







Effect on Yield and Some Quality Characteristics of Seed Harvest at Different Stages of Maturity in *Nigella sativa* L.

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ABSTRACT

Nigella sativa L. is a significant medicinal and aromatic plant due to the usage of both its seed and fixed oil. The aim of the study is to investigate the effects on seed yield, various yield components, fixed and essential oil content, and chemical composition (2018 year) of black cummin when harvesting the seed at four different stages of maturity. These stages were 25% (SH₁), 50% (SH₂), 75% (SH₃) and 100% (SH₄) browning of capsules. Two-year field experiments (2017 and 2018) were set up according to a randomized complete block design with triplicate, using a black cummin population obtained from the Burdur province under Isparta ecological conditions.

Significant statistical differences were found among the harvest stages in regards to the seed yield, plant height and the thousand-seed weight, while no differences were found in the numbers of capsules and branches, essential oil and fixed oil contents in both experimental years. Seed yield and its components increased during the harvest stage up to SH₃, while essential oil and fixed oil contents decreased insignificantly

from SH₁ to SH₄ in both years. According to the combined years; plant height, the number of capsules, the number of branches, the 1000 seed weight, seed yields, essential oil and fixed oil contents varied between 38.3-42.5 cm, 6.03-6.85 capsule plant⁻¹, 6.50-6.91 branches plant⁻¹, 2.30-2.57 g, 307.3-542.3 kg ha⁻¹, 0.087-0.101% and 31.14-32.69%, respectively.

The main components of black cummin essential oil were characterized by cymol (25.01-26.90%), thymoquinone (2.39-4.41%), carvacrol (10.12-10.41%), junipene (5.33-6.66%), Δ-3-carene (5.55-8.71%), β-pinene (2.98-3.65), trans-sabinene hydrate (8.02-11.93%) and α-thujene (7.82-9.42%) according to harvest stages in the 2018 season.

Considering the present results, SH₃ stage was advised because of its higher seed yield. The contents of essential oil composition of black cummin varied according to harvest stages.

Keywords: Harvest stages, Seed yield, Thymoquinone, Essential oil composition

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1. Introduction

Black cummin is an annual herbaceous plant which is grown widely in Southwest Asia, Europe and North Africa, and the Afyon, Isparta, Burdur and Konya provinces in Turkey (Baytop 1984). The species of *Nigella sativa* L., *Nigella damascena* L. and *Nigella arvensis* L. are traded and cultivated in Turkey because they are widely used in folk medicine and as a culinary spice (Kar et al. 2007). Black cummin is used in the food industry as an ingredient in food products, herbal teas and bakery products, and it is also used in the pharmaceutical, cosmetic and dyeing sectors (Baytop 1984). *Nigella* seeds contain protein, alkaloid, saponin, fixed and essential oil (Işık et al. 2017), crude fiber, minerals and vitamins (Güler et al. 2006).

Generally, black cummin has been grown based on natural precipitation (without watering) in continental climatic conditions. A higher seed yield can also be obtained under irrigated conditions. However, farmers prefer higher yielding plants such as fruits, vegetables, corn, potato, sugar beet etc. in irrigated conditions. In order to compete with these crops, it is of great importance to investigate the factors affecting the yield and quality characteristics of black cummin, one of these being harvest time. An early harvest of black cummin may cause a loss of yield due to non-uniform ripening, and a late harvest may cause the seed spilling as a result of capsule dehiscence. In addition to the amount of thymoquinone, which is one of the main active compounds of black cummin, essential oil can also be influenced by the maturity stages. There are a few studies in which a proper harvest stage was determined for high seed yield and quality in non-uniform ripening plants such as black cummin. Özel (2008) stated that the yield and quality of anise were affected by the harvest stage, and the highest seed yield, essential oil and anethole were obtained from the primary umbel completely mature stage. Similarly, Omidbaigi et al. (2003) determined that anise harvested in different levels of maturation caused changes in the yield and the essential oil composition of the crop. Kara (2017) reported that the highest grain yield of buckwheat occurred when 80% of the grains had turned brown. There are some research findings indicating that the chemical composition of black cummin is affected by both genetic and environmental factors (Arslan et al. 2012; Ertaş 2016).

The purpose of the present study was to investigate the effect of change on seed yield and yield components, fixed and essential oil contents and composition of black cumin when harvesting the seed at different stages of maturity.

