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MORPHOMETRIC AND BIOCHEMICAL ASSESSMENT OF *NIGELLA* L. GENOTYPES OF EUROPEAN-ASIAN ORIGIN

V.I. NEMTINOV[1], Y.N. KOSTANCHUK[1*], S.M. MOTYLEVA[2], O.A. PEKHOVA[1], L.A. TIMASHEVA[1], V.S. PASHTETSKIY[1], and A.G. KATSKAYA[1]

[1]Federal State Budget Scientific Institution, Research Institute of Agriculture of Crimea, Simferopol, Russia
[2]Federal Horticultural Research Center for Breeding, Agrotechnology, and Nursery (FHRCBAN), Moscow, Russia
*Corresponding author's email: kostanchuk_yu@niishk.ru
Email addresses of co-authors: nemtinov@mail.ru, motyleva_svetlana@mail.ru, isocrimea@gmail.com, pvs98a@gmail.com, kackaja_a@niishk.ru

SUMMARY

A recent study in 2019-2020 assessed the productivity, the quality of fatty and essential oils, and the mineral composition of eight nigella genotypes at the Federal State Budget Scientific Institution (FSBSI), Research Institute of Agriculture of Crimea, Simferopol, Russia. Of the eight genotypes, two cultivars originated from Crimea, Russia (*Nigella sativa* cv. 'Krymchanka,' and *Nigella damascena* cv. 'Yalita'), and one each from six European-Asian countries, i.e., Dagestan, Uzbekistan, and Sweden (*Nigella sativa*), Pakistan and India (*Nigella indica*), and Belgium (*Nigella damascena*). The Russian nigella genotypes served as control. Genotypes from three European-Asian countries (Sweden, Pakistan, and Dagestan) distinguished from the rest by their highest seed productivity, i.e., 1.0-1.6 g plant^{-1}, which was 1.7-2.7 times higher than the control cultivar 'Krymchanka.' The seed productivity of the genotype *N. damascena* cv. 'Yalita' control was 1.5 times greater than the nigella genotype from Belgium. The *N. indica* produced the highest fatty oil content (29.9%), which exceeded two other species, i.e., *N. sativa* and *N. damascena* by 16%–22%. Fatty oils of nigella also contained essential oils of 0.5% for *N. sativa* and 1.2% for *N. damascena*. The essential oil of *N. sativa* contained dominant components, such as, p-cymene (53.5%) and thymoquinone (19.2%), while *N. damascena* contained p-cymol (82.2%) and other principal components. The identified samples with the maximum accumulation of fatty and essential oils, and macro and microelements can be used to treat and replenish the deficient elements in the human body. Nigella genotype samples exhibited high accumulation of microelements, viz., potassium, calcium, manganese, iron, copper, zinc, and molybdenum in the leaves and seeds.

Keywords: Nigella genotypes, leaves, seeds, fatty acids and essential oils, mineral elements, submicroscopic scanning, energy-dispersive X-ray diffraction analysis

Key findings: The latest study aimed to assess the productivity, the quality of fatty acids and essential oils, and the mineral composition of eight nigella genotypes. The identified samples with the maximum accumulation of fatty acids, essential oils, and macro and microelements can effectively treat and replenish the deficient elements in the human body.

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INTRODUCTION

The genus Nigella belongs to the family Ranunculaceae and unites about 20 species, wherein the seeds and young leaves of four species, i.e., *N. damascena* L., *N. sativa* L., grown in Europe, Transcaucasia, and Central Asia; *N. indica* L. grown in India, Afghanistan, and Pakistan; and *N. grandulifera* found in Turkmenistan and Western regions of China are mainly used for food and medicinal needs (Rybak *et al*., 1989; Popova and Litvinenko, 2008). With the spicy-aromatic properties of *N. sativa* L. and *N. damascena* L., their leaves and seeds are used for salads, bread, and cheese, and in fermenting cabbage, where adding a consortium of microorganisms improves the product fermentation (Posokina *et al*., 2018). It is also used for pickling cucumbers and watermelons, as well as, in perfumes and medicines.

In Russia, based on the raw material of Damascus nigella, the preparation "Nigedaza" has been registered and used for the treatment of the gastrointestinal tract (cholecystitis, enterocolitis, hepatitis, and pancreatitis). The seeds of nigella contain lipolytic enzymes, which help increase the raw material base needed for the said preparation (Orlovskaya, 2002). In Russia, the nigella seed is registered as a homeopathic remedy, and the pharmacopoeia of China regulates the quality of nigella glandular seeds. Past studies reported the biological properties of nigella seed oil, which has anti-sclerotic, vasodilating, antibacterial, and choleretic effects (Orlovskaya *et al.,* 2011). In the seeds of these species, the content of essential oil (0.92%) determines their high efficiency and consumption properties (Nemtinov, 2016). Nevertheless, through cold pressing of nigella seeds, the yield of fatty oil is 18%–20%, and the rest of the mass falls in the cake. Relatedly, studies can continue the following example of Shevtsov *et al*. (2020) as feed additives of nigella cake with phytobiotic properties to animals.

