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Evaluation of Fixed Oil and Fatty Acids of Black Cumin under Cobalt or Kinetin Treatments

K. A. Khalid*

Medicinal and Aromatic Plants Department, National Research Centre, El Buhouth St., 12311, Dokki, Cairo, Egypt.

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K. A. Khalid

ahmed490@gmaiil.com
+201117727586

Abstract

Fixed oil of black cumin used for different cases of medicines and food. Cobalt ion (Co^{2+}) is considered as a beneficial element for higher plants. Lipid and fatty acids are influenced by cytokinins. Black cumin plants were subjected to different levels of Co^{2+} (0, 25, 50 and 75 mg L^{1}) or Kinetin (0, 10, 20 and 40 mg L^{-1}) during two successive seasons. The highest fixed oil contents [29.6, 28.8% and yield (2.4, 2.3 g $Plant^{-1}$)] were recorded at 75 mg L^{-1} Co^{2+} level during the first and second seasons respectively. Major fatty acids (linoleic and oleic) increased with various treatments of Co^{2+} levels compared with control treatment. The changes in fixed oil and all fatty acid constituents were highly significant for Co^{2+} levels. 60 mg L^{-1} of kinetin produced the highest contents of fixed oil with values of 33.3, 36.7 % and 6.6, 7.3 g $Plant^{-1}$ during both seasons. The same treatment (60 mg L^{-1} of kinetin) produced the highest values of main fatty acids [linolenic, linoleic and oleic]. The changes in the most of fatty acids were highly significant for Kinetin treatments.

1. Introduction

Fixed oil of black cumin (*Nigella sativa* L) seeds have a long history of folklore usage in various systems of medicines and food [1]. In Egypt the fixed oil of black cumin seeds used for certain cases such as chronic cough and bronchial asthma [2]. Fatty acids of linoleic (59.6%), oleic (23.8%), palmitic (12.4%) and stearic (<1%) were detected in the fixed oil extracted from Egyptian variety of black cumin [3]. Black cumin fatty acids have an antimicrobial role [4]. *N. sativa* oil has the potential to be used as a natural adjuvant to conservative treatment in the management of diabetic nephropathy. As *N. sativa* has very low cost and side effects, the cost benefit ratio will be in favor of using it, if its usefulness is proved by further studies. Therefore, add on therapy of *N. sativa* oil boosted the therapeutic advantage of conservative management in patients of diabetic nephropathy [5].

Heavy metals are prevalent in municipal and industrial effluents; they are modifying the structure and productivity of aquatic ecosystems [6]. Cobalt (Co^{2+}) is considered as a beneficial element for higher plants due to its direct role in their metabolism. Co^{2+} promoted many developmental processes including stem and coleoptile elongation opening of hypocotyl, leaf expansion and bud development [7].

Some reports have shown that fixed oils of medicinal plants were significantly increased under the treatments of Co^{2+}. Gad [8] reported that Co^{2+}has a highly significant effect for increase fixed oil of olive fruits. Gad [9] revealed that application of Co^{2+} (12.5 mg L^{-1}) resulted in the highest amounts of fixed oil content (1671 kg ha^{-1}) of canola plant. The compositions of fatty acids in canola were affected by different levels of Co^{2+} [9].

Cytokinin (CYT) is a phytohormone that participates in events in the course of whole plant ontogeny, from fecundated ovule to senescence and death. It is present in processes such as cell division, shoot initiation and growth, senescence delay and photomorphogenic development, control of chloroplast division and growth, modulation of metabolism and morphogenesis in response to environmental stimulus [10-12]. Lipid and fatty acids are influenced by cytokinins [13]. Kull [13] indicted that kinetin application caused an increase in lipid

values of different plant species. Kinetin increased the linolenic acid and decreased the palmitic acid percentages of soybean, *Coleus blumei* Benth and *Impatiens sultani* Hook [13-14]. While CYT caused a significant increase in palmitic acid although linolenic acid decreased [15].
In this study, investigated the possible effect of Co^{2+} or kinetin on the fixed oil and fatty acids composition of Egyptian black cumin as an important medicinal plant.

