International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.11, pp 85-92, 2015

Effect of cobalt on growth and yield of fenugreek plants

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Abstract: Two field experiments were carried out to evaluate cobalt role on fenugreek growth, yield quantity and quality. Experiments were conducted at Research and production Station, National Research Centre, El-Nubaria site, El-Beheara Governorate – Delta Egypt. Fenugreek Seedling at (third truly leaves) treated once, with cobalt sulphate, with the different cobalt concentrations (0.0,4,8,12,16 and 20 ppm) under drip irrigation system during 2011 and 2012 seasons.

The obtained results are summarized in the following:-

Cobalt application levels significantly increase growth and yield parameters, nutritional status and chemical constituents of fenugreek seeds comparison with control.

Cobalt at 12 ppm has a superior values.

Increasing cobalt level in plant media more than 12 ppm, reduces the pormotive effect.

Key words:- Fenugreek, Cobalt, grains and oil yield.

1-Introduction

Fenugreek (*Trigonella foenum- graceum*) is an annual crop belonging to the legumacea. It is commonly growing in the Mediterranean regions of the world. Its seeds and leaves are primary used as a culinary spice. In Egypt, Greece, in Italy and South Asia it is also used to treat some healthy problems[1]. Fenugreek seed contains 20 % protein, 50 % carbohydrates, 5 % fat, dietary fibers lipids, cellulose, starch, ash, iron, calcium and B-carotene[2], Also, it has been found to contain vitamin "C", niacin, potassium and diosgenin (which are a compound that has properties similar to esterogen). Other active constituents in fendgreek are alkaloids (lysine and L- tryptophan) as well as steroidal saponins[3]. Additionally, green fenugreek is a good source of iron for human [4]. Fenugreek is a medicinally important plant possessing anti-diobetic, anticancerous, anti- microbial and hypocholesterolaemic properties [5]. The multiple uses for this plant, in foods, as a spice and medicine, as colic flatulence in dysentery, diarrhea, as galactagolat, dyspepsia, with loss of appetite, chronic cough, enlargement of liver and spleen[6].

Cobalt is considered to be a 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 lower plants such as euglena gracilis. Cobalt is an essential element for certain micro-organisms particularly those fixing atmospheric nitrogen, its deficiency seems to depress the efficiency of nitrogen fixation [7]. Addition of cobalt to highly purified nutrient solution substantially increased host-plant growth and alleviated symptoms of nitrogen deficiency. Cobalt requirements for nitrogen fixation are greater than those for host-plant growth [8]. Cobalt was directly proportional to vitamin B_{12} which plays an important role in enhancement fixation of atmospheric nitrogen. Cobalt is essential for growth rhizobia, the specific bacteria involved inlegume nodulation and nitrogen fixation[9].[10,11] found that cobalt recorded the maximum leaf area index, dry matter accumulation in aerial parts of the plants, root dry weight, plant height as well as pods yield in both cowpea and groundnuts compared with the control. [12,13] demonstrated that cobalt at 50 mg/kg soil increased growth parameters such as plant height, root length, shoots and roots dry wight along with yield parameters such as seedling vigor, number of pods per plant in green gram (*Vigna radiate* L.) and maize (*Zea mays* L.) plants. [14] stated that the amendment of cobalt at 12 ppm to the soil improved the growth parameters, nodulation rate nitrogenase activity, seed yield and minerals composition content in faba bean. Recently, [15] showed that cobalt at 12 ppm significantly increased nitrogenase activity which was parallel related to the increase nodules numbers and weights, growth and yield parameters, minerals composition and chemical constituents in soybean seeds specially with 100 % and 75 % nitrogen. Finally, the addition of cobalt at 12 ppm to the soil save 75 % nitrogen fertilizer compared with untreated plants. Thus the aim of this investigation is to study the effect of cobalt supplement on fenugreek growth, nodulation rate, seed yield quantity, minerals content and chemical constituents as well as seed oil percentage.

