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Physiological and Chemical Response of Groundnut (*Arachis hypogaea*) to Cobalt Nutrition

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Abstract: Two field experiments were carried out to study groundnut physiological and chemical response to cobalt nutrition. Experiments were conducted at Research and production statation, National Research Centre, El-Nobaria Beheara Governorate, Delta, Egypt under drip irrigation system. Cobalt added once i.e. 0.0, 4, 6, 8, 10 and 12 ppm.
The obtained results indicate that:

- Cobalt concentrations significantly improve all growth and yield quantity and quality compared with control.
- Cobalt at 8 ppm gave the highest figures of all nodulation, growth, yield parameters as well as minerals composition endugennous hormone and chemical constituents.
- Increasing cobalt concentration more than 8 ppm excerted the adverse promotive effect.

Key words: Groundnut plants · Cobalt · Nitrogen fixation

INTRODUCTION

Cobalt is considered to be beneficial element for higher plants in spite of the absence of evidence for direct role in their metabolism. This is true in spite of essentiality for photosynthetic activities of low plants such as euglena gracilis; it was frequently reported to be localized in various sub-cellular fractions as in chloroplasts [1]. Cobalt is an essential element for certain microorganisms particularly those fixing atmospheric nitrogen, it is deficiency seems to depress the efficiency of N_2 fixation. The Co-enzyme cobalamin has cobalt (III) as a metal component, chelated to four nitrogen atoms at the center of a prophyrin structure simslar to that of iron in hemin. In rhizobium species, three enzymes are known to be cobalamin dependent with cobalt induced changes in their activities are primarily responsible for the relationship to nodulation and nitrogen fixation in legumes [2]. These enzymes are: methionine synthase, ribonucleatide reductase and methylmalonyl Co-enzyme A mulase [3].

Groundnut is one of most important leguminous crops in Egypt as well as in many parts of the world. It is used for human consumption, oil production, food industries and animal feeding. [4] Showed that the importance of cobalt on growth and development of leguminasea plants was mainly due to the effect of cobalt on the activity and population of both atmospheric nitrogen of *Azotobacter and Nitrobacter.* [5] found that cobalt is an essential element for the synthesis of vitamin B_{12} which is required for animal and human nutrition. [6] Stated that cobalt at the rate of 2.0 mg/kg soil gave the highest nodulation rate, fresh and dry weights of phasselus volgaris compared with the control plants [7]. Stated that seeds oil and protein contents as well as the concentration of N, P and K of groundnut seeds were increased by 1.0 ppm cobalt sulphate and rhisobium. Authors [8, 9] reported that cobalt at 0.21 kg/ha increased plant height, number of branches and leaves, leaf area index, dry weight of shoots as well as pods yield of groundnut, [10, 11] pointed that cobalt recorded the maximum leaf area index, dry matter accumulation in aerial parts of the plants, root dry weight, plant height, nodules number as well as pods yield in both cowpea and groundnuts compare with the control.

Recently, [12, 13] showed that cobalt at 50 mg/kg soil increased growth parameters such as plant height, root length, total leaf area, nodules number and rate, stem

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diameter, number of branches and leaves, shoots and roots biomass and pods yield of groundnut and soybean compared with the control.

More recently, [14] added that the amendment of cobalt at 12 ppm to the soil improved the growth parameters, nodules number and weight, nitrogenase activity, seeds yield as well as minerals composition and chemical contents of faba bean seeds compared with untreated plants.

Unlike other heavy metals, cobalt is save for human consumption up to 8 ppm can be consumed on a daily basis without health hazard [15].

MATERIALS AND METHODS

Soil Analysis: Physical and chemical properties of Nubaria Soil were determined and particle size distributions along with soil moisture were determined as described by [16]. Soil pH, EC, cations and anions, organic matter, $CaCO_3$, total nitrogen and available P, K, Fe, Mn, Zn, Cu were run according to [17]. Determination of soluble, available and total cobalt was determined according to method described by [18]. Some physical and chemical properties of Nubaria soil are shown in Table (1).

