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Research Article

Changes in N, K, and Fatty Acid Composition of Black Cumin Seeds Affected by Nitrogen Doses under Supplemental Potassium Application

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This study was carried out to determine the efficiency of nitrogen (N) doses (0, 30, 60, and 90 kg N ha^{-1}) under supplemental potassium (K) application (50 kg K_2O ha^{-1}) on black cumin in 2011 and 2012. The results showed that increased N levels resulted in increasing seed yield and N and K contents in seed, while oil content decreased. The seed yield and oil yield were peaked at the doses of 60 kg N ha^{-1} and 50 kg K ha^{-1}. An increase in N doses caused a reduction in oil content regardless of K supply. Saturated fatty acids and oleic acid were slightly increased by K application, while minor changes in linoleic acid were detected. It was concluded that 60 kg N ha^{-1} with supplemental K application should be advised for enhancement in seed yield, oil yield, and N and K contents in seeds of black cumin without significant changes in fatty acid composition.

1. Introduction

Black cumin (*Nigella sativa* L.), an annual herbaceous plant, has been used for centuries for spice, medicinal, and culinary purposes. It is extensively cultivated from Morocco to northern India and Bangladesh and in East Africa and Russia for spice, while it is grown on a minor scale mainly for medicinal purposes in North America, Europe, and Southeast Asia [1]. Its seeds have been utilized as a food preservative to flavor sauces, pickles, and meat dishes and are sprinkled on bread and cheese [2]. The seeds and extracts have antioxidant [3], antidiabetic [4], antihistaminic [5], antihypertensive [6], anti-inflammatory, analgesic [7], antimicrobial [8], and antitumor [9] effects. In addition, the oil extracted from seeds of black cumin is rich in linoleic, oleic, and palmitic acids [10–12].

Black cumin is generally adapted to disturbed soils and semiarid regions [13]. In these areas, low soil fertility due to insufficient organic matter is the main obstacle limiting high yielding productivity, and consequently N fertilization must be applied for improvement in biomass and seed yield. Nitrogen is one of the essential nutrients affecting the plant growth and development, being an essential component of the proteins which builds cell materials and plant tissue [14]. Several findings have shown the positive effects of N fertilization on the seed yield and seed oil quality of black cumin by Ashraf et al. [15], Özgüven and Sekeroğlu [16], and Shah [17].

Potassium, which is known to affect several physiological activities in the plant, withstands water stress, increases resistance to diseases, and enhances protein synthesis and

Table 1: Monthly climatic data of the experimental field and long-term average during growing seasons.

Month	Mean temperature (°C)			Total precipitation (mm)		
	2011	2012	Long-term	2011	2012	Long-term
March	4.8	1.5	4.9	16.6	56.4	29.6
April	8.0	11.9	9.7	60.8	22.1	44.3
May	13.7	14.4	14.9	92.3	80.9	39.4
June	18.1	20.1	19.2	32.0	0.0	24.4
July	23.4	22.8	22.0	20.0	5.5	13.4
August	20.6	20.8	22.0	2.2	3.5	9.0
Mean/total	14.8	15.3	15.5	223.9	168.4	160.1

quality. However, deficient K concentration in plant inhibited N uptake and photosynthetic carbon assimilation [18]. Nitrogen and K are required by crops especially during the beginning of growth to maintain development and expansion of the leaf canopy [19]. The optimum yield and crop quality can be obtained at optimal N and K rates. Insufficient K may lead to reduced N uptake, less developed roots, susceptibility to water loss, wilting, and lodging [20]. Moderate N fertilization results in higher yield increases in many crops after K application compared to N and K applied separately [21]. The increase in N uptake through K application ensures increased N use efficiency [22]. The efficiency of N and K fertilizer application is affected by numerous factors, including soil type, the soil's original N and K supplying capacity, crop cultivars, organic matter, and the levels of other nutrients [23, 24]. The interactions between N and K on crop growth and final yield which occur at the agronomic level are due to their underlying physiological interactions on tissue hydration and osmotic adjustment. On the other hand, the optimum level of N and K to be applied should be determined due to unnecessary expenses and environmental pollution like nitrate leaching, water contamination, and eutrophication in case of excessive N uses [25, 26]. Contrarily, K does not cause a significant environmental pollution by leaching or contamination, while K leaching, which affects adversely plant growth and quality, may usually occur in sandy soils and high rainfall areas [27, 28].

