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Effect of Cobalt on Growth, Yield and Chemical Constituents of *Nigella sativa* L.

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Abstract

Cobalt ion (Co^{2+}) is considered a beneficial element for higher plants due to its direct role in their metabolism. *Nigella sativa* L. (*N. sativa*) is a widely used medicinal plant throughout the world. The aim of this study was to evaluate the influence of Co^{2+} on *N. sativa* growth, yield and chemical constituents. *Nigella sativa* L. plants were subjected to different rates of Co^{2+} to study the influence of Co^{2+} on growth, yield and chemical constituents. The growth characters [Plant height (cm), number of leaves ($plant^{-1}$), number of branches ($plant^{-1}$), number of capsules ($plant^{-1}$), herb dry weight ($plant^{-1}$) and seed yield ($plant^{-1}$)] increased as the concentration of Co^{2+} increased. The highest values of growth characters (45.8, 55.8, 13.7, 39.1, 19.6 and 9.7) were observed at 30 mg L^{-1}. The highest essential and fixed oil contents (0.5% and 24.1%) were recorded at 40 mg L^{-1}. The highest values of total carbohydrate, soluble sugars and protein contents resulted from the treatment of 30 mg L^{-1}, the increases in total carbohydrate, soluble sugars and protein were 47%, 140% and 44.4% higher than the control treatment. The treatment with 30 mg L^{-1} resulted in the highest NPK accumulation (3.9, 0.6 and1.4%). The highest N and K uptakes (0.8% and 0.3%) were recorded in the treatment of 30 mg L^{-1}. No changes occurred in P uptake under Co^{2+} concentrations. The lowest values of growth characters and chemical constituents were observed in control treatment (0.0 mg L^{-1}).

Key words: Nigella sativa L., Growth characters, Chemical constituents.

1. Introduction

Nigella sativa L. (*N. sativa*) belongs to *Ranunculaceae* family is a widely used medicinal plant throughout the world. The seeds of *N. sativa* have widely been used in the treatment of different diseases and ailments. It has been widely used as antihypertensive, liver tonics, diuretics, digestive, antidiarrheal, appetite stimulant, analgesics, antibacterial and in skin disorders [1].

Cobalt ion (Co^{2+}) is considered a beneficial element for higher plants due to its direct role in their metabolism. Co^{2+} promoted many developmental processes including stem and coleoptiles elongation, opening of hypocotyls, leaf expansion and bud development [2]. Vegetative growth characters, fixed oil and NPK contents of canola were affected differently by the Co^{2+} levels [3]. Helmy and Gad [4] indicated that by adding the ion of Co^{2+} plant growth characters (plant height, leaf number, fresh and dry weights of levees, fresh and dry weights of roots) and essential oil yield of parsley were significantly increased. The same trend was found for the fresh and dry weights of herb, essential oil yield and NPK content of peppermint plant which increased with Co^{2+}

levels [5]. Increasing Co^{2+} concentrations strongly increased coriander herb yield and essential oil biosynthesis [6]. The highest values of herb fresh weight, herb dry weight, essential oil, total protein, fixed oil and NPK content of lemongrass plants were observed within plants treated with 22.5 ppm of Co^{2+} [7]. Aziz and Gad [8] have shown a positive effect of Co^{2+} on herb yield and essential oil of sweet basil. Gad et al [9] demonstrated that increasing Co^{2+} levels significantly increased plant growth measurements and oil yield of Rosemary.
The objective of this study was to evaluate the influence of Co^{2+} on growth, yield and chemical constituents of *N. sativa*.

2. Materials and methods

2.1. Plant material

The experiments were conducted during the successive seasons, 2013/2014 and 2014/2015. *Nigella sativa* L. seeds were obtained from the Department of Medicinal and Aromatic Plants Institute, Ministry of Agriculture, Egypt and were sown in plastic pots (30 cm diameter) during the first week of October at both seasons. After 45 days, the seedlings (3 Pot^{-1}) were transferred and maintained in a greenhouse at National Research Centre (NRC) under the following conditions: mean maximum and minimum air temperatures of 31.5°C and 21.2°C, respectively, and mean relative humidity of 24.2%, until the harvests. Physical and chemical properties of the soil used in this study were determined according to Jackson and Cottenie et al [10, 11] and are presented in Table 1. The plants were cultivated using complete solution [12]. The control (0.0 mg L^{-1}) contained only the complete nutrient solution in the absence of the cobalt ion; the treatments contained the cobalt ion at a concentration of 10, 20, 30 and 40 mg L^{-1} in the complete nutrient solution. The solutions were prepared using de-ionized water, were continuously aerated using a rotary blower and were renewed every two weeks, based on the pH. The nutrient solution was maintained at pH 5.5–6.0, which was monitored daily using a Digimed DMPH-3 pH meter. The electrical conductivity was maintained at 1.5–2.5 mS/cm using a Digimed CD-21. The constituents of solution used were N (210 ml L^{-1}), K (235 ml L^{-1}), Ca (200 ml L^{-1}), P (31 ml L^{-1}), S (64 ml L^{-1}), Mg (48 ml L^{-1}), B (0.5 ml L^{-1}), Fe (1 to 5 ml L^{-1}), Mn (0.5 ml L^{-1}), Zn (0.05 ml L^{-1}), Cu (0.02 ml L^{-1}) and Mo (0.01 ml L^{-1}). Source of Co2+ was cobalt chloride ($CoCl_2$).

