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Genetic diversity in fatty acid composition and antioxidant capacity of *Nigella sativa* L. genotypes

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ABSTRACT

A study was conducted to explore the suitability of *Nigella sativa* L. oil for human consumption on the basis of fatty acid (FA) composition in twenty three selected genotypes. Total oil content ranged from 147 to 270 ml/kg. Eleven fatty acids including palmitic, oleic, and linoleic acids were found during fatty acid methyl esters (FAME) analysis. Linoleic acid was major contributor in the range of 608.9 ml/l (AN-15) to 713.9 ml/l (AN-8). Saturated fatty acids ranged from 121 to 181 ml/l in genotype AN-23 and AN-3, respectively. Significant genetic variation was observed with respect to mono-unsaturated fatty acids. Poly-unsaturated fatty acid being dominant existed in the range of 651–771 ml/l. Metabolic capacity to inter-conversion of fatty acids and nutritive value of *Nigella* oil is described on the basis of various fatty acid ratios. Multivariate analysis revealed that, oil content has positive correlation with linoleic acid. The Euclidean based clustering revealed, that genotypes AN-23, AN-5, AN-8, AN-9, AN-10, AN-11, AN-19, AN-21 and AN-24 are suitable for trait specific breeding programme for higher oil content, increased linolenic and reduced palmitic acid with higher nutritive value. Genotype AN-4 exhibited good combination of higher polyunsaturated fatty acids (PUFA) as well as oxidative stability.

1. Introduction

Plants seed are major sources of oils that have nutritional, industrial and pharmaceutical importance. Fatty acids (FAs) composition is an important indicator for suitability of oil for a particular purpose. Interest in the specific fatty acids composition for ideal edible oils has been emerging with the growing scientific views, that all fats are not equivalent with regard to consumer health. Till date seed oil of several thousand plant species have been chemically analysed and a few of them are taken into cultivation as an oil crop. The variation in fatty acids composition within a species also proved to be useful in chemotaxonomy and phylogenetic studies (Velasco & Goffman, 2000). Exploitation of lesser known crops is a possible solution for growing and diversified nutritional needs of growing population. Black cumin (*Nigella sativa* L.) commonly known as *Nigella*, belongs to the family *Ranunculaceae,* is a well known herb cultivated from the Southern and Eastern-rim of the Mediterranean basin to Iran, Pakistan, and India. *Nigella* seeds are rich in nutritional values and are used as seasoning and flavoring of food, bread, pickles and bakery products. It is important for both oil and bioactive compounds. Studies have shown a wide spectrum of properties curing various diseases (Dubey, Singh, Mishra, Kant, & Solanki, 2016; Iqbal et al., 2011). The seeds as whole or their extracts have antitumor (Khan, Sharma, & Sultana, 2009), antidiabetics (Fararh, Atoji, Shimizu, & Takewaki, 2008), antibacterial, antiviral (Edris, 2007; Mashhadian & Rakhshandeh, 2005), antioxidant, galactagogue, carminative, laxative and antiparasitic properties (Kanter et al., 2003). The oil of *Nigella* possesses phenolic, flavonoids and other related compounds which increased its antioxidant activity (Badary, Taha, Ayman, El-Din, & Abdel-Wahab, 2003; Kruk, Michalska, & Klanda, 2000) and is considered highly prized nutritive oil. Inspite of the above facts, *Nigella* seed oil does not really have a significant economic market share (Padhye, Banerjee, Ahmad, Mohammad, & Sarkar, 2008).

Oil seed crops rich in essential polyunsaturated fatty acids (PUFA) can be more economic alternatives compared to traditional crops. Linoleic acid (LA) is one of the important PUFA, referred as essential fatty acid (EFA) and recognized as essential biochemical

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components of human diet (Gomez, Bermejo, & Kohen, 2011). Though, sufficient work has been done on medicinal properties of *Nigella* seeds, there are meagre reports on extent of genotypic variability in cultivated genotypes of *Nigella* in India with reference to fatty acid composition. To formulate an effective breeding programme for desirable fatty acid composition, information regarding heritability, genetic advance and correlation among fatty acids is a pre requisite. Hence, the present study has been conducted to explore the genetic variation in selected genotypes of *N. sativa* based on analysis of oil composition, antioxidant potential and its suitability for human consumption by blending with other food oils.

2. Materials and methods

2.1. Plant material

Seeds of twenty three genotypes of *Nigella* were collected from different *Nigella* growing parts of India. Crop has been raised in farm of ICAR-NRCSS, Ajmer (26° 27′ 0″ N, 74° 38′ 24″ E), India during *rabi* season (winter season which commences from November first week up to February) of year 2012–13 and 2013–14 under standard package and practices. A detailed description of the materials used in this study is shown in Table 1.

2.2. Chemicals

All reagents and fatty acid standards were procured from Sigma-Aldrich,St. Louis, Missouri, USA and were analytical or HPLC grade.

