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Evaluation of Black Cumin Oils under Various Nitrogen Treatments

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Key words

- ✓ Nigella sativa L.,
- Essential oil,
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- ✓ Oleic and
- ✓ Linoleic.
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Essential oil (EO) and fixed oil (FO) isolated from the seeds of Nigella sativa L. (N. sativa)

Abstract

used in various aspects of traditional medicine and different food prducts. The bilogical activities such as antimicrobial of *N. sativa* oils were reported by previous investigators. There are tow major nitrogen (N) sources i.e Urea (UR) and ammonium sulfate (AS). The UR and AS contain 46 and 21% of N respectively. In addition, AS also contains about 24% of sulfur (S). Nutrition with more N content are preferred over low-N nutrition because of the lower cost of transport and application. The objective of this study was to evaluate effect of AS and UR on EO and FO composition of Black cumin. Experimental areas were divided into 3 main groups. The first group was subjected to no rate of N (control). The second group was subjected to different doses of N (50, 100 and 150 kg N ha⁻¹) as AS (20.5 % N). The third group was subjected to the same treatments of N but UR (46 % N) was added. The EO and FO were isolated from black cumin seeds of all treatments and analyzed by GC- MS. Data were statistically analyzed using 1-way analysis of variance (ANOVA-1). Significant rates were identified according to P values (P < 0.05 = significant, P < 0.01 = moderate significant and P < 0.001 = highly significant). The highest amounts of EO contents were recorded under at 100 kg N ha⁻¹ rate (as UR) with the values of 0.4 - 0.5%; 11.6 -14.5 g (100 plant)⁻¹. Greatest amounts of main components were produced from 100 kg N ha⁻¹ (as AS) with the values of 51.7% (p-cymene) and 15.9 (α -thujene). Treatment 50 kg N ha⁻¹ (as UR) produced the highest contents of FO which recoded 31.8 and 33.6%; 699.6 and 739.2 g (100 plant)⁻¹. Greatest amounts of major FA were obtained from the treatment of 150 kg N ha⁻¹ (as AS) with the values of 22.9% (oleic) and 74.7% (linoleic). Different nitrogen sources caused significant changes (P < 0.05) in EO, FO, main compounds of EO and major FA.

1. Introduction

N. sativa (family *Ranunculaceae*) grow widely in various places of the world such as Mediterranean, European and Asian regions. Biological and therapeutic characters of the seeds of N. sativa were known in several civilizations. Essential oil (EO) isolated from N. sativa seeds used in many medical purposes and food industries [1]. Wajs[1] indicted that p-cymene, α -thujene, γ -terpinene, carvacrol, α -pinene and β -pinene were the major constituents of the EO extracted from N sativa seeds. Many biological and pharmaceutical activities were found in N. sativa fixed oil (FO); it used in canned food and drug industries (cough and bronchial asthma) [2, 3]. Different fatty acids (FA) were identified in N. sativa FO such ad linoleic, oleic, palmitic as well as stearic [4], which have antibacterial and antifungul activities [5].

Addition of nitrogen (N) element can be in several forms such as Urea (UR) and ammonium sulfate (AS) which are the major inorganic N sources [6]. UR provides the crops by 46% of inorganic N, while AS can prduce 21%. Also AS has about 24% sulfur (S). Supply agricultural crops with high sources of N and low cost are required [6]. The N value is an important criterion; however, other factors should also be taken into consideration when choosing a fertilizer carrier [6]. These factors can increase nutrient availability to plant and reduce the costs of fertilizers [6]. At present, the environmental as well as financial impact of N fertilizer use deserves increased attention. Field data on changes of soil acidity indices such as pH, calcium (Ca), and magnesium (Mg) saturation, base saturation, aluminum (Al) saturation, and acidity (H + Al) saturation with the application of AS and UR, two major N sources, are scarce for Egyptian soils.