Table 1. Soil properties

Item	Value	Item	Value (meq l^{-1})	Item	Value (meq l^{-1})
Sand	75.5%	Ca	4.3	Zn	0.3
Silt	12.5%	Mg	4.2	Mn	1.1
Clay	2.5%	Na	5.1	CO_3	1.7
Gravel	10.5%	K	0.6	HCO_3	1.5
pH	7.3	Fe	4.4	Cl	2.1
EC (dS m^{-1})	1.5	Cu	0.4	SO_4	4.2

2.2. Harvesting

Plants were harvested after 260 days from sowing date in both seasons. Vegetative growth characters measurements [plant height (cm), number of leaves ($plant^{-1}$), number of branches ($plant^{-1}$), number of capsules ($plant^{-1}$), herb dry weight ($plant^{-1}$) and seed yield ($plant^{-1}$)] were recorded.

2.3. Essential oil isolation

Dry seeds were collected from each treatment. Then 50 g from each replicate of all treatments was subjected to hydro-distillation for 3h using a Clevenger-type apparatus [13]. The essential oil content was calculated as a relative percentage (v/w).

2.4. Fixed oil Extraction

The seeds (50 g) were powdered mechanically and extracted with light petroleum ether (40 - 60°C) for 4h in a Soxhlet apparatus. Removal of the solvent under reduced pressure gave the fixed oils [14].

2.5. Determination of total carbohydrates and soluble sugars

Total carbohydrates and soluble sugars contents were determined from plant material (young leaves) collected from each treatment. The method of Dubois et al [15] was used.

2.6. Determination of mineral content and protein

Total nitrogen (protein) and phosphorus in leaves were determined using the methods described by the Association of Official Agricultural Chemists [14]. The samples of leaves were dried, ground and K extracted [11]. Concentrations were determined by atomic absorption spectrophotometer using a Perkin-Elmer method [16].

2.7. Statistical analysis

In this experiment, one factor was considered: Co^{2+} treatments (0, 10, 20, 30 and 40 mg L^{-1}). For each treatment there were 4 replicates, each of which had 10 pots; in each pot 3 individual plants were planted. The experimental design followed a complete random block design [17]. The averages of data of both seasons were statistically analyzed using 1-way analysis of variance (ANOVA-1). Significant values were determined according to P values ($P < 0.05$ = significant, $P < 0.01$ = moderate significant and $P < 0.001$ = highly significant). The applications of that technique were according to the STAT-ITCF program [18].

3. Results

3.1. Effect of Co^{2+} on growth characters

The growth characters [Plant height (cm), number of leaves (plant^{-1}), number of branches (plant^{-1}), number of capsules (plant^{-1}), herb dry weight (plant^{-1}) and seed yield (plant^{-1})] of *N. sativa* plants under Co^{2+} treatments are presented in Table 2. The growth characters of *N. sativa* increased as the concentration of Co^{2+} increased. The highest values of growth characters (45.6, 55.8, 13.7, 39.1, 19.6 and 19.7) were observed at 30 mg L^{-1}. The lowest values of growth characters (20.9, 22.7, 7.4, 17.5, 7.9 and 7.8) were observed at 0.0 mg L^{-1}. The increases in growth characters were highly significant for Co^{2+} treatments.

Table 2. Effect of Co^{2+} treatments on plant growth characters

Cobalt levels (mg L^{-1})	Plant height (Cm)		Leaf number (Plant $^{-1}$)		Branch number (Plant $^{-1}$)		Capsule number (Plant $^{-1}$)		Dry weight (g Plant $^{-1}$)		Seed yield (g Plant $^{-1}$)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	20.9	0.9	22.7	0.7	7.4	0.4	17.5	0.5	7.9	0.9	7.8	0.8
10	23.8	0.8	31.6	0.6	9.5	0.5	22.8	0.8	12.8	0.8	11.9	0.9
20	32.8	0.8	42.6	0.6	11.6	0.6	31.2	0.2	17.2	0.2	15.8	0.8
30	45.6	0.6	55.8	0.8	13.7	0.7	39.1	0.1	19.6	0.6	19.7	0.7
40	41.7	0.7	48.2	0.2	12.8	0.8	32.5	0.5	15.7	0.7	16.8	0.8
F Ratio	572.5***		1411.3***		53.0***		910.9***		130***		99.7***	
* P ≤0.05, ** P < 0.01 and *** P < 0.001.												

