Journal of Herbal Drug

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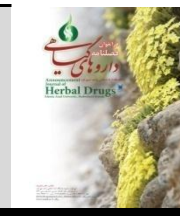

Genetic improvement of quantity/quality yield of black cumin (*Nigella sativa* L.) ecotypes cultivated in Iran climatic conditions

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ARTICLE INFO

Type: *Short Original Research*

Topic: *Medicinal Plants*

Received *January 5th 2014*

Accepted *April 5th 2014*

Key words:

- ✓ *Nigella sativa* L.
- ✓ *Genetic variation*
- ✓ *Selection criteria*
- ✓ *Regression*
- ✓ *Path analysis*

ABSTRACT

Background & Aim: Black seed or black cumin (*Nigella sativa* L.) belongs the family Ranunculaceae. Black seed is one of these species, which is naturally distributed in different parts of the country. It is extensively cultivated in various regions of Iran.

Experimental: The experiment was achieved using randomized complete block design with three replications in the research field of Islamic Azad University of Isfahan Branch during 2013. Seed of ten ecotypes namely; Semirom, Zawareh, Golpayegan, Fereydan, Meymeh, Kashan, Khansar, Daran, Ardestan and Isfahan were cultivated in the plots comprising four rows. The essential oil was extracted by a Clevenger type apparatus and analyzed by using GC/MS.

Results & Discussion: Statistical analysis indicated that there was significant difference among all traits. The results of regression and path analysis indicated that the number of branches, grain filling rate and days to blooming traits were the best indirect selection criteria to improve yield in black cumin. According to the results of this research, Fereydan and Daran ecotypes showed the optimum amount of essential oil content and morphological traits. Furthermore, cross between these ecotypes has suitable advantage to increase seed and oil yield in black cumin breeding programs.

Industrial and practical recommendations: There are few researches about breeding of black cumin. The results of present study could be applied in research centers as well by farmers to cultivate commercially and production black cumin especially for seed.

1. Introduction

Black cumin (*Nigella sativa* L.) is plant belongs the family Ranunculaceae. The body of this annual plants is 20-40 cm height, straight, and lightly feathered, with finely divided. The flowers are delicate and usually coloured pale blue and white, with five to ten petals. Seeds flattened, oblong, small, black in colour and inflated capsule composed of three to seven united follicles, each containing numerous seeds and about eight species in Iran (Mozaffarian, 1998). Black cumin is native in Europe, Middle East and Western Asia (Iqbal *et al*., 2010). It also grows in most parts of Iran such as Arak, Kermanshah, Isfahan and other parts of the country. Black cumin originally grown in arid and semi-arid regions (Moody and Mohasel, 1998; D'Antuno *et al*., 2002). The basic chromosomes number of X = 6 have been found of *Nigella sativa* L. The diploid chromosome complement was 2n =12 (Jabeen *et al*., 2012). The seeds of *Nigella sativa* contain thymoquinone, monoterpens such as p-cymene and pinene, nigellidine, nigellimine and saponin (Ansari and Sadiy, 1989). The seeds have also been found to contain fats, crude fiber, minerals; e.g. Fe, Na, Cu, Zn, P, Ca and vitamins like ascorbic acid, thiamine, niacin, pyridoxine and folic acid (Takruri *et al*., 1998). Nickavar *et al*. (2003) reported the chemical composition of the extracted fixed oil and volatile oil of *Nigella sativa* L. seeds grown in Iran were eight fatty acids (99.5%) and thirty- two compounds (86.7%) have been identified in the fixed and volatile oils, respectively. The main fatty acids of the fixed oil were linoleic acid (55.6%), oleic acid (23.4%), and palmitic acid (12.5%). The major compounds of the volatile oil were trans-anethole (38.3%), p-cymene (14.8%), limonene (4.3%), and carvone (4.0%). The seeds have the anthelmintic, insecticidal, antimalarial, antibacterial, antifungal and antitumor activates. The seeds are also reported to have antispasmodia, diuretic, carminative, digestive and antiseptic properties (Ali *et al*., 2008). Salamati and Zeinali (2013) reported that investigate genetic diversity and relationships among morphological traits in 21 genotypes of (*Nigella sativa* L.) in Iran, seed yield varied from 63.34 g in genotype of Shiraz to (147.36 g) in genotype of Zabol.

