nitrogen level in plant media decreased. These results are agree with those obtained by Ota and Zabunoglu (1991) who pointed that, as nitrogen in plant media increased, the content of calcium oxalate in spinach leaves significantly increased.

(ii) Effect of cobalt

Data presented in Table (3) indicate that cobalt resulted adverse noticeable effect on the content of NO₃-N, NO₂-N and calcium oxalate in spinach leaves with all nitrogen rates. Cobalt significantly reduced Nitrate, Nitrite and oxalate contents. Increasing the nitrate, nitrite and oxalate contents in spinach leaves decreased the leaves quality. These results may go along with results of Wang *et al.* (1998) who found that, lowering NO₃-N and No₂,-N in spinach leaves increased leaves quality. Cobalt hence the quality of spinach leaves. Compared with untreated plants. This was confirmed by Nadia Gad *et al.* (2009) who pointed that cobalt gave noticeable decrease on the content of both NO₃-N and NO₂-N as well as calcium oxalate in spinach leaves.

Chlorophyll content

(i) Effect of Nitrogen

It is obvious from data in Table (4), that as increasing nitrogen level in plant media, the chlorophyll content in spinach leaves significantly increased. Data clearly indicate that chlorophyll contents in spinach leaves recorded the greatest values with 100% followed by 75% N followed by 50% N while 25% N gave the lowest ones. These results are in harmony with those obtained by Kulsum et al. (2007) who pointed that a best-fit positive linear relationship exited between cowpea leaf chlorophyll and leaf nitrogen content under different nitrogen doses. The trend of cowpea leaves, chlorophyll content gradually decreased as nitrogen in plant media decreased. The mention data go along with those obtained by Jamaati et al. (2009) who stated with increasing nitrogen content in durum wheat leaf chlorophyll content was increased. Also, with increasing plant population, chlorophyll content was decreased.

(ii) Effect of cobalt

The present data in Table (4) exhibits the effect of cobalt on chlorophyll content of spinach leaves under different nitrogen levels. Data reveal that cobalt significantly enhanced the content of chlorophyll in spinach leaves in the two seasons comparison with untreated plants. These results are agree with those obtained by Jaya Kumar *et al.* (2009b) who pointed that cobalt at 50 mg/kg soil had a beneficial effect on photosynthetic pigments as chlorophyll-a, chlorophyll-b and total chlorophyll content with both 100% and 75% nitrogen while 25% nitrogen gave the lowest figures. The mentioned data go along with those obtained by Abdul Jaleel *et al* (2009 a,b) who stated that cobalt increased photosynthetic pigments as chlorophyll-b and total chlorophyll-a, chlorophyll-b and total chlorophyll-a and green gram leaves.

Confirm these results Danesh *et al.* (2012) who pointed that nitrogen fertilizer enhance plant growth, leaf chlorophyll content, yield and its components.

Chemical constituents

Effect of nitrogen

Obtained results in Table (4) show that, total proteins, total carbohydrates, vitamin "A" along with vitamin "C" were reduced as nitrogen in plant media was reduced. This was confirmed by Marchner, (1995) who found that nitrogen has many functions in plant life being responsible for the biosynthesis of enzymes, nucleoproteins, amino acids, proteins, sugars, polypeptides, chlorophyll content, yield and its components.

Effect of cobalt

The amount total proteins, total carbohydrates, vitamin "A" along with vitamin "C" in spinach leaves as affected by cobalt under different nitrogen levels are given in Table (4). Results indicate that all mentioned parameters were significantly increased by cobalt addition with all nitrogen rates.

Nutritional Status

control.

Effect of Nitrogen

Data presented in Table (5) show that as nitrogen fertilizer in plant media decreased, the contents of macronutrients (N, P and K) significantly decreased. This is true in spite of results obtained by El-Desuki *et al.* (2010) who showed that, the content of N, P and K as well as total proteins of pea plants significantly increased as increasing nitrogen rate in plant media.

well as chemical constituents in leaves compared with

Presented data in Table (5) show similar trend of N,P and K in spinach leaves, the contents of Fe, Mn, Zn and Cu significantly reduced as nitrogen fertilizer in plant media was reduced. This results are agree with those obtained by Marraz *et al.* (2014) who found that, spirulina platensis is a rich source of potassium, Ca, P, Mg as well as Fe, Mn, zn and Cu which have a great role in cell division and enlargement as well as induce the photosynthesis and in turn reflected a great shoot growth. Confirm these results Lopez *et al.* (2008).

Effect of cobalt

Macronutrients Content (N, P and K)

Results presented in Table (5) show the effect of cobalt on the macronutrients content of spinach leaves. Data revealed that all cobalt levels significantly increased the content of N, P and K under different nitrogen rates compared with the control plants. Confirm these results Basu *et al.* (2006). The highest values of N, P and K content in peppermint herbs were obtained by using cobalt until 15 ppm, as compared with control and other used levels. These results are agreement with those obtained by Eman Aziz *et al.* (2011) who stated that cobalt had a promotive effect on N, P and K content of peppermint herb. While increasing cobalt dose in plant media reduce the promotive effect.

Manganese, Zn and Cu Content:

Presented data in Table (5) revealed that cobalt gave the highest values of Mn, Zn and Cu of spinach leaves under different nitrogen rates compared with the control. Confirm these results Laila Helmy and Nadia Gad (2002) who indicated that addition of cobalt had a significant promotive effect for better status of Mn, Zn and Cu in coriander herbs. Eman Aziz *et al.* (2007) stated that low cobalt dose (20mg kg⁻¹soil) posses a synergistic effect on the status of Mn, Zn and Cu in leaves and calyces of roselle plants.