The given example used amaranth cake (Shevtsova *et al*., 2018) as a feed product, similarly, nigella seed meal can be a promising animal feeds additive. However, increasing the effective use of Damascus Nigella to interact with microbial preparations have not been studied. A report established that it has a positive effect on pre-sowing inoculation of seeds with microbial preparations (KMP) on seed yield and on increasing fatty and essential oils (Nemtinov *et al*., 2019a; Baranskaya *et al*., 2020). Researchers like Kulakova *et al*. (2005), Demin *et al.* (2010), and Stepychev and Fudko (2011) also evaluated the high biological activity of the lipids composition in the species, *N. sativa* and *N. damascena*. Still, studies to identify the useful properties in raw materials of various nigella types and their comparison has not been extensive.

Assessing the nutritional and medicinal properties of various nigella genotypes, the Pyatigorsk Medical and Pharmaceutical Institute has discovered 27 elements in the seeds of *N. damascena* (Mashirova, 2012). Most of them are known to be part of enzymes that participate in their activation, affect human health, and improve plant growth and development. The trace elements, Fe, Mn, Zn, and Cu enter the active center of the enzymes in redox reactions. Manganese acts as a reducing agent of enzymes that restore molecular oxygen. Zinc is part of the active center of more than 100 enzymes, particularly in RNA polymerase, carbonyl anhydrase, Cu, and Zn superoxide dismutase.

Zinc stabilizes the structure of molecules, in the metabolism of DNA and RNA, in protein synthesis, and in cell division to transmit a signal inside the cell. Interestingly, the identification of Zn concentrator plants that accumulate it can be used for the treatment and prevention of zinc deficiency in the human body. Deficiency of Fe, Mn, Zn, and Cu can lead to iron deficiency anemia, impaired development of bone tissues (Mn, Cu), decreased immunity and weakening of the antioxidant defense of the body (Zn), impaired brain function (Zn, Mn), and hematopoiesis (Cu). Medical studies confirmed the important role of trace elements in metabolic reactions and sub-molecular processes, the activity of which depends on the presence of certain micronutrients in the daily human diet (Avtsyn *et al.,* 1991; Kavita and Puneet, 2017; Motyleva *et al*., 2017).

Since there exists no comparative past data relating to the biochemical parameters of the seeds of different nigella genotypes, the latest study used samples from six countries of the world grown in Crimea. In particular, the purpose of the research work was a comparative morphometric biochemical assessment (fatty and essential oils) and the accumulation of minerals in eight nigella cultivars of European-Asian origin, including two from the FSBIS "Research Institute of Agriculture of Crimea" to determine the edible medicinal properties of leaves and seeds and for use in future breeding programs.

MATERIALS AND METHODS

Plant material and procedure

Research in 2019–2020 undertook experiments on eight nigella genotypes at the Federal State Budget Scientific Institution (FSBSI), Research Institute of Agriculture of Crimea, Simferopol, Russia on soil not contaminated with heavy metals. These eight genotypes comprised two cultivars originating from Crimea, Russia (*Nigella sativa* cv. 'Krymchanka' and *Nigella damascena* cv. 'Yalita'), and one each from six European-Asian countries, i.e., Dagestan, Uzbekistan, and Sweden (*Nigella sativa*), Pakistan and India (*Nigella indica*), and Belgium (*Nigella damascena*). The Russian nigella genotypes served as control. The soil characteristics included humus content (4.5%–5.3%), pH (7.8), and mineral content—N-NO_3 (6.3 mg/100 g), P_2O_5 (18.4 mg/100 g), and K_2O (73.0 mg/100 g). The temperature and rainfall are expressed as the average values for two years of research. i.e., May (16.2°C and 32.8 mm), June (22.4°C and 50.4 mm), and July (23.0°C and 27.8 mm). The experimental technology consisted of sowing seeds in the open ground according to common standards at 45 cm depth. The distance between plants was 8–10 cm, with drip irrigation. Mass flowering was observed in the nigella genotypes, i.e., *N. sativa* and *N. damascena* in 72 days, *N. indica* in 63 days, and seed maturation in 104 and 91 days, respectively.

Morphometric studies of nigella genotypes were carried out in a collection nursery in three repetitions on accounting plots with an area of 1.5–1.8 m^2. The fruits from which the seeds ripened were divided by size: large > 1 cm, medium from 0.8 to 1 cm, and small > 0.8 cm. The fertilizers application ratio consisted of 60:60:45 kg NPK ha^{-1} following EU Regulation 834/2007. Full doses of phosphorus and potassium, and 50% nitrogen were applied during early spring cultivation, before sowing the seeds, while the remaining 50% nitrogen was applied during intensive growth and flowering of nigella with 11% nitrogen fertilizer. Irrigation was carried out at 70% of the soil water capacity. Researchers assessed the various element contents assessed during the period of intensive growth of leaves and seeds after harvest.

