Sowing window, plant density, nitrogen and phosphorus influence fatty acid profile of *Nigella sativa* L.

K. Giridhar[1,*], G. Sathyanarayana Reddy[2], S. Surya Kumari[1], A. Lalitha Kumari[3] and A. Sivasankar[4] and Praduman Yadav[5]
[1]Dr. Y.S.R. Horticultural University, AICRP on Spices, Guntur, Andhra Pradesh, India.
[2]Sri Konda Lakshman Telangana State Horticultural University, Rajendranagar, Hyderabad, India - 500030
[3]Regional Research Unit (SSAC), Regional Agricultural Research Station, ANGRAU, Lam, Guntur, India - 522034
[4]Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad, India - 500030
[5]Directorate of Oilseeds Research, Rajendranagar, Hyderabad, India - 500030
*gkalidasu@yahoo.com

Abstract

Nigella (*Nigella sativa* L.) belongs to the family *Ranunculaceae*, the butter cup family. The plant is regarded highly in unani, ayurveda, siddha and other ethnomedicine systems. The pressed seed oil is used as topical and oral medical applications. In two separate studies conducted, the changes in the fatty acid profile of nigella seed were determined at a varied sowing window (staggered by 15 days from 1st fortnight of October to 1st fortnight of December), plant densities (50, 33.3 and 25 plants m^{-2}), graded levels of nitrogen (0 to 60 kg ha^{-1}) and phosphorus (0 to 45 kg ha^{-1}). The experiment was conducted with cv. Ajmer Nigella-1 in vertisols of Andhra Pradesh, India. The fatty acid composition of the composite sample of each treatment was assayed. Irrespective of the sowing window and plant densities, linoleic acid content was highest (52.44 to 58.4 %) followed by oleic acid (23.51 to 30.70 %), palmitic acid (10.93 to 12.57 %), stearic acid (2.55 to 3.15 %) and eicosatrienoic acid (2.12 to 2.96 %). The biplot analysis revealed positive correlation between the pairs – oleic acid and stearic acid and palmitic acid and eicosatrienoic acid. The discriminant analysis revealed that fatty acid profiles of the crop differed with varying sowing window. Linoleic acid production was favoured in the crop sown during October but it was discouraged in the crop sown between 2nd fortnight of November and 1st fortnight of December. The fatty acid profile of the plant densities 50 and 33.3 plants m^{-2} was similar and favoured oleic acid production with associated decrease in linoleic acid content. In fertilizer treatments, irrespective of the treatments, linoleic acid content was highest (55.60 % to 58.30 %) followed by oleic acid (24.47 to 27.32 %), palmitic acid (11.38 to 12.39 %), stearic acid (2.66 to 2.94 %) and eicosatrienoic acid (2.33 to 2.85 %). Correlations among fatty acids were similar to the sowing window and plant densities. The biplot clearly depicted the association between production of linoleic acid and low levels of N application in the present test environment.

