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Research Article
Impact of Cobalt Ion on Essential Oil Composition Extracted from *Nigella sativa* L.

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Abstract
Background: Essential oil of *Nigella sativa* L. (*N. sativa*) used in traditional medicine and food additives. Essential oil content was generally proportional to cobalt ion (Co^{2+}). **Materials and Methods:** *Nigella sativa* plants were subjected to different levels of Co^{2+} i.e., 0, 25, 50 and 75 mg L^{-1}. Seeds of *N. sativa* were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. The essential oil content was calculated as a relative percentage (v/w). The qualitative analysis of the essential oil compounds was performed on a Gas Chromatograph (GC) coupled to a Mass Spectrometer (MS). **Results:** Different levels of Co^{2+} promoted the percentage and yield of *N. sativa* essential oil. The greatest essential oil percentage or yields were resulted under 50 mg L^{-1} Co^{2+} level with the values of 0.3% and 1.1 mL/100 plant, respectively. The major constituents were p-cymene, α-thujene and γ-terpinene. The levels of 25 and 50 mg L^{-1} Co^{2+} gave the highest amounts of major constituents with the values of 59.6, 12 and 9%. The major fraction of *N. sativa* essential oil was monoterpene hydrocarbons (MCH). Oxygenated monoterpenes (MCHO), sesquiterpene hydrocarbons (SCH) and oxygenated sesquiterpenes (SCHO) formed the minor fractions. Different levels of Co^{2+} increased the MCHO and SCHO fractions. The MCH increased towards 50 mg L^{-1} Co^{2+} level and then decreased with 75 mg L^{-1} Co^{2+} level. The SCH increased with 25 mg L^{-1} Co^{2+} level while it was decreased with 50 mg L^{-1} Co^{2+} level then stabilized with 75 mg L^{-1} Co^{2+} level. **Conclusion:** The quantity and quality of *N. sativa* essential oil were proportional to Co^{2+} levels. These results show that Co^{2+} should be considered in the chemical characterization of the oil produced from essential oil-bearing plants when treating by Co^{2+}.

Key words: Cobalt, essential oil, p-cymene, α-thujene, γ-terpinene

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Black cumin (*N. sativa*) belongs to Ranunculaceae family. It is widely distributed and cultivated in Mediterranean, Europe and Asia regions. Essential oil of *N. sativa* seeds used in folk medicine, as food additives and as spice[1]. The main constituents of essential oil isolated from *N. sativa* seeds (p-cymene, α-thujene, γ-terpinene, carvacrol, α-pinene and β-pinene) belongs to monoterpenes class[1]. Essential oil compositions affected by various factors such as fertility of soil[2], light[3], parts of plants[4], climate[5], seasons[6] and different locations[7,8].

Heavy metals are prevalent in municipal and industrial effluents; they are modifying the structure and productivity of aquatic ecosystems[9]. Cobalt ion (Co^{2+}) is considered a beneficial element for higher plants due to its direct role in their metabolism. Cobalt ion promoted many developmental processes including stem and coleoptile elongation opening of hypocotyl, leaf expansion and bud development[10]. Essential oil content was generally proportional to Co^{2+} concentration. Composition of parsley and rosemary essential oils increased with Co^{2+} treatments[11,12]. Essential oil contents in *Cymbopogon citrates* increased from 43.0-59.2 L ha^{-1} with Co^{2+} treatments (7.5-15.0 mg L^{-1}) compared with non-treatment[13]. Aziz *et al.*[14] indicated that essential oil extracted from basil increased from 94.7-181.5 L ha^{-1} with Co^{2+} treatments (7.5-22.5 mg L^{-1}) compared with control. The maximum peppermint essential oil value (39.0 L ha^{-1}) obtained at 15 mg L^{-1} Co^{2+} treatment[13]. The highest amount of major compounds of peppermint essential oil [menthone (37.84%) and isomenthone (15.19%)] and lemon grass essential oil [neral (36.17%) and geranial (29.26%)] resulted from 30.0 mg L^{-1} of Co^{2+} treatment.