Experimental Works: A Preliminary pot experiment was conduct at wire house of National Research Cetre to define cobalt concentrations range, which gave growth and yield.

Seedlings of groundnut (at the third true leaves) irrigated once with cobalt concentrations: 0.0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 ppm. According to the preliminary experiment results, the concentration range of cobalt, which gave the groundnut response growth and yield, were 0.0, 4, 6, 8, 10 and 12 ppm. Cobalt at 8 ppm gave the best growth and yield parameters of groundnuts.

Two Field experiments were carried out to evaluate groundnut physiological and chemical response to cobalt nutrition with different levels of nitrogen. The sandy loam soil with plot area consists of five ridges, 3.5 meter in length and 60 cm width ($10.5m^2 = 1/400$ fed). Calcium super phosphate (15.5 %) at the rate of 200 kg P_2O_5/fed, chicken manure at the rate of 15 m^3/fed (Table 2) and potassium sulphate (48% k_2O) at the rate of 100 kg /fed were added during soil preparation. Seeds of groundnut (*Arachis hypogaea* mill, cv. Giza- 6) were inoculated prior to sowing with a specific strain of rhizobium (*bradyr hizobium sp*.). Seeds were sown on April, 2011 and 2012 summer seasons.

Seedling the seedlings (at the third true leaves) were irrigated once with six concentrations of cobalt (0.0, 4, 6, 8, 10 and 12 ppm) as a cobalt sulphate form. All required agricultural managements for plants growth and production were carried out as recommended by Ministry of Agriculture.

Measurements of Vegetative Growth: After 80 days from sowing, all growth parameters of groundnut plants such as plant height, root length, number of branches and leaves as well shoot and root fresh and dry weights were recorded according to [19].

Nodulation and Nitrogenase Activity: Nodules number, fresh and dry weights were recorded after 50 days from sowing. Nitrogenase activity was determined according to [20]. Groundnut plants were gently uprooted then the root nodules were placed in 500 ml serum bottles and

Table 1: Some physical and chemical properties of Nubaria soil

Physical properties

Particle size distribution (%)			Soil moisture constant (%)				
Sand	Silt	Clay	Soil texture	Saturation	FC	WP	AW
70.8	25.6	3.6	Sandy loam	32.0	19.2	6.1	13.1

Chemical properties				Soluble cations (meq^1L)				Soluble anions ($meq^{-1}L$)			
pH 1:2.5	EC ($dS\ m^{-1}$)	CaCO3 %	OM %	Ca^{++}	Mg^{++}	K^+	Na^+	HCO_3^-	CO_3	Cl^-	$SO_4^=$
8.49	1.74	3.4	0.20	0.8	0.5	1.6	1.80	0.3	-	1.9	0.5

Cobalt (ppm)	Total	Available	Available micronutrients ppm						
	-------mg 100 g^{-1} soil---------								
Soluble	Available	Total	N	P	K	Fe	Mn	Zn	Cu
0.35	4.88	9.88	15.1	13.3	4.49	4.46	2.71	4.52	5.2

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

Table 2: Some properties of chicken manure

					Available nutrients (%)		DTPA-extractable (ppm)			
O.M (%)	Total N (%)	C/N ratio	pH (1:25)	EC dsm^{-1}	P	K	Fe	Mn	Zn	Cu
36.0	2.96	7.07	6.40	8.85	0.72	0.93	566	36.8	28.2	34.7

were sealed with suba-seal rubbers and 10 % of the gas phase was replaced by C_2H_2 then bottles were incubated in dark at room temperature for 2 hr. production of C_2H_4 was measured by injecting one ml gas sample into (GC). Nitrogenase activity values were recorded as µmol C_2H_4 /g/h.