2. Materials and Methods:

2.1. Soil analysis:

Particle size distribution along with soil moisture constants of used soil sample, as described by [16] were determined. Contents of $CaCO_3$, Organic matter, pH and EC as well as Soluble Cations and Anions were assayed according to **[17].** Total and available macro and micro nutrients were determined according to [18]. Total cobalt was determined in Aqua regain extract, soluble and available cobalt being assayed according to [19]. Some physical and chemical properties of Nubaria soil sample are shown in Table (1).

Table (1). Some physical and chemical properties of El-Nobaria soil

<table>
<tr><td colspan="12">Physical properties</td></tr>
<tr><td colspan="4">Particle size distribution %</td><td colspan="8">Soil moisture constant %</td></tr>
<tr><td>Sand</td><td>Silt</td><td>Clay</td><td>Soil texture</td><td colspan="2">Saturation</td><td colspan="2">FC</td><td colspan="2">WP</td><td colspan="2">AW</td></tr>
<tr><td>70.8</td><td>25.6</td><td>3.6</td><td>Sandy loam</td><td colspan="2">32.0</td><td colspan="2">19.2</td><td colspan="2">6.1</td><td colspan="2">13.1</td></tr>
<tr><td colspan="12">Chemical properties</td></tr>
<tr><td colspan="4"></td><td colspan="4">Soluble cations ($meq^{-1}L$)</td><td colspan="4">Soluble anions ($meq^{-1}L$)</td></tr>
<tr><td>pH 1:2.5</td><td>EC ($dS\ m^{-1}$)</td><td>CaCO3 %</td><td>OM %</td><td>Ca^{++}</td><td>Mg^{++}</td><td>K^{+}</td><td>Na^{+}</td><td>HCO_3^{-}</td><td>CO_3</td><td>Cl^{-}</td><td>$SO_4^{=}$</td></tr>
<tr><td>8.49</td><td>1.74</td><td>3.4</td><td>0.20</td><td>0.8</td><td>0.5</td><td>1.6</td><td>1.80</td><td>0.3</td><td>0.0</td><td>1.9</td><td>0.5</td></tr>
<tr><td colspan="4">Cobalt</td><td>Total</td><td colspan="2">Available</td><td colspan="5">Available micronutriments</td></tr>
<tr><td colspan="4">Ppm</td><td colspan="3">mg 100 g^{-1} soil</td><td colspan="5">Ppm</td></tr>
<tr><td>Soluble</td><td colspan="2">Available</td><td>Total</td><td>N</td><td>P</td><td>K</td><td>Fe</td><td>Mn</td><td>Zn</td><td colspan="2">Cu</td></tr>
<tr><td>0.35</td><td colspan="2">4.88</td><td>9.88</td><td>15.1</td><td>13.3</td><td>4.49</td><td>4.46</td><td>2.71</td><td>4.52</td><td colspan="2">5.2</td></tr>
</table>

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

2.2. Experimental works:

A preliminary experiment was carried out to define the concentrations range of cobalt which gave growth and yield response of fenugreek plants. Using plastic pots (10 Kg capacity) filled with sandy loam soil from Nubaria farm, in Wire house, National Research Centre, El- Bohooth St., Dokki, Cairo, Egypt. Two field experiments were carried out to evaluate the response of fenugreek to cobalt levels. Experiments were conducted in the Research and Production Station, National Research Centre, El-Nubaria Site, El- Beheara Governorat, Delta Egypt, under drip irrigation system, during the two successive seasons of 2012 and 2013.

Grains of fenugreek (*Trigonella foenum-graecum* L.cv. Giza-30) were inoculation prior to sowing with a specific strain of *Rhizobium moltiti*. Grains were sown on 4 and 6 November in the two experimental seasons with all agricultural managements required for production of seedlings as usually recommended. The experimental unit consist of 6 treatments. Each treatment was represented by 3 plots. Each plot area was 5x3 meter, consisting of three rows. Ten plants in each row (50 cm apart) were planted. The seedlings (at the third truly leaves) were irrigated with cobalt sulphate once, with the different cobalt concentrations (0.0, 4, 8, 12, 16 and 20 ppm). All the plants received natural agricultural practices whenever they needed.