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Nitrogen source	Nitrogen rate (%)	Plant height (cm)	Leaves number Per plant	Leaf area index (cm ²)	Leaves fresh weight/plant (g)	Leaves dry weight /plant (g)	Fresh yield (fed.)			
				Control						
um te	100	49.3	12	454	79.2	4.81	8.2			
mmoniı Sulphat	75	48.6	11	418	76.8	4.48	7.9			
	50	41.7	10	379	68.7	4.07	7.3			
Ā.	25	34.5	8	305	59.9	3.76	6.6			
Ammonium Sulphate	With cobalt (9 ppm)									
	100	54.6	14	598	85.8	5.56	10.0			
	75	52.9	14	589	81.4	5.34	9.4			
	50	47.5	11	472	75.7	4.75	8.6			
	25	41.4	10	411	68.5	4.02	7.9			
LSD 0.05	Ν	3.1	1.3	17.2	4.3	0.63	0.83			
	N x Co	2.7	1.1	15.2	3.7	0.57	0.75			

 Table 3 : Effect of cobalt on NO₃-N, NO₂-N and calcium oxalate contents of spinach leaves under different levels of nitrogen.

			Harvest					
Nitrogen	Nitrogen	10	-am	7-	pm	Calcium	Oxalate	
source	(%)	NO ₃ -N NO ₂ -N		NO ₃ -N	NO ₂ -N	(,0)	(7/100g)	
			Con	trol				
e m	100	1461	7.62	1258	4.88	0.691	49.80	
oniu	75	1319	6.98	1165	3.56	0.686	48.49	
Amme Sulp	50	1285	4.86	1137	2.93	0.679	45.22	
	25	1184	2.78	1062	1.89	0.671	44.53	
			With coba	lt (9 ppm)			•	
Ammonium Sulphate	100	1458	7.25	1252	4.76	0.695	49.8	
	75	1312	6.67	1148	3.43	0.691	48.4	
	50	1277	4.61	1114	2.82	0.686	46.2	
	25	1183	2.54	1051	1.83	0.681	44.5	
LSD	N	57.1	1.1	49.2	0.95	0.006	1.7	
0.05	N x co	54.6	0.95	47.5	0.81	0.005	1.3	

 Table 4: Effect of cobalt on chemical constituents of spinach leaves under different nitrogen levels.

Nitrogen source	Nitrogen rate (%)	Chlorophyll content	Total proteins	Total carbohydrates	Vitamin ''A''	Vitamin "C"			
		(Spad)		(%)	mg/100g fresh tissue				
Control									
te m	100	48.3	23.8	3.98	32.19	7.85			
ioni	75	46.2	20.1	3.90	31.41	7.49			
Int	50	43.6	14.8s	3.83	30.59	7.21			
A 3	25	39.7	10.4	3.66	29.74	6.90			
With Control (9 ppm)									
te II.	100	50.2	24.9	4.22	34.30	8.11			
noni	75	48.5	21.3	4.14	33.55	7.77			
Inn	50	46.0	18.3	3.98	32.64	7.46			
A S	25	42.4	13.3	3.74	31.59	7.12			
LSD	Ν	3.7	3.0	0.12	1.7	0.21			
0.05	Co N x Co	29	2.7	0.10	1.4	0.17			

Nitrogen	Nitrogen rate	Μ	lacronutrier	Micronutrients				Cobalt	
source	(%)	(%)				(ppm)			
		Ν	Р	K	Mn	Zn	Cu	Fe	Со
	Control								
te m	100	3.80	0.630	2.25	43.7	35.6	29.4	485	0.96
non	75	3.21	0.624	2.11	43.2	35.1	28.9	482	0.96
Inn	50	2.37	0.583	1.89	40.9	32.3	25.2	479	0.95
A 27	25	1.66	0.556	1.67	376	29.0	22.8	476	0.95
	With Control (9 ppm)								
Ammoinm Sulphate	100	3.98	0.675	2.86	45.1	37.0	30.3	480	3.67
	75	3.40	0.669	2.78	44.9	35.9	29.8	484	3.63
	50	2.13	0.597	1.94	43.0	34.8	28.6	487	3.59
	25	1.79	0.568	1.79	40.7	32.9	27.1	491	3.55
LSD	Ν	0.45	0.011	0.23	1.2	1.7	1.3	3.7	0.05
0.05	N x Co	0.41	0.009	0.18	1.1	1.3	0.9	2.5	0.03

Table 5: Effect of cobalt on nutritional status of spinach leaves under different nitrogen levels.

Iron Content:

Results present in Table (5) indicated that, increasing cobalt concentration in the plant media resulted in a progressive depression effect on iron content in the spinach leaves for two seasons under different nitrogen rates. This may be explained on the basis of obtained results by Blaylock (1993) who showed certain antagonistic relationships between the two elements (Fe and Co).

Cobalt Content:

Increasing cobalt concentration in plant media increased cobalt content in spinach leaves under different nitrogen levels for two seasons as compared with untreated plants (Table 5). These results clearly indicated that cobalt content goes along with the concentration of added cobalt. The obtained results are in harmony with those obtained by Nadia Gad and Hala Kandil (2012) how found that increasing cobalt in plant media increased cobalt content in coriander herbs.

Conclusion

It could be concluded that cobalt at 9 ppm has a promotive effect on spinach growth yield, mineral composition as well as chemical constituents under different nitrogen levels. Cobalt save 25% from nitrogen fertilizer compared with control (100% Nitrogen alone).

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