This study was aimed at determining the effects of increasing N doses on seed yield, oil content, and fatty acid composition of black cumin with or without supplemental K fertilization.

2. Materials and Methods

This research was carried out during the two years of 2011 and 2012 at the Faculty of Agriculture, Eskisehir Osmangazi University, located at an altitude of 789 m and between 39°48′N and 30°31′E. A local population (Dereyalak village, İnönü, Eskisehir) of black cumin was used as seed material. The soil of the experimental area was alkaline (soil/water-1/2.5 pH: 8.09) loam textured and calcareous with a low content (0.91%) of organic matter. Phosphorus content (6.4 mg kg^{-1}) of the soil was insufficient but the K content (160 mg K kg^{-1}) of the soil was sufficient.

As shown in Table 1, the total precipitation in 2011 (223.9 mm) was higher than that in 2012 (168.4 mm); however, the average temperature was lower in the first year (14.8°C) than in the second year (15.3°C). The long-term average temperature (15.5°C) and the total precipitation (160.1 mm) were approximately equal to those of the second year.

The experiment was arranged in a split plot design with three replications (K to main plots and N doses to subplots), and the plot size was 5.0 m × 1.8 m, consisting of 6 rows. Seeds were sown at the rate of 15 kg ha^{-1} at the end of March in both years using rows with 30 cm spacing on a well-prepared seedbed. Nitrogen fertilization was applied as ammonium nitrate (33% N) at four doses (control, 30, 60, and 90 kg N ha^{-1}), and all of the plots received half of N before sowing with the remaining being applied at flowering stage. Phosphorus fertilization was applied at sowing (40 kg ha^{-1}) in the form of triple superphosphate (TSP 42% P_2O_5). The plots fertilized with K were not fertilized with TSP because the K fertilization was applied in the form of monopotassium phosphate (0-52-34) at a dose of 50 kg K_2O ha^{-1}. Potassium and N fertilizers were not applied to the control plots. The weeds were controlled manually by hoeing. The plants were not irrigated in the first year because of sufficient precipitation, while the plants were irrigated twice in the second year after sowing for seedling emergence and during the flowering period. Harvest and handling were performed manually at the beginning of August.

The seeds after harvesting and cleaning were finely ground using an electrical grinder (Bosch, Germany) for oil content. The samples were dried at 105°C for 3 hours in a drying oven (Binder ED 115, Germany). The grounded 10 g sample was fed into a Soxhlet apparatus fitted with a 500 mL round-bottom flask and a condenser. The extraction was performed on a water bath at 40–60°C for 4 h with 200 mL petroleum ether. The solvent was distilled off under vacuum in a rotary evaporator. The seed oil was weighed and transferred into glass sealed amber dark bottles. The solvent residue in the seed oil was removed and stored at −18°C until analysis. The percentage of seed oil content was calculated by gravimetric methodology on the basis of dry matter [29]. The oil yield was calculated by seed oil content (%) × seed yield (kg ha^{-1}).

The fatty acid composition of the oil samples extracted was analyzed after derivatization to fatty acid methyl esters

Table 2: Main effects of N doses and supplemental K on seed yield, seed oil content, oil yield, and N and K contents in seeds of black cumin.

Year	Seed yield (kg ha^{-1})	Seed oil content (%)	Oil yield (kg ha^{-1})	N content (%)	K content (%)
2011	1080^{a}	43.2^{a}	466^{a}	3.18	1.68^{a†}
2012	886^{b}	32.6^{b}	288^{b}	3.11	1.61^{b}
			Potassium (kg K_2O ha^{-1})		
Control	950^{b}	37.3^{b}	359^{b}	2.99^{b}	1.57^{b}
50	1015^{a}	38.5^{a}	395^{a}	3.30^{a}	1.72^{a}
			Nitrogen (kg N ha^{-1})		
Control	909^{c}	39.7^{a}	365^{b}	2.70^{b}	1.52^{c}
30	948^{b}	38.6^{b}	371^{b}	2.91^{b}	1.61^{b}
60	1030^{a}	37.2^{c}	390^{a}	3.36^{a}	1.70^{a}
90	1043^{a}	36.1^{d}	383^{ab}	3.60^{a}	1.75^{a}
			Analysis of variance		
Year (Y)	**	**	**	ns	**
Potassium (K)	**	**	**	**	**
Nitrogen (N)	**	**	**	**	**
K × N	**	*	**	ns	ns
Y × N	ns	*	*	ns	ns
Y × K	ns	**	*	ns	ns
Y × K × N	**	ns	*	ns	ns