3.2. Effect of Co^{2+} on essential oil and fixed oil

Essential and fixed oils contents were increased with various treatments of Co^{2+}. The highest essential and fixed oil contents (0.5% and 24.1%) were recorded at 40 mg L^{-1} (Table 3). The lowest essential and fixed oil contents (0.1% and 15.7%) were recorded at control. The increases in essential oil content were moderate significant while the increases in fixed oil were highly significant with as a result of Co^{2+} treatments.

3.3. Effect of Co^{2+} on total carbohydrate, soluble sugars and protein

Total carbohydrate, soluble sugars and protein content increased with Co^{2} treatments (Table 4). However, the highest values of total carbohydrate, soluble sugars and protein contents resulted from 30 mg L^{-1}, the increases in total carbohydrate, soluble sugars and protein were 47%, 140% and 44.4% higher than the control respectively. The increases in total carbohydrate, soluble sugars and protein contents were highly significant in plants treated with Co^{2+} treatments.

Table 3. Effect of Co^{2+} treatments on oils content

Cobalt levels (mg L^{-1})	Essential oil (%)		Fixed oil (%)	
	Mean	SD	Mean	SD
0	0.1	0.0	15.7	0.7
10	0.2	0.1	16.9	0.9
20	0.3	0.1	22.8	0.9
30	0.4	0.1	23.9	0.9
40	0.5	0.1	24.1	0.1
F Ratio	9.3**		89.2***	
* P ≤0.05, ** P < 0.01 and *** P < 0.001.				

Table 4. Effect of Co^{2+} treatments on biochemical constituents

Cobalt levels (mg L^{-1})	Total Carbohydrates (%)		Soluble sugars (%)		Protein (%)	
	Mean	SD	Mean	SD	Mean	SD
0	11.5	0.5	3.2	0.2	16.9	0.9
10	13.7	0.7	5.3	0.3	18.1	0.1
20	13.9	0.9	5.9	0.4	23.8	0.8
30	16,9	0.9	7.7	0.2	24.4	0.4
40	14.2	0.2	6.4	0.4	17.5	0.5
F Ratio	23.1***		83.7***		106.6***	
* P ≤0.05, ** P < 0.01 and *** P < 0.001.						

3.4. Effect of Co^{2+} on nutrient contents and its uptake

An increase in Co^{2+} level caused increase in measured nutrient content such as macro elements (NPK) (Table 5). Co^{2+} (30 mg L^{-1}) resulted in the highest nutrient accumulation (3.9, 0.6 and1.4%) while the lowest mineral contents (2.7, 0.2 and 0.8%) were observed in control treatment. The increases in N were highly significant and the increases in P were moderate significant while the increases in K were significant as a result of Co^{2+} treatments.

Table 5. Effect of Co^{2+} treatments on nutrient content

Cobalt levels (mg L^{-1})	Nutrient content (%)					
	N		P		K	
	Mean	SD	Mean	SD	Mean	SD
0	2.7	0.2	0.2	0.1	0.8	0.2
10	2.9	0.1	0.4	0.1	0.9	0.1
20	3.8	0.3	0.5	0.1	1.1	0.1
30	3.9	0.1	0.6	0.1	1.4	0.4
40	2.8	0.2	0.5	0.1	1..1	0.1
F Ratio	26.6***		6.9**		3.5*	
* P ≤0.05, ** P < 0.01 and *** P < 0.001.						

The uptake of N and K were increased with various treatments of Co^{2+}. The highest N and K uptake (0.8% and 0.3%) were recorded at 30 mg L^{-1} (Table 6). The lowest N and K uptake (0.1%) were recorded in control. The increases in N uptake were highly significant and the increases in K uptake were insignificant for Co^{2+} treatments. On the other hand no changes in P uptake were found with Co^{2+} treatments compared with control.

Table 6. Effect of Co^{2+} treatments on nutrient uptake

Cobalt levels (mg L^{-1})	Nutrient uptake (g $Plant^{-1}$)					
	N		P		K	
	Mean	SD	Mean	SD	Mean	SD
0	0.1	0.0	0.1	0.0	0.1	0.0
10	0.4	0.1	0.1	0.0	0.2	0.0
20	0.7	0.2	0.1	0.0	0.2	0.1
30	0.8	0.2	0.1	0.0	0.3	0.1
40	0.4	0.1	0.1	0.0	0.2	0.1
F Ratio	11.5***		0.0		0.8	
* P ≤0.05, ** P < 0.01 and *** P < 0.001.						