Talebi Kouyokhi *et al*. (2008) reported that phytochemical variations were not only found among samples of different regions but also among samples of the same region with different altitude reflecting the effect of environment on essential oil components. It is well known that yield and yield components of plants are determined by a series of factors including plant genetic (Shafiee*et al*., 2009), climate, edaphic, elevation, topography and also an interaction of various factors (Rahimmalek *et al*., 2009). Therefore, the aim of this study was to the role of genetic distance and effective traits on the quantity/quality ecotypes yield of black cumin (*Nigella sativa* L.) cultivated in Iran climatic conditions.

2. Materials and Methods

2.1. Plant Material

This experiment has been conducted in the research field of Islamic Azad University of Isfahan, in center Iran (32°, 38_ N and 51°, 47_ E, 1550 m above sea level), during 2013. The experiment was achieved using randomized complete block design with three replications. This location is arid and warm area (according to the Koppen climate classification) characterized by warm and dry summers. The long-term (30 years) mean annual rainfall and temperature of area was 121.1 mm and 33.4 centigrade degrees, respectively. The soil texture of the experimental field was clay loam type with pH 7.37, contains total N (0.75 %), total P_2O_5 (35 ppm) and total K_2O (452 ppm) with an EC of 4.69(ds/m). Weeds were removed by hand weeding during growth stages. Seed of ten black seed ecotypes (Semirom, Zawareh, Golpayegan, Fereydan, Meymeh, Kashan, Khansar, Daran, Ardestan and Isfahan) were cultivated the plots comprising four rows. Seeds were planted in rows 30 cm apart with inter-row spacing 30 cm. The measured characters involved; plant height (cm), number of branches, number of capsules, days to beginning of blooming, days to full blooming, 1000-seed weight (g), grain filling rate (g/m^2/day), essential oil percent (%), essential oil yield (g/m^2), thymoquinone content (%), thymoquinone yield (g/m^2), γ-terpinene content (%) and γ-terpinene yield (g/m^2).

2.2. Extraction of essential oil

The essential oil was extracted from 100 g of powdered seeds in 1 L of water contained in a 2 L flask and heated by heating jacket at 100 °C for 4 h in a Clevenger–type apparatus, according to producers outlined British Pharmacopoeia. The collected essential oil was dried over anhydrous sodium sulphate and stored at 4 °C until analyzed.

2.3. Composition and identification of the constituents of the essential oils

GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. HP-5MS column (30 m x 0.25

mm, 0.25 µm film thickness) was used with helium as carrier gas with flow rate of 1.0 mL/min. The oven temperature was kept 20 °C at 50 °C for 4 min and programmed to 280 °C at a rate of 5 °C /min, and kept 20 °C constant at 280 °C for 5 min, at split mode. The injector temperature was at 20°C at 280 °C. Transfer 20 line temperatures 280 °C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 450.

2.4. Statistical analysis

Analysis of variance of the collected data was conducted based on randomized complete blocks design model. Then existence of significant difference between means of different parameters were evaluated using Duncan's new multiple range test (DNMRT) at 5% probability level. SPSS16 program was used to statistical analysis.

3. Results and discussion

3.1. Growth characteristics

Analysis of variance (Table 1) indicated the significant difference between ecotypes for plant height, number of branches, number of capsules, days to beginning of blooming, days to full blooming, 1000-seed weight and grain filling rate.