2. Material and Methods

The experiment was carried out during the 2017 and 2018 years under the Isparta ecological conditions. From March to the end of August of 2017 and 2018, there was a total precipitation of 213.9 and 198.2 mm and an average temperature of 17.0 and 18.2 °C (Table 1).

Table 1- Temperature and precipitation data of the experiment area*

Climatic factors	Years	Months						Total or Average
		March	April	May	June	July	August	
Average Temperature (°C)	2017	7.3	10.6	14.9	20.1	25.1	24.0	17.0
	2018	9.2	14.8	16.8	20.3	24.3	24.1	18.2
	Long Years	9.3	10.8	15.6	20.1	23.4	25.8	17.5
Precipitation (mm)	2017	74.4	25.6	49.5	30.9	13.1	20.4	213.9
	2018	65.9	51.0	43.3	30.8	4.0	3.2	198.2
	Long Years	42.9	56.6	50.8	24.4	12.8	0.3	187.8

*; Data were taken from Isparta Meteorological Station

In the years 2017 and 2018, the experimental soil had a sandy-loamy, low organic matter (1.75% and 1.68%, respectively), slightly alkaline (pH 7.86 and 7.51, respectively) and limy (15.47% and 13.16% CaCO₃, respectively).

In this study, a local black cumin population, which is cultivated in the Burdur province, was used as genetic material. Harvest stages were arranged according to the randomized complete block design, with three replicates. Seeds were sown in 20 cm x 5 cm on row spacing. Each plot area was 5 m² (5.0 m x 1.0 m) and consisted of 5 rows. Seeds were sowed by hand at 1-2 cm depths using a dibbler in the first week of March in both experimental years. The total quantity of phosphorus with 46% P₂O₅ and half of ammonium sulphate with 21% N fertilizers were applied at the time of sowing at a rate of 60 kg ha⁻¹ and 40 kg ha⁻¹, respectively, and the other half of ammonium sulphate was applied when the plant was at a height of 15-20 cm (Tunçtürk et al. 2012). The plants were non-irrigated at any growing periods, and all the cultural practices were applied in both years.

In order to determine the seed yield and quality properties of black cumin, the plants were harvested from 3 rows in the center of each plot at four different stages of seed maturity as follows; SH₁ (Seed harvest): browning of 25% of capsules, SH₂: browning of 50% of capsules, SH₃: browning of 75% of capsules and SH₄: browning of 100% of capsules. After the harvest, the capsules were dried for one week on wire racks and the seeds in the dry capsules were then manually blended. Seed yield (kg ha⁻¹), plant height (cm), the number of branches (branches plant⁻¹), the number of capsules (capsule plant⁻¹) and the 1000-seed weight (g) were determined as described by Telci (1995).

For each of the harvest stages, 100 g of powdered black cumin samples in 0.5 L of water were hydro-distilled using Clevenger apparatus for 3 hours according to the standard procedure described in the European Pharmacopoeia for determining the essential oil content (v/w, %). The fixed oil content (%) of black cumin seeds belonging to each harvest stage were determined using NMR (Nuclear Magnetic Resonance, Bruker mqone) apparatus. The measurement was conditioned for 30 minutes at 20 °C and 35 °C in NMR, and the results are presented as a percentage (%). The chemical composition of the essential oil was identified by GC-MS (Gas Chromatography-Mass Spectrometry, Shimadzu 2010 Plus GC).

All the data were evaluated with analysis of variance (ANOVA) using an SAS Statistics Package Program. Means were compared using the LSD (Least Significant Difference) test.

3. Results and Discussions

3.1. Seed yield and its components

Significant statistical differences were found among the harvest stages of black cumin in relation to seed yield, plant height and the thousand-seed weight. However, no differences were found in the number of capsules, the number of branches, or the essential oil and fixed oil contents in either year or combined years (Table 2). Significant statistical differences (except for essential and fixed oil content) were found between the mean of the years. The plant height, number of capsules, number of branches and seed yield were higher in the first year, while the thousand-seed weight was higher in the second year. Essential oil and fixed contents were not changed significantly according to the mean the years. Differences in the yield and some yield components between the years might be due to higher raining during the growing period in the second year (Table 1). Sufficient moisture in the soil promotes the nutrition uptake of the plants, so rainfall in the first year increased the seed yield. Similar results that were obtained in other studies have also reported that seed yield and other plant characteristics varied depending on the climatic conditions of the year (Sadeghi et al. 2009; Ghamarnia & Jalili, 2013; Kara et al. 2015).