Significant increments (P < 0.05) were found in basil EO under UR doses [7]. EO yield and constituents of lemon balm were significantly (P < 0.05) affected by the application of UR [8]. The UR resulted in significant changes in menthone (major constituent of peppermint) while no significant changes were found in EO yield [9]. The constituents of thyme EO (p-cymene, γ -terpinene and carvacrol) were increased under UR treatments, but thymol amounts were decreased [10]. The EO and its major compounds were enhanced significantly with the treatments of UR [11]. Yields, major constituents (thymol, γ -terpinene and p-cymene) and chemical classes {(monoterpene hydrocarbons (MCH), oxygenated monoterpenes (MCHO), sesquiterpene hydrocarbons (SCH) and oxygenated sesquiterpenes (SCHO)} of EO extracted from thyme herb were increased under UR doses [12]. Many variations were recorded in FO and FA (saturated and unsaturated) of sunflower with application of UR [13].

Significant changes (P < 0.05) were produced in basil EO (%) with AS levels [7]. Application of AS at the level of 150 kg N ha⁻¹ could be recommended for maximizing EO yields of American basil [14]. Dragonhead plants treated with AS at 100 kg N/ha increased the values of EO yield, EO constituents such as neral, geranyl acetate, and geraniol [15]. Positive changes (P < 0.05) were found in EO accumulation, major components, mono and sesquiterpenes of *Pimpinella anisum*, *Coriandrum sativum* and *Foeniculum vulgare* var dulce fruits with AS rates [16]. The main constituents of EO extracted from coriander, sweet fennel, anise and sweet basil were increased by the levels of AS increased [17-20]. The FO and FA of anise, coriander, sweet fennel, black cumin, artichoke and rapeseed were significantly increased with different rates of AS [21-24]. The research findings can contribute among the farmer's communities /researchers both in vitro & vivo for detection and production of EO, FO composition of *N. sativa*.

2. Material and Methods

2.1. Field site

This investigations were conducted during two seasons (2013/2014 and 2014/2015) at the National Research Centre station that located at Nubaria city, Egypt. The Jackson [25] and Cottenie [26] methods were used to determine the soil characters (**Table 1**).

Item	Values
Sand	81%
Silt	13.5%
Clay	3.5%
Gravel	2%
pН	7.9
EC	1.2 dsm^{-1}
OM	0.3%
Anions (mg 100 g ⁻¹ Soil)	
SO_4^{-2}	1.1
Cl ⁻¹	19.1
HCO ₃ ⁻¹	0.2
Cations (mg 100 g ⁻¹ Soil)	
Na ⁺	12.2
Mg^{++}	0.5
Ca ⁺⁺	0.1
\mathbf{K}^+	0.4

 Table 1. Soil analysis

2.2. seed source

The *N. sativa* seeds were obtained from the Agricultural Research Centre, Egypt. During the third week of October at the first and second seasons seed were sown in the field. Complete randomized block design with 4 relicates were used in this study; plot area was $4m^2$ which containing four rows. The sowing distance was 25x50 cm. After 56 days from sowing thinning with 3 plants/hill was made. Seed viability was more than 90% [27]. All expremental plots were divided into three parts: The first part was untreated with N (0.0 kg N ha⁻¹). The second one was treated with 50, 100 and 150 kg N ha⁻¹ of AS while the third part was subjected to the same N rates but UR was used. the recommendations of Ministry of Agriculture, Egypt (EMinAgric) were used.

2.3. Plant collection

Plants were collected during the ripening phase at both seasons and the yield of seeds was recorded as g plant⁻¹.

2.4. Extraction and analysis of the EO

The collected seeds of each treatment were used to isolate the EO by hydro-distillation method with Clevengertype apparatus [28]. Relative percentage (v/w) and yield of isolated EO (ml 100 Plant)⁻¹ were calculated by using seed dry weights.