2.2.1 Measurement of nodulation rate and nitrogenase activity:

After 50 days from sowing, nodules number, nodules fresh weight and biomass were recorded to [20]. Nitrogenase activity was determined according to [21]. Fenugreek plants were gently uprooted then the root nodules were placed in 500 ml serum bottles and were sealed with subaseal 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_2 was measured by injecting one ml gas sample into (GC). Nitrogenase activity value were recorded as Umol/C_2H_2/g/h.

2.2.2 Measurement of plant growth parameters:

After 75 days from swing, some growth parameters such as plant height number of both branches and leaves per plant, leaf area index, shoots and roots fresh weight per plant and the biomass of both shoots and roots per plant were recorded by [22].

2.2.3 Measurement of yield characteristics:

After 110 days from sowing, yield parameters such as pods number per plant, seeds weight per plant, seeds yield per feddan, seeds oil percentage, seeds oil per plant as well as seed oil yield per feddan were recorded according to [20].

2.2.4 Nutritional status:

Macronutrients (N, P and K) and micronutrients (Mn, Fe, Zn and Cu) as well as cobalt content were determined in fenugreek grains sampled either from the intact plant for each treatment of both seasons according to [19].

2.2.5 Chemical constituents:

Total carbohydrates, total proteins, vitamin "C" as L- Ascorbic acid and vitamin "A" as a carotenoids were determined in fenugreek grains according to standard methods described by [23].

Mucilage percentage in fenugreek grains was determined according to the method described by [24].

The trigonellin content in fenugreek grains was determined according to the equation: trigonellin alkaloid = absorbance of test at 268 nm/Absorbance of standard by [25].

2.2.6 Statistical analysis:

All data were subjected to statistical analysis according to procedure outlined by 26 computer program and means were compared by LSD method according to [27].

3. Results and Discussion

3.1 Nodulation and nitrogen fixation:-

Table (2) indicate that cobalt significantly increased nodules number per plant, nodules fresh and dry weights per plant compared with control. Cobalt at 12 ppm gave the greatest values. Increasing cobalt level in the plant media above 12 ppm reduce the promotive effect. These results are in harmony with those obtained by [10, 28] they pointed that cobalt significantly improved total nodules number and root dry weight on both cowpea and pea plants compared with control.

Table (2): Fenugreek nodulation and nitrogenase activity as affected by cobalt levels after 50 days from sowing (mean of two seasons).

Cobalt treatment (ppm)	Nodules number/plant	Nodules fresh weight (g)	N0dules dry weight (g)	Nitrogenase activity (μ mol C_2H_2/g/H)
Control	17.32	1.85	0.178	19.3
4	20.13	2.08	0.190	21.4
8	24.60	2.20	0.217	24.6
12	30.00	2.65	0.325	28.9
16	28.21	2.89	0.286	26.7
20	26.01	2.78	0.257	24.9
LSD 5 %	1.89	0.8	0.2	1.8

Table (2) also reflected that cobalt can play a vital role in increasing nitrogenase enzyme activity with all cobalt concentrations of fenugreek root nodulation after 50 days from sowing in the two season. Cobalt at 12 ppm recorded the best rate of the nitrogenase enzyme activity. These results are agree with those obtained by [29] who found that root nodules parameters and nitrogenase enzyme activity in nodules of cowpea was significant influenced by cobalt addition. [15] added that cobalt at 12 ppm had a significant positive effect on soybean root nodules parameters such as number of total nodules per plant, fresh and dry weights of nodules. Cobalt at 12 ppm recorded the highest rate of nitrogense activity.

3.2 Vegetative growth:

Fenugreek growth parameters as affected by cobalt rates after 75 days from sowing are given in Table (3). Data indicate that cobalt at 12ppm has a significant promotive effect on all studied growth parameters such as plant height, number of branches and leaves per plant, shoot and root fresh weights, leaf area per plant as well as biomass of shoots and roots.

Table (3): Effect of cobalt on fenugreek growth parameters (mean of two seasons).