* and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively; ns: not significant. †: means followed by the same letter(s) in each column are not significantly different at $p < 0.05$.

Table 3: Effects of N doses and K application on seed yield, oil content, and oil yield of black cumin in 2011 and 2012.

K application	N level	Seed yield (kg ha^{-1})		Oil content (%)		Oil yield (kg ha^{-1})	
		2011	2012	2011	2012	2011	2012
Control	Control	976	797	44.3	34.5	433	275
	30	1053	851	42.6	32.6	448	277
	60	1037	887	41.9	31.4	434	279
	90	1115	885	40.8	30.3	455	269
	Mean	*1045*	*855*	*42.4*	*32.2*	*443*	*275*
K+	Control	1001	863	45.5	34.6	455	299
	30	1027	859	44.9	34.4	462	296
	60	1245	950	43.1	32.6	536	310
	90	1183	991	42.8	30.3	506	300
	Mean	*1114*	*916*	*44.1*	*33.0*	*490*	*301*
$LSD_{0.05}$		ns	34.5	ns	ns	ns	ns

ns: not significant.

(FAMEs) with 2 N KOH in methanol at room temperature [30]. Analysis of FAMEs was carried out with a gas chromatograph (Agilent 6850, USA) equipped with an Agilent 5975 N mass selective detector and a capillary column: (30 m length, 0.25 mm ID, and 0.25 μm film). The injector temperature was 250°C. The oven temperature was held at 150°C (hold for 1 min), ramped to 200°C at the rate of 2.0°C/min (hold for 1 min), and finally increased to 250°C at the rate of 25°C/min (hold for 1 min). The carrier gas was helium with a flow rate of 2 mL/min; the split rate was 1/50. The fatty acids were identified by the comparison of retention times to known standards. The results were expressed as percentage (g 100 g^{-1}) of total fatty acid.

The experiment was established as two factors (N and K applications) and as randomized complete block design combined over years with three replications. The data were subjected to analysis of variance (ANOVA) using SPSS for Windows (versions 20.0). The differences among the means were compared using the LSD values ($p < 0.05$ and $p < 0.01$).

3. Results and Discussion

The main effects of year, K, and N application on the investigated parameters were shown in Table 2, and the results obtained from each experimental year were given separately in Tables 3, 4, 5, and 6. A significant difference between the

Table 4: Fatty acid composition of black cumin oil extracted from seeds under supplemental K in 2011 and 2012.

Fatty acids	2011		2012	
	Control	K+	Control	K+
Myristic (14 : 0)	0.180	0.183	0.180^{b}	0.205^{a*}
Palmitic (16 : 0)	13.40^{b}	13.56^{a}	13.20^{b}	13.87^{a}
Palmitoleic (16 : 1)	0.175	0.170	0.170	0.217
Stearic (18 : 0)	3.515	3.630	2.807^{b}	3.180^{a}
Oleic (18 : 1)	24.06^{b}	25.19^{a}	21.85^{b}	23.89^{a}
Linoleic (18 : 2)	53.96^{a}	53.12^{b}	57.21^{a}	54.21^{b}
Linolenic (18 : 3)	0.524	0.514	0.576	0.567
Arachidic (20 : 0)	0.177	0.177	0.175	0.190
Eicosenoic (20 : 1)	0.385	0.400	0.360	0.347
Eicosadienoic (20 : 2)	2.425^{a}	2.290^{b}	2.720	2.843
Total	98.80	99.23	99.25	99.52
SAFAs	17.27	17.55	16.37	17.45
MUFAs	24.62	25.76	22.38	24.45
PUFAs	57.08	56.10	60.68	57.81
Total UFAs	81.71	81.86	83.06	82.26

∗ shows comparison between K applications in each year and means followed by the same line with different letters are significant at $p < 0.05$. SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids; K+: 50 kg K ha^{-1}.