Chemical analyses

The content and quality of fatty and essential oils were determined in the Department of Processing and Standardization of Essential Oil Raw Materials of the FSBIS Research Institute of Agriculture of Crimea. From nigella seeds, exhaustive extraction (Soxhlet) removed fatty oils, while hydrodistillation (Clevenger) took out the essential oil. The fatty acid composition of fatty oils and the chemical composition of essential oils were determined using the gas chromatographic method (GC) on an Agilent 7820A GC (Agilent Technologies, USA), equipped with a flame ionization detector and an HP-Innowax 30 mm × 0.25 mm × 0.25 μm (polyethylene glycol) capillary column. The analysis took place at a helium flow rate through the column: 1.36 ml/min, injector temperature (250°C), detector temperature (275°C), with the column temperature at 150°C for one min. The column temperature was then increased at the rate of 2.9°C/min to 250°C and incubated for three min. The volume of the analyzed sample was 1 μl. The identification of MEGCs identified MEJK, which was performed by the retention time at the separation of standard mixtures of these substances (AccuStandart, USA) and were estimated as the percentage of the total content weight relative to the internal standard.

Basic preparations

The research obtained data of the quantitative elemental composition from the Laboratory of Biochemistry and Physiology of the FHRCBAN. The original research was carried out using modern analytical equipment. The leaves and seeds of nigella samples were mineralized in a Naberterm muffle furnace (Germany) at t = 400°C. The resulting ash was dispersed by ultrasound at a frequency of 18 kHz for 15 min. An even layer of dispersant was applied to the slide table covered with carbon tape.

Energy dispersion spectrometry (EDS) analysis

The energy dispersion spectrometry (EDS) determined the chemical composition of the main ash components (K, Mn, Ca, Fe, Cu, Mo, and Zn) on an analytical scanning electron microscope JEOL JSM 6090 LA. The resolution of the microscope was 4 nm at an accelerating voltage of 20 kV (image of secondary electrons), scaling from × 10 to × 10000. During the elemental analysis, the working distance (WD) was 10 mm. The X-ray microanalysis data was placed on the table after weighing the correlation and mass percent (%).

Statistical analyses

The biochemical analysis repeatability was four-fold, and the results were expressed as an average value of ±standard deviation. The recorded data for various parameters were presented as mean values (n = 10) in standard error (S×), and coefficient of variation (CV). Statistical analyses were performed with an Excel package (Statistica v. 7.0). The treatments, which showed significant differences for drought-stress-dependent characteristics, were further determined by t-test.

RESULTS AND DISCUSSION

In 2017 and 2019, two cultivars were developed at the FSBSI-Research Institute of Agriculture of Crimea—*N. sativa* cv. 'Krymchanka' and *N. damascena* cv. 'Yalita' (Nemtinov and Glumova, 2017; Nemtinov *et al*., 2019b), which served and evaluated as a control in the study (Figure 1). The *N. sativa* has seeds in the fruit boxes, the leaflets of which are fused to the top. The seeds are triangular, ovoid, wrinkled, and black, with a spicy aroma and a burning, peppery taste.

Figure 1. Nigella samples from: a) Russia (cv. Krymchanka control), b) Dagestan, c) Uzbekistan, d) Sweden, e) Pakistan, f) India, g) Russia (cv. Yalita control), and h) Belgium.

Table 1. Morphometry of nigella genotypes from European-Asian countries, $\overline{X}$ 2019–2020.

No.	Country of origin	Linear dimensions of plants (cm)		Number of lateral shoots on the plant of different order (pcs)			
		Height	Diameter	Total	1st	2nd	3rd
		Nigella sativa					
1	Russia (cv. Krymchanka control)	56.7±1.7	21.3±0.9	38.6±1.2	10.0±0.6	24.3±0.9	4.3±0.3
2	Dagestan	47.7±0.9	20.3±0.9	38.0±0.9	8.0±0.6	24.7±0.9	5.3±0.3
3	Uzbekistan	27.0±0.6	15.7±1.5	18.0±0.6	2.0±0.6	4.7±0.3	3.0±0.6
4	Sweden	36.0±1.2	18.3±1.3	36.3±0.3	10.0±0.6	21.0±1.5	5.3±0.9
	$\overline{X} \pm S\overline{x}$	42.1±1.1	18.9±1.2	32.7±0.8	7.5±0.6	18.7±1.0	4.5±0.5
		Nigella indica					
5	Pakistan	37.7±1.2	14.0±0.6	29.6±0.9	6.3±0.9	19.0±0.6	4.3±0.3
6	India	27.7±0.3	10.3±0.9	19.3±1.2	5.0±0.6	12.0±1.2	2.3±0.3
	$\overline{X} \pm S\overline{x}$	32.7±0.8	12.2±0.8	24.4±1.1	5.6±0.6	15.5±0.9	3.3±0.3
		Nigella damascena					
7	Russia (cv. Yalita control)	27.0±0.6	14.0±1.0	18.0±0.6	2.0±0.6	13.0±0.3	3.0±0.6
8	Belgium	28.0±0.6	11.7±0.9	13.0±0.6	5.6±0.3	5.7±0.3	1.7±0.3
	$\overline{X} \pm S\overline{x}$	27.5±0.6	12.8±1.0	15.5±0.6	3.8±0.4	9.3±0.3	2.4±0.4

However, in *N. damascena*, the seeds are wedge-shaped, less often wedge-triangular, black, with a strawberry smell and spicy taste, and the fruit formed as five leaflets fused to the top. These control cultivars are represented in the genotypes of Eurasian countries.