2. Experimental details

2.1. *Experimental site*

The Egyptian black cumin plants were grown during two successive seasons (2010:2011 and 2011:2012) in sandy soil. Black cumin seeds were obtained from the Department of Medicinal and Aromatic Plants Institute, Egypt. Seeds were sown in plastic pots (30 cm diameter). After forty five days, the seedlings (three per pot) were transferred and maintained in a greenhouse at National Research Centre (NRC) under the following conditions: maximum and minimum air temperatures were 31.5°C and 21.2°C, respectively, and relative humidity of 24.2%, until the harvests.

2.2. *1st Experiment*

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 Co^{2+}; the treatments contained the Co^{2+} ion (as cobalt chloride) at low 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 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.

2.3. *2nd Experiment*

Pots were divided into fifth main groups. The first group was not subjected to any kinetin treatment (control). The second, third and fourth groups were subjected to the treatments of kinetin [Super-grow Kinetin 99 % 50 g Tamper Evident High Purity (6-Furfurylaminopurine)] by 20, 40, 60 and 80 mg L^{-1} as foliar spray respectively. The foliar spray was done after 45 days from sowing. Mechanical and chemical properties of the soil used in this experiment were done according to Jackson [17] and Cottenie [18] and are resented in Table 1.

Table 1: Physical and chemical properties of the soil used

Clay	Silt	Sand	OM	N	P	K	pH	EC (dsm^{-1})
(%)								
38.0	36.0	26.0	1.3	0.3	0.1	0.1	7.7	0.6
Note: OM= organic matter, EC= electronic conductivity.								

2.4. *Harvesting*

At fruiting stage (270 days from sowing), plants were harvested (at the end of season). Seed yields (g $Plant^{-1}$) were recorded.

2.5. *Extraction of fixed oil*

Ten gram were powdered mechanically and extracted with light petroleum ether (40 - 60°C) for 4 h in a Soxhlet apparatus. Removal of the solvent under reduced pressure gave the fixed oils [19]. In addition, total fixed oil yields (% and g $Plants^{-1}$) were calculated by using the dry seeds of both seasons. The fixed oils extracted from black cumin seeds were collected in both seasons from each treatment to identify the fatty acids constituents.

2.6. *Gas chromatography*

The fatty acid content of the fixed oils was investigated by GC analysis of their methyl esters. Oil (0.5 g) was dissolved in 20 ml light petroleum ether (60 - 80 °C) and 2 ml 2 M methanolic KOH was added. The mixture was shaken for 2 min and allowed to stand for 10 min. The upper layer was removed, was hed with water, and 1 ml used for GC analysis [19].

GC analyses were performed using an HP 6890 gas chromatograph with a Supelco SP23 80 capillary column (60 m X 0.25 mm X 0.20 µm) and helium as the carrier gas. The oven temperature was kept at 140 °C for 5 min, programmed to 165 °C @ 5 °C/ min and kept at 165 °C for 10 min, then programmed to 190 °C @ 5 °C/min and kept at 190 °C for 20 min. Inject or and detector (FID) temperatures were k pt at 250 °C. The split ratio was 70:1. Relative percentage amounts were calculated from total area under peaks by the software of apparatus.

2.7.*Gas chromatography mass spectrometry (GC- MS)*

GC- MS analyses of the oils were carried out on an HP GC- MS 6890-5 973 model instruments. The GC column used was a Supelco SP23 80 capillary column (60m X 0.25mm X 0.20 µm). Oven temperature was as above; transfer line temperature 280 °C; ion source temperature 230 °C; carrier gas helium; splitting ratio 1:10; ionization energy 70 eV; scan range 15 - 550 amu.

2.8. *Qualitative and quantitative analyses*

Compounds were identified by comparison of their GC retention times with those of reference solutions of 1% w/ v of the methyl esters of the fatty acid and also by comparison of their mass spectra with either known compounds or published spectra (Wiley 275 L) . Quantified ion of fatty acid methy lesters was obtained directly from GC peak area using Chemstation 8.02 software and expressed as percent ages.