On other hand the highest amount of menthol (28.5%) (Obtained from peppermint essential oil) resulted from 7.3 mg L^{-1} of Co^{2+} treatment[13,15]. According to Aziz *et al.*[14], the highest values of major compounds of basil essential oil [linalool (35.46%), 1,8 cineol (9.65%), linalyl acetate (8.7%) and benzyl acetate (8.1)] obtained from 15.0 mg L^{-1} of Co^{2+} treatment.

The objective of this study was to evaluate influence of Co^{2+} levels on essential oil composition of *N. sativa* seeds.

MATERIALS AND METHODS

Plant material: The *N. sativa* plants were grown between October, 2009 and June, 2010 in sand soil. *Nigella sativa* seeds were obtained from the Department of Medicinal and Aromatic Plants Institute (Kalubiya, Egypt). Seeds were sown in plastic pots (30 cm diameter). After 45 days, the seedlings (three per pot) were transferred and maintained in a greenhouse at National Research Centre under the following conditions; mean maximum and minimum air temperatures of 31.5 and 21.2°C, respectively and mean relative humidity of 24.2% until the harvests. The plants were cultivated using complete nutrient solution[16]. The control (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 25, 50 and 75 mg L^{-1} in the complete nutrient solution. The solutions were prepared using deionized water were continuously aerated using a rotary blower and were renewed every 2 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^{-1} using a Digimed CD-21.

Extraction and analysis of the essential oils: The seeds of the *N. sativa* were collected from each treatment and were subjected[17] to hydro-distillation in a Clevenger-type apparatus for 3 h.

The qualitative analysis of the essential oil compounds was performed on a Gas Chromatograph (GC) coupled to a Mass Spectrometer (MS) (GC–MS; Shimadzu QP5000), operating at an MS ionization voltage of 70 eV.

The chromatography was equipped with a fused silica capillary column DB-5 (J and W Scientific; 30 m length×0.25 mm diameter×0.25 µm film thickness) and helium was used as the carrier gas. The following chromatography conditions were used: Injector at 240°C, detector at 230°C, carrier gas flow at 1 mL min^{-1} with 1:20 split ratio, The following temperature program was used: 60°C increased to 200°C at a rate of 3°C min^{-1} and a 1 µL injection of solution (1 mg of essential oil and 1 mL of ethyl acetate).

The compounds were identified using the comparative analysis of the acquired mass spectra with those stored in the GC-MS database of the system (Nist 62.Lib), the literature[18] and retention indices[19] which were obtained from the injection of a mixture of n-alcanes (C9-C25, Sigma Aldrich, 99%) employing a column temperature program as follows: 60-240°C at a rate of 3°C min^{-1}. Separation and quantification (normalization area method) of the substances were carried out by GC (Shimadzu GC-2010), equipped with a flame ionization (GC-FID), using a DB-5 (J and W Scientific; 30 m×0.25 mm×0.25 mm×0.25 µm) capillary column and helium was used as the carrier gas, temperature injector at 240°C, detector at 230°C, gas flow 1.0 mL min^{-1}, split 1/20. The following chromatography conditions were used:

60-135°C at a rate of 5°C min^{-1}, then 135-240°C at a rate of 8°C min^{-1} and 60-240°C at a rate of 3°C min^{-1}; 1 μL of solution was injected (1 mg of essential oil and 1 mL of ethyl acetate). Same conditions used in GC-MS.

Statistical analysis: In this experiment, one factor was considered: Co^{2+} treatments (0, 25, 50 and 75 mg L^{-1}). For each treatment there were four replicates, each of which had 10 pots; in each pot three individual plants were planted. The number of experimental pots was 200 pots. The experimental design followed a complete random block design. According to Snedecor and Cochran[20] the averages of data were statistically analyzed using one-way analysis of variance (ANOVA-1). Significant values 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[21].