The biological features and the state of morphometry of the gene pool of nigella origin from various countries got evaluated, i.e., the analysis of the height and diameter of the plants, and the load of shoots and fruits on the plants, which are the basis of productivity and yield. The assessment of the morphometry of *N. sativa* plants showed that the cultivar Krymchanka was 1.3 times higher in height than the average value (42.1±1.1) of three samples from different countries (Table 1). The genotype *N. indica's* (obtained from Pakistan) plant height was 10 cm higher than similar plants from India. Nonsignificant differences were found for *N. damascena* for plant height. The evaluation of the diameter (crown) of plants involves the use of technology in the scheme of their placement in the sowing area. In diameter, the cultivar Krymchanka was 1.1 times higher than the average value (18.9±1.2) of three samples of these botanical species *N. sativa* from European-Asian countries; the cultivar Yalita of *N. damascena* was also 1.2 times larger in plant diameter than the sample from Belgium, which must be considered when forming the density of sowing plants.

An increase in lateral shoots in favor of cultivars from Russia was notable on both types of nigella compared with the samples from European-Asian countries. Samples 1 to 8 formed the largest number of shoots from the second order against the first and third orders. But, more concerning the shoots of the 1st order: sowing of nigella 2.5 times; Indian at 2.8, and Damascus at 2.4 times when these exceeded the average values of the genotypes, i.e., 18.7±1.0, 15.5±0.9, and 9.3±0.3 of the various botanical species. The largest role in establishing the productivity of the genotypes of *N. sativa* plants was determined by large fruits, which correspond to the total number of fruits according to the genotype from Sweden (48.2%) and Dagestan (40.7%) with seed productivity of 1.0-1.6 g plant^{-1} (Table 2). The vital importance of large fruits was also noted in the genotype of *N. indica*, 65.7% of these were from Pakistan with seed productivity of 1.2 g plant^{-1}. Notably, the genotype *N. damascena*, 86.4%–88.2% corresponded to the total number of large fruits, but the productivity of plants due to the frailty of seeds was 2.5–2.7 times less.

For genotype *N. sativa* from Dagestan, only 36.1 fruits came out, comprising of large (14.7), medium (14.7), and small (6.7) pieces that corresponded to 100% (40.7%, 40.7%, and 18.6%). In the genotype from Sweden, it is lesser at 27.6 fruits, consisting of large (13.3), medium (8.3), and small (6.0) pieces,

Table 2. Characteristics of fruits and productivity of the nigella genotypes from European-Asian countries $\overline{X}$ 2019–2020.

No.	Country of origin	Number of fruits per plant (pcs)				Seed productivity from one plant (g)
		Total	Large	Medium	Small	
		Nigella sativa				
1	Russia (cv. Krymchanka control)	30.3±2.4	14.3±1.2	10.0±1.0	6.0±0.6	0.6±0.0
2	Dagestan	36.1±1.0	14.7±0.9	14.7±0.9	6.7±0.9	1.6±0.2
3	Uzbekistan	24.0±0.7	9.7±0.7	8.3±0.3	6.0±0.6	0.5±0.0
4	Sweden	27.6±0.9	13.3±0.9	8.3±0.3	6.0±0.6	1.0±0.1
	$\overline{X} \pm S\overline{x}$	29.5±1.2	13.0±1.0	10.3±0.6	6.2±0.7	1.0±0.1
		Nigella indica				
5	Pakistan	23.3±1.2	15.3±0.9	4.0±0.6	4.0±0.6	1.2±0.1
6	India	16.7±1.2	7.3±0.9	5.7±0.3	3.7±0.3	0.6±0.5
	$\overline{X} \pm S\overline{x}$	20.0±1.2	11.3±0.9	4.8±0.4	3.8±0.4	0.9±0.3
		Nigella damascena				
7	Russia (cv. Yalita control)	16.2±0.7	14.0±1.0	1.3±0.3	0.9±0.1	0.6±0.0
8	Belgium	12.8±0.3	11.7±0.9	0.6±0.1	0.5±0.1	0.4±0.1
	$\overline{X} \pm S\overline{x}$	14.5±0.5	12.8±1.0	1.0±0.2	0.7±0.1	0.5±0.05

which corresponded to 100% (48.2%, 30.0%, and 21.8%). The genotype *N. indica* from Pakistan had a similar distribution of fruits with a total of 22.3 fruits, comprising of large (15.3), medium (4.0), and small (4.0) pieces, which corresponded to 100% (65.6%, 17.2%, and 17.2%). Thus, the genotypes from three European-Asian countries— Sweden, Pakistan and Dagestan — gave the highest seed productivity, i.e., 1.0–1.6 g plant^{-1}, which was 1.7–2.7 times more than the control cultivar Krymchanka. The seed productivity of the control genotype *N. damascena* cv. 'Yalita' was 1.5 times greater than the sample obtained from Belgium.