Measurements of Plant Yield: After 120 days from sowing groundnut yield parameters such as pods number/ plant, weight of pods/plant, weight of seeds/plant, 100 seeds weight, total pods yield (kg/fed), total pods yield (Ardab/fed) were recorded according to [21]. Ardab = 75 kg

Nutritional Status: Groundnut, seeds sampled either from the intact plant for each treatment of both seasons was oven dried at 70°C for 48 hr ground and kept to chemical determinations. For extraction a weight of 0.2 g finely powdered dry sample and digested using a mixture of sulfuric acid (H_2SO_4) with hydrogen peroxide (H_2O_2). Macronutrients (N, P and K) as well as micronutrients (Fe, Mn, Zn, Cu, along with cobalt were determined according to the method described by [18].

Chemical Constituents: The percent of total carbohydrates, total soluble sugars, total soluble solids, of groundnut seeds were determined according to [22]. Also total phenoles Aqueous acetone (70%) was determined according to [23].

Seeds Oil Fatly Acids Content: Groundnut Seeds oil fatly acids content was determined using soxhelt apparatus and n-Hexan as solvent, according to [24].

Statistical Analysis: All data were subjected to statistical analysis according to procedure outlined by [25] computer program and means were compared by LSD method according to [26].

RESULTS AND DISCUSSION

Vegetative Characteristics: Groundnut growth parameters as affected by cobalt nutrition are given in Table (3). Data indicated that all cobalt levels significantly increased the growth parameters of groundnut compared with control plants. Cobalt at 8 ppm had a significant promotive effect on all growth parameters of groundnut after 80 days from sowing. It is clear that cobalt enhances all growth parameters such as plant hight, number of branches and leaves per plant, leaf area index, root length as well as shoot and root biomass. When cobalt addition increased more than 8 ppm (10 and 12 ppm) the promotive effect reduced all growth figures. These observations are consistent with previous reports obtained by [10, 11] who stated that cobalt recorded the maximum leaf area index, dry matter accumulation in shoot and root, plant hight as well as pods yield in both cowpea and groundnuts compared with control. [12, 13] added that cobalt at 50 mg/kg soil growth parameters such as plant hight, root length, total leaf area, shoots and roots biomass and pod yield of groundnuts and soybean compared with the control.

Nodulation and Nitrogen Fixation: Present data in Table (4) indicate that cobalt had a significant positive effect on groundnut root nodules parameters compared with untreated plants. Cobalt at 8 ppm recorded the maximum nodules number, fresh and dry weights. In addition the encourage happened in the nodule formation process resulted in increasing the efficiency of rhizobium bacteria to perform with N_2 fixation at high capacity to produce healthy plants. These results are in harmony with those obtained by [10] and [27], they pointed that cobalt significantly improved total nodules number and weights as well as root dry weight in both cowpea and pea plants compared with control. Similar results are reported by [28] who found that the presence of cobalamin Co-enzyme in the nodules of several legumes confirmed the role of cobalt in nitrogen fixation. Thus a deficiency in cobalt is shown in reduced vitamin B_{12} production and lower nitrogen fixation. The obtained data in Table (4) reflected that cobalt can play a vital role in increasing nitrogenase enzyme activity of groundnut root nodulation after 50 days from sowing compared with untreated plants. These results are agree with those obtained by [29-30], they pointed that cobalt stimulated the growth and development of plants, nodules and increased the N accumulated in leguminous plants. It was also observed

Table 3: Groundnut growth parameters as affected by cobalt after 80 days from sowing (mean of two seasons)

Cobalt treatments (ppm)	Plant high (cm)	Number/plant Branches	Number/plant Leaves	Leaf area (cm^2)	Root length (cm)	Dry weight (g) Shoot	Dry weight (g) Root
Control	23.6	7	61	1468	13.3	27.6	1.25
4	27.2	9	72	1679	14.7	29.0	2.39
6	29.9	11	79	1988	15.7	31.4	2.95
8	33.5	13	86	2345	17.2	35.0	4.67
10	13.3	12	80	2216	16.5	33.2	4.18
12	30.4	11	78	1965	16.0	31.4	3.78
LSD 5%	0.9	1	2	22	0.5	1.1	0.45