RESULTS

Response of essential oil (percentage and yield) to various levels of Co^{2+}: Different levels of Co^{2+} increased the percentage and yield of *N. sativa* essential oil. Level of 50 mg L^{-1} Co^{2+} gave the greatest essential oil percentage and yield with the values of 0.3% and 1.1 mL/100 plant, respectively (Table 1). Control treatment (0 mg L^{-1} Co^{2+}) resulted the lowest essential oil percentage and yield that recorded 0.1% and 0.4 mL/100 plant, respectively. The changes in essential oil percentage were insignificant while essential oil yield was moderate significant in Co^{2+} levels.

Effect of Co^{2+} levels on essential oil constituents: Sixteen components were identified by GC-MS (Table 2). The major components were p-cymene, α-thujene and γ-terpinene of all levels of Co^{2+}. Constituents were identified in essential oil extracted from *N. sativa* seeds belong to four chemical classes. The major one was monoterpene hydrocarbons (MCH). The minor classes were oxygenated monoterpenes (MHO), sesquiterpene hydrocarbons (SCH) and oxygenated sesquiterpenes (SCHO). The highest amounts of major compounds [p-cymene (59.6%), α-thujene (12%) and γ-terpinene (9%)] were resulted from the 25 and 50 mg L^{-1}

Table 1: Response of essential oil percentage and yield to Co^{2+} levels

	Essential oil content			
	Percentage		Yield (mL 100 $plant^{-1}$)	
Co^{2+} levels (mg L^{-1})	Mean	SD	Mean	SD
Control	0.1	±0.0	0.4	±0.1
25	0.2	±0.1	0.8	±0.2
50	0.3	±0.1	1.1	±0.1
75	0.2	±0.1	0.6	±0.2
F-value	2.7		10.7**	

*p≤0.05, **p<0.01 and *** p<0.001

Table 2: Effect of Co^{2+} levels on essential oil constituents

			Co^{2+} levels (mg L^{-1})								
			Control		25		50		75		
Constituents (%)	[a]RI	[b]RI	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F-value
β-pinene	939	939	1.7	±0.2	1.0	±0.2	1.4	±0.2	1.8	±0.3	7.4*
Sabinene	976	977	0.9	±0.2	1.2	±0.2	1.7	±0.1	1.1	±0.1	33.9***
β-pinene	980	982	2.4	±0.2	2.2	±0.2	2.2	±0.2	2.1	±0.1	1.5
Myrcene	991	991	0.1	±0.0	0.2	±0.1	0.2	±0.1	0.4	±0.1	6.4*
α-thujen	1005	1005	11.9	±0.9	12.0	±0.2	12.0	±0.2	11.8	±0.9	0.1
γ-terpinen	1018	1018	1.0	±0.0	1.7	±0.2	1.6	±0.2	1.5	±0.3	40.2***
p-cymene	1026	1028	59.5	±0.5	59.6	±0.6	59.6	±0.6	57.9	±0.9	4.7*
Limonene	1031	1031	1.4	±0.2	1.6	±0.1	1.5	±0.5	1.6	±0.1	0.4
1,8-cineol	1033	1033	0.1	±0.0	0.4	±0.2	0.3	±0.2	0.6	±0.2	4.3*
γ-terpinene	1062	1064	8.5	±0.5	8.9	±0.9	9.0	±0.5	8.9	±0.9	1.0
Terpinen-4-ol	1177	1179	2.1	±0.1	2.1	±0.1	2.4	±0.4	2.1	±0.1	1.4
p-cymen-8-ol	1183	1185	0.2	±0.1	0.4	±0.1	0.3	±0.1	0.7	±0.2	8.0**
Thymoquinone	1249	1250	3.0	±0.1	3.0	±0.1	3.1	±0.1	3.2	±0.2	0.1
Carvacrol	1298	1300	2.4	±0.4	2.4	±0.4	2.5	±0.5	2.6	±0.6	0.1
Longfolene	1402	1406	0.9	±0.1	1.1	±0.1	0.8	±0.3	0.9	±0.1	2.4
Thymohydroquinone	1509	1510	0.4	±0.1	0.5	±0.2	0.8	±0.3	0.7	±0.2	2.2
Monoterpene hydrocarbons (MCH)			87.4	±0.2	88.4	±0.5	89.2	±0.1	87.1	±0.2	0.6
Oxygenated monoterpenes (MCHO)			7.8	±0.2	8.3	±0.1	8.6	±0.6	9.2	±0.2	0.8
Sesquiterpene hydrocarbons (SCH)			0.9	±0.1	1.1	±0.1	0.8	±0.2	0.9	±0.1	2.7
Oxygenated sesquiterpene (SCHO)			0.4	±0.2	0.5	±0.2	0.8	±0.5	0.7	±0.2	0.5
Total identified			96.5		98.3		99.4		97.9		