According to chemical composition, the *N. sativa* cultivar includes steroids (six biologically active compounds, including cholesterol), alkaloids (three compounds, including nigerin), the enzyme lipase, essential and fatty oils, triterpene saponins, coumarins, flavonoids, phenol carbonic acids, amino acids, carbohydrates, proteins, minerals, glycoside melanin, bitters, tannins, and vitamins (Cheikh *et al*., 2007; Bourgou *et al*., 2008). Unsaturated fatty acids of nigella are highly biologically active in terms of fatty and essential oils content. Linoleic acid stabilizes cell membranes, eicosenoic and eicosadienoic acids are precursors of prostaglandins, which prevent the development of inflammatory processes in the body (Mahmmoud and Christensen, 2011).

The study determined that out of four samples of *N. sativa*, the fatty oil in seeds from Dagestan exceeded the control cultivar Krymchanka by 17.3% (Table 3). An increase of 15.9% was also noted concerning the average value of the nigella genotype sowing (25.21 ± 1.3). In *N. indica* seeds, the average index for fatty oil (28.50 ±1.4) was also 13.4%−15.6% higher than samples of *N. sativa* from Russia, Uzbekistan, and Sweden. The seeds of *N. indica* provided the highest fatty oil (29.9%) as observed, which exceeded other species, i.e., *N. sativa* and *N. damascena* by 16%-22%. A correlation was observed between the mass fraction of fatty oil and the highest seed productivity in the genotypes, i.e., from Dagestan r = -0.5 (medium), from Pakistan r = 0.9 (strong), and from Sweden r = 0.6 (medium).

According to Dehkordi and Kamkhah (2008) and Bamosa *et al*. (2010), the nigella seed oil exhibits hypoglycemic activity, reduces cholesterol concentration, reduces blood pressure, and increases respiratory rate, which got confirmed by testing nigella seed extracts on rats. Nigella oil is a diuretic, mild laxative, and immunostimulant agent (acts on the thymus glands by stimulating them). It optimizes the work of the intestines by eliminating the phenomena of dysbiosis, prevents obesity, promotes weight loss, serves as an antidote to alcohol, and helps to increase appetite. It is used in cases of mental illnesses or for insomnia (Taşkin *et al*., 2005).

Results of the determination of the fatty acid composition of oils in genotypes of *N. sativa* and other European and Asian origins have shown that the composition of fatty oils is dominated by the following acids, i.e., palmitic (10.56%−11.20%), oleic (17.43%−26.94%), and linoleic (44.54%−57.89%); covering

Table 3. The mass fraction of fatty oil in the seeds of nigella genotypes of European-Asian origin.

No.	Country of origin	Repetitions				Average
		1	2	3	4	
	Nigella sativa					
1	Russia (cv. Krymchanka control)	25.20	23.81	24.12	25.63	24.69±0.43
2	Dagestan	29.08	28.65	28.31	29.84	28.97±0.33
3	Uzbekistan	23.70	24.06 21.62	23.01	21.62	23.10±0.54
4	Sweden	23.88	24.21	24.82	23.33	24.06±0.31
	$\overline{X} \pm S\overline{x}$	25.47±1.25	25.18±1.16	25.07±1.14	25.11±1.78	25.21±1.30
	Nigella indica					
5	Pakistan	26.84	27.32	27.45	26.79	27.10±0.17
6	India	31.15	29.54	30.00	28.91	29.90±0.47
	$\overline{X} \pm S\overline{x}$	29.00±2.16	28.43±1.11	28.73±1.28	27.85±1.06	28.50±1.40
	Nigella damascena					
7	Russia (cv. Yalita control)	23.62	22.75	23.00	23.31	23.17±0.19

Table 4. The mass fraction of fatty acids in the seed oil of nigella genotypes of European-Asian origin (%, to the sum of fatty acids).

