International Journal of Basic and Applied Sciences, *3 (4) (2014) 527-531*
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www.sciencepubco.com/index.php/IJBAS
doi: 10.14419/ijbas.v3i4.3712
Research Paper

Effect of cobalt supplement on rosemary *(rosmarinus officinals L)* A- herb yield, essential oil content and its composition

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Abstract

Two field experiments were conducted in Research and Production Station, National Research Centre, Nobaria Location, Beheara Governorate, Delta Egypt. Experiments were carried out to study the effect of cobalt concentrations (0.0, 5.0, 7.5, 10.0, 12.5 and 15.0 ppm) on Rosemary herb yield, essential oil content and its composition under drip irrigation system during 2012 and 2013 seasons.
The obtained results indicate that:-

- All cobalt concentrations significantly increased all Rosemary growth and herb yield parameters compared with untreated plants.
- Cobalt at 10 ppm gave the maximum values of fresh herb and its biomass, essential oil and its composition.
- Increasing cobalt levels above 10 ppm in plant growing media cobalt promotive effect reduced.

Keywords: *Rosemary, Cobalt, Herb Yield, Essential Oil.*

1. Introduction

Rosemary (Rosmarinus officinalis L.), Family Lamiaceae, is an evergreen subshrub with erect or ascending branches, which are bale green and downy when young, later brown and woody. Rosemary is native to the Mediterranean region. It is now widely cultivated for ornamental, culinary, medicinal and perfumery purposes. The leaves and young shoots are used medicinally. The constituents include an essential oil (up to 2%) with cineole and borneol, camhor and borneol as the main components, plus tannins, saponin and organic acids. These substances give Rosemary a pronounced rubefacient action and dried herb and oil, obtained by steam distillation from fresh parts, are components of antiheumatic liniments and aintments. Rosemary also has sedactive, diuretic, stomachic, cholagogic, tonic, aromatic, antispasmodic and antiseperties. It is especially beneficial for fatigue and neuralgia [1].
Cobalt is beneficial element for plant growth, in higher plants. Cobalt is an essential element for the synthesis of vitamin B12 which is required for human and animal nutrition [2].
Laila Helmy and Nadia Gad [3] reported that cobalt at 25 mg Kg-1 soil significantly increased parsley growth expressed as plant height, leaf fresh and dry weights, and number of leaves per plant as well as root fresh and dry weights. Cobalt fertilization also significantly increased essential oil yield of parsley leaves. The main aroma constituent, 1,3,8-p-menthatriene which forms about (76%) of leaves essential oil, showed about (10%) increase over than of control with 50 mg Kg^{-1} soil. Eman, Aziz et al [4] stated that cobalt at 15 ppm gave the greatest peppermint fresh and dry weight herb yield, the highest essential oil yield as well as improve the status of macronutrient (N, P and K) and micronutrient (Mn, Zn and Cu) content. The highest level of cobalt (30 ppm) increased the principal components of menthone (37.84%) and isomenthone (15.19%) and this effect was companied with decreasing the relative content of L-(-)-menthol (20.54%). While cobalt at 20 mg/Kg soil recorded the highest content of L-(-)- menthol (28.54%) as compared with other cobalt levels of peppermint essential oil could be successfully produced in newly reclaimed land of Egypt. Eman, Aziz and Nadia Gad [5] showed that all the previous cobalt treatments significantly increased all growth and yield parameters compared with control of lemongrass. Cobalt at 22.5 ppm gave the highest values of fresh and dry herb yields (8.97 and 2.66 ton^{-1}) as well as recorded the greatest increase in the essential oil yield (63.07 Lh^{-1}). While the