2.9. *Statistical analysis*

In both experiments, two factors were considered: Co^{2+} or kinetin levels. For each treatment there were 4 replicates, each of which had 8 pots; in each pot 3 individual plants. The experimental design followed a complete random block design. According to Snedecor [21], 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 [22].

3. Results and discussion

3.1. *Effect of Co^{2+} levels on fixed oil content and fatty acids constituents*

Fixed oil contents [% and yield (g $Plants^{-1}$)] were increased with various levels of Co^{2+}. The highest fixed oil contents [29.6, 28.8 % and yield (2.4, 2.3 g $Plant^{-1}$)] were recorded at 75 mg L^{-1} Co^{2+} level during the first and second seasons respectively **(**Table 2**).** The lowest fixed oil contents [18.6, 16.8 % and yield (0.9, 0.8 g $Plant^{-1}$)] were recorded at control during the first and second seasons respectively. ANOVA indicated fixed oil contents were highly significant in Co^{2+} levels of both seasons.

Table 2: Effect of cobalt on fixed oil contents

Cobalt treatments (mg L^{-1})	Fixed oil							
	(%)				g $Plant^{-1}$			
	First season		Second season		First season		Second season	
	M	SD	M	SD	M	SD	M	SD
0	18.6	±0.6	16.8	±0.8	0.9	±0.1	0.8	±0.1
25	22.9	±0.9	18.4	±0.3	2.1	±0.1	1.1	±0.1
50	24.6	±0.8	22.7	±0.1	1.5	±0.5	1.6	±0.7
75	29.6	±0.8	28.8	±0.8	2.4	±0.4	2.3	±0.3
F ratio	109.7 ***		178.8 ***		12.3 ***		43.0 ***	
Note: M= mean; SD= standard deviation								

Fatty acids analysis revealed the presence of nine different fatty acids were identified under Co^{2+} treatments (Table 3), in this study, linoleic and oleic were detected as the major fatty acids which gave the highest percentages of the total fatty acids in all treatments that changed under different Co^{2+} levels (Table 3). Fatty acids Constituents were identified in fixed isolated from black cumin seeds belong to two chemical classes.

Unsaturated fatty acids was the major one, the remaining fractions as saturated fatty acids formed the minor classes (Table 2). The highest amount of major fatty acids [linoleic (62.1%) and oleic (25.8%)] resulted from the 75 mg L^{-1} Co^{2+} level compared with other levels. Linoleic and oleic increased with various treatments of Co^{2+}

levels compared with control treatment. ANOVA indicated that the changes in all fatty acid constituents were highly significant for Co^{2+} levels (Table 3).

Table 3: Effect of cobalt on fatty acids constituents

Fatty acids	RT	Cobalt treatments (mg L^{-1})								F ratio
		0		25		50		75		
		M	SD	M	SD	M	SD	M	SD	
Saturated fatty acids										
Caprylic (C8:0)	5.2	0.5	±0.1	0.4	±0.1	0.1	±0.0	0.2	±0.1	12.3***
Capric (C10:0)	11.6	1.7	±0.2	2.3	±0.3	2.1	±0.1	1.2	±0.2	15.7***
Lauric (C12:0)	14.8	2.8	±0.2	1.1	±0.1	1.3	±0.3	1.1	±0.1	54.1***
Myristic (C14:0)	17.5	9.2	±0.2	9.5	±0.5	3.7	±0.2	2.6	±0.1	459.9***
Stearic (C18:0)	19.7	8.3	±0.3	6.2	±0.2	3.6	±0.1	3.1	±0.1	467.7***
Arachidic (C20:0)	24.7	2.1	±0.1	1.2	±0.2	2.4	±0.4	2.1	±0.1	14.7***
T. saturated fatty acids		24.6	±0.3	20.7	±0.3	13.2	±0.3	10.3	±0.1	17.3***
Unsaturated fatty acids										
Oleic (C18:1)	21.8	22.4	±0.4	22.9	±0.9	23.1	±0.1	25.8	±0.8	17.3***
Linoleic (C18:2)	22.9	50.6	±0.6	55.1	±0.3	61.5	±0.5	62.1	±0.1	574.0***
Linolenic (C18:3)	23.1	2.1	±0.1	0.5	±0.1	1.5	±0.5	1.2	±0.2	17.1***
T. unsaturated fatty acids		75.1	±0.5	78.5	±0.4	86.1	±0.4	89.1	±0.5	892.1***
Total fatty acids		99.7		99.2		99.3		99.4		
Note: M= mean; SD= standard deviation; T= total.										