2.5. EO analysis

Gas chromatograph, GC with mass spectrometer, MS (GC/MS) was used to identified the EO components as well as retention indices (RI) was used to quantity and quality analysis [29, 30].

2.6. FO isolation

Soxhlet apparatus was used to isolate FO (total lipids) from *N. sativa* seeds and the solvent was petroleum ether, $40 - 60^{\circ}$ C [31]. FO was calculated as percentage and g Plants⁻¹.

2.7. FA identification

FA were detected by GC and GC- MS analysis according to Houghton [32].

2.8. Analyzed of Data

Analysis of variance (ANOVA-1) was used in this study [33, 34].

3. Results and discussion

3.1. Response of EO contents to N sources

Various N sources (AS & UR) and rates resulted in different increments of EO contents {% or yield (g 100 plant)⁻¹} at the first and second seasons (**Table 2**). The highest amounts of EO content were obtained at 100 kg N ha⁻¹ rate (as UR) with the values of 0.4 and 0.5%; 11.6 and 14.5 g (100 plant)⁻¹ at the two seasons. The increases in EO (%) were non significant during the singular seasons but significant (P < 0.05) during second season. The increases in EO yield were highly significant (P < 0.001).

3.2. Effect of N sources on FO contents

The FO contents {% or yield $(g100 \text{ plant})^{-1}$ } were affected by AS and UR treatments (**Table 2**). Various AS and UR rates caused highly significant (P < 0.001) increases in FO contents at both seasons. Treatment 50 kg N ha⁻¹ (as UR) produced the highest contents of FO which recoded 31.8 and 33.6%; 699.6 and 739.2 g 100 plant⁻¹ at both seasons.

N sources]	EO		FO				
(kg N ha^{-1})		%		Yield {(g 100 plant) ⁻¹ }		%		Yield {g (10	$00 \text{ plant})^{-1}$	
					S	Seasons				
		First	Second	First	Second	First	Second	First	Second	
Contro	1	0.1±0.0	0.1±0.0	1.5±0.7	1.5±0.7	15.7±0.3	16.2±0.3	235.5±0.7	243.0±0.9	
AS	AS 50		0.3±0.1	3.4±0.4	5.1±0.1	18.7±0.7	18.9±0.1	317.9±0.1	321.3±0.3	
	100	0.3±0.1	0.3±0.1	5.1±0.1	7.2±0.2	21.6±0.4	22.1±0.1	518.4±0.4	530.4±0.4	
	150		0.2±0.1	4.4±0.4	4.4±0.4	22.7±0.2	24.8±0.2	499.4±0.4	545.6±0.3	
UR 50		0.2±0.1	0.3±0.1	4.4±0.4	6.6±0.4	31.8±0.3	33.6±0.4	699.6±0.4	739.2±0.2	
	100	0.4±0.2	0.5±0.2	11.6±0.6	14.5±0.5	23.4±0.4	24.5±0.5	678.6±0.4	710.5±0.5	
	150	0.3±0.1	0.3±0.1	7.5±0.5	7.5±0.5	24.1±0.1	25.3±0.3	602.5±0.5	632.5±0.5	
F values		NS	2.7*	132.3***	240.3***	425.8***	855.3***	448909.7***	235.9***	
Note: UR, u	Note: UR, urea; AS; N, nitrogen; EO, essential oil; FO, fixed oil; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; $p\geq0.05$, NS.									

Table 2. Effect of UR and AS on EO and FO contents (Mean ± SD).