Table 4: Groundnut nodulation parameters as affected by cobalt levels after 50 days from sowing (mean of two seasons)

Cobalt treatments (ppm)	Nodules No./plant Nodule	Nodules fresh weight (g)	Nodules dry weight (g)	N- ase activity U mol C_2H_2/g/h
Control	78.5	8.61	1.78	15.2
4	102.6	10.3	2.98	16.5
6	121.8	11.3	3.74	17.8
8	140.5	12.5	4.98	20.9
10	132.4	11.4	4.74	18.6
12	129.6	11.0	4.61	17.5
LSD 5%	3.2	0.3	0.13	1.0

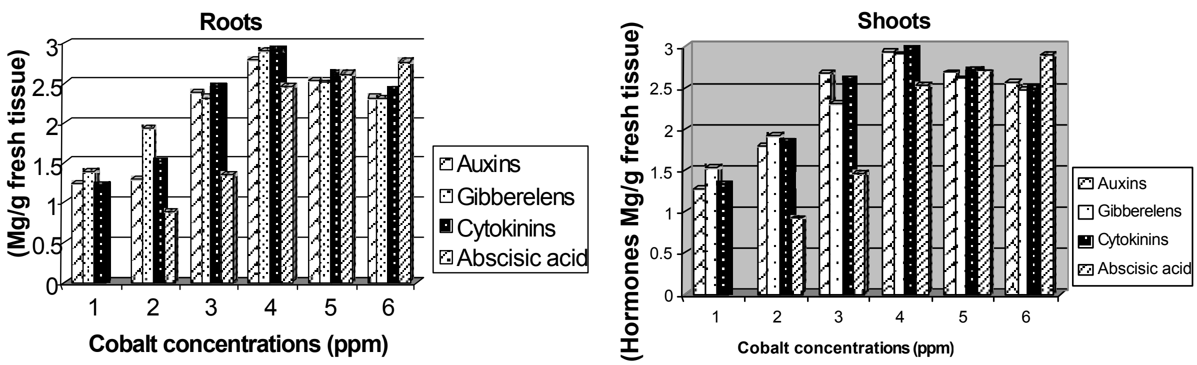

Fig. 1: Groundnut endogenous hormones (Auxins, Gibberelens, Cytokinins and Abscisic acid) as affected by cobalt concentrations after 80 days from sowing

that the increase in nitrogenase activity was parallel and related to the increase in nodules number and efficiency. As well known, the nitrogenase is a complex enzyme which enable fixation of atmospheric nitrogen. In nitrogen-fixing bacteria, the nitrogenase enzyme derives the reaction of atmospheric dinitrogen fixation in presence of ATP. These results are in harmony with those obtained by [31] who reported that, in bacteria the requirement for cobalt is primiraly due to its presence in vitamin B_{12}, which plays an important role in methionine biosynthesis. The increase in nitrogenase activity reached its maximum using cobalt dose of 8 ppm. It is appearantly from the data that this dose of cobalt (8 ppm) in case on groundnut plants in the marginal concentration for all estimated values. Confirm these data [9] stated that, root nodules parameters and nitrogenase enzyme activity in nodules of cowpea was significant influenced by cobalt.