The increases in fixed oil under Co^{2+} treatments may be due to an increase seed yield, so that fixed oil yields increased under Co^{2+} treatments [23]. $Co2^{+}$ is considered a beneficial element for higher plants due to its direct role in their metabolism. $Co2^{+}$promoted many developmental processes including stem and coleoptile elongation opening of hypocotyl, leaf expansion and bud development [7]. The effect of different treatments (Co^{2+}) on fixed oil and fatty acids constituents may be due to its effect on enzyme activity and metabolism of fixed oil and fatty acids constituents' production [24]. These results are in accordance with those obtained by Khalid [23]; Gad [8] pointed that Co^{2+} had a significant primitive effect on the fixed oil content of *N. sativa* and olive fruits. The highest values of fixed oil content of canola plants resulted from treating plants with 12.5 mg L^{-1} Co^{2+}[8]. On the other hand these results agree with those obtained by Khalid [23 & 25], they reported that *Nigella sativa* plants treated with saline irrigation water containing cobalt resulted in higher fixed oil content than those treated with saline irrigation water alone. The quantity and quality of *N. Sativa* oil were proportional to Co2+levels [26].

3.2. *Effect of kinetin levels on fixed oil content and fatty acids constituents*

Data presented in Table 4 shows that Kinetin levels caused an increase in fixed oil content [% and yield (g $Plants^{-1}$)] during both seasons as compared with control. The highest amounts of fixed oil contents were recorded with 60 mg L^{-1} of kinetin with values of 33.3, 36.7 % and 6.6, 7.3 g $Plant^{1}$ during the first and second seasons respectively. The increases in fixed oil under different Kinetin treatments were highly significant.

Table 4: Effect of Kinetin on fixed oil contents

Kinetin treatments (mg L^{-1})	Fixed oil							
	(%)				g $Plant^{-1}$			
	First season		Second season		First season		Second season	
	M	SD	M	SD	M	SD	M	SD
Control	8.1	±0.3	12.6	±0.6	0.7	±0.2	0.9	±0.1
20	12.5	±0.5	17.8	±0.8	1.5	±0.5	2.1	±0.1
40	22.9	±0.9	27.9	±0.9	3.7	±0.2	4.5	±0.4
60	33.2	±0.2	36.7	±0.7	6.6	±0.7	7.3	±0.3
80	27.3	±0.3	28.4	±0.4	4.6	±0.6	4.5	±0.5
F ratio	1308.9***		551.8***		77.1***		150.7***	
Note: M= mean; SD= standard deviation								

Ten fatty acids (saturated and unsaturated) were identified in the of black cumin fixed oil under Kinetin treatments (Table 5). The maim fatty acids under Kinetin conditions were Linolenic (27 – 29.6%), Linoleic (23.9 – 26.3%) and Oleic (24.7 – 27.4). Kinetin levels caused an increase in the values of main fatty acids compared with control. Plants treated with 60 mg L^{-1} of kinetin produced the highest values of the three main fatty acids. Statistical analysis reported that the changes in most of fatty acids were highly significant for kinetin treatments.