Cobalt treatment (ppm)	Plant high (cm)	Number/plant		Leaf area plant	Fresh weight per plant (g)		Dry weight per plant (g)	
		Branches	Leaves	Cm^3/plant	Shoot	Root	Shoot	Root
Control	35.23	10.05	73.40	263.4	39.06	4.82	12.97	1.61
4	37.08	10.66	76.71	269.0	43.68	5.02	14.77	1.67
8	39.80	11.30	8.91	276.2	49.89	5.89	17.80	1.96
12	43.52	12.50	87.60	283.5	56.34	6.06	23.28	2.02
16	42.19	12.25	87.14	280.4	56.00	5.93	23.00	1.89
20	40.43	11.61	85.25	275.2	54.90	5.24	22.15	1.78
LSD 5 %	0.43	0.25	0.46	3.1	0.34	0.22	0.28	0.4

[30] found that cobalt being with positive effect due to several induced effect in hormonal synthesis and metabolic activity, while its reduce the activity of some enzymes such as peroxidase and catalase in tomato plants and hence increasing the catabolism rather than anabolism. Data also indicate that increasing cobalt level in plant growing media above 12 ppm, the promotive effect reduce. These results are agree with those obtained by [31] who stated that cobalt concentrations (0.0, 2, 4, 6, 8, 10 and 12 ppm) significantly improve all growth and yield parameters of groundnut compared with control. Cobalt at 8 ppm gave the highest figures. Also, [11] stated that cobalt recorded the maximum leaf area index, dry matter accumulation in aerial parts, root dry weights and plant height in summer groundnut plants compared with control.

3.3 Yield characteristics:

Data in Table (4) show that cobalt has a significantly promotive effect on all yield parameters of fenugreek such as pods number per plant, grains weight per plant, grains yield per feddan, grains oil percentage and grains oil yield per plant as well as grains oil yield per feddan compared with untreated plants. Cobalt at 12 ppm gave the superior values. Increasing cobalt rate more than 12 ppm reduced the promotive effect. These observations are consistent with previous reports obtained by [12,13] who stated that cobalt addition in soil increased all growth parameters along with yield parameters such as seedling vigour, number and weight of pods and grains yield per plant in both green gram (*Vigna radiate* L.) and maize (*Zea maiz* L.) plant. Confirm these results [15] who indicated that cobalt at 12 ppm significantly increased all yield parameters of soybean. The results in Table (4) show also the relative calculated values as percentage from control. It is evident that cobalt rate at 12 ppm increased yield parameters: pods number per plant 42.8 %, grains weight per plant 39.5 %, grains yield per feddan 40,2 %, grains oil percentage 22.8, grains oil yield per plant 21.6 %, grains oil yield per feddan 20.3 %, respectively in the two seasons.

Table (4): Effect of cobalt on fenugreek yield parameters (mean of two seasons).

Cobalt treatment (ppm)	Pods number per plant	Grains weight per plant (g)	Grains yield per feddan (Kg)	Grains oil percentage %	Grains oil yield per plant (g)	Grains oil yield per fed (Kg)
Control	27.74	6.38	425.36	10.13	0.89	60.65
4	34.67	7.87	524.68	10.75	0.95	64.74
8	36.19	8.28	553.11	11.53	1.02	66.70
12	39.60	8.90	596.34	12.32	1.09	71.88
16	38.46	8.67	576.83	11.93	1.05	68.66
20	36.74	8.31	552.96	11.39	1.01	66.05
LSD 5 %	0.55	0.3	0.05	0.54	0.04	1.21

These results are good agreement with those obtained by [32] who reported that cobalt at 0.21 kg/ha gave higher oil content in groundnut seeds compared with control and other doses of cobalt. Confirm these results [33] who found that cobalt significantly increased groundnut yield parameters such as pods number and weight per plant, 100 seeds weight, pods yield per feddan, seed oil percentage and oil yield per feddan.

3.4 Nutritional status:

3.4.1 Nitrogen, P and K content:

Results in Table (5) reveal the effect of cobalt on macronutrients content in fenugreek seeds. Cobalt has a beneficial effect on N, P and K of fenugreek grains compared with control. Cobalt at 12 ppm gave the maximum values. These data are in harmony with those obtained by [34] who pointed that cobalt increased the concentration of N, P and k of groundnut seeds.