Endogenous Hormones: The presented data in Fig. (1) outline the response of groundnut endogenous hormones to different cobalt levels. Data indicate that all cobalt levels significantly increased the content of endogenous hormones such as Auxins, Gibberllins and Cytokinins compared to control in both shoots and roots. Cobalt at 8 ppm resulted the highest figures of groundnut phytohormones. As cobalt concentrations were ranged above 8 ppm, the promotive effect significantly reduced. Plant hormones (Auxins, Gibberllins and Cytokinins) are natural products, they stimulate the physiological response of plant growth. Different strategic are being employed to maximize plant growth and yield. Theses results are in harmony with those obtained by [32] who stated that, both Auxins and Gibberllins enhancement the activation of specific enzyme which participates RNA and protein synthesis in rice plants. Confirm, these reslts [27], found that cobalt at 7.5 ppm had a significant positive effect in tomatoes hormonal synthesis and metabolic activity and resulted in maximum growth and yield. She added that cobalt may be attributed to catalase and peroxidase activities which were found to decrease with low levels of cobalt and increase with higher ones. Moreover, higher cobalt concentration were found increase the activity of peroxidase and catalase in plant and hence increasing the catabolism rather than anatolism. [33] pointed that cobalt addition of 22.5 ppm had a significant promotive effect of lemongrass endogenous hormones (Auxins, Gibberllins and Cytokinens). These hormones resulted high herb yields quantity and quality. Higher concentrations more than 22.5 ppm exerted advers effect.

Figure (1) also indicate a gradual Abscisic acid synthesis with cobalt addition in plant media and increased a cobalt concentrations increased. Under newly reclaimed soils, cobalt gradually increase dramatically Abscisic acid which was pereviously supposed to blay a central role in hormonal control of water balance and help plants to tolerate the drought. These results are good agreement with those obtained by [34] who stated that cobalt application reduced water loss as well as water consumption by tomato and squash plants, symptoms of willting being revealed with cobalt application which significantly increased the percentage of stomatal closure and reduced the transpiration rate. Confirm [35], stomatal resistance was significantly correlated with both leaf water potential and net photosynthesis of peach seedlings.

Yield Characteristics: Obtained results in Table (5) revealed that cobalt significantly improvement all yield parameters such as pods number per plant, pods weight per plant, weight of 100 seeds, seeds and oil yield per Fadden of groundnut. All cobalt levels significantly increased groundnut yield parameters compared with control plants.

The highest recored figures of the mentioned parameters of groundnut were obtained in plants treated with 8 ppm cobalt. When cobalt concentration increased more than 8 ppm, the promotive effect of all yield parameters reduced. These observations are consistent with previous reports obtained by [10, 11] they found that cobalt recorded the maximum pods yield in both cowpea and groundnut compared with control. Data in Table (5) also indicated that cobalt at 8 ppm increased pods and oil yield up to 34.4% and 43.6% respectively. According to, [36-37] cobalt addition in soil increased all yield parameters such as seedling vigour, number and weights of pods and seeds yield per plant in green gram (*Vigna radiate* L.) and maize (*Zea maiz* L.) plants. Finally, [14] added that cobalt at 12 ppm resulted in maximizing all faba bean growth and yield parameters, seeds quantity and quality.

Nutritional Status

Macronutrients (N, P and K) Contents: Presented data in Table (6) show the effect of different cobalt levels (0.0, 4, 6, 8, 10 and 12 ppm) on macronutrients in groundnut seeds for two seasons. Data reveale that all the cobalt levels significantly increased the content of N, P and K as

Table 5: Groundnut yield parameters as affected by cobalt after 120 days from sowing (mean of two seasons)

Cobalt treatments (ppm)	Pods number/plant (pod)	Pods weight/plant (g)	100 seeds weight (g)	Peds yield ardat/fed	Oil %	Oil yield (Kg/fed)
Control	15.0	18.3	51.2	18.0	42.6	627.9
4	22.7	19.9	53.1	21.3	45.7	632.1
6	25.2	23.8	54.6	22.9	47.1	811.5
8	27.5	27.2	56.0	24.2	49.5	901.4
10	24.8	25.5	55.4	23.9	49.0	881.5
12	23.5	23.4	53.8	23.0	48.3	850.4
LSD 5%	1.2	0.4	0.6	0.3	0.5	24.0