[a]RI: Retention index calculated, [b]RI: Retention index literature, *p≤0.05, **p<0.01 and ***p<0.001

Co^{2+} levels compared with other treatments. The MCHO and SCHO were increased with various levels of Co^{2+} compared with control. The MCH was increased towards 50 mg L^{-1} Co^{2+} level and then was decreased with 75 mg L^{-1} Co^{2+} level. The SCH was increased with 25 mg L^{-1} Co^{2+} level while, it was decrease with 50 mg L^{-1} Co^{2+} level then stabilized with 75 mg L^{-1} Co^{2+} level. The changes in all chemical classes and the most of constituents were insignificant for Co^{2+} levels except the components of α-pinene, myrcene, p-cymene and 1,8-cineol were significant, sabinene and β-terpinene were highly significant, p-cymen-8-ol were moderate significant (Table 2).

DISCUSSION

According to Bakkali *et al.*[22], essential oils are complex natural mixtures that can contain 20-60 components at different concentrations. The oils are characterized by two or three major components that generally determine the biological properties of the essential oils. For example, one study observed that *M. piperita* essential oil contains 59% menthol and 19% menthone[22]. The quality of essential oil used in this investigation was somewhat similar to that of *N. sativa* growing in Poland whose essential oil composition was rich in p-cymene major constituents[1]. Changes observed were related to the relative proportions of constituents and not to the presence of new or the absence of particular ones. In this study, Burbott and Loomis[23] reported that variations in essential oil content and composition could be due to its effect of Co^{2+} treatments on enzymes activity and metabolism improvements. The study results agree with the previous results of Helmy and Gad[11] and Gad *et al.*[12] indicated that composition of parsley and rosemary essential oils increased with Co^{2+} treatments. Misra *et al.*[24] on geranium (*Pelargonium graveolens* L. Her.' ex. Ait.) plants who indicated that heavy metals effect on the CO_2 assimilation rate, photosynthetic pigments content and ultimately the accumulation of geranium essential monoterpenes oil(s) and the production of biomolecule geraniol (main component of geranium essential oil). Khalid and Ahmed[25] indicted that the highest percentage of *N. sativa* essential oil was recorded at 40 mg L^{-1} of Co^{2+} when the plants treated with different levels of Co^{2+} (0, 10, 20, 30 and 40 mg L^{-1}). This study described that the changes in the chemical constituents of *N. sativa* essential oil were confirmed by previous study[24] they claimed that within the essential oil, the relative level of various constituents increased, decreased or did not change at all in geranium (*Pelargonium graveolens* L. Her.' ex. Ait.) plants under heavy metals treatments as compared with untreated control plants. The type of elements in the production system should be considered in the chemical characterization of the essential oil produced from bearing plants[26] when treating with Co^{2+}.

CONCLUSION

It may be concluded that the greatest essential oil percentage or yields were resulted under 50 mg L^{-1} Co^{2+} level. The levels of 25 and 50 mg L^{-1} Co^{2+} gave the highest amounts of major constituents (p-cymene, α-thujene and γ-terpinene). Different levels of Co^{2+} increased the MCHO and SCHO fractions MCH was increased towards 50 mg L^{-1} Co^{2+} level and then was decreased with 75 mg L^{-1} Co^{2+} level. The SCH was increased with 25 mg L^{-1} Co^{2+} level while it was decrease with 50 mg L^{-1} Co^{2+} level, then, stabilized with 75 mg L^{-1} Co^{2+} level.

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