Introduction

The genus *Nigella*, which belongs to the butter cup family (Ranunculaceae), consists 14 annual plant species distributed from the Mediterranean regions to West Asia, but some species transcended most of Europe, northern and southern Africa, western and eastern Asia. The genus embraces several taxa of ethnopharmacological importance, the herbs and seeds used in an extensive array of medicinal systems. Among these, *Nigella sativa* L., which is commonly known as black cumin or nigella acquired the status of industrial crop due to development of several herbal drugs of commercial interest. It is believed to have originated in the Mediterranean region and subsequently spread to Europe, Asia and Africa (Zohary *et al.*, 2012). The use of vernacular names in different countries shows prevalent use of nigella in more than 100 countries. The crop is known by many common names viz. black-caraway, black-cumin, fennel-flower, nigella, nutmeg-flower and roman-coriander. It is cultivated in many parts of Asia, Africa, and Europe and recently entered the American continents. The seeds are black in colour, resemble onion seed and bitter in taste. The seed was highly regarded for its medicinal values in Greco Arab or Unani Tibb system of medicine, which originated from Hippocrates and is regarded highly in unani, ayurveda, siddha and other ethnomedicine systems. Hence, it is appropriately known as seed of blessing (habbatul barakah). The spice was attributed with numerous medicinal properties and is widely used in unani, ayurveda, siddha and other ethnomedicine systems across the world (Padmaa, 2010). The medicinal value of the spice is immense and numerous workers appreciated its unique, varied and powerful pharmacological traits. Its pharmacological action such as anti-tumour, anti-diabetic, cardioprotective, gastroprotective, antiasthmatic, nephroprotective, hepatoprotective, antiinflmmatory, immunomodulatory, neuroprotective, anticonvulsant, anxiolytic, antioxidant, antinociceptive, antioxytocic, contraceptive, abortifacient, antinociceptive, antiimplantation, diuretic, antiurolithiatic, antispasmolytic, antibacterial, antifungal, anti-schistosomiasis and anthelmintic activities were immensely appreciated. The numerous pharmacological consequences, which cannot be neglected, might contribute to the above-mentioned effects. Apart from its medicinal uses, the seeds of nigella are used as spice and condiment in various recipes due to their characteristic aroma and bitter and peppery taste (Hedrick and Sturtevant, 1972). The seed oil is considered as highly prized nutritive oil. The dethymquinated seed oil is considered as a valuable source of medically important vegetable oil. The fatty acid composition of seed oil is reported to vary with geographical origin (Cheikh-Rouhou *et al.*, 2007). However, little information is known regarding the influence of sowing window, plant density and nutrient management except for a few studies. There is absolutely, no information from India which is the largest producer and exporter of Nigella. In view of this present investigation was carried out. In the present investigation consisting two separate studies, the changes in the fatty acid profile of nigella seed were determined at a varied sowing window (staggered by 15 days from 1st fortnight of October to 1st fortnight of December), plant densities (50, 33.3 and 25 plants m^{-2}), graded levels of nitrogen (0 to 60 kg ha^{-1}) and phosphorus (0 to 45 kg ha^{-1}).

Material and methods

This study was performed in an experimental area of the Horticultural Research Station (Dr. YSR Horticultural University) at Lam, Guntur in the state of Andhra Pradesh (India). The experimental soil is texturally classified as vertisols, having a composition of sand (24.0 %), silt (18.0 %), and clay (58.0 %) with a bulk density of 1.22 g cm^{-3}. The water holding capacity of the soil is 47 % (by volume) with a hydraulic conductivity of 1.4 cm hour^{-1}. The climate is humid tropics, the weekly mean maximum temperatures ranged from 29.9 to 34.5 °C during crop growing period. The mean minimum temperature ranged from 16.2 to 24.4 °C. The weekly mean relative humidity (afternoon) ranged from 36 to 98 %. A total rainfall of 331.1 mm was received during the *Rabi* season. The test variety was Ajmer Nigella-1. The fixed oil content of the variety is ~ 30 % (Krishna Kant *et al.*, 2009). The crop was raised by dibbling seed during the winter season of 2013-14. An irrigation level equivalent to the 60% of the corresponding evapotranspiration (ETo) was applied using sprinkler irrigation system. The present investigation consisted of two separate studies. The first study was conducted with five different sowing dates (1st fortnight of October, 2nd fortnight of October, 1st fortnight of November, 2nd fortnight of November and 1st fortnight of December) as main plots with three plant densities (25, 33.3 and 50 plants m^{-2}) as sub plots. The sowings were taken as per the sowing window specified. In the second study, influence of four levels of nitrogen (0, 20, 40 and 60 kg nitrogen (N) ha^{-1}) as main plots and four levels of phosphorus (0, 15, 30 and 45 kg phosphorus (P) ha^{-1}) as sub plots was studied. The sowing of the experiment was taken up during the third week of October. The experiments were laid out according to the split plot design as suggested by Gomez and Gomez (1984). Upon crop maturity, the freshly harvested seed was dried to 7% moisture level and used for carrying out the assays. The composite sample of each date of sowing was used for the extraction and analysis of fatty acid profile.