highest cobalt level (30 ppm) increased the principal components of neral (36.17%) and geranial (29.26%), which represented about 65.43%of lemongrass oil as well as the quality is generally determined by its content of citral (neral and geranial isomers). This effect was compainied with decreasing the relative content of limonene and citronellol. Nadia Gad and Eman, Aziz [6] demonstrated that cobalt at 22.5 ppm had a significant promotive effect of the lemongrass endogenous hormones (Auxins, Gibberlins and Cytokinens), herbs quality such as total soluble solids, total protein, total lipids, total phenols and the contents of N, P, K, Mn, Zn and Cu as compared with other doses. With increasing cobalt, abscisic acid increased while Fe decreased. Higher cobalt concentration more than 22.5 ppm decreased the promotive effect. Generally, the obtained results showed that cobalt had a positive role on herbs of lemongrass. Recently, [7] reported that cobalt at 15 ppm gave the highest fresh and dry herb yield (66.20 and 13.19 ton ha^{-1}) of Ocimumbalsilicum. Increasing cobalt from 0.0 to 7.5, 15.0 and 22.5 ppm significantly increased the essential oil yield from 38.39 to 94.67, 266.76 and 181, 49 L ha^{-}1. While the highest level of cobalt (30 ppm) recorded 91.15 L ha^{-1}. The essential oil of Ocimumbasilicum were characterized by high content of linalool (23.43-35.46%), methyl chavecol (27.68-29.77%), followed by eugenol (6.76-10.54%) then 1,8 cineol (2.34-9.65%) cobalt at 15 ppm increased the principal components of linaloal (35.46%), 1,8 cineol (9.65%), linalyl acetate (8.71%) and benzyl acetate (8.14%) while cobalt at 30 ppm have possible role in the essential oil compounds and recorded the highest content of methyl chavedol and eugenol. Which were considered the main contributors of the antioxidant activity of volatile extract of sweet basil? Nadia Gad et al [8] stated that applying cobalt at 15 ppm gave a significant increasing in endogenous hormones such as Auxins, Gibberllins, Cytokinens and abscisic acid compared with control. Cobalt at 15 ppm also gave the synergistic effect on chemical constituents as well as nutritional status of sweet basil herbs.

2. Experimental

2.1. Materials and methods

Two field experiments were conducted in Research and Production Station, National Research Centre, Nobaria Location, Beheara Governorate, Delta Egypt.

2.2. Soil analysis

Physical and chemical properties of Nobaria Soil were determined and particle size distributions along with soil moisture were determined as described by Blackmore [9]. Soil pH, EC, cations and anions, organic matter, $CaCO_3$, total nitrogen and available P, K, Fe, Mn, Zn, Cu were run according to Black et al., [10]. Determination of soluble, available and total cobalt was determined according to method described by Cottenie et al., [11]. Some physical and chemical properties of Nubaria soil are shown in Table (1).

Table 1: Some Physical and Chemical Properties of 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 L^{-1})</td><td colspan="4">Soluble anions (meq L^{-1})</td></tr>
<tr><td>pH 1:2.5</td><td>EC (dS m^{-1})</td><td>$CaCO_3$ %</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>Available</td><td colspan="2">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>4.88</td><td colspan="2">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).

2.3. Plant material and experimental work

Experimental were carried out the effect of cobalt nutrition on Rosemary herb yield quantity and quality.

A preliminary pot experiment was conducted at Wire house of National Research Centre during 2011 season to define cobalt concentrations range which gave growth and yield response. Seedlings of Rosemary (at the true leaves) irrigated once with cobalt concentrations: 0.0, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 22.5 and 25.0 ppm according to the preliminary experiment results, the concentrations range of cobalt which gave the Rosemary response 0.0, 5.0, 7.5, 10.0, 12.5 and 15.0 ppm. Cobalt at 10 ppm gave the best growth and yield parameters of Rosemary.

Seeds of Rosemary *(Rosmarinus officinals L.)* were sown in second week of August during both 2012 and 2013 successive seasons. After one month from sowing, seedlings were irrigated once with cobalt sulphate (0.0, 5.0, 7.5, 10.0, 12.5 and 15.0 ppm Cobalt). Each experiment consisting of 6 treatments. Each treatment represented by three plots. Each plot area was 5x3 meters consisting of three rows. Twenty five plants in each row (20 cm a part) were planted. All the plants received natural agriculture practices whenever they needed. One month after transplanting were harvested (10 cm above soil surface), and then plants re-harvested second harvest after one month from first harvest.

2.4. Measurement of herb growth and yield parameters

At each harvesting time, all growth and yield parameters as well as fresh and dry weights of herb were recorded according to FAO [12] and Gabal et al [13].