The tallest plant height (74 cm) was obtained in Daran province and the shortest (52 cm) in Isfahan province (Table 2). The maximum number of branches (192.6) was showed in Fereydan province ecotype and the minimum (164) in Ardestan (Table 2). The highest number of capsules (85) was obtained in Daran province ecotype wheaeas the lowest (66.6) in Meymeh (Table 2). Days to beginning of blooming revealed the maximum (172.67) in Zawareh province although the minimum days to beginning of blooming (154) was observed in Kashan province (Table 2). The highest mean of days to full blooming (198.67) was obtained in Zawareh province ecotype and the lowest in Kashan with 165 days (Table 2). The maximum mean for 1000-seed weight (4.47 g) was obtained in Isfahan province ecotype whereas the minimum (2.4 g) in Meymeh. On the other hand, the highest amount of grain filling rate (296 g/m^2) was showed in Fereydan province and the lowest (265.6 g/m^2) in Ardestan (Table 2).

The reason might be the better remobilization that occurs in black cumin ecotypes and causes the seeds to be filled better. Although, for considering the traits such as the seed yield as selection criteria more care must be taken. Because, if the plant is too dwarf, it might lead to reduction of the competition of plant to get light and other environmental parameters, thus the seed yield would diminish. Therefore, it is necessary that the best morphologic traits is studied more carefully (Golparvar and Hejazi Dehaghani, 2012). Faravani *et al*. (2006) reported that significant differences were observed in plant height, weight of 1000 kernel, seed yield, number of stem branches, vascular bundles, weight of capsule and xylem and phloem diameter on the 28 landraces of *Nigella sativa* were collected from Khorasan province in Iran. Black cumin seeds are important in herbal medicines commonly employed in Iran. The environmental factors like climate and location might be a possible reason for these variations. Moreover, difference in genetic makeup could also be a contributing factor as indigenous variety was tested in the trial. *Nigella sativa* L., is a new crop in the country and the first step for successful introduction is evaluation of diverse germplasm that not only helps to identify the superior genotypes, but selected genotypes could also be used in agronomic experiments (Shadia *et al*., 1998).

3.2. Essential oil yield

Analysis of variance for essential oil yield (Table 1) indicated that there was significant difference among ecotypes for essential oil content and yield. The results obtained in our study showed that the highest essential oil content (1.7%) was extracted in Fereydan, Kashan and Daran province ecotypes and the lowest (1.22%) in Semirom province. The highest essential oil yield (4.694 g/m^2) was obtained in Fereydan province ecotype whereas the lowest (3.29 g/m^2) in Isfahan province ecotype (Table 2).

Some solvent extracted oil yields from Nigella seeds of different countries such as; Iran (40%) Cheikh-Rouhou *et al*. (2007), Yemen (36.8–38.4%), Saudi-Arabia (38.2%), Morocco (37%) Gharby *et al*. (2013), Egypt (34.8%) Ali and Blunden (2003), Turkey (30–36%) Matthaus and Ozcan (2011), Bangladesh (32%) Ali *et al*. (2012), Pakistan (31.2%) Sultan *et al*. (2009), Tunisia (28%) Cheikh-Rouhou *et al*. (2007) and Italy (13–23 %) D'Antuono *et al*. (2002).

3.3. Chemical constituents

Analysis of variance (Table 1) indicated that there was significant difference for thymoquinone content and yield, γ-terpinene content and yield among ecotypes.

The highest of thymoquinone content (0.74 %) was obtained in Daran province ecotype and the lowest (0.34 %) in Ardestan (Table 2). The highest thymoquinone yield (2.033 g/m^2) was extracted in Daran province ecotype whereas the lowest (0.834 g/m^2) in Ardestan (Table 2). The highest γ-terpinene content (0.557 %) was obtained in Zawareh province ecotype and the lowest (0.244 %) in Golpayegan province ecotype (Table 2). The highest amount of γ-terpinene yield (1.423 g/m^2) was showed in Zawareh province ecotype and the lowest (0.76 g/m^2) in Daran province ecotype (Table 2). The essential oil was analysed by Burits and Bucar (2000) using GC-MS. Many components were characterized, but the major ones were thymoquinone (27.8%–57.0%), ρ-cymene (7.1%–15.5%), carvacrol (5.8%–11.6%), t-anethole (0.25%–2.3%), 4-terpineol (2.0%–6.6%) and longifoline (1.0%–8.0%).

