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The Effect of Micronutrients (Fe, Zn, B, and Mn) Applied to the Leaf on Oil Content and Fatty Acid Composition of Black Cumin (*Nigella sativa* L.) in Winter Sowing Condition

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Abstract

Oil contents and fatty acid composition of black cumin treated with some micronutrients (Fe (3 g Lit^{-1}), Zn (2 g Lit^{-1}), B (4 g Lit^{-1}), and Mn (3 g Lit^{-1})) were determined. Oil contents of the plant seeds ranged from 31.64% (Mn) to 40.00% (Fe). Saturated fatty acids and unsaturated fatty acids ranged from 16.71%-17.54% and 80.86%-82.00%, respectively. The seed oils of all black cumin treated with fertilizers have high unsaturated fatty acid content as well as a balanced fatty acid composition. The major fatty acid in the oils was linoleic acid which ranged 56.85% (Zn)–58.85% (control). Oleic and palmitic acid contents of oils ranged from 22.80%–24.28% and 11.60%–11.77%, respectively. These results also suggest that these seed oils have a potential as a vegetable oil source for food industry.

Keywords: Black Cumin, micronutrient, fatty acid composition, oil content.

Öz

Yapraktan Uygulanan Mikro Elementlerin (Fe, Zn, B ve Mn) Çörekotu'nun (*Nigella sativa* L.) Kışlık Ekimde Yağ Oranı ve Yağ Asitleri Bileşimi Üzerine Etkisi

Çalışmada, kışlık ekilen ve faklı mikro besin elementleri (Fe, Zn, B ve Mn) uygulanmış çörekotu tohumlarında yağ oranı ve yağ asitleri bileşimleri araştırılmıştır. Çörekotu tohumlarının yağ içeriği %31.64 (Mn) ile %40.00 (Fe) arasında belirlenmiştir. Doymuş ve doymamış yağ asitleri oranları sırasıyla %16.71–%17.54 ve %80.86–%82.00 arasında bulunmuştur. Farklı mikro besin elementleri uygulanmış çörekotu tohumlarının dengeli bir yağ asidi dağılımına sahip olmasının yanında, yüksek oranda doymamış yağ asidi içeriğine sahip olduğunu göstermektedir. Başlıca yağ asidi linoleik asit olup, örneklerdeki içeriğinin %56.85 (Zn)–%58.85 (kontrol) arasında değiştiği belirlenmiştir. Oleik ve palmitik asit oranları sırasıyla %22.80–%24.28 ve %11.60–%11.77, arasında olduğu görülmüştür. Ayrıca, elde edilen sonuçlara göre bu tohumların gıda endüstrisi için yemeklik yağ kaynağı olma potansiyeli bulunmaktadır.

Anahtar Kelimeler: Çörekotu, mikro besin elementler, yağ asitleri kompozisyonu, yağ oranı.