2.5. Measuring of essential oil

The essential oil percentage of fresh herb was determined by hydro distillation in Clevenger's apparatus for 3 hours according to A.O.A.S [14]. The essential oil yield (ml plant^{-1} and L ha^{-1}) was calculated.

2.6. Measuring of essential oil components

Percentage of fresh herb was determined by hydro-distillation in Clevenger's apparatus for 3 hours according to the Egyptian harvest at monthly intervals. The essential oil yield (ml plant^{-1}and L ha^{-1}) was calculated. The resulted oil was dehydrated over anhydrous sodium sulphate in glass vials. The GLC analysis of the oil samples was carried out using HEWIETT PACKARD quipped with FID, HP 6890 Series System, and USA according to Bunzenet al [15].

2.7. Statistical analysis

The obtained data were statistically analyzed of variance procedure outlined by SAS [16] computer program and means were compared by LSD method according to Snedecor and Cochran [17].

3. Results and discussion

3.1. Herb yield

Data presented in Table (2) show that the fresh and dry herb yield of Rosemary (g plant^{-1} and ton ha^{-1}) increased gradually by increasing cobalt levels from 0.0 to 10.0 ppm. Cobalt at 10 ppm gave the maximum values of fresh herb and biomass (53.79 and 11.96 ton ha^{-1}). When cobalt addition increased more than 10 ppm (12.5 and 15.0 ppm) the promotive effect reduced all growth and yield parameters of Rosemary compared with the level of 10 ppm cobalt. These observation are consistent with previous obtained by Nadia Gad et al., [18], who stated that cobalt at 15 ppm resulted the greatest growth and yield of cucumber plants as compared with the higher ones. Eman, Aziz et al [4] added that, low level of cobalt (7.5 ppm) recorded the highest fresh and dry weight of peppermint herb.

3.2. Essential oil yield

Data in Table (3), show that, all cobalt doses had a significant promotive effect on the Rosemary essential oil percent and yield (ml plant-1and L ha-1) compared with control. In the first and second cuts, cobalt at 10 ppm recorded the highest values of essential oil percent (0.21 and 0.23), content (2.31 and 2.38 ml plant^{-1}) and yield (122.46 L ha^{-1}). Increasing cobalt level in Rosemary plant growing media above 10 ppm, the promotive effect was reduced. These results are good agreement with those obtained by Liala Helmy and Nadia Gad [3] who found that cobalt at 25 mg Kg^{-1} soil significantly increased essential oil content in parsley leaves. Nadia Gad et al [19] added that, cobalt is a promising element in the newly reclaimed soils such as Rass Seder, Sinai, Egypt.

Table 2: Effect of Cobalt on Herb Growth and Yield of Rosemary Her (Mean of Two Seasons).

Cobalt treatment (ppm)	Plant hight (cm)		Branch no. plant^{-1}		Herb fresh weight plant^{-1} (g)		Herb dry weight plant^{-1} (g)		Total fresh weight Per plant	Total dry weight Per plant	Fresh herb Ton fed^{-1}	Dry herb Ton fed^{-1}
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	(g)		1st cut	2nd cut
Control	41.11	44.19	9.44	10.15	412.33	418.40	105.11	111.2	830.73	216.13	17.95	4.62
5.0	52.24	56.08	12.67	13.04	720.71	726.52	182.04	188.8	1447.23	370.82	30.26	7.93
7.5	58.87	62.01	15.82	16.12	1184.01	1191.25	291.07	304.47	2375.26	595.54	46.04	11.40
10.0	63.91	67.25	16.91	17.33	1222.14	1239.97	302.26	318.92	2462.11	621.18	53.79	11.96
12.5	60.43	63.14	16.04	16.94	1188.61	1216.22	294.02	311.26	2404.83	605.28	50.42	11.56
15.0	56.14	58.87	15.66	16.19	1143.02	1187.61	285.64	302.83	2330.63	588.47	48.48	11.26
LSD 5%	1.55	1.12	0.16	0.07	4.6	3.62	0.95	1.64	44.63	15.70	2.0	0.30

Table 3: Effect of Cobalt on Essential Oil Content of Rosemary Herb (Mean of Two Seasons).