Table 5: Effects of N doses and K application on stearic, palmitic, and oleic acid of black cumin in 2011 and 2012.

K application	N level	Stearic acid (%)		Palmitic acid (%)		Oleic acid (%)	
		2011	2012	2011	2012	2011	2012
Control	Control	3.52	2.81	13.4	13.2	24.1	21.7
	30	3.54	2.89	13.9	13.2	24.5	22.0
	60	3.55	2.84	14.2	13.1	25.4	22.6
	90	3.55	2.99	13.6	13.2	25.9	22.5
	Mean	*3.54*	*2.88*	*13.8*	*13.2*	*25.0*	*22.1*
K+	Control	3.30	2.91	13.5	13.9	25.2	23.9
	30	3.69	3.09	13.6	13.9	25.7	24.0
	60	3.71	2.67	13.5	14.0	26.0	24.0
	90	3.51	2.36	13.7	13.8	25.9	24.1
	Mean	*3.55*	*2.76*	*13.6*	*13.9*	*25.7*	*24.0*
$LSD_{0.05}$		ns	ns	ns	ns	ns	ns

ns: not significant.

Table 6: Effects of N doses and K application on linoleic, linolenic, and eicosadienoic acid of black cumin in 2011 and 2012.

K application	N level	Linoleic acid (%)		Linolenic acid (%)		Eicosadienoic acid (%)	
		2011	2012	2011	2012	2011	2012
Control	Control	54.0	57.2	0.49	0.58	2.43	2.72
	30	54.7	58.2	0.49	0.58	2.44	2.75
	60	55.3	58.3	0.53	0.61	2.48	2.80
	90	55.3	58.6	0.57	0.61	2.49	2.81
	Mean	*54.8*	*58.1*	*0.52*	*0.60*	*2.46*	*2.62*
K+	Control	54.3	54.2	0.51	0.57	2.29	2.84
	30	54.9	55.6	0.52	0.57	2.31	2.90
	60	55.0	55.7	0.52	0.57	2.32	2.90
	90	55.3	55.7	0.53	0.58	2.30	3.02
	Mean	*54.9*	*55.3*	*0.52*	*0.57*	*2.31*	*2.92*
$LSD_{0.05}$		ns	ns	ns	ns	ns	ns

ns: not significant.