According to the combined years, the highest plant height (43.1 cm), number of branches (6.91 branches plant⁻¹), number of capsules (6.85 capsule plant⁻¹), 1000 seed weight (2.57 g) and seed yield (542.3 kg ha⁻¹) were obtained from the SH₃ stage. The lowest values of these characteristics were determined from the SH₁ stage (Table 2). The plant height slightly increased by delaying the harvest stage, but there weren't significant statistical differences among SH₂, SH₃ and SH₄. Similarly, there weren't significant differences between all harvest stages in the number of branches and capsules per plant in both years. These results can be explained by the determinate growth of black cumin, due to the ceasing of growth after the flowering stage. The 1000 seed weight decreased at the last harvest stage (SH₄). This could be as a result of the respiration losses which occurred in the seed storage. The reason for this is that when the plant reaches harvest maturity, the photosynthesis decreases, but respiration continues (Bugbee & Salisbury 1988). The seed yield increased up to the SH₃ stage, and it decreased in following harvest stage (SH₄). This decrease may have occurred due to the decrease of the 1000 seed weight at the SH₄ (Table 2). Özgüven & Şekeroğlu (2007) and Sadeghi et al. (2009) informed that there was a positive relationship between seed yield and its such as the number of branches, number of capsules and the 1000 seed weight. In studies conducted on another plant by Özel (2008) and Kara (2017) they reported that seed yields of anise and buckwheat with non-uniform ripening increased up to a certain ripening stage, and then it decreased. In other studies that were conducted, black cumin seed yield varied between 367.8-527.3 kg ha⁻¹ (Telci 1995), 166.7- 600.0 kg ha⁻¹ (Arslan et al. 2012), 676.6-903.3 kg ha⁻¹ (Kulan et al. 2012) and 325.9-416.3 kg ha⁻¹ (Seyyedani et al. 2014). In comparison with the above studies, the differences in seed yield could be as a result of a variety of characteristics, maturity periods of genotypes, climatic factors and agricultural practices (Karim et al. 2017; Selicioğlu 2018; Sultana et al. 2018).

Table 2- Effect on yield, some yield characteristics, essential oil and fixed oil content of seed harvest at different stages of maturity in black cumin

Harvest stages	Plant height (cm)			Number of branches (branches plant ⁻¹)		
	2017	2018	Combined years	2017	2018	Combined years
SH ₁	43.3 b	33.4 b	38.3 b	6.96	6.03	6.50
SH ₂	48.1 a	33.5 b	40.8 ab	6.80	6.30	6.55
SH ₃	48.4 a	34.8 a	41.6 a	7.26	6.56	6.91
SH ₄	49.8 a	35.4 a	42.5 a	7.20	6.35	6.76
LSD _{Harvet stages}	4.63	1.20	2.65	-	-	-
F value	12.81**	9.11*	20.90**	0.52 ns	0.55 ns	0.88 ns
CV (%)	3.27	3.04	3.22	7.13	8.07	7.58
Years	47.4 A	34.2 B	LSD _{years} : 2.17**	7.05 A	6.30 B	LSD _{years} : 0.45*

Harvest stages	Number of capsule (capsule plant ⁻¹)			1000 seed weight (g)		
	2017	2018	Combined years	2017	2018	Combined years
SH ₁	6.70	5.63	6.16	2.20 b	2.40 b	2.30 b
SH ₂	6.80	6.46	6.63	2.30 ab	2.50 ab	2.40 b
SH ₃	7.13	6.56	6.85	2.48 a	2.66 a	2.57 a
SH ₄	6.70	5.36	6.03	2.43 ab	2.56 ab	2.45 ab
LSD _{Harvet stages}	-	-	0.55	0.261	0.252	0.158
F value	1.63 ns	3.50 ns	2.62 ns	6.56 *	5.56 *	7.87 *
CV (%)	4.07	7.21	6.82	3.81	3.32	3.56
Years	6.83 A	6.01 B	LSD _{years} : 0.39*	2.35 B	2.50 A	LSD _{years} : 0.07*