3.3. Effect of N sources on EO components

Various sixteen compounds were identified by GC–MS analysis in *N. sativa* EO (**Table 3**). This investigation indicated that p-cymene as well as α -thujene were detected as the main constituents which produced the highest amounts (> 60%) of the EO in all treatments that increased under different AS and UR treated application (**Table 3**). Various components were detected in *N. sativa* EO divided into four chemical classes. Monoterpene hydrocarbons (MCH) was the major one, the remaining fractions as oxygenated monoterpenes (MCHO), sesquiterpene hydrocarbons (SCH) and oxygenated sesquiterpenes (SCHO) formed the minor classes (**Table 3**). Greatest amounts of main components were obtained from the treatment of 100 kg N ha⁻¹ (in case of AS) with the values of 51.7% (p-cymene) and 15.9 (α -thujene). The highest values of MCH (83.2%) and SCHO (4.5%) were resulted from the treatment of 100 kg N ha⁻¹ (AS) and 50 kg N ha⁻¹

(UR) resulted the highest values of MCHO (13.1%) and SCH (2.3%). It was found that highly significant (P < 0.00) changes in β -pinene, myrcene, a-thujene, a-terpinene, ρ -cymene, γ -terpinen-4-ol, thymoquinone, MCH and MCHO. The changes in a-pinene were moderate significant (P < 0.01) but it was significant in ρ -cymen-8-ol, longifolene and SCH. Insignificant variations was found in sabinene, limonene, 2-hydroxy 1,8 cineole, γ -terpinene, carvacrol, thymohydroquinone and SCHO.

Table 3. Effect of UF	and AS on EO	constituents	$(Mean \pm SD)$).
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			N sources (kg N ha ⁻¹)							F
No Compounds		RI	Control		AS		UR			- r values
			Control	50	100	150	50	100	150	values
1	a-Pinene	939	0.9±0.1	0.9±0.1	0.8±0.2	0.5±0.1	0.6±0.1	0.7±0.3	1.1±0.1	4.6**
2	Sabinene	977	4.7±0.5	4.8±0.2	4.9±0.1	4.8±0.2	4.8±0.2	4.8±0.2	4.8±0.2	^{ns} 0.2
3	β-Pinene	982	2.9 ± 0.1	2.5±0.5	2.1 ± 0.1	1.7±0.3	1.6±0.4	1.8±0.2	1.7±0.3	6.2***
4	Myrcene	991	2.9 ± 0.1	2.8 ± 0.2	1.5 ± 0.5	1.3±0.3	1.5±0.5	1.6 ± 0.4	1.5±0.5	7.3 ***
5	a-Thujene	1005	13.5±0.7	14.7±0.3	15.9±0.1	14.8±0.2	13.6±0.4	13.8±0.2	13.8±0.2	20.3***
6	a-Terpinene	1018	1.8 ± 0.3	1.9 ± 0.1	1.3±0.3	2.1±0.1	2.2±0.1	2.2 ± 0.1	2.3±0.3	6.8***
7	ρ-Cymene	1028	48.7±0.3	48.9±0.1	51.7±0.3	51.1±0.1	48.8±0.2	48.9±0.1	48.9±0.1	136.7***
8	Limonene	1031	2.7 ± 0.4	2.5±0.5	2.4 ± 0.1	2.2 ± 0.2	2.1±0.1	2.3±0.3	2.4±0.4	NS
9	2-Hydroxy 1,8 Cineole	1033	2.8 ± 0.3	2.9±0.1	2.8 ± 0.2	2.5 ± 0.5	2.7±0.3	2.8 ± 0.2	2.9±0.1	NS
10	γ-Terpinene	1064	2.8 ± 0.3	2.7±0.1	2.6±0.4	2.6±0.4	2.7±0.3	2.5±0.5	2.6±0.4	NS
11	γ-Terpinen-4-ol	1179	1.6 ± 0.6	1.5±0.5	1.5 ± 0.5	1.1 ± 0.1	2.7±0.3	2.5±0.5	2.6±0.1	6.9***
12	ρ-Cymen-8-ol	1185	2.8 ± 0.3	2.9±0.1	2.4 ± 0.4	2.2 ± 0.2	2.4 ± 0.4	2.1±0.1	2.4±0.4	2.5*
13	Thymoquinon	1250	1.1 ± 0.1	1.3±0.4	1.4 ± 0.3	1.5±0.5	2.9±0.1	2.3±0.3	2.5±0.5	8.1***
14	Carvacrol	1300	2.9 ± 0.1	2.8 ± 0.2	2.4 ± 0.4	2.2 ± 0.2	2.4 ± 0.4	2.3±0.3	2.4±0.4	NS
15	Longifolene	1406	1.9 ± 0.1	2.1±0.1	1.5 ± 0.5	2.3±0.3	2.2±0.2	2.1±0.1	2.1±0.1	2.7^{*}
16	Thymohydroquinone	1510	4.3±0.4	4.4 ± 0.4	4.5±0.5	4.4 ± 0.4	4.4 ± 0.4	4.4 ± 0.4	4.4 ± 0.4	NS
17	MCH		80.9±2.1	81.7±1.0	83.2±0.2	81.1±0.1	77.9±0.9	78.6±2.0	79.1±0.1	6.7***
18	MCHO		11.2 ± 0.3	11.4 ± 0.4	10.5±0.5	9.5±0.5	13.1±0.1	12.0±2.0	12.8 ± 0.2	5.6***
19	SCH		1.9±0.1	2.1±0.1	1.5 ± 0.5	2.3±0.3	2.2±0.2	2.1±0.6	2.1±0.6	2.8^{*}
20	SCHO		4.3±0.4	4.4 ± 0.4	4.5±0.5	4.4 ± 0.4	4.4 ± 0.4	4.4 ± 0.4	4.4 ± 0.4	NS
Total identified 98.3 99.6 99.7 97.3 97.6 97.1 98.4										
Note: UR, urea; AS; N, nitrogen; RI, retention index; M, mean; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; $p \ge 0.05$, NS.										