Introduction

The dependence of humankind on medicinal and aromatic plants to treatment various pathologies is as old as their long history and developments in the field of nutrition during last few decades uncovered therapeutic potential of various culinary crops (Hameed et al., 2008; Jabeen et al., 2008). Black cumin (*Nigella sativa* L.) is an annual herbaceous plant belonging to the Ranunculaceae family. The seed components have also been used to make functional cosmetic and dietary supplemental products (Lutterodt et al., 2010; Ramadan, 2007). Oils are the major components of human diet. High quantities of oils may be found in some plant seeds, distributed in many regions all over the world. They can probably provide oils with a high concentration of monounsaturated fatty acids that prevent cardiovascular diseases by several mechanisms in our body (López–Miranda et al., 2006). The fixed oil of the plant seed is considered as one among newer sources of edible oils, thanks to its important role in human suitable nutrition and health. The plant fixed oil is a valuable source of essential fatty acids, glycolipids, phospholipids, and bioactive phytosterols (Ramadan, 2007; Ramadan et al., 2012a). Besides better fatty acid profile, it also contains considerable quantities of tocopherols and allied bioactive compounds that are important in attenuating the overall antioxidant capabilities of the body (Cheikh–Rouhou et al., 2008; Valko et al., 2007; Ramadan and Moersel, 2003). The plant is good source of nutritionally essential oil components (Black et al., 2006). This essential oil has been reported to possess antitumor activity, antioxidant activity, anti–inflammatory activity, antibacterial activity and a stimulatory effect on the immune system (Cheikh–Rouhou et al., 2007). Similarly, pharmacological studies explored the efficiency of essential oil and its active ingredient against various maladies like oxidative stress, cancer, immune dysfunction and diabetic complications (Gali–Muhtasib et al., 2004; Hussein et al., 2005). The plant has various different chemical ingredients including thymoquinone (30‒48%), flavonoids, anthocyanins, alkaloids and essential fatty acids, particularly linoleic and oleic acid. It has been traditionally used for the treatment of different diseases such as respiratory and digestive disorders, kidney and liver dysfunction and rheumatism (Ahmad et al., 2013). Different scientific investigations have depicted the plant seed composition i.e., moisture, oil, proteins, ash and total carbohydrates contents in the range of 3.8–7.0%, 22.0–40.4%, 20.9–31.2%, 3.7–4.7% and 24.9–40.0%, respectively (Atta, 2003). Human health enhancing potential of the plant seed has been attributed to the active ingredients which are mainly concentrated in edible and essential oil (Ramadan, 2007). The oil content of most black cumin seeds (produced in different conditions) investigated, so far, is higher than 30 percentage (Matthaus et al., 2011) and possibly up to 40 percentage (Cheikh–Rouhou *et al*., 2007; Tembhurne *et al*., 2014). Edible oil of black cumin seeds contains appreciable quantities of unsaturated especially polyunsaturated fatty acids; constitute the bulk of oil ranging from 48–70%, while monounsaturated (18–29%) and saturated fatty acids (12–25%) are in lesser proportions (Nickavar et al., 2003; Cheikh–Rouhou et al., 2007). The edible oil of the seeds reported by researchers to contain a fatty acid rich in unsaturated fatty acids, mainly linoleic acid (50– 60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%). Saturated fatty acids such as palmitic acid and stearic acid content to about 30% or less than (Mehta et al., 2008; Nickavar et al., 2003).

Comprehensive nutrition of plants is one of the key factors influencing the yield, yield component, and quality of crop productions from different aspects (Sharma, 2003). Besides the macronutrients (N, P and K), micronutrient elements also play a vital role as essential elements in deciding the growth and development of plants; macronutrients are needed in high quantities whereas micronutrients are needed in small quantities for normal plant growth and development (Ali et al., 2001; Sindhu and Tiwari, 1993). According to the results of researches, many of micronutrient elements have received a great deal of importance in all strategic crop production during recent years because of the widespread occurrences of their deficiencies from different parts of agricultural lands in all countries. Researchers from the countries all over the world have also reported significant responses of many crops to micronutrient elements fertilization (Aravind and Prasad, 2005). Application of micronutrients plays an important role in the production of high quality and yield of plants as crops (Rashid et al., 2004). The importance and role of micronutrients in photosynthesis, N–fixation, respiration, chlorophyll, stimulation of many enzymes and other metabolic processes of the plants are well indicated and documented (Hussain et al., 2006; Whitehead, 2000 and Pariari et al., 2003). Micronutrients such as Fe, Zn, B, Mn, and etc., due to their stimulatory and catalytic effects on metabolic processes and ultimately on yield and yield components, play vigorous and vital roles in the plants growth and development (Lahijie, 2012) and quality (Khosa et al., 2011). Due to the quick and simple absorption of nutrients (macro and micro) by plants leaves, foliar fertilization apply is a mean of increasing crop production (Ali et al., 2001).

For successful production of each crop, planting time is very important. Shortening of the growing cycle decreases the amount of radiation intercepted during the growing season and biomass of plants (Moosavi et al., 2012; Kaleem et al., 2011). With delayed sowing, development is accelerated because the crops encounter higher temperatures during the vegetative growth (Moosavi, 2014). It is believed that with winter sowing, some quantitative and qualitative properties can be increased by long of plant cycle in absence of suddenly chilling.

It is believed that this study will be a good source for future studies and contribute to determine micronutrients application in black cumin. The main objectives of the submitted work were to evaluate the effect of some micronutrients (Fe, Zn, B and Mn) on oil content and fatty acid composition of black cumin under Urmia condition, West Azerbayjan, Iran.