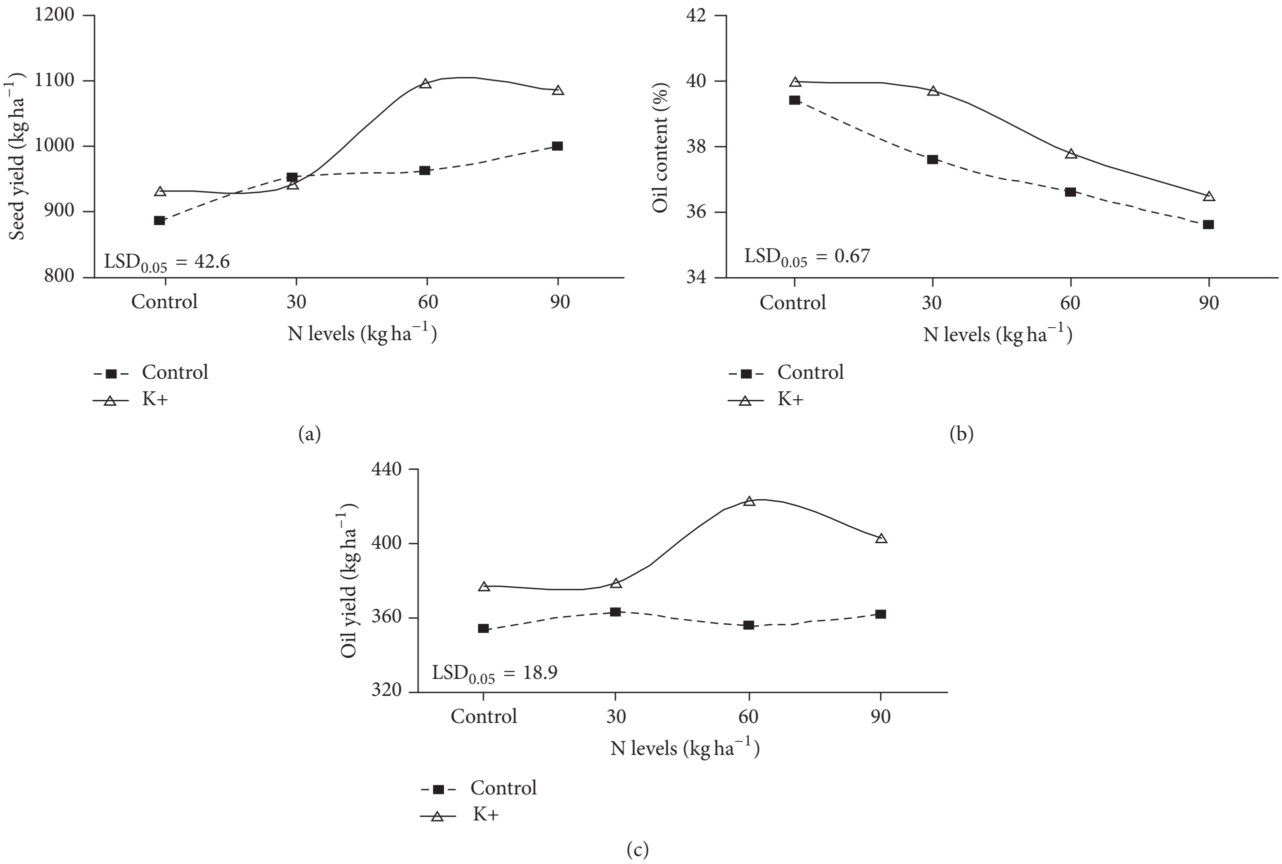

Figure 1: Changes in seed yield (a), oil content (b), and oil yield (c) of black cumin as affected by N doses and supplemental K application.

experimental years was determined in seed yield, oil content, and oil yield (Table 2). Mean seed yield, oil content, and oil yield were higher in the first year compared to the second year. This may be explained by higher precipitation and lower temperature in 2011 relative to 2012. The average temperature was 14.8°C in 2011 and 15.3°C in 2012. The total precipitation amount in the first experimental season (March–August) was higher (223.9 mm), and its distribution was more uniform than that in the second year (168.4 mm). Thus, higher seed yield, oil content, and oil yield were obtained in 2011.

The investigated characters except for fatty acid composition were significantly higher in the plants with supplemental K compared to the control (Table 2). The beneficial effects of K on seed yield of black cumin were detected. Likely, Sale and Campbell [31] found increases in soybean fruit yield and Rao et al. [32] in basil herb yield with increasing K supply. Also, increased N produced an increase in seed yield and oil yield, while it reduced oil content. The highest mean seed yield was obtained from 60 kg N ha^{-1} but the oil content reached the minimum level in 90 kg N ha^{-1}. It was determined that there was no benefit for seed yield and oil content when 90 kg N ha^{-1} was applied.

A significant two-way interaction between N and K applications was found for seed yield, seed oil content, and oil yield of black cumin (Table 3). The highest seed yield was obtained from the application of 50 kg K ha^{-1} with 60 kg N ha^{-1} as 1245 kg ha^{-1} in 2011, whereas seed yield was higher in K application with 90 kg N ha^{-1} in 2012. Usually, the uptake of K by crops closely correlated with N uptake. Better N uptake and utilization with adequate K means improved the N use and resulted in higher yields. When adequate K is available, the addition of N and/or other nutrients increases K uptake, as the yields are increased [18]. Similarly, increasing doses of N and K resulted in improving seed yield of cottonseed [33], rosemary [34], rice, and barley [35]. Puttanna et al. [34] stated that K fertilization was necessary to obtain an optimum rosemary yield even if the soil was able to meet the crop requirements for K. Our findings showed similar results: higher seed yields in the black cumin plants with supplemental K were observed even if the K content of the soil of the experiment was sufficient.