2.3. Oil extraction

The *Nigella* seeds were ground in a grinder before oil extraction. Twenty gram ground seeds were homogenized and oil was extracted with 150 ml of *n*-hexane following the procedure of Soxhlet extraction. Six extraction cycles were performed for maximum recovery of oil. Each cycle took 1 h, thus total duration of extraction was 6 h. The oil extract was concentrated under vacuum in a rotary evaporator (JSGW, Amballa Cantt, India) at 35 °C. Solvent free oil was weighed to determine the oil content and then transferred to amber coloured glass vials and stored at −20 °C until further analysis.

Table 1
List of *Nigella* genotypes used in this study.

Genotype	Location
AN-1	NRCSS, Tabiji, Ajmer, Rajasthan
AN-2	Beawar, Ajmer, Rajasthan
AN-3	Kekri, Ajmer, Rajasthan
AN-4	Pisangan, Ajmer, Rajasthan
AN-5	Pushkar, Ajmer, Rajasthan
AN-6	Digod, Kota, Rajasthan
AN-7	Anta, Kota, Rajasthan
AN-8	Local market, Jaipur, Rajasthan
AN-9	Local market, Nagaur, Rajasthan
AN-10	Phalodi, Jodhpur, Rajasthan
AN-11	Masodha, Faizabad, Uttar Pradesh
AN-13	Kumargang, Faizabad, Uttar Pradesh
AN-14	Kanpur, Uttar Pradesh
AN-15	Chaubepur, Kanpur, Uttar Pradesh
AN-16	Ludhiana, Punjab
AN-17	Mandsaur, Madhya Pradesh
AN-18	Neemuch, Madhya Pradesh
AN-19	Ratlam, Madhya Pradesh
AN-20	Sangod, Kota
AN-21	Digod, Kota
AN-22	Banaskantha, Gujarat
AN-23	Pushkar, Ajmer, Rajasthan
AN-24	Ranakpur, Gujarat

2.4. FAME analysis

Fatty Acid Methyl Esters (FAME) were prepared according to AOCS Method CE 1–62. Diluted FAME were separated on an Agilent Series GC-MS (Agilent Technologies, Santa Clara, California, USA; GC -7820 A, MS-5975) equipped with an HP5 (Universal column) (30 m × 0.325 mm x 0.25 μm); Agilent J&W GC column with an auto sampler. A sample of 1 μl was used in split mode (20:1) with an auto sampler. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The column temperature was programmed from 50 °C to 280 °C with equilibrium time of 3 min, held for 30 min. Injector temperatures were set at 250 °C. The fatty acids were identified by a comparison of their retention indices and their identification was confirmed by computer matching of their mass spectral fragmentation patterns of compounds in the NIST-MS library and published mass spectra with the help of Chemtation software (Agilent Technologies, Santa Clara, California, USA).

2.5. Estimation of phenolics and antioxidant capacity

Total phenol concentrations were determined using a Folin-Ciocalteu assay, as described by Amin, Norazaidah, and Hainida (2006). An aliquot of 0.1 ml from extracted oil was taken in a test tube and made the volume 1 ml by adding *n*-hexane solvent. Three ml of 100 g/l sodium carbonate was added. Previously 10-fold diluted Folin-Ciocalteu reagent was added to the mixture. The mixture was allowed to stand at room temperature for 90 min and then absorbance was measured at 710 nm. Gallic acid was used as the standard phenol. The amount of phenolic content was calculated by using the standard curve of Gallic acid having R^2 (a statistical measure of how close the data are to the fitted regression line) value ranged from 0.96 to 0.99 and was expressed as μg Gallic Acid Equivalents/ml oil (GAE/ml).

Total flavonoid concentration was determined by using previously reported method by Chang, Yang, Wen, and Chern (2002). An aliquot of 0.1 ml from extracted oil was taken in a test tube and 100 μl aluminium chloride (1 mol/l) solution was added carefully from the side wall of the test tube followed by addition of 100 μl potassium acetate. The total volume was made 4 ml by adding 2.8 ml of *n*-hexane solvent in the test tube. After 30 min incubation of reaction mixture at room temperature, stable yellow colour was developed. Absorbance was measured at 415 nm. Quercetin was used as the standard flavonoids. The amount of flavonoid was calculated by using the standard curve of quercetin having R^2 value ranging from 0.96 to 0.99 and was expressed as μg Quercetin Equivalents/ml (QE/ml) extracted oil.

The antioxidant activity of oil extract was evaluated on the basis of its activity in scavenging the stable DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) radical using the method described by Shimada, Fujikawa, Yahara, and Nakamura (1992). Oil extract was diluted in methanol to give at least 5 different concentrations. An aliquot (1, 1.5, 2, 2.5 ml) of the oil extract of each concentration was mixed with 1 ml of 1 mol/l DPPH solution. The mixture was then homogenized and left to stand for 30 min in the dark. The absorbance was measured at 517 nm against a blank of methanol using a spectrophotometer. DPPH solution plus methanol was used as control and Butyl hydroxyl toluene (BHT) was used as a standard reference synthetic antioxidant with R^2 value ranging from 0.95 to 0.99. Results were expressed as μg Butyl hydroxyl toluene (BHT) Equivalent/ml oil. Results were expressed as a mean ± standard deviation from three replicate measurements. The percent scavenging effect (capacity of scavenging DPPH free radicals) was