Table 5: Effect of kinetin on fatty acids constituents

Fatty acids	RT	Kinetin treatments (mg L^{-1})										F ratio
		Control		20		40		60		80		
		M	SD	M	SD	M	SD	M	SD	M	SD	
Saturated fatty acids												
Caprylic (C8:0)	5.2	0.4	±0.1	0.3	±0.1	0.1	±0.1	0.3	±0.1	0.2	±0.1	4.9**
Capric (C10:0)	11.6	1.8	±0.2	1.6	±0.1	1.5	±0.2	1.7	±0.2	1.9	±0.1	NS
Lauric (C12:0)	14.8	2.5	±0.5	2.6	±0.6	2.7	±0.1	0.9	±0.1	3.1	±0.1	16.1***
Myristic (C14:0)	17.5	8.5	±0.5	8.6	±0.1	7.9	±1.2	6.1	±1.2	8.6	±0.6	3.1*
Stearic (C18:0)	19.7	7.2	±0.2	6.1	±0.1	5.4	±0.5	4.5	±0.5	5.4	±0.4	24.4***
Arachidic (C20:0)	24.7	2.9	±0.5	2.8	±0.8	2.7	±0.4	2.8	±0.4	2.9	±0.1	NS
T. saturated fatty acids		23.3	±0.3	22.0	±0.1	20.3	±0.3	16.3	±0.3	22.1	±0.1	87.5***
Unsaturated fatty acids												
Oleic (C18:1)	21.8	24.7	±0.7	24.8	±0.8	25.1	±0.4	27.4	±0.4	25.3	±0.3	13.3***
Linoleic (C18:2)	22.9	23.9	±0.9	24.1	±0.1	25.6	±0.3	26.3	±0.3	25.1	±0.1	12.0***
Linolenic (C18:3)	23.1	27.5	±0.5	28.1	±0.1	28.6	±0.6	29.6	±0.6	27.0	±0.2	NS
T. unsaturated fatty acids		76.1	±0.1	77.0	±0.2	79.3	±0.3	83.3	±0.3	77.4	±0.4	28.3***
Total fatty acids		99.4		99.0		99.6		99.6		99.5		
Note: M= mean; SD= standard deviation; T= total.												

The increases in fixed oil under Kinetin treatments may be due to an increase seed yield, so that fixed oil yields increased under Kinetin treatments [27]. The effect of different treatments (Kinetin) on fixed oil and fatty acids constituents may be due to its effect on enzyme activity and metabolism of fixed oil and fatty acids constituents' production [24]. Our results are in accordance with those obtained by Hook [13-14]. They indicated that kinetin increased the linolenic acid and decreased the palmitic acid percentages of soybean, *Coleus blumei* Benth and *Impatiens sultani* .

Changes in cytokinin levels are generally positively correlated with levels of mineral nutrients, especially nitrogenous nutrients [28]. These results are in accordance with those obtained by Shah [29, 30] and reported that kinetin increased the vegetative growth characters such as shoot length, leaf number, leaf area, branch number and dry weight, capsule number and seed yield of *Nigella sativa* (L.), so the kinetin increased the fixed oil content. Kinetin had a significant effect on nitrogen metabolism of *N. sativa* (L.) [29]. Youssef [31] reported that kinetin increased plant height, branch number, herb dry weight, carbohydrate, soluble sugars , NPK and oil of lavender plants. Moussa [32, 33] reported that kinetin treatments had a significant increase in plant growth characters and yield of *Hordeum vulgare* plants.

Generally, environmental and agricultural factors caused significant effects on the chemical composition of *N. sativa*. NPK x foliar nutrition led to higher biochemical contents of *N. sativa* than the control [34]. Salinity levels resulted in significant changes on oil composition isolated from *N. sativa* seeds [35, 36]. Foliar nutrition and ammonium sulfate caused a highly significant increase in *N. sativa* fixed oil [37].

Conclusions

It may be concluded that the greatest fixed oil and main fatty acids contents of Egyptian black cumin seeds were resulted with 75 mg L^{-1} Co^{2+} or 60 mg L^{-1} of kinetin. The changes in fixed oil and most fatty acid constituents were highly significant for Co^{2+} or kinetin levels.

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