Materials and Method

The trial was done at the experimental fields of the Department of Agronomy, Faculty of Agriculture (latitude 37.53° N, 45.08° E, and 1320 m above sea level) and the Lab of Jahade Daneshghahi, Urmia University, Urmia, Iran, during 2015–2016, based on randomized complete block design and three replications in plots of an area of 4.20 m^2. The seeds for sowing were obtained from Bukan, West Azerbaijan, Iran. West Azerbaijan Province is located in the utmost end of Iran's northwest, between 35 degrees 58 minutes and 46 degrees northern Latitude, and also between 44 degrees 3 minutes and 47 degrees 23 minutes longitude. This province covers an area of 37614 km^2 which includes the 23 percent of the whole country's area (Najafi and Darvishzadeh Sherafatmand, 2013). The long term outdoors climatic data of the experimental city (Table 1.) are shown.

Table 1. The long term outdoors climatic data of the experimental city*.

Months	Rainfall (mm)	Temperature (C°) (Average)	Temperature (C°) (Lowest)	Temperature (C°) (Highest)	Wind speed (Knots)
January	29.3	19.3	-22.8	16.4	2.0
February	33.2	13.4	-22.0	21.0	2.5
March	51.5	6.8	-19.0	26.0	3.3
April	61.3	1.3	-12.0	30.8	4.0
May	44.3	-1.8	-1.6	31.8	3.5
June	14.2	0.1	4.0	36.2	3.4
July	5.5	5.3	9.8	38.0	3.1
August	2.4	11.0	8.0	39.2	3.0
September	4.7	15.7	2.2	36.0	3.0
October	24.3	20.3	-5.0	30.0	2.6
November	39.6	23.9	-13.4	23.0	2.2
December	28.6	23.5	-20.0	21.4	2.0

* The government meteorological association of Iran

In the trial the treatments included: control, Fe, Zn, B, and Mn. Foliar application of Fe (3 g Lit^{-1} per m^2), Zn (2 g Lit^{-1} per m^2), B (4 g Lit^{-1} per m^2), and Mn (3 g Lit^{-1} per m^2) were separately used for two times in vegetative phase. Soil samples (0–30 cm) were taken in autumn before application of fertilizers. Soil analysis results of the experimental soil samples in the field (Table 2.) are shown.

Table 2. Soil analyses results of the experimental soil samples in the field before corm sowing.

EC	1.42 dSm^{-1}	**O.C**	1.27%
$CaCO_3$	14.2%	**pH**	7.3
B.S	51%	**K**	312.0 mg kg-1
F.C	28%	**P**	11.2 mg kg-1
Clay	42%	**Fe**	17.0 mg kg-1
Loam	37%	**Zn**	1.8 mg kg-1
Sand	21%	**B**	0.4 mg kg-1
Texture	Clay-Loam	**Mn**	16.0 mg kg-1

The land was plowed at the optimum moisture level (field capacity) and leveled. Phosphorus (40 kg ha^{-1}) and Potassium (20 kg ha^{-1}) fertilizers were used at pre–sowing in Autumn, according to soil analysis and farrowed in 30 cm. Sowings were done on 15 November 2015. Nitrogen (80 kg ha^{-1}) fertilizer was used in spring, according to soil analysis. Thinning was done (10 cm) on 10 April 2016 to grow better. Irrigation was conducted depending on plants need. The crop harvested in first month of summer 2016.

The dried seeds (~1 g) were ground and extracted with n–hexane by soxhalet apparatus (60–80 °C for 6 h) in Agronomy Laboratory, Department of Agronomy, Faculty of Agriculture, Urmia University. The solvent were removed in rotary evaporator and oil content calculated as weight percent of dried seeds. A sample preparation step was necessary prior to introduction of the extracts into the gas chromatograph (GC) for the determination of fatty acids compositions. Fatty acid methyl esters were obtained by transesterification with potassium hydroxide in methanol [7]. One ml of a 2.0 M solution of potassium hydroxide in methanol and 0.2 g of the seed oil were mixed and shaken vigorously for 5 min in a vortex mixer. Then 1.0 ml of n–heptane was added to extract fatty acid methyl esters. The n–heptane phase was separated and passed through anhydride sodium sulfate. Fatty acid methyl esters were separated on an Agilent 6890N Gas Chromatograph equipped with a DB–wax capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness), a FID detector, split/splitless injector and chemstation software. The initial column temperature was maintained at 60 °C for 3 min and then raised at 25 °C /min to 190 °C and held for 10 minutes. Nitrogen was used as carrier and makeup gas, which their flow rates are 1.0 ml/min and 25 ml/min respectively. The injector and detector temperature were held at 250 °C and 260 °C, respectively. Injection of analyses were made in split mode with 50:1 split ratio (Barthet *et al.*, 2002; AOAC, 2000a; AOAC, 2000b). The fatty acid identification was based on the comparison of their relative retention times with the corresponding fatty acid methyl ester standards. Individual reference methyl ester standards (myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), gadoleic acid (C20:1), behenic acid (C22:0) and lignoceric acid (C24:0) and as well as fatty acid methyl ester mix (37 components FAME mix) were purchased from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany).