Harvest stages	Seed yields (kg ha ⁻¹)			Essential oil content (%)		
	2017	2018	Combined years	2017	2018	Combined years
SH ₁	311.7 c	303.2 c	307.3 d	0.099	0.103	0.101
SH ₂	479.5 b	451.0 b	465.2 b	0.096	0.102	0.099
SH ₃	564.9 a	519.8 a	542.3 a	0.088	0.096	0.092
SH ₄	441.6 b	434.1 b	437.8 c	0.085	0.089	0.087
LSD _{Harvet stages}	39.50	23.8	13.5	-	-	-
F value	195.630**	397.45**	488.94**	3.29 ns	2.12 ns	6.41 ns
CV (%)	4.90	3.84	2.45	5.44	6.27	5.59
Years	449.4 A	426.7 B	LSD _{years} : 9.58*	0.092	0.097	LSD _{years} : ns

Harvest stages	Fixed oil content (%)		
	2017	2018	Combined years
SH ₁	32.36	33.02	32.69
SH ₂	31.38	33.71	32.54
SH ₃	32.20	31.48	31.53
SH ₄	30.81	30.87	31.14
LSD _{Harvet stages}	-	-	-
F value	2.67 ns	3.65 ns	2.32 ns
CV (%)	2.50	2.98	2.75
Years	31.68	32.27	LSD _{years} : ns

SH; Seed harvest, *, **, significant at P<0.05 and P<0.01 probability levels, respectively, ns; non significant

3.2. Essential oil and fixed oil content

Considering the combined years in the present study, the essential oil and fixed oil content varied between 0.087-0.101% and 31.14-32.69%, respectively. The essential oil and fixed oil content tended to decrease only slightly from SH₁ to SH₄ (Table 2). However, differences among in the harvest stages in both years weren't statistically significant.

El-Gamal & Ahmed (2017) reported that the essential oil content of fennel slowly decreased depending on delaying harvest time in the seed maturity period, while it was higher in early harvest stages. Telci et al. (2009) stated that the essential oil content of fennel was higher in the early stages of fruit development than the advanced stages. However, in our study, seeds were harvested at different stages of maturity, therefore, the rate of decrease in essential oil content was lower. In the plants harvested of seed, the synthesis of essential oil and fixed oil can be completed when it reaches the stage of maturity. In comparison to previous studies, the essential oil content of the present results were lower than the values reported: 0.5% (Bourgou et al. 2010), 0.5-1.6% (Ramadan 2007), 0.27-0.35% (Toncer & Kızıl, 2004), while showing parallels with the findings of Benkaci et al. (2013) and Kara et al. (2015). Kara et al. (2015), Mohammadi et al. (2016) and Mazaheri et al. (2019) reported that fixed oil content varied between 26.0-32.5%, 31.6-40.0% and 34.0-39.0%, respectively. These differences might have been due to air temperature, precipitation, soil fertility, agronomic conditions and harvest stages (Telci 1995; Kulan et al. 2012; Kara et al. 2015)

3.3. Chemical composition of essential oil

Essential oil compositions in black cumin oil were shown in Table 3. Cymol, thymoquinone, carvacrol, junipene, Δ -3-carene, β -pinene, trans-sabinene hydrate and α -thujene were determined as the main components in the essential oil of black cumin. According to the harvest stages, the cymol, thymoquinone, carvacrol, junipene, Δ -3-carene, β -pinene, trans-sabinene hydrate and α -thujene varied between 25.01-26.90%, 2.39-4.41%, 10.12-10.41%, 5.33-6.66%, 5.55-8.71%, 2.98-3.65, 8.02-11.93% and 7.82-9.42%, respectively. The rates of these components varied according to harvest stages. Generally, α -thujene, α -pinene, β -pinene, Δ -3-carene, cyclopropane, 4-terpineol and thymoquinone content decreased, while cymol, trans-sabinene hydrate, cyclohexen and carvacrol contents were increased by delaying harvest stages up to SH₄.