The fatty acid profile of composite samples was analyzed by GC equipped with capillary column at oilseeds section of Department of Biochemistry, Directorate of Oilseeds Research, Hyderabad. The seeds were oven-dried at 40 °C for 4 hours, using a hot air oven, up to moisture content of about 5 %. Then the seeds were powdered in mortar and pestle and 100 mg powder was esterified using 4 ml methenolic-HCL and extracted in 3 ml hexane. The hexane extracts were dried in argon current and reconstituted in 1:50 volumes of chloroform (Manku *et al.*, 1983). 1 µl of this extract was injected in Autosystem XL GC with SP-2330 Supelco capillary column, 30 meter long and 0.32 mm diameter. The temperature program was 150 °C for 10 min, followed by a gradient of 10 °C min^{-1} up to 220 °C and held for 10 min. Helium 1 ml min^{-1} was used as the carrier gas. The injector port was maintained at 240 °C and FID detector at 275 °C. Appropriate fatty acid standards were procured from and the fatty acid peaks were identified by integrating them with the satndards' profiles. The area under the peak was expressed as percentage fatty acid content. Estimation of each sample was repeated minimum three times to ascertain the integrity of the results.

To investigate the chemical variability, the fatty acid data obtained were submitted to principal component analysis (PCA), in order to detect pattern distribution of samples and to identify which constituents can differentiate between the different dates of sowing, plant densities, nitrogen and phosphorous application. Biplots were generated from PCA output for better interpretation of PCA output. PCA and Biplot analyses were carried out with R-3.1.1 software (Chambers, 2008) for windows using the method as suggested by Jolliffe (2005).

Results and discussion

The black cumin fixed oil in all the samples from two experiments comprised two major saturated fatty acids, i.e. palmitic acid (16:0), stearic acid (18:0), palmitic acid being dominant. Among the unsaturated fatty acids in the fixed oil, all the samples comprised three fatty acids i.e. linoleic acid (18:2), oleic acid (18:1) and eicosatrienoic acid (20:3), linoleic acid being dominant followed by oleic and eicosatrienoic acids. These observations agree with those reported in some previous studies on black cumin from different regions (Babayan *et al.*, 1978; Nickavar *et al.*, 2003; Kaskoos, 2011; Aftab *et al.*, 2014).

Influence of sowing window and plant density

The presence, yield and composition of fixed oils in plants, from their formation in plants to their final isolation, are influenced by environmental factors. It is well known that the genetic constitution and prevailing environmental conditions influence the oil yield of many crops, however it is unknown the extent of their influence on the fatty acid profile of nigella. Irrespective of the sowing window and plant densities, linoleic acid content was highest (52.44 to 58.4 %) followed by oleic acid (23.51 to 30.70 %), palmitic acid (10.93 to 12.57 %), stearic acid (2.55 to 3.15 %) and eicosatrienoic acid (2.12 to 2.96 %) (Table 1).

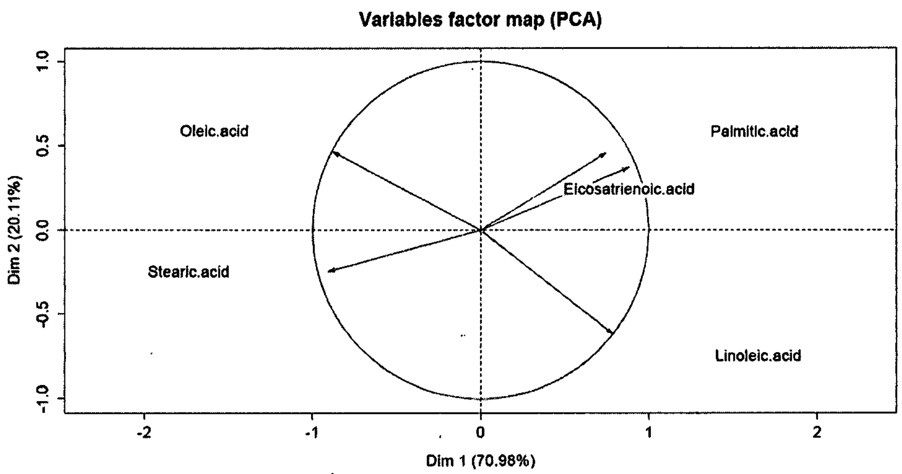

Figure 1.Correlation circle of variables of fatty acid profile measured in the first two principal components of PCA