Results and Discussion

Oil content

The average of oil contents from different treatments are presented in Table 5. The average of oil content was the highest (40.0%) in the samples of Fe treatment, whereas samples treated with Mn had the lowest oil content (31.6%). Gharby et al., (2015) indicated that black cumin seeds from Morocco afforded 37% and 27% of oil after hexane– or cold press–extraction, respectively. Atta (2003) reported that the extraction of oil with petroleum ether from the seeds of black cumin originated from Egypt yields 34.78% of crude oil. Oil content in most seeds of the plant investigated, so far, is higher than 30% (Akram Khan, 1999; Matthäus and Özcan, 2011) and possibly up to 40% (Cheikh–Rouhou et al., 2007). Ebrahimian et al., (2010) reported that using Fe and Zn are more beneficial to oil biosynthesis in sunflower, as well as oil content is increased by. Eslami et al., (2015) indicated that spraying zinc sulfate in sunflower increased oil content. Rahimizadeh et al., (2010) showed that micronutrient treatments increased oil content in sunflower.

Fatty acids composition

Fatty acid composition of the plant seed oils is presented in the Table 5 and 6. The linoleic, oleic and palmitic acids was determined as major fatty acids in the seed oils. Sum of unsaturated fatty acids of black cumin seed oils in different treatments ranged from 80.86% (Fe) to 82.00% (control), made up mainly linoleic acid (C18:2n). The ratio of saturated to unsaturated fatty acids (S/U%) ranged from 20.37% (control) to 21.56% (Mn and Zn). These ratios were lower than that reported by Ramadan and Morsel (2003) for black cumin seed oil (25.7%). Major fatty acid linoleic acid which is polyunsaturated fatty acid and essential for human diet ranged from 56.85% (Mn) to 58.85% (control). In terms of linoleic acid the difference among control and other treatments are significant. Linoleic acid is responsible for the biosynthesis of arachidonic acid and some prostaglandins. This result agreed with those reported in *Nigella sativa* oil (C18:2 = 52%, C18:1 = 25%) (Ramadan and Morsel, 2002). Oleic acid (C18:1) ranged from 22.80% (Fe) to 24.28% (Zn), the fatty acid which is the second most abundant unsaturated fatty acid.

In terms of oleic acid the difference among Zn and other treatments (control, Fe and B despite Mn) are significant. Atta (2003), showed that the oils of black cumin varieties contained oleic and linoleic acids at relatively high levels ranged 18.09–20.10% and 47.50–49.00%, respectively. It is well known that edible oils, rich in linoleic acid, prevent cardiovascular disorders such as coronary heart diseases, atherosclerosis and high blood pressure. Also, it was reported that the nutritional value of linoleic acid is because of its metabolism at the tissue levels, which produces the long–chain polyunsaturated fatty acids and prostaglandins (Sayanova et al., 2003). Linolenic acid (C18:3) as unsaturated fatty acid, were also present in small concentrations usually accounting for less than 0.28% of the oil composition. High linolenic content is undesirable for vegetable oils as it is prone to auto–oxidation causing off–flavor compounds in oils (Yazicioğlu and Karaali, 1983). Saturated fatty acids of the plant seed oils in different treatments ranged 16.71% (control) to 17.54% (Zn), made up mainly palmitic acid (C16:0). Palmitic (C16:0), stearic acid (C18:0) and arachidic acid (C20:0) were found as major saturated fatty acids in all treatments. The percentage of palmitic acid in the oils ranged from 11.60% (control) to 11.77% (Mn). In terms of this fatty acid the difference among Mn and other treatments are significant. Stearic acid as saturated fatty acid varied between 2.52% (control) and 2.99% (Zn). Arachidic acid, another saturated fatty acid in black cumin seed oils ranged 2.37%