These changes could be as a result of climatic (especially temperature) differences between each harvest stage. The amount of temperature until the next harvest stage may affect the synthesis of essential oil composition. For example, there were about 26 days in the first year and 24 days in the second year between the first harvest and the last harvest. Synthesis of secondary metabolites in plants can be effected from biotic and abiotic factors as well as genetic traits (Telci et al. 2014). In comparison to previous studies, rates of main essential oil components varied: 42.4% thymoquinone and 10.3% carvacrol (Mahmoudvand et al. 2014), 67.7% thymoquinone, 8.4 % carvacrol, 4.8% junipene, 2.3% p-cymen and 1.9% 4-terpineol (Palabıyık & Aytaç 2018). These differences might have been due to variety, various ecological conditions including air temperature, radiation, precipitation and soil fertility affecting the production of secondary metabolites of black cumin (Benkaci et al. 2007; Mahmoudvand et al. 2014; Kara et al. 2015).

Table 3- Chemical composition contents in different harvest stage of black cumin

Chemical components*	RI	Harvest stages (%)			
		SH ₁	SH ₂	SH ₃	SH ₄
α -Thujene	927	9.42	8.37	7.82	8.71
α -Pinene	933	3.37	2.73	3.58	2.09
3-Hexanol (CAS) Hexan-3-ol	948	0.03	0.03	1.68	0.13
2-Pentanol, 4-methyl- (CAS) 4-Methyl-2-pentanol	957	-	-	1.91	-
Sabinene	981	1.29	1.40	1.42	1.47
β -Phellandrene	991	-	-	1.69	1.55
β -Pinene	997	3.65	3.60	3.06	2.98
Pyridinepropanoic acid, α -methyl-.beta.-oxo-, thyl es	998	0.03	-	0.12	-
α -Terpinene	1001	0.07	0.42	0.52	0.65
Cymol	1025	25.62	25.01	26.90	26.70
Limonene	1031	1.20	1.49	2.41	1.73
Eucalyptol (1,8-Cineole)	1035	0.11	0.12	0.14	0.11
2-methyl-5-(1-methylethyl)-, (1. α -,2.al)	1041	0.06	1.36	-	-
γ -Terpinene	1053	2.16	1.41	1.16	2.69
Linalool	1062	0.58	-	-	-
Δ -3-Carene	1073	8.71	5.55	-	-
Trans-sabinene hydrate	1100	9.15	8.02	10.85	11.93
Cyclopropane, 1,1-dimethyl-2-(3-methyl-1)	1102	-	3.99	3.89	3.45
4-Terpeneol	1190	3.93	3.06	-	2.19
Cyclohexen-1, 4-methyl-1-(1-methylethyl)- 4-Terp.	1194	-	-	3.98	5.11
Benzeneethanol, α -, α -dimethyl acetate	1198	0.12	0.13	-	-
Cis-p-Mentha-2,8-dien-1-ol	1209	2.21	2.66	-	1.04
β -Cyclocitral	1214	-	-	1.15	-
2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)	1255	0.21	2.44	2.15	4.26
Thymoquinone	1261	4.41	2.70	2.82	2.39
Carvacrol	1313	10.12	10.13	10.41	10.43
(1S,4R)-p-Mentha-2,8-diene, 1-hydroperoxide	1326	-	3.17	-	-
α -Longipinene	1361	0.73	0.86	-	1.07
Junipene	1419	6.66	6.45	5.33	6.56
(-)-Caryophyllene oxide	1749	-	1.99	-	-
(-)-Caryophyllene oxide	1982	1.16	-	-	-
Dimethoxy-Cis-9-Octadecene	2113	-	-	2.37	-

SH; Seed harvest, RI; Retention Indices, *; Contents of chemical compounds belong to 2018

4. Conclusions

As a results of the research: i) The SH₃ harvest stage had the highest seed yield, with a mean of 542.3 kg h⁻¹ so that in compared to the other harvest stages, it increased by 14.2-43.3%. The yield and its components increased by delaying the harvest stage up to SH₃, and decreased in the following harvest period (SH₄). Therefore, under Isparta's ecological conditions, black cumin should be harvested in the SH₃ stage due to the higher seed yield.

ii) Differences among the harvest stages in relation to essential and fixed oil content in both years weren't statistically significant.

iii) Cymol, thymoquinone, carvacrol, junipene, Δ -3-Carene, β -pinene, trans-sabinene hydrate and α -thujene were identified as major compounds of black cumin's essential oil, and rates of these components varied according to the harvest stages.

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