Fatty acids	*Nigella sativa*					*Nigella indica*			*Nigella damascena* Russia (Yalita)
	Russia (Krym-chanka)	Dagestan	Uzbekistan	Sweden	Average $\overline{X} \pm S\overline{x}$	Pakistan	India	Average $\overline{X} \pm S\overline{x}$	
Palmitic $C_{16:0}$	6.69	11.48	12.06	12.00	10.56±1.30	10.91	11.48	11.2±0.29	10.86
Palmitoleic $C_{16:1}$	0.25	0.16	0.30	0.26	0.24±0.03	0.18	0.20	0.19±0.01	0.21
Stearic $C_{18:0}$	3.75	1.49	1.64	2.18	2.27±0.52	2.49	2.42	2.46±0.04	2.85
Oleic $C_{18:1}$ (omega-9)/	15.99	20.52	17.73	15.47	17.43±1.14	19.98	22.35	21.17±1.19	26.94
Linoleic $C_{18:2}$(ω−6)	46.50	55.32	56.07	57.89	53.95±2.54	56.36	57.03	56.7±0.34	44.54
Linolenic $C_{18.3}$(ω−3)	0.06	0.16	0.11	0.17	0.13±0.03	0.17	0.16	0.17±0.01	0.80
Gondoinic $C_{20:1}$	0.90	0.33	0.31	0.37	0.48±0.14	0.35	0.17	0.26±0.09	0.47
Amount	74.14	89.46	88.22	88.34	85.06±3.64	90.44	93.81	92.13±1.69	86.67

81.9% to 89.1% of the total content of fatty acids, while other components were present in trace amounts (Table 4). As for the *N. indica* genotype, the above three most important fatty acids prevailed on average over the genotypes of *N. sativa* and *N. damascena* in palmitic acid by 5.7% and 3.0% and oleic acid by 17.6%, where *N. damascena* contained it by 27.4% more. Linoleic acid in the *N. indica* genotype also exceeded the samples of *N. sativa* and *N. damascena* by 4.3% and 21.4%. Markedly, the fatty oil of two nigella types (*N. sativa* and *N. damascena*) contains eicosadienoic acid that varies between 2.3%–2.6%. This component can be considered one of the markers of authenticity of oils from black cumin seeds (nigella) (Goryainov *et al*., 2020). The sum of fatty acids of the genotypes ranked as, i.e., 93.8 > 90.4 > 89.5 > 88.3 - 88.2 > 86.6 > 74.1 (obtained from *N. indica*, *N. damascena,* and *N. sativa*). For comparison, the fatty oil of milk thistle contains palmitic acid (8%), oleic acid (26%), and linoleic acid (53%) of the total amount (Kleymenova, 2020).

Several researchers determined the effectiveness of nigella essential oil and its active component, thymoquinone, as an anti-inflammatory and analgesic agent, which protects against asthma attacks, bronchitis, and cough, and as a natural antioxidant (Kanter *et al*., 2005; Abdalbasit *et al*., 2009; Ravindran *et al*., 2010; Al-Ghamdi, 2011). It has been established that the anti-inflammatory effect of the oil is also due to the antioxidant properties of p-cimene and limonene. Budantsev and Lesiovskaya (2001), Cheikh *et al*. (2007), and Parhizkar *et al*. (2011) determined that the alcohol extract of nigella seeds has an estrogenic, antispasmodic effect, and a choleretic effect, which is significant in fat metabolism and detoxification. The hepatoprotective effect is based on the antioxidant properties of thymoquinone and dithymoquinone (Kanter *et al*., 2005; Bamosa *et al*., 2010). In Turkey, nigella essential oil is used as an antibacterial and antifungal agent (Mashirova, 2012). In particular, the cream with nigella oil "Baraka" is recommended for skin care against dermatitis, eczema, and psoriasis, and relieves allergic reactions. According to Budantsev and Lesiovskaya (2001) the essential oil is also an antiviral agent.

The chromatography conditions experimentally determined the chemical composition of nigella essential oils. Nigella fatty oils obtained by cold pressing contain essential oils of 0.5% (*N. sativa*) and 1.2% (*N. damascena*). Table 5 presents the results of chromatographic analysis of essential oils extracted from the fatty oil of two types of nigella by hydrodistillation method. From the given data of said table, the chemical composition of the essential oils of the two types of nigella differs. Thus, in *N. sativa* essential oil, the dominant components consisted of p-cimene (53.49%) and thymoquinone (19.24%), with no terpinen-4-ol. The main component of *N. damascena* essential oil is p-cymol, whose content is 82.2%. Some indicators of *N. damascena* exceeded the components of *N. sativa* essential oil, in limonene by 5.2 times, γ -terpinene by 1.6, and p-cimene by 1.5 times, where terpinen -4-ol was found in a fraction of 1.85%.

Table 5. Analysis of the chemical composition of essential oils extracted from fatty oils.

Component name	Mass fraction of essential oil component, %	
	N. sativa (Krymchanka cultivar)	*N. damascena* (Yalita cultivar)
A-pinene	4.75	-
Camphene	2.36	1.54
B-pinene	0.04	-
Limonene	0.35	1.81
Linalool	2.91	-
Г-terpinene	1.17	1.89
terpinen-4-ol	-	1.85
r-cimen (r-cimol)	53.49	82.2
Thymoquinone	19.24	-

In addition to biologically active compounds of fatty and essential oils, a treasure of macro and microelements has been found in the ash of leaves and seeds, many of which are part of enzymes and participate in their activation, affecting human health, and improving plant growth and development. The features of the accumulation of trace elements depend on many factors, including specific plant genes (Kabata-Pendías and Pendías, 2000). The trace elements, i.e., K, Ca, Mn, Fe, Cu, Zn, and Mo, and their accumulation have been studied in various onion genotypes in the Crimea (Nemtinov *et al*., 2020). Under the same conditions, the accumulation of these elements in nigella genotypes are studied as follows.