Table 6: Mineral composition in groundnut seeds as affected by cobalt (mean of two seasons)

	Macronutrients (%)			Micronutrients (ppm)				
Cobalt treatments (ppm)	N	P	K	Mn	Zn	Cu	Fe	Cobalt (ppm)
Control	0.68	0.078	0.788	19.3	16.7	15.9	44.8	0.97
4	0.97	0.118	0.965	21.1	17.0	17.6	42.0	1.05
6	1.24	0.169	1.217	23.5	18.6	19.2	39.8	2.30
8	1.65	0.240	1.605	25.8	19.5	20.6	36.4	4.76
10	1.58	0.240	1.522	24.2	19.0	20.1	34.5	6.0
12	1.47	0.233	1.468	24.2	18.4	19.7	32.2	7.12
LSD 5%	0.10	0.08	0.54	1.6	0.2	0.5	2.2	0.08

compared with control treatment. The highest values of N, P and K content were obtained by cobalt rate at 8 ppm compared with other cobalt doses. Increasing cobalt concentration in plant media above 8 ppm the positive effect was significantly reduced. These results are in harmony with those obtained by [7] who found that the concentrations of N, P and K of groundnut seeds were increased by 1.0 ppm cobalt sulphate and rhizobium. Confirm, [9] who stated that cobalt gave a promotive the status of N, P and K groundnut productivity. Finally, [27] who pointed that cobalt had a positive effect on contents of N, P and K of pea plants. While increasing cobalt concentrations exerted the promotive effect adverse one.

Micronutrients (Mn, Zn and Cu) Contents: Data in Table (6) indicate the effect of cobalt on micronutrients (Mn, Zn and Cu) in groundnut seeds in two seasons. Data show that cobalt at 8 ppm gave the highest Mn, Zn and Cu content compared to control. Increasing cobalt addition in plant media more than 8 ppm resulted in proportion significant the promotive effect reduction. These results are good agreement with those of [38] who showed that, cobalt level of 2.5 ppm in solution culture exerted a promotive effect on Mn, Zn and Cu content in tomatoes. [12] added that all minerals content of blackgram were increased with cobalt at 50 mg/kg soil when compared with the control. Confirm these results, [14] stated that cobalt at 12 ppm increased Mn, Zn and Cu contents in faba bean compared with the control. While increasing cobalt levels in plant media being the promotive effect reduction.

Iron Content: Presented data in Table (6) also show that, Fe content in groundnut seeds significantly decreased with the increasing cobalt concentration in plant media. These results are in harmony with those obtained by [39] who found certain antagonistic relationship between both Fe and Co elements. Confirm [27] who indicted that increasing cobalt doses in plant media resulted in a progressive depression effect on iron content in pea plants.

Cobalt Content: Data in Table (6) also reveal that cobalt content in groundnut seeds significantly increased when cobalt addition increasing in plant media. These results are good agreement with those obtained by [14] who found that increasing cobalt concentration in plant media significantly increased cobalt content in faba bean plants compared with control.

Chemical Constituents: The amount of total protein, total carbohydrates, total soluble sugars, total soluble solids, oil and total phenols percentage in groundnut seeds as affected by different cobalt levels are given in Table (7). Results indicate that all the mentioned parameters were significantly increased by the addition of cobalt levels (4, 6, 8, 10 and 12 ppm) as compared with those obtained by control treatment. In this concern, [40] who stated that all cobalt treatments significantly increased all chemical contents in sugar beet roots (total soluble solids, carbohydrates, proteins and vitamin C) compared to control. Cobalt dose at 7.5 ppm gave the highest values in all chemical constituents for sugar beet roots compared with other cobalt concentrations.

Data in Table (7) also indicate that increasing cobalt levels in groundnut plant media significantly increased total phenols percentage (1.94%) of seeds. The percentage (1.94%) less 2% safty human health. These results are agree with those obtained by [23] and [41].