Harami *et al*. (2010) reported the volatile oils from the seed of *Nigella sativa* were nine volatile oils were identified and 2-methyl-5(1-methyl ethyl)-Bicyclo [3.1.0] hex-2-ene was the major constituent (62.28%) while α-pinene was the minor (2.28%).

3.4. Regression analysis

Step-wise regression analysis for seed yield showed that the number of branches, γ-terpinene yield, days to full blooming and essential oil yield accounted for 75.2 %of variation exist in seed yield (Table 3). The γ-terpinene yield showed negative effect but other traits had positive effect on seed yield. The results obtained in our study showed that increase in γ-terpinene content might be decrease in seed yield amount.

The results obtained in regression analysis of essential oil yield showed that the plant height, days to beginning of blooming, grain filling rate, number of capsules and 1000-seed weight could be accounted for 78.9% variation of essential oil yield. The highest plant height and days to beginning of blooming had the maximum effect on this dependent variable. The plant height and grain filling rate regression coefficient was positive but other traits have the negative (Table 4).

Iqbal *et al*. (2010) reported the high genetic variability was recorded for plant height, days to first flower, days to 50% flowers, days to maturity, biomass, capsule weight, yield, 1000-seed weight and harvest index, whereas for other characters low to medium variability was observed. Iqbal *et al*. (2010) reported the high CV for biomass, grain yield and 1000 seed weight indicated the diverse nature of germplasm for these characters. Acceptance range of CV for most of the characters enhanced the validity of experiment. Based on breeding nature, some of these accessions might be mixture because this germplasm was mostly collected from market sources and better plants could be selected and bulked for developing superior cultivars. *Nigella sativa* L., is a new crop in the country and the first step for successful introduction is evaluation of diverse germplasm that not only helps to identify the superior genotypes, but selected genotypes could also be used in agronomic experiments (Shadia *et al.*, 1998). The 1000-seed weight is an important yield contributing traits in most of the crops as reported by Ghafoor & Arshad (2008) and Iqbal *et al*., (2003a), but negative association of 1000-seed weight with yield and its components is suggested to break through mutation, selective diallel or back cross method.

Richards (1996) reported the days to full flowering and head diameter have a negative correlation as well as direct and negative effect on seed yield. Thus, selection for the lowest amounts of this trait can increase the seed yield. In other words, it is possible that the choice of genotype that enter the generative stage sooner and also terminate the flowering stage faster, causes the plant to escape preventing it from encountering the heat of end of the season. This mechanism leads to enhancement of the yield in stress conditions of the end of season.

3.5. Path analysis

The results obtained in path analysis of seed yield showed that number of branches had the highest and positive direct effect on seed yield. Days to full blooming revealed the positive direct effect on seed yield. The γ-terpinene yield had negative direct effect on seed yield. Also, essential oil yield showed the negative direct effect on seed yield.

Therefore, the highest number of branches and the lowest amount of γ-terpinene yield could be cause of improvement seed yield in black cumin (Table 5).

The results obtained in path analysis of essential oil yield showed that plant height and grain filling rate had the highest positive direct effect on essential oil yield. Whitherward, the days to beginning of blooming have the highest negative direct effect on essential oil yield. Therefore, plant height and grain filling rate were recommended as the best indirect selection criteria to improve essential oil yield in ecotypes of black cumin. The trait 1000-seed weight although has negative direct effect on essential oil yield but its indirect effect on essential oil yield from other traits were considerable.

Notice that this genome-wide estimate of nucleotide diversity is obtained from a sample derived from a single diploid individually. Develops similar methods for estimating gametic disequilibrium as a function of genetic distance for a sample from a single diploid individual. He also extends that method to samples from a pair of individuals, and he describes how to estimate mutation rates by comparing sequences derived from individuals in mutation accumulation lines with consensus sequences (Lynch, 2008).