Table (5): Effect of cobalt on nutritional status of fenugreek grains (mean of two seasons).

Cobalt treatment (ppm)	Macronutrients (%)			Micronutrients (ppm)				Cobalt (ppm)
	N	**P**	**K**	**Mn**	**Zn**	**Cu**	**Fe**	
Control	**3.12**	**0.446**	**2.59**	**22.4**	**17.3**	**14.2**	**48.2**	**0.76**
4	**3.40**	**0.449**	**2.65**	**23.9**	**17.9**	**15.5**	**47.7**	**0.85**
8	**3.66**	**0.486**	**2.76**	**25.0**	**19.6**	**16.8**	**27.1**	**1.37**
12	**3.75**	**0.539**	**2.98**	**26.7**	**21.0**	**18.2**	**25.8**	**2.65**
16	**3.72**	**0.523**	**2.95**	**26.7**	**20.3**	**17.8**	**25.0**	**4.09**
20	**3.69**	**0.519**	**2.78**	**25.1**	**19.8**	**16.9**	**23.9**	**6.11**
LSD 5 %	**0.3**	**0.5**	**0.3**	**1.1**	**0.6**	**0.4**	**0.6**	**0.9**

Confirm these results [35] who stated that cobalt at 8 ppm increased N, P, and K content in both shoots and roots of cowpea compared with control.

3.4.2 Manganese, Zn and Cu content:

Data in Table (5) clearly indicate that all cobalt treatments significantly increase the content of Mn, Zn and Cu compared with control. Cobalt at 12 ppm gave the highest figures. Increasing cobalt level in plant growing media reduce the positive effect. These results are agree with those obtained by [33] who found that cobalt concentrations significantly increased the content of Mn, Zn and Cu in groundnut seeds in two seasons. Cobalt at 8 ppm gave the greatest values. As cobalt addition in plant media more than 8 ppm the promotive effect reduced.

3.4.3 Iron content:

Data in Table (5) also indicate that increasing cobalt rates in plant growing media, iron content in fenugreek grains exerted the adverse effect. These results are good agreement with those obtained by [36] who showed certain antagonistic relationship between both Fe and Co elements. Confirm these results [15] who stated that increasing cobalt doses in plant media resulted in a progressive depression effect on iron content in soybean seeds.

3.4.4 Cobalt status:

Data in Table (5) show that cobalt content in fenugreek grains significantly increased when cobalt addition increasing in plant growing media. These results are agree 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.

3.5 Chemical constituents:

The amount of total proteins, total carbohydrates, mucilage and trigonellin as well as vitamin "C" and vitamin "A" in fenugreek grains as affected by different cobalt rates are given in Table (6). Data indicate that all the mention parameters are significantly increase by the addition of cobalt rates (4, 8, 12, 16 and 20 ppm) as compared with those obtained by control plants.

Table (6): Effect of cobalt on chemical constituents of fenugreek grains (mean of two seasons).

Cobalt treatment (ppm)	Total proteins	Total carbohydrates	Mucilage	Trigonellin	Vitamin "C"	Vitamin "A"
	%			(mg/100g)	Mg/100g f.w	
Control	19.50	36.17	0.30	0.31	8.06	6.22
4	21.25	36.87	0.31	0.33	8.23	6.48
8	22.87	38.06	0.33	0.36	8.42	6.59
12	23.44	39.65	0.36	0.40	8.66	6.76
16	23.25	39.22	0.35	0.40	8.49	6.63
20	23.06	38.96	0.33	0.39	8.40	6.63
LSD 5 %	0.19	0.43	0.01	0.02	0.9	0.11

In this concern, [37] who stated that cobalt had a significant favorable effect on chemical constituents of okra pods compared with untreated plants.

Conclusions:

Cobalt at 12 ppm has the superior growth, grains yield, nutritional status and chemical constituents of fenugreek. Cobalt levels of tomato fruits in the highest cobalt treatment (20 ppm) is 6.11 ppm. 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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