Table 4. Effect of UR and AS on FA constituents (Mean \pm SD).

		N sources (kg N ha ⁻¹)							
FA (%)	RT	Control		AS			F values		
		Control	50	100	150	50	100	150	values
SFA									
Caprylic (C8:0)	10.4	4.8±0.3	4.7±0.3	4.7±0.3	4.6±0.4	4.7±0.2	4.3±0.3	3.8±0.2	4.0^{**}
Capric (C10:0)	15.7	3.1±0.1	2.9 ± 0.1	2.8±0.2	2.5±0.5	2.5±0.1	2.3±0.2	2.2±0.2	4.7**
Lauric (C12:0)	18.9	4.4±0.1	4.1±0.1	3.3±0.3	3.1±0.1	2.9±0.1	2.8±0.2	2.5±0.5	19.5***
Myristic (C14:0)	22.7	8.5±0.7	8.6±0.4	8.7±0.3	8.9±0.1	$10.0{\pm}1.0$	10.2±0.2	10.9 ± 0.1	12.9***
Stearic (C18:0)	24.8	6.6±0.6	6.7±0.3	5.8±0.2	5.7±0.3	5.5±0.5	5.4±0.3	5.2±0.2	61.8***
Arachidic (C20:0)	27.9	1.2 ± 0.3	1.4 ± 0.4	1.0±0.0	0.5±0.1	2.5±0.5	2.7±0.2	2.8±0.2	26.3***
TSFA		28.6±0.6	28.4 ± 0.4	26.3±0.3	25.3±0.3	28.1±0.1	27.7±0.2	27.4±0.4	31.7***
UFA									
Oleic (C18:1)	31.8	21.8±0.3	21.9±0.1	22.5±0.5	22.9±0.1	22.0±1.0	22.1±0.1	22.2±0.2	NS
Linoleic (C18:2)	36.8	44.7±0.5	44.9±0.1	46.5±0.5	47.8±0.2	46.3±0.1	46.6±0.4	47.1±0.1	31.2***
Linolenic (C18:3)	37.9	4.9±0.1	4.8±0.2	4.7±0.3	4.0 ± 1.0	3.6±0.1	3.6±0.1	3.3±0.3	5.9***
TUFA		71.4±0.6	71.6±0.4	73.7±0.4	74.7±0.4	71.9±0.1	72.3±0.3	72.6±0.3	23.9***
TFA		100	100	100	100	100	100	100	100
Note: UR, urea; AS; N, nitrogen; RT, retention time; FA, fatty acids; M, mean; SFA, saturated fatty acids; TSFA,									
total saturated fatty acids; UFA, unsaturated fatty acids; TUFA, total unsaturated fatty acids, TFA; total fatty									
acids; ∗, p<0.05; ∗∗, p<0.01; ∗∗∗, p<0.001; p≥ 0.05, NS.									