Potassium (K) is necessary for muscle contractions, normalization of the heart muscle, and activity of nerve cells, osmotic blood concentration, acid-base, and water balance. It controls the transmembrane potential of osmotic pressure, the cathode-anion balance, and the pH of cell hemostasis. In ionic form, potassium increases the concentration of other ions and is found in all organs of the human body (Meathnis *et al*., 1997). The highest amount of potassium (26.5–23.5 wt. %) was accumulated in the leaves of two genotypes of *N. sativa* and *N. damascena* belonging to Uzbekistan and Belgium, with an average correlation, *r* = -0.4 and 0.7. However, the accumulation of potassium in the seeds (13.1–14.2 wt. %) was greater in the samples obtained from Uzbekistan, Sweden, and Belgium. The remaining samples were characterized by medium and low potassium content (Table 6).

Most of the human skeleton and teeth consist of calcium (Ca). Calcium ions are involved in blood clotting processes, as well as, in providing constant osmotic pressure. It participates in the processes of cell growth and development, is part of enzymes, and affects metabolism and immunity (Gins and Gins, 2011; Gins *et al*., 2018). The range of 13.9...16.4 wt. % of calcium accumulated in leaf ash was more in two nigella samples from

Table 6. Chemical elements in leaves and seeds of nigella genotypes of European-Asian origin (wt. %).

Elements		*Nigella sativa*				*Nigella indica*		*Nigella damascena*	
Symbols	Designations	Russia (Krymchanka cultivar)	Dagestan	Uzbekistan	Sweden	Pakistan	India	Russia (Yalita cultivar)	Belgium
	L	15.0	11.6	26.5	5.3	6.2	11.5	14.4	23.5
K	C	11.6	10.9	13.1	13.2	12.1	12.0	12.8	14.2
	r	0.5	0.2	-0.4	0.4	0.9	-0.01	0.9	0.7
	L	11.9	12.5	5.9	8.8	13.9	7.2	16.4	6.0
Ca	C	9.4	11.4	11.1	8.4	12.6	13.1	11.2	10.8
	r	0.2	-0.1	-0.4	-0.4	0.8	0.1	0.4	0.2
	L	0.2	*	0.3	*	*	0.4	0.1	0.7
Mn	C	0.2	0.4	0.04	0.4	0.5	*	*	0.2
	r	0.8	–	-1	–	–	–	–	1
	L	0.5	0.5	0.3	0.5	0.4	1.3	*	0.9
Fe	C	*	1.0	*	0.3	1.0	0.5	*	1.3
	r	-	1	–	-0.3	-0.9	0.1	–	-0.3
	L	0.2	0.7	1.4	0.1	1.4	0.5	0.9	0.2
Cu	C	0.4	0.5	0.5	0.4	0.4	0.4	0.4	0.1
	r	0.9	0.3	-0.1	0.1	-0.8	-0.3	0.3	0.4
	L	1.5	2.8	2.0	0.7	5.4	1.3	6.1	2.6
Zn	C	1.6	1.3	1.6	1.2	1.4	1.2	1.3	0.3
	r	0.9	-0.5	0.6	0.1	-0.5	-0.9	0.6	-0.3
	L	4.5	2.6	5.8	2.4	1.9	2.5	4.4	2.1
Mo	C	3.5	2.7	3.3	2.3	4.0	2.3	2.4	2.4
	r	0.8	-0.7	-0.2	-0.3	-0.5	-0.8	0.9	-0.8

Note: *: Uneven distribution of elements, L: Leaves, C: Seeds, r: Correlation

Pakistan and Russia cv. Yalita at r = 0.8 and 0.4 in leaves and seeds. A high accumulation of Ca (range of 12.6...13.1 wt. %) was observed in the seed ash in two samples from Pakistan and India at r = 0.8 and 0.1 in the leaves and seeds. The remaining calcium indicators gave medium and low values.

Manganese (Mn) participates in immune reactions, blood circulation, and cell respiration, in the regulation of carbohydrate and lipid metabolism, and supports reproductive function. It is necessary for growth, affects the skeleton development, and participates in the process of osteogenesis. The daily requirement of an adult for Mn is 2-10 mg. It is found in legumes, nuts, and vegetables, including lettuce and sea products. In the ash of nigella leaves, genotype one from Belgium showed an increased accumulation of Mn (0.7 wt. %) (Table 6). The high accumulation of Mn in seeds included three samples with a content (0.4–0.5 wt. %) obtained from Dagestan, Sweden, and Pakistan. The correlation between leaves and seeds was high in samples from Russia cv. Krymchanka, Uzbekistan, and Belgium (r = 0.8...-1 and 1). The remaining manganese indicators showed medium and low values.