The biplot analysis revealed positive correlation between the pairs – oleic acid and stearic acid and palmitic acid and eicosatrienoic acid (Fig. 1). The first and second principal components (labeled as Dim 1 and Dim 2) accounted for 91.1 % of the variation, revealing very good discrimination of the treatments. The discriminant analysis revealed that fatty acid profiles of the crop differed with varying sowing window. Linoleic acid production was favoured in the crop sown during October but it was discouraged in the crop sown between 2nd fortnight of November and 1st fortnight of December. The fatty acid content in nigella was certainly influenced by sowing window and plant density, varying with the change in the effected factor. This is the first report of such nature on nigella. Under the conditions of the present investigation, nigella seeds from all treatments contained normal to moderate amount of linoleic acid.

High linoleic acid content is desirable as the nigella seed oil is considered as a valuable natural source of linoleic acid (C18:2 acid), which belongs to essential fatty acid family. The decrease in oleic-linoleic acid ratio in the early sowing suggests a possible role of temperature on the activity of oleate desaturase in the developing seeds. Kizil *et al.* (2008) compared the grain quality of nigella during winter (November) and spring (March) sowings in clay loams of Turkey and reported that maximum increase in linoleic (C:18:2) acid was recorded from winter crop (51.37 %) than spring crop (44.3 %).

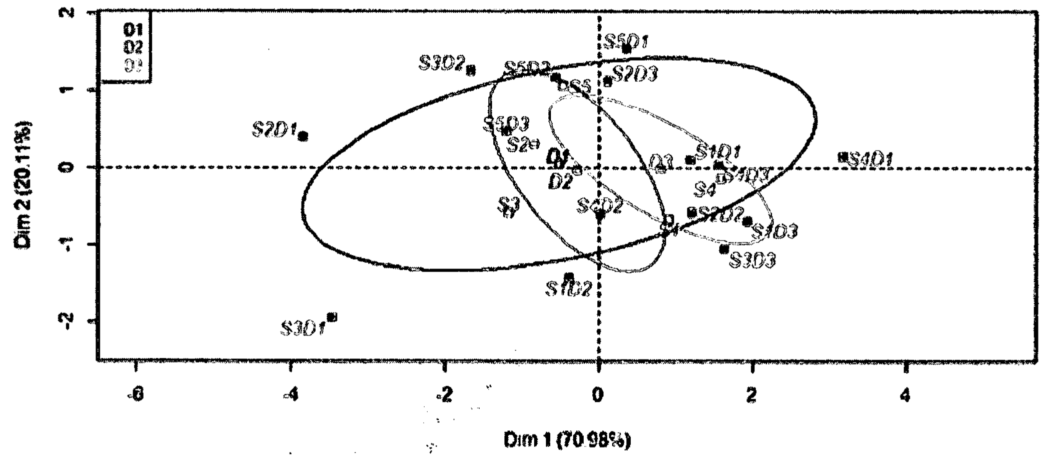

Figure 1. Discriminant analysis of fatty acid profile as affected by dates of sowing and plant densities. (*Coloured ellipses that enclose swarms of data points represent regions that include 95% of the theoretical distribution of canonical scores for each environment.)

The fatty acid profile of the plant densities 50 and 33.3 plants m^{-2} was similar and favoured oleic acid production with associated decrease in linoleic acid content **(Fig. 2)**. A decrease in oleic acid with lower plant densities may be because of a decrease in the activity of Desaturase-9 enzyme due to varied competition among crop plants during crop growth. The variation in fatty acid content as a product of plant density was reported in linseed (Agegenehu and Honermeier, 1997), lesquerella (Brahim *et al.*, 1998), soybean (Fasoula and Boerma, 2005) and amaranthus (Ardali, 2014).