Cobalt treatment (ppm)	1st cut		2nd cut		Total oil yield	oil yield
	Oil (%)	ml plant^{-1}	Oil (%)	ml plant^{-1}	ml plant^{-1}	L ha^{-1}
Control	0.12	0.73	0.13	0.74	1.47	31.70
5.0	0.18	0.89	0.20	0.96	1.85	39.86
7.5	0.21	1.08	0.23	1.13	2.21	58.45
10.0	0.26	2.31	0.27	2.38	4.69	122.46
12.5	0.24	2.03	0.25	2.05	4.08	88.05
15.0	0.22	1.78	0.24	1.88	3.66	78.94
LSD 5%	0.3	0.16	0.20	0.17	0.42	8.16

It`s had a significant positive effect on olive (Manzanello and Arbicon) fruits yield quantity and oil percent especially with organic fertilization. Confirm these results [20] who demonstrated that cobalt at 12.5 ppm gave a beneficial effect of canola oil compared to untreated plants.

3.3. Essential oil composition

Data presented in Table (4) obviously reveal that Rosemary oil composition responded greatly to cobalt nutrition, phenylalanine and ornithine at different rate of application. The GLC analysis of essential oil extracted from Rosemary plants which treated with cobalt reveal that 13 peaks were identified, 5 hydrocarbons and 8 oxygenated compounds were detected.

Table 4: Effect of Cobalt on Essential Oil Composition of Rosemary Herbs in 2nd Cut (Mean of Two Seasons).

	Control	5.0	7.5	10.0	12.5	15.0
Cobalt treatment	(ppm)					
Oil constituents	Hydrocarbon compounds (%)					
a-Pinene	0.68	0.62	0.59	0.48	0.45	0.41
Camphene	12.19	12.34	12.47	12.85	11.89	11.23
B-Pinene	0.34	0.75	0.84	0.95	0.62	0.59
Limonine	1.15	2.09	2.45	3.22	3.10	2.88
P-cymene	6.68	5.64	5.89	5.42	5.08	4.87
Total hydeocarbons	21.04	21.44	22.24	22.92	21.14	19.98
Oxygenated compounds (%)						
1.8-Cineole	10.26	11.28	12.56	13.45	12.65	12.21
Camphor	27.01	26.10	25.33	24.19	23.78	22.43
Linalool	7.82	9.59	10.83	12.44	11.82	11.69
Boranylocetate	14.69	14.51	14.39	14.02	12.20	11.76
Isobomeol	0.16	0.22	0.48	0.78	0.75	0.66
Bomeol	5.41	5.22	4.28	3.95	3.69	3.45
Geranyl acetate	0.20	0.22	0.26	0.35	0.31	0.27
Geraniol	0.99	1.29	1.57	1.62	1.46	1.28
Total Oxygenated	66.54	68.43	69.69	70.80	66.66	63.75
Total identified	87.58	89.87	91.93	93.72	87.70	83.73
Unidentifide	12.42	10.13	8.07	6.28	12.30	16.27

Total hydrocarbons ranged from 19.98 to 22.92% while total oxygenated compounds ranged from 63.75 to 70.80%. The 1, 8- cineole oil ranged from 10.26 to 13.45 %, Camphor (22.43 to 26.10%), Boranyl acetate (11.76 to 14.69%) are the major constituents (more than 9%) in all cobalt treatments. The higher content of both 1-8- cineole and camphor oils gave the better quality of Rosemary essential oil. In this concern, it is obvious from the obtained results that cobalt at 5 ppm followed by 7.5 ppm. Increasing cobalt applications in plant media reduce the promotive effect. These results are in harmony with those obtained by Eman, Aziz et al [4] who found that cobalt at 15 ppm recorded the highest essential oil values of peppermint while the high cobalt levels above 15 ppm reduced the positive effect.

4. Conclusion

Cobalt had a significant effect of Rosemary growth, herb yield, herb essential oil yield and its fatty acids components. From this study it could be suggested that cobalt is consider a beneficial element for higher plants. Therefore, considerable attention should be taken concerning applying this element (Co) as a fertilizer, but further studies are needed to learn more about this element and its mechanisms in soil and plant.

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