4. Discussion

The increases in growth characters under Co^{2+} treatments may be due to an increase in chlorophyll content caused by Co^{2+}, and consequently, photosynthesis efficiency on the other hand, Co^{2+} increases the total carbohydrates (source of energy) and mineral content, so that plant growth parameters increased under Co^{2+} treatments [7]. The effect of different treatments (Co^{2+}) on essential and fixed oil may be due to its effect on enzyme activity and metabolism of essential and fixed oil production [19]. In this study, the Co^{2+} treatments enhanced the plant to preserve carbohydrates and soluble sugars for sustained metabolism are prolonged energy supply [20]. The increase of nutrients and protein under Co^{2+} treatments probably due to more availability of these elements to plants [21]. Also El-Sherif et el [21] reported that Co^{2+} treatments caused an increase in the accumulation of protein and nutrients in tomato plants.

Our results are in accordance with those obtained by Jayakumar et al [22] who indicated that low concentrations (less than 50 mg L^{-1}) of Co^{2+} caused an increase in growth and biochemical constituents of Radish (*Raphanus sativus*). Helmy and Gad [4], they reported that Co^{2+} (30 mg L^{-1}) increased the vegetative growth characters, yield and essential oil content of parsley plant. Aziz et al [23] indicated that Co^{2+} (40 mg L^{-1}) had a positive effect on the growth characters of Roselle (*Hibiscus sabdariffa*). Gad et al [24] pointed that Co^{2+} (15 mg L^{-1}) had a significant primitive effect on the growth, yield and fixed oil content of olive trees. The highest values of growth measurements, seed yield, fixed oil yield and NPK content of Canola plants resulted from treating plants with 12.5 mg L^{-1} Co^{2+} [3]. On the other hand the our results agree with those obtained by Khalid and Shedeed [20], they reported that *N. sativa* plants treated with saline irrigation water containing cobalt resulted in higher plant growth characters [plant height (cm), number of leaves ($plant^{-1}$), number of branches ($plant^{-1}$), number of capsules ($plant^{-1}$), herb dry weight ($plant^{-1}$) and seed yield ($plant^{-1}$)] and chemical constituent's values (fixed oil, soluble sugars, proline, N,P,K and protein) than those treated with saline irrigation water alone.
It can be noted that Co^{2+} treatments increased the essential oil of *N. sativa*. Essential oils and their components are becoming increasingly popular as naturally occurring antimicrobial agents. In this work the chemical composition and the antibacterial properties of *N. sativa* essential oils and of their main components were determined. The essential oil components were identified by GC -MS analysis. The antibacterial activity of the essential oil was determined against a panel of strains bacteria, using a broth microdilution method. The GC – MS analysis showed that the major constituents of the oil were monoterpene hydrocarbons and phenolic monoterpenes, and results of antibacterial activity confirmed the possibility of using *N. sativa* essential oils or some of their components in biology and pharmaceutical preparations [25]. On the other hand the growth characters also affected by other nutrients such as NPK and foliar nutrition. The effect of NPK and foliar nutrition on the growth [Plant height (cm), leaf number ($plant^{-1}$), branch number ($plant^{-1}$), capsule number ($plant^{-1}$), herb dry weight ($plant^{-1}$) and seed yield ($plant^{-1}$)] was measured and quantitative analysis of fixed oil, total carbohydrate, soluble sugars and nutrient content were performed. The most effective rate was $N_3P_3K_3$ x foliar nutrition interaction, resulting in a positive increase in vegetative growth. The highest values of vegetative growth characters were 27.7, 41.4 cm (plant height); 55.4, 51.9 (leaf number); 10.2, 11.7 $plant^{-1}$ (branch number); 15.5, 20.8 $plant^{-1}$ (capsule number); 47.1, 49.4 g $plant^{-1}$ (herb dry weight); 4.3, 4, 7 g $plant^{-1}$ (seed yield) during the first (2006 / 2007) and second (2007 / 2008) seasons respectively. As well as $N_3P_3K_3$ x foliar nutrition led to higher biochemical contents than the control. The highest values of chemical contents were 22.9 and 25.1% (fixed oil); 33.0, 30.1 % (total carbohydrate); 16.9, 8% (soluble sugars); 23.7 and 24.8 % (protein); 3.8 and 4 % (N); 0.4 and 0.4 % (P); 1.2 and 1.8 % (K) during the first and second seasons respectively [26].

Conclusion

It may be concluded that all Co^{2+} treatments caused positive increases and significantly improved plant growth characters and chemical composition of *N. sativa* plants compared to control. The highest percentages of growth characters, total carbohydrate, soluble sugars, NPK and protein were observed in plants treated with 30 mg L^{-1}. While the highest essential and fixed oil contents were recorded in plants treated with 40 mg L^{-1} Co^{2+}.

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