Influence of nitrogen and phosphorus

Irrespective of the fertilizer treatments, linoleic acid content was highest (55.60 % to 58.30 %) followed by oleic acid (24.47 to 27.32 %), palmitic acid (11.38 to 12.39 %), stearic acid (2.66 to 2.94 %) and eicosatrienoic acid (2.33 to 2.85 %) (Table 2). The biplot analysis revealed positive correlation between the pairs - oleic acid with stearic acid and palmitic acid with eicosatrienoic acid. Further, the effects of N and P were found independent (Fig. 3). The first and second principal components (labeled as Dim 1 and Dim 2) accounted for 95.5 % of the variation, revealing very good discrimination of the treatments.The biplot clearly depicted the association between linoleic acid production with low levels of N application in the present test environment. Correlations among fatty acids were similar to the sowing window and plant densities.

Variables factor map (PCA)

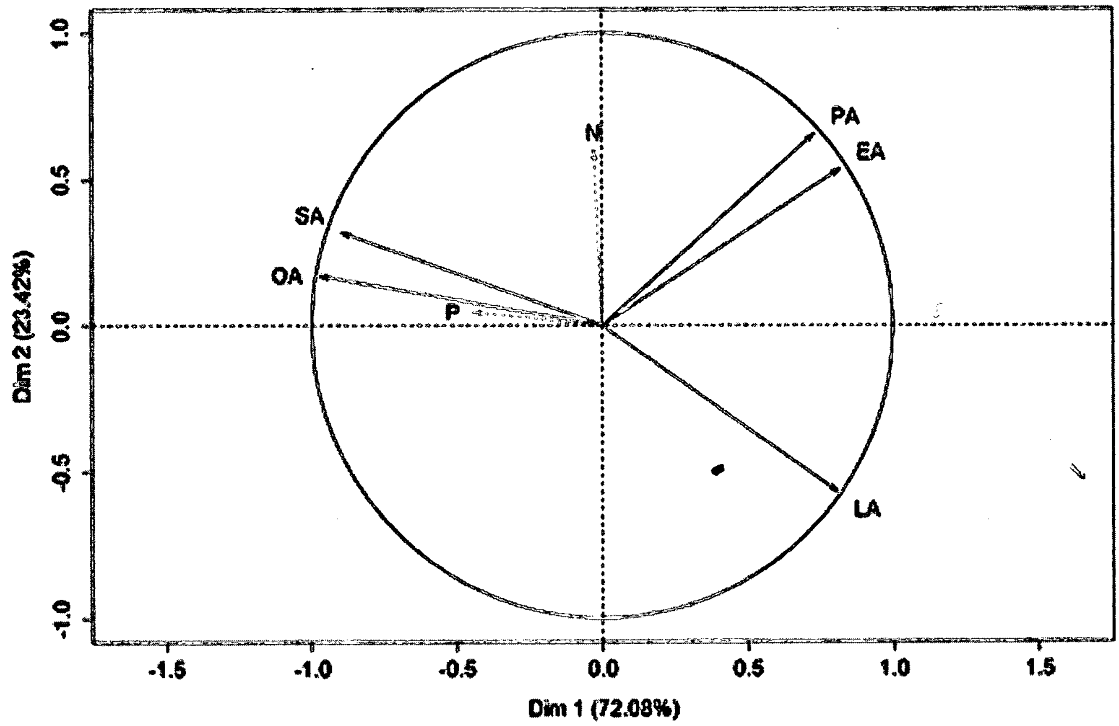

Figure 1.Biplot of fatty acids as affected by N and P application

Legend: LA: Linoleic acid, OA: Oleic acid, PA: Palmitic acid, EA: **Eicosatrienoic acid and SA: Stearic acid**