The oil content changed between 30.3 and 45.5% through the different N applications (Table 3). The oil content increased significantly with K application compared to the control but decreased with N doses in both years (Figure 1). The highest seed oil content was determined as 45.5% in 2011 and 34.6% in 2012 by 50 kg K ha^{-1} without N application. These results were in accordance with those of Ashraf et al. [15], who found that seed oil content was significantly higher at the control and at the lowest N dose (30 kg N ha^{-1}). Moreover, Özgüven and Sekeroğlu [16] and Shah [17] did not find any significant differences among N doses on the seed oil content of black cumin. Many studies on other oily seeds have shown that increasing the N dose resulted in reduction in seed oil content [33, 36–38]. On the other hand, the oil content in seeds is usually low at suboptimal K supply, as found by Sale and Campbell [31] and Sawan et al. [33], because K plays a key role in fatty acid and lipid metabolism.

In the study, the oil yield ranged between 310 and 536 kg ha^{-1} for the combination of K and N applications (Figure 1). The application of N and K significantly improved oil yield due to mainly enhancement in seed yield (Table 3). Higher oil yield was received from the plants with K application compared to the control; consequently, the maximum oil yield was detected in the interaction between 50 kg K ha^{-1} and 60 kg N ha^{-1}.

The application of N and supplemental K caused a considerable increase in N and K content in seeds. Supplemental K improved both N and K content of seeds and each increase in N dose resulted in enhanced N content in seeds of black cumin. However, no significant difference between 60 and 90 kg N ha^{-1} for N and K contents in seeds existed; they peaked at 90 kg N ha^{-1}.

The effect of K application on the fatty acid composition was significant ($p < 0.05$, Table 4). K application caused an increase in oleic and palmitic acid in both years and additionally stearic and myristic acids in 2012. Saturated fatty acids (SAFAs) such as palmitic, stearic, and myristic acids increased in small increments in both years with K application. For unsaturated fatty acids (UFAs), oleic acid (25.19 and 23.89%) increased, while linoleic acid (53.12 and 54.21%) declined in small increments with K application in either year (Table 5). Gaydou and Arrivets [39] found also an increase in oleic acid and a decrease in linoleic and linolenic acids in soybean by K application. They also determined a positive correlation between linoleic and linolenic acids and a negative correlation between linoleic and oleic acids. But Sawan et al. [33] reported that saturated fatty acids diminished, while unsaturated fatty acids enhanced with increasing K and N doses. Our findings revealed that N doses with or without K application had limited effects on fatty acid composition of black cumin as indicated by Ashraf et al. [15].

The oil of black cumin contains a high amount of linoleic acid (53.1 to 57.2%) (Table 6). Linoleic acid, the main polyunsaturated fatty acid (PUFA), was found to be high in many studies [11, 40, 41]. The results showed that UFAs (palmitoleic acid, oleic acid, linoleic acid, linolenic acid, eicosenoic acid, and eicosadienoic acid) account for between 81.71 and 83.06%; the main monounsaturated fatty acid (MUFA) was oleic acid, and the main SAFA was palmitic acid. The total MUFAs, PUFAs, and SAFAs compositions were between 22.38 and 25.76%, between 56.10 and 60.68%, and between 16.36 and 17.55%, respectively. The ratio of linoleic acid to oleic acid was almost 2 : 1. These results are in accordance with Cheikh-Rouhou et al. [11] and Al-Naqeeb et al. [40].

This study revealed that the highest seed yield and oil yield in black cumin were obtained from the applications of 60 kg N ha^{-1} with supplemental K in both years, while oil content reduced by increasing N doses. The main components of oil were linoleic (54.5%), oleic (23.5%), and palmitic acids (13.5%), which were not clearly influenced by fertilization. SAFAs, palmitic acid, stearic acid, and myristic acid, were slightly increased by K application. Oleic acid increased, whereas linoleic acid decreased with K application in either year, but these differences were too small to evaluate for practical purposes. In terms of reducing environmental risks such as leaching, contamination, and excessive nitrate accumulation in plant parts, application of 90 kg N ha^{-1} should be avoided. It was concluded that K application should be advised for enhancement of seed yield, oil content, and oil yield of black cumin and the effective N dose was determined as 60 kg N ha^{-1}.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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