Fatty Acid Content in Groundnut Oil: The effect of cobalt on fatty acids content of groundnut oil is given in Table (8). Data indicate that all cobalt concentration decreased in the principal saturated fatty acid is palmitic.

Table 7: Chemical content of groundnut seeds as affected by Cobalt (mean of two seasons)

Cobalt treatments (ppm)	Protein	Total carbohydrates	Total soluble sugars	Total soluble solids	Oil	Total phenoles
	(%)					
Control	4.24	25.0	9.21	36.8	42.6	1.08
4	6.05	25.8	9.69	37.4	45.7	1.25
6	7.74	26.3	9.87	38.0	47.1	1.44
8	10.3	27.5	10.5	38.7	49.5	1.69
10	9.86	27.2	10.2	38.4	49.0	1.86
12	9.17	27.0	10.2	38.0	48.3	1.94
LSD 5%	0.12	0.2	0.3	0.3	0.5	0.08

Table 8: Oil fatty acids in groundnut seeds as affected by cobalt (mean of two seasons)

	Cobalt treatments (ppm)					
Fatty of groundnut oil (%)	control	4	6	8	10	12
Palmitic (C16:0)	9.08	8.13	7.74	7.71	7.48	7.29
Stearic (C18:0)	2.31	2.26	1.95	1.83	1.49	1.17
Arachidic (C20:0)	0.83	0.81	0.75	0.69	0.64	0.61
Behenic (C22:0)	6.63	6.42	6.05	5.91	5.7	5.66
Lignoceric (C24:0)	1.26	1.2	1.16	1.13	1.07	1.07
Total saturated fatty acids (TS)	20.11	18.82	17.65	17.27	16.42	15.8
Palmitoletic (C16:1)	-	-	-	-	-	-
Oleic (C18:1)	30.67	30.9	31.09	31.25	31.96	33.05
Linoleie (C18:2)	12.76	13.26	13.86	14.11	14.28	15.34
Linolenic (C18:3)	4.18	4.84	5.05	5.89	6.16	6.49
Eicosonole (C20:1)	0.39	0.44	0.49	0.55	0.59	0.64
Erucic (C22:1)	-	-	-	-	-	-
Total unsat. Fatty acids (TU)	48	49.44	50.49	51.8	52.99	55.52
TU/TS	2.39	2.63	2.86	3	3.23	3.51

Increasing cobalt level in plant media resulted in a progressive effect. Cobalt redused other saturation fatty acids such as stearic, Arachenic, Behenic and Lignoceric acids. It is worthy to mention that the decrease in the percentage of saturated fatty acids is profitable in human nutrition.

Data in Table (8) shows that all cobalt concentrations ranged from 4 to 12 ppm gave the major constituent of unsaturated fatty acids is Oleic acid (C 18: 1). Data also indicate that cobalt increased in the percentage in the other unsaturated fatty acids i.e. Linoleic, Linolenic, Eicosonole and Erucic acids. Linoleic acid consists of Omega 3 and Omega 6 which processes an antioxidant properties. It is actually super food and is necessary to several metabolic processes [42]. Results in Table (8) also revealed cobalt with all concentrations used caused an increase in the ratio of total unsaturated fatty acids to the saturated one. This effect is known to give the oil better quality and profitable in human health. These results are in harmony with those obtained by [43] who found that cobalt increased the percentages of unsaturated fatty acids while induced a reduction in the percentage of total saturated one of canola seeds.

CONCLUSION

Cobalt is a promising element in the newly reclaimed soils. It is used to reduce the harmfull effect of high temperature, drought and salinity in these soils. Cobalt is a assential element in both human and animal nutrition. The daily cobalt requirements for human nutrition could reach 8 ppm depending on cobalt levels in the local supply of drinking water without health hazard.

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