El-Sayed *et al.* (2000) reported that crop nutrition significantly affects the fatty acid composition of nigella and observed that the lower N nutrition promoted the production of linoleic acid. Ashraf *et al.* (2006) evaluated the influence of graded levels of nitrogen (0, 30, 60, and 90 kg N ha^{-1} soil) on the fatty acid composition of nigella seed and reported that linoleic acid showed no change in their amounts at varying levels of N, but in contrast stearic acid decreased considerably at 60 kg N ha^{-1}. In many oil bearing spice crops, application of nitrogen modified the composition in a significant way (Leiva-Candia *et al.*, 2013; Brito *et al.*, 2012; Keshavarz Afshar *et al.*, 2015). Regarding the phosphorus influence, application of phosphorus was observed to be positively correlated with the production of oleic acid and stearic acids. The production of linoleic acid, palmitic acid and eicosatrienoic acid were negatively correlated. El-Sayed *et al.* (2000) reported that phosphorus nutrition significantly affects the fatty acid composition of nigella and observed that the higher P nutrition in combination with higher N promoted the production of 11, 14-eicosadienoic acid. Seyyedi *et al.* (2015) evaluated three levels of phosphorus (0, 30 and 60 kg ha^{-1}) in combination with biofertilizers + manures and observed that soluble P as basal application had significant decreasing effect on linolenic acid content of nigella seed oil.

Conclusion

Fatty acids composition of nigella seed oil was observed to change with varying sowing times, plant densities and graded levels of N and P application. As nigella has become a valuable natural source of medicinally important bioactive fatty acids, it is very crucial for further study about the accumulation of these compounds during the various seed development phases against array of situations. This will benefit the knowledge accumulation as well as determining the precise conditions for achieving high quality produce from the field.

Table 1. Fatty acid composition of fixed oil as influenced by dates of sowing and plant densities

	Linoleic acid (18:2)				Oleic acid (18:1)				Palmitic acid (16:0)				Stearic acid (18:0)				Eicosatrienoic acid (20:3) acid			
	Plant density (plants m^{-2})				Plant density (plants m^{-2})				Plant density (plants m^{-2})				Plant density (plants m^{-2})				Plant density (plants m^{-2})			
Date of sowing	D_1-50	D_2-33.3	D_3-25	Mean	D_1-50	D_2-33.3	D_3-25	Mean	D_1-50	D_2-33.3	D_3-25	Mean	D_1-50	D_2-33.3	D_3-25	Mean	D_1-50	D_2-33.3	D_3-25	Mean
S_1 (1st FN of October)	56.97	57.60	58.54	57.70	25.53	24.97	23.93	24.81	12.16	11.95	12.13	12.08	2.66	3.04	2.67	2.79	2.68	2.45	2.73	2.62
S_2 (2nd FN of October)	58.55	56.88	57.30	57.58	23.51	25.82	25.24	24.86	12.42	11.97	11.96	12.12	2.55	2.83	2.65	2.68	2.96	2.50	2.85	2.77
S_3 (1st FN of November)	55.60	53.12	58.74	55.82	28.25	29.98	23.84	27.36	10.93	11.49	12.01	11.48	3.11	2.78	2.72	2.87	2.12	2.64	2.69	2.48
S_4 (2nd FN of November)	52.44	57.82	54.85	55.04	30.70	24.72	27.39	27.60	11.37	12.03	12.26	11.89	3.15	2.74	2.80	2.90	2.34	2.69	2.71	2.58
S_5 (1st FN of December)	54.58	54.25	54.60	54.48	27.41	28.02	27.91	27.78	12.57	12.30	12.14	12.34	2.77	2.85	2.93	2.85	2.68	2.58	2.43	2.56
Mean	55.63	55.93	56.81	56.12	27.08	26.70	25.66	26.48	11.89	11.95	12.10	11.98	2.85	2.85	2.75	2.82	2.56	2.57	2.68	2.60
$\sigma_{\bar{x}}$				2.05				2.23				0.42				0.17				0.21
CV %				3.66				8.43				3.54				6.19				8.03