Table 1. Analysis of variance for the measured characteristics.

S.V	d.f	PH	NB	NC	DBB	DFB	SW	SFS	EP	EY	TC	TY	γ-TC	γ-TY
Block	2	1.43	37.43	1.43	55.03	5.70	0.11	32.53	0.054**	0.435**	0.035*	0.21	0.001	0.009
Ecotype	9	174.76**	425.72**	101.66**	114.13**	350.13**	1.85**	434.09**	0.117**	0.984**	0.051**	0.44**	0.034**	0.197**
Error	18	14.43	12.06	10.62	21.48	13.59	0.44	41.64	0.008	0.062	0.009	0.063	0.001	0.006

*,**: Significant at 0.05 and 0.01 probability levels, respectively, PH, (Plant height), NB, (Number of branches), NC, (Number of capsules), DBB, (Days to beginning of blooming), BFB, (Days to full blooming), SW, (1000-seed weight), SFS, (grain filling rate), EP, (Essential oil percent), EY, (Essential oil yield), TC, (Thymoquinone content), TY, (Thymoquinone yield), γ-TYC, (γ-Terpinene content) and γ-TY, (γ-Terpinene yield).

Table 2. Mean comparison for the measured characteristics.

	PH	NB	NC	DBB	DFB	SW	SFS	EP	EY	TC	TY	γ-TC	γ-TY
Meymeh	56^{cd}	173.3^{c}	66.6^{d}	168^{ab}	177.3^{b}	2.4^{b}	267.3^{d}	1.4^{cd}	3.47^{bc}	0.5^{bcd}	1.237^{bcd}	0.49^{bc}	1.212^{b}
Kashan	64.33^{b}	192^{ab}	76.3^{bc}	154^{d}	165^{d}	3.37^{ab}	275.6^{cd}	1.7^{a}	4.348^{a}	0.65^{ab}	1.662^{ab}	0.547^{ab}	1.398^{a}
Semirom	57^{cd}	189^{ab}	83.6^{a}	165.67^{abc}	193.3^{a}	4.33^{a}	293^{ab}	1.227^{e}	3.349^{c}	0.693^{a}	1.893^{a}	0.477^{c}	1.298^{ab}
Isfahan	52^{d}	174.3^{c}	73.6^{c}	170.67^{a}	187^{b}	4.47^{a}	283.3^{bc}	1.25^{de}	3.290^{c}	0.51^{bcd}	1.335^{bc}	0.387^{d}	1.012^{c}
Ardestan	63^{bc}	164^{d}	76^{bc}	164.67^{abc}	176^{c}	3.6^{ab}	265.6^{d}	1.33^{cde}	3.275^{c}	0.34^{d}	0.834^{d}	0.48^{c}	1.18^{b}
Daran	74^{a}	172^{c}	85^{a}	165.67^{abc}	193.67^{a}	2.73^{b}	294.6^{ab}	1.7^{a}	4.669^{a}	0.74^{a}	2.033^{a}	0.277^{e}	0.76^{d}
Fereydan	63^{bc}	192.6^{a}	72^{cd}	155.67^{d}	174.67^{c}	2.57^{b}	296^{a}	1.7^{a}	4.694^{a}	0.473^{bcd}	1.303^{bc}	0.437^{cd}	1.206^{b}
Khansar	58.67^{bcd}	158^{e}	76.6^{bc}	159^{cd}	185^{b}	3.27$_{ab}$	266^{d}	1.57^{ab}	3.857^{b}	0.463^{cd}	1.139^{cd}	0.497^{abc}	1.22^{b}
Golpayegan	53.33^{d}	174^{c}	83.6^{a}	161.3^{bcd}	173.3^{c}	4.3^{a}	287.3^{abc}	1.24^{de}	3.315^{c}	0.706^{a}	1.887^{a}	0.244^{e}	0.650^{d}
Zawareh	73.33^{a}	186^{b}	80^{ab}	172.67^{a}	198.67^{a}	2.67^{b}	275.6^{cd}	1.45^{bc}	3.708^{bc}	0.623^{abc}	1.594^{abc}	0.557$_{a}$	1.423^{a}