Iron (Fe), as a main active element of blood hemoglobin, is part of the enzymes of other cells, and catalyzes the respiration processes in them. Organic Fe is an essential compound in the body and is part of many redox enzymes. In the ash of nigella leaves, two genotypes had an increased accumulation of Fe–from India and Belgium (0.9–1.3 wt. %). The increased accumulation of Fe in seeds included three samples with a content of 1.0–1.3 wt. %: from Dagestan, Pakistan, and Belgium. The correlation between their leaves and seeds was recorded as direct and high (r = 1 and -0.9) and medium (r = -0.3). The remaining iron indicators exhibited medium and low values.

Copper (Cu) is a vital irreplaceable trace element in human metabolism, as it is associated with enzymes, hormones, and vitamins (Fraga, 2005). According to the US Institute of Medicine, and the Scientific Committee on Food Products of the European Union, the daily Cu requirement of an adult is 1-1.5 mg. Copper carries out the biological mechanism of enzyme biocatalysis, electron transfer, and interaction with Fe. It participates in the formation of generative organs and hemoglobin, and in the growth and development as a part of melanin (Avtsyn *et al*., 1991). The highest accumulation of Cu (0.9–1.4 wt. %) was observed in the leaves of three samples of nigella from Uzbekistan, Pakistan, and Russia cv. Yalita. From seed samples, seven of the nigella isolated from the genotypes of *N. sativa* and *N. indica,* and the genotype *N. damascena* from Russia cv. Yalita produced the highest accumulation of Cu (range of 0.4...0.5 wt. %). The leaves-seeds correlation was high (*r* = 0.9 and -0.8) in samples from Russia cv. Krymchanka and Pakistan, medium (*r* = -0.3...0.4) in samples from Dagestan, India, Russia cv. Yalita, and Belgium, and very low (*r* = -0.1...0.1) in samples from Uzbekistan and Sweden. The remaining copper indicators revealed medium and low values.

Zinc (Zn) stabilizes the structure of molecules and plays an important role in the DNA and RNA metabolism, protein synthesis, cell division, and the process of signal transmission inside the cell (Nechaev *et al*., 2007). Noteworthy is the fact of the identification of concentrator plants, and even over-concentrators, which accumulate trace elements and can be used for the treatment and prevention of zinc deficiency in the human body. In the latest study, the leaves of two nigella samples of *N. indica* and *N. damascena* from Pakistan and Russia cv. Yalita accumulated a high content of Zn (range of 5.4...6.1 wt. %). The highest accumulation of Zn in seeds (range of 1.2...1.6 wt. %) was observed in seven isolated nigella samples, i.e., four of these belong to the *N. sativa* genotype, two from *N. indica*, and one from *N. damascena* cv. Yalita from Russia. The leaves-seeds correlation was high (*r* = 0.9 and -0.9) in samples from Russia cv. Krymchanka and India, medium (*r* = -0.5) in samples from Dagestan and Pakistan, and also medium (*r* = 0.6) in samples from Uzbekistan and Russia cv. Yalita. The remaining zinc indicators got marked with medium and low values.

Molybdenum (Mo) provides mechanisms of enzyme catalysis, as well as, electron transfer, and participates in the synthesis of amino acids, and in the exchange of vitamins C, E, and B12 (Avtsyn *et al.*, 1991). The daily human demand for Mo is 0.5 mg. Molybdenum accumulates mainly in the liver, kidneys, endocrine glands, and skin. The highest accumulation of Mo in leaves (4.4–5.8 wt. %) was found in three samples of nigella with the highest value from Uzbekistan. For high Mo (3.3–4.0 wt. %) accumulation in seeds, three samples were isolated from *N. sativa* from Russia cv. Krymchanka, Uzbekistan, and *N. indica* from Pakistan. The leaves-seeds correlation was medium and high (*r* = -0.7–0.8 and 0.8–0.9) in five nigella samples obtained from Dagestan, India, Belgium, and Russian cultivars Krymchanka and Yalita, medium (*r* = 0.5–0.3) for samples from Pakistan and Sweden, while low (*r* = -0.2) for a sample from Uzbekistan. The remaining molybdenum indicators displayed medium and low values.

CONCLUSIONS

Nigella sativa L. samples with the concentrator onion cultivars and other genotypes from European-Asian countries were studied—concentrators with the greatest accumulation of fatty and essential oils in seeds, and macro- and microelements in leaves and seeds. The studied samples of *N. sativa* from Dagestan and *N. indi*ca from India come highly recommended for manufacturing fatty and essential oils for medicinal and nutritional purposes. The identified genotypes need more utilization to further breeding programs and prevent the deficiency of elements in the human body. The study recorded the following number of nigella samples with the highest accumulation of elements in leaves and seeds: for K (two and two), Ca (two and two), Mn (one and three), Fe (two and three), Cu (one and seven), Zn (two and seven), and Mo (three and three).

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