(control) to 2.62% (Zn). Myristic (C14:0) were also present in small concentrations usually accounting for less than 0.24% of the oil composition. Few amounts of myristoleic (C14:1) = 0.18% and lignoceric (C24:0) = 1.08% acids were detected by Saleh Al–Jassir (1992) in the plant seeds from Saudi Arabia. In our study, a few amount of eicosenoic acid was detected (0.4%) in the chloroform–methanol extract and reported by Babayan *et al*. (1978). Few trials have considered physicochemical treats of the plant oil. Chemical analysis of whole mature seeds of black cumin showed that the ether–extractable crude oil ranged from 34.49% to 38.72% (Salem, 2001).

Table 1. The variance analysis saturated fatty acids (Myristic acid, Palmitic acid, Stearic acid and Arachidic acid) and unsaturated fatty acids (Oleic acid, Linoleic acid, Linolenic acid) of Nigella sativa applied micro–nutrients.

Source	**Df**	**Myristic acid**	**Palmitic acid**	**Stearic acid**	**Arachidic Acid**	**∑saturated Fatty acid**
Rep	2	0.00002	0.00002	0.0001	0.00002	0.067
Applies	4	0.0005*	0.016**	0.12**	0.027**	0.505**
Error	8	0.0001	0.0004	0.0004	0.0006	0.068
CV (%)		5.16	0.17	0.72	0.99	0.32

Source	**df**	**Oil (%)**	**Oleic acid**	**Linoleic acid**	**Linolenic acid**	**∑Unsaturated Fatty acid**	**(S/U)**
Rep	2	0.61	0.067	0.0001	0.0001	0.000	0.004
Applies	4	28.64**	1.22**	1.83**	0.0005*	0.338**	0.712**
Error	8	1.28	0.067	0.0003	0.0008	0.001	0.008
CV (%)		3.25	1.10	0.03	3.49	0.24	0.42

Table 2. Average compares the fatty acid components of Nigella sativa applied micro–nutrients.

Micronutrients	**Oil content (%)**	**Oleic acid (%)**	**Linoleic acid (%)**	**Linolenic acid (%)**	**∑Unsaturated Fatty acid (%)**	**(S/U) (%)**
Control	34.35 b	22.90 b	58.85 a	0.25 a	82.00 a	20.37c
Fe	40.00 a	22.80 b	57.79 c	0.27 a	80.86 c	21.31b
Zn	34.54 b	24.28 a	56.85 e	0.24 b	81.37 bc	21.55a
B	34.35 b	23.12 b	58.03 b	0.25 b	81.40 b	21.26b
Mn	31.64 c	23.83 a	57.16 d	0.27 a	81.26 bc	21.56a

Micronutrients	**Myristic acid (%)**	**Palmitic acid (%)**	**Stearic acid (%)**	**Arachidic acid (%)**	**∑Saturated Fatty acid (%)**
Control	0.22ab	11.60d	2.52b	2.37d	16.71d
Fe	0.21ab	11.61d	2.95a	2.46c	17.23c
Zn	0.20b	11.73b	2.99a	2.62a	17.54a
B	0.20b	11.68c	2.96a	2.47c	17.31b
Mn	0.23a	11.77a	2.97a	2.55b	17.52a

Conclusions

In conclusion as the results of the trial, oil content of black cumin significantly affected by foliar application of Fe than control, as well as increased oil content in the seeds of the plant. Despite oil content, using Fe reduced unsaturated fatty acids. Mn treatment reduced oil content than control. The major fatty acids in black cumin seed oil were linoleic acid, oleic acid and palmitic acid. The seed oils of the plant had a balanced fatty acid distribution having a high content of unsaturated fatty acids as linoleic and oleic acids. These fatty acids are two important in human diet. Increase in their concentration relates to the quality of related oil. Linoleic acid has some abilities to lower blood cholesterol levels in humans, and thus may contribute to the reduced risk for atherosclerosis and other cardiovascular diseases. Furthermore, with a balanced fatty acid composition, the seeds of black cumin could be used in some foods to improve their nutritional value.

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