PH, (Plant height (cm)), NB, (Number of branches), NC, (Number of capsules), DBB, (Days to beginning of blooming), BFB, (Days to full blooming), SW, (1000-seed weight (g)), SFS, (grain filling rate (g/m^2/day)), EP, (Essential oil percent (%)), EY, (Essential oil yield (g/m^2)), TC, (Thymoquinone content (%)), TY, (Thymoquinone yield (g/m^2)), γ-TYC, (γ-Terpinene content (%)) and γ-TY, (γ-Terpinene yield (g/m^2)).

Table 3. Step-wise regression analysis of seed yield in black cumin ecotypes.

Traits	Regression coefficient	t-statistic	R^2
Number of branches	0.733	5.910**	23
Thymoquinone yield (g/m^2)	-34.029	-6.280**	64.1
Days to full blooming	0.324	2.276*	70.9
Essential oil yield (g/m^2)	4.660	2.081*	75.2
Intercept	92.101	3.053**	

Table 4. Step-wise regression analysis of essential oil yield in black cumin ecotypes

Traits	Regression coefficient	t-statistic	R^2
Plant height (cm)	0.035	3.882**	31.1
Days to beginning of blooming	-0.037	-4.626**	51.9
Grain filling rate (g/m^2/day)	0.024	5.005**	65.4
Number of capsules	-0.026	-2.377*	74.6
1000-seed weight (g)	-0.167	-2.197*	78.9
Intercept	3.513	1.789	

Table 5. Path analysis of seed yield as dependent variable in ecotypes of black cumin

Traits	Number of branches	γ-Terpinene yield (g/m^2)	Days to full blooming	Essential oil yield (g/m^2)	Total
Number of branches	<u>0.788</u>	-0.287	-0.026	0.062	0.538
γ-Terpinene yield (g/m^2)	0.308	<u>-0.733</u>	0.006	0.004	-0.413
Days to full blooming	-0.064	-0.016	<u>0.317</u>	-0.019	0.219
Essential oil yield (g/m^2)	0.275	-0.020	0.179	<u>-0.034</u>	0.402
Residual effects	0.360				

Table 6. Path analysis oil yield as dependent variable in ecotypes of black cumin.

Traits	Plant height (cm)	Days to beginning of blooming	Grain filling rate (g/m^2/day)	Number of capsules	1000-seed weight (g)	Total
Plant height (cm)	<u>0.548</u>	-0.032	0.044	-0.077	0.135	0.620
Days to beginning of blooming	0.034	<u>-0.500</u>	-0.046	-0.015	-0.014	-0.538
Grain filling rate (g/m^2/day)	0.054	0.050	<u>0.446</u>	-0.113	-0.037	0.402
Number of capsules	0.177	-0.030	0.212	<u>-0.236</u>	-0.082	0.043
1000-seed weight (g)	-0.345	-0.032	0.075	-0.090	<u>-0.216</u>	-0.607
Residual effects	0.301					

Recent findings indicated that some of the medicinal plant characteristics can be affected by genetic and ecological factors, including precipitation, temperature and plant competition. Since essential oils are the product of a predominantly biological process further studies are needed to evaluate if the reported characteristics of each population are maintained at the level of individual plants and along the breeding and selection program when grown under climatic conditions (Ghasemi Pirbalouti and Mohammadi, 2013).

4. Conclusion

According to the results of this project the optimum seed and oil yield of black cumin (*Nigella sativa* L.) were obtained at Fereydan and Daran province climatic conditions. Black cumin are cultivated under semi-arid climatic conditions of Isfahan regions of Iran. Therefore, differences among different Nigella seed yield and oil could be explained by various factors including seed genetic variations, oil processing differences, or different harvest dates.

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