Table 2. Fatty acid composition of fixed oil as influenced by nitrogen and phosphorus levels

N (kg ha^{-1})	Linoleic acid (18:2) %				Mean	Oleic acid (18:1) %				Mean	Palmitic acid (16:0) %				Mean	Stearic acid (18:0) %				Mean	Eicosatrienoic acid (20:3) %				Mean
	P levels (kg ha^{-1})					P levels (kg ha^{-1})					P levels (kg ha^{-1})					P levels (kg ha^{-1})					P levels (kg ha^{-1})				
	P_0-0	P_1-15	P_2-30	P_3-45		P_0-0	P_1-15	P_2-30	P_3-45		P_0-0	P_1-15	P_2-30	P_3-45		P_0-0	P_1-15	P_2-30	P_3-45		P_0-0	P_1-15	P_2-30	P_3-45	
N_0-0	57.45	58.30	57.92	56.05	57.43	25.53	24.74	24.62	27.32	25.55	11.76	11.81	12.10	11.38	11.76	2.69	2.66	2.67	2.94	2.74	2.56	2.49	2.69	2.33	2.52
N_1-20	57.53	57.46	56.82	57.84	57.41	24.79	24.58	25.62	25.19	25.05	12.25	12.39	12.06	11.78	12.12	2.72	2.75	2.77	2.72	2.74	2.71	2.83	2.73	2.46	2.68
N_2-40	57.34	57.38	55.73	57.70	57.04	24.80	25.63	27.02	24.47	25.48	12.39	11.75	11.89	12.29	12.08	2.72	2.78	2.85	2.69	2.76	2.75	2.46	2.50	2.85	2.64
N_3-60	57.16	57.05	56.72	55.60	56.63	25.18	25.61	25.62	26.99	25.85	12.16	11.99	12.22	12.04	12.10	2.73	2.71	2.70	2.91	2.76	2.77	2.61	2.74	2.46	2.65
Mean	57.37	57.55	56.80	56.79	57.13	25.08	25.14	25.72	25.99	25.48	12.14	11.99	12.07	11.87	12.02	2.72	2.73	2.75	2.82	2.75	2.70	2.60	2.67	2.53	2.62
$\sigma_{\bar{x}}$					0.78					0.91					0.28					0.08					0.16
CV %					1.36					3.56					2.29					3.00					6.01

References

Aftab, A.K., Mahesar, S.A., Khaskheli, A.R., Sherazi, S.T.H., Sofia, Q. and Zakia, K., 2014. Gas chromatographic coupled mass spectroscopic study of fatty acids composition of Nigella sativa L.(KALONJI) oil commercially available in Pakistan. *International Food Research Journal*, *21*(4), pp.1533-1537.

Agegenehu, M. and Honermeier, B. (1997) Effects of seeding rates and nitrogen fertilization on seed yield, seed quality and yield components of false flax. *Die Bodenkultur*. 48 (1): 15-20.

Ardali, S.A. (2014) Effects of plant density and nitrogen rate on Fatty acids profile of grain of Amaranth (*Amaranthus hypocondriacos* L.). *Intl J Agri Crop Sci.* 7 (7): 390-392.

Ashraf, M., Ali, Q. and Iqbal, Z. (2006) Effect of nitrogen application rate on the content and composition of oil, essential oil and minerals in black cumin (*Nigella sativa* L.) seeds. *Journal of the Science of Food and Agriculture*. 86 (6): 871-876.

Babayan, V.K., Koottungal, D. and Halaby, G.A., (1978) Proximate analysis, fatty acid and amino acid composition of Nigella sativa L. seeds. *Journal of Food Science*, *43*(4), pp.1314-1315.

Brahim, K., Ray, D.T. and Dierig, D.A. (1998) Growth and yield characteristics of *Lesquerella fendleri* as a function of plant density. *Industrial Crops and Products*. 9 (1): 63-71.