3.4. Effect of N sources on FA constituents

Data presented in Table 4 indicated that nine FA were detected in *N. sativa* FO with the treatments of AS and UR. The major FA were oleic and linoleic (recorded more than 60 %). All FA of *N. sativa* FO were divided into two fractions. Unsaturated FA (UFA) was the main fraction and saturated FA (SFA) was the minor one. UFA

were oleic, linoleic and linolenic while caprylic, capric, lauric, myristic, stearic and arachidic were the SFA. All FA resulted in different variations with UA and AS. The rates of AS and UR caused various increases in major FA compared with control. Greatest amounts of major FA were formed with the dose of 150 kg N ha⁻¹ (as AS) with the values of 22.9% (oleic) and 74.7% (linoleic). Caprylic, capric, lauric, linolenic and TSFA were decreased under AS or UR treatments, while myristic, stearic and arachidic were changed (increased or decreased), on the other hand TUFA were increased. The variations in various FA were highly significant (P < 0.001) for AS or UR rates except caprylic, capric (were significant (P < 0.01) and oleic (were non significant).

The positive effect of AS and UR may be due to decrease in soil pH which produce a good feeding for *N. sativa* plants and increasing EO and FO production [35].

N has a major physiological role in the development of plants especially in porphyrin structure which has various metabolic activities in photosynthetic pigments and cytochromes that are basic in respiration, photosynthesis and protein synthesis [36] that resulted in an increase of plant growth, yield EO and FO of some medicinal and aromatic plants such as anise, coriander, sweet fennel and *N. sativa* plants [21, 37].

Obtained results agreed with some previous research work, that UR caused significant increases in EO content of basil [7], EO yield and constituents of lemon balm [8] and menthone (major constituent of EO extracted from peppermint [9]. Significant variations were found in EO yield, thymol, γ -terpinene, p-cymene, MCH, MCHO, SCH and SCHO of thyme herb under UR treatments [12]. The UR application resulted in various changes in FO and FA contents of sunflower [13]. The AS produced a significant increment in EO of Egyptian basil [7], American basil [14], major constituents of dragonhead (neral, geranyl acetate, and geraniol) [17] and main constituents of EO extracted from coriander, sweet fennel, anise and sweet basil were increased by the levels of AS increased [17-20]. The FO and FA of anise, coriander, sweet fennel, black cumin, artichoke, rapeseed and dill were significantly increased with different rates of AS [21-24, 38].

Similar results were observed by Khalid and Shedded [39] in *N. sativa* EO; sixteen compounds were detected in EO extracted from *N. sativa* subjected to four chemical groups i.e. MCH, MCHO, SCH and SCHO. Also, similar FA constituents were obtained from *N. sativa* FO [40]; it was revealed that nine FA were detected in *N. sativa* FO belongs to two chemical classes i.e. UFA and SFA.

Conclusion

It has been concluded On the basis of results AS and UR prduced a significant variation of *N. sativa* EO and FO components. The major component of EO (p-cymene and α -thujene) and main FA (oleic and linoleic) were increased under AS and UR levels. Highest values of major components of EO and main FA were resulted from 100 or 150 kg N ha⁻¹ (as AS) rates respectively.

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