Brito, D.M., Barreto, S.C., Goncalves, F.V., Castro, R.N. and Souza, S.R., (2014) Productivity, fatty acid profiles and nitrogen metabolism of crambe under varied nitrate levels. *Agricultural Journal*, *9*(1), pp.38-44.

Chambers, J. (2008) *Software for data analysis: programming with R*. Springer Science & Business Media.

Cheikh-Rouhou, S., Besbes, S., Hentati, B., Blecker, C., Deroanne, C. and Attia, H., (2007) Nigella sativa L.: Chemical composition and physicochemical characteristics of lipid fraction. *Food chemistry*, *101*(2), pp.673-681.

El-Sayed, K.A., Ross, S.A., El-Sohly, M.A., Khalafalla, M.M., Abdel-Halim, O.B. and Ikegami, F. (2000) Effect of different fertilizers on the amino acid, fatty acid, and essential oil composition of *Nigella sativa* seeds. *Saudi Pharmaceutical Journal*. 8 (4): 175-182.

Fasoula, V.A. and Boerma, H.R. (2005) Divergent selection at ultra-low plant density for seed protein and oil content within soybean cultivars. *Field crops research*. 91 (2): 217-229.

Gomez, K.A. and Gomez, A.A. (1984) Statistical procedures for Agricultural Research. Second Edition. A Wiley inter-Science Publications, John Wiley and Sons, New York, Chichester, Torento, Singapore. 680 p.

Hedrick, U.P. and Sturtevant, E.L. (1972) Sturtevant's Edible Plants of the World. Dover Publication Inc., New York. pp. 388–389.

Jolliffe, I.T. (2005) *Principal component analysis*. John Wiley & Sons, Ltd.

Kaskoos, R.A., (2011) Fatty acid composition of black cumin oil from Iraq. *Res J Med Plant*, *5*, pp.85-89.

Keshavarz Afshar, R., Chaichi, M.R., Rezaei, K., Asareh, M.H., Karimi, M. and Hashemi, M., (2015) Irrigation regime and organic fertilizers influence on oil content and fatty acid composition of milk thistle seeds. *Agronomy Journal*, *107*(1), pp.187-194.

Kizil, S., Kirici, S., Çakmak, Ö. and Khawar, K.M. (20080 Effects of sowing periods and P application rates on yield and oil composition of black cumin (*Nigella sativa* L.). *Journal of Food Agriculture and Environment*. 6 (2): 242-246.

Krishna Kant, Anwer, M.M., Meena, S.R. and Mehta, R.S. (2009) Advance production technology of Nigella. National Research Centre on Seed Spices, Ajmer-305206 (Rajasthan).

Leiva-Candia, D.E., Ruz-Ruiz, M.F., Pinzi, S., García-Ruiz, J.R., Domínguez, J., García, I.L. and Dorado, M.P., (2013). Influence of nitrogen fertilization on physical and chemical properties of fatty acid methyl esters from Brassica napus oil. *Fuel*, *111*, pp.865-871.

Manku, M.S., Horrobin, D.F., Huang, Y.S. and Morse, N. (1983) Fatty acids in plasma and red cell membranes in normal humans. *Lipids*. 18: 906-908.

Nickavar, B., Mojab, F., Javidnia, K. and Amoli, M. R. (2003) Chemical composition of the fixed and volatile oils of Nigella sativa L. from Iran. *Zeitschrift Fur Naturforschung C*, *58*(9/10), 629-631.

Padmaa, P.M. (2010) *Nigella sativa* Linn.–A comprehensive review. *Indian journal of natural products and resources*. 1 (4): 409-429.

Seyyedi, S.M., Khajeh-Hosseini, M., Moghaddam, P.R. and Shahandeh, H., (2015) Effects of phosphorus and seed priming on seed vigor, fatty acids composition and heterotrophic seedling growth of black seed (Nigella sativa L.) grown in a calcareous soil. *Industrial Crops and Products*, *74*, pp.939-949.

Zohary, D., Hopf, M. and Weiss, E. (2012) *Domestication of Plants in the Old World: The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin*. Oxford University Press.