



## Research article

Volatile compounds of black cumin (*Nigella sativa* L.) seeds cultivated in Bangladesh and IndiaYearul Kabir<sup>a,\*</sup>, Yoko Akasaka-Hashimoto<sup>b</sup>, Kikue Kubota<sup>b</sup>, Michio Komai<sup>c</sup><sup>a</sup> Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka, Bangladesh<sup>b</sup> Department of Nutrition and Food Science, Ochanomizu University, Tokyo, Japan<sup>c</sup> Graduate School of Agricultural Sciences, Tohoku University, Sendai, Japan

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## ABSTRACT

The compositional analysis of volatile compounds of *Nigella sativa* L. seeds obtained from India and Bangladesh was carried out in this study. Apart from the proportion of volatile compounds, the chemical composition of seeds from both sources were similar. The major volatile compounds in Bangladesh seeds were *p*-cymene (36.35%), thymoquinone (29.77%),  $\alpha$ -thujene (12.40%), carvacrol (2.85%),  $\beta$ -pinene (2.41%), limonene (1.64%), methyl linoleate (1.33%) and sabinene (1.18%), contribution of these is 87.93% of the total volatile oil. On the other hand, the major volatile compounds in Indian seeds were *p*-cymene (41.80%),  $\alpha$ -thujene (13.93%), thymoquinone (10.27%), methyl linoleate (4.02%), carvacrol (3.65%),  $\beta$ -pinene (2.96%), *d*-limonene (2.11%), 4,5-epoxy-1-iso-propyl-4-methyl-1-cyclohexene (1.80%), sabinene (1.50%) and 4-terpineol (1.22%); contribution of these were 83.24% of the total volatile oil. In both seeds, *p*-cymene, thymoquinone, and  $\alpha$ -thujene were the major components. Importantly, *N. sativa* seeds of Bangladesh contained almost 3-fold thymoquinone compared to Indian seeds. In conclusion, the seeds from Bangladesh contain a higher amount of terpene ketones (29.86%) represented by thymoquinone in comparison to Indian seeds (10.61%); on the other hand, Indian seeds contained a higher amount of terpene hydrocarbons (63.18%) mainly *p*-cymene, compared to Bangladesh seeds (54.53%). This is the first study to report detailed compositional analysis and comparison of *Nigella sativa* L. seeds from Bangladesh and India.

## 1. Introduction

In Bangladesh and India, Black cumin (*Nigella sativa* L.) seed is cultivated for use in traditional medicine and as a spice for long time [1]. Moreover, its application as a food additive and flavoring agent have been reported in various countries around the world [2, 3]. Mature seeds of *N. sativa* are extensively utilized in bakery goods, including preparation of pickles, Indian cuisines, and traditional dishes [4]. Depending on the method and the region of cultivation *N. sativa* seeds contain over a hundred different volatile components with distinctive aroma and taste [5].

Previous studies have identified many volatile components present in *N. sativa* seeds, including thymoquinone, thymohydroquinone, dithymoquinone, *p*-cymene,  $\alpha$ -thujene,  $\gamma$ -terpinene, carvacrol,  $\alpha$ -pinene,  $\beta$ -pinene, 4-terpineol, and sesquiterpene longifolene, carvone, limonene, and citronellol [6, 7, 8, 9, 10, 11]. However, depending on the region of the seeds were cultivated, there are still many unknown volatile

components to be characterized [6, 9, 12]. Many of these compounds can induce pharmacological effects and have therapeutic potential in humans [13, 14, 15]. Researchers have previously reported thymoquinone, a main compound in *N. sativa*, to have anti-inflammatory [16, 17, 18, 19], antimicrobial [20], and anti-cancer activity [21, 22, 23, 24] as well as asthma alleviating properties [25]. Inhibition of neuroinflammation and the protective effect of thymoquinone against Ischemia-reperfusion (I/R) injury of the spinal cord was also reported [26, 27]. Furthermore, Sahak et al. [28] reported that *N. sativa* and/or its bioactive constituents can enhance learning and memory in animals and humans.

Effectiveness of black cumin seed and its volatile component thymoquinone in anticonvulsive, pain protection, and prevention of rheumatoid arthritis in rat models have also been reported [18, 29]. Overall, several bodies of work suggest these seeds hold diverse therapeutic potentials. The contribution of minor compounds present in the seeds warrants investigation, as they might be responsible for the previously mentioned effects. Therefore, the compositional variations in *N. sativa*

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seeds and presence of beneficial compounds requires exploration for distinctive medicinal application. Like most herbs, compositional variation depends on the region of collection, time of harvest and agronomic practices that have been reported for *Nigella sativa* seeds [30, 31]. Bourgo et al. [32] reported a distinctive type of *p*-cymene from Tunisian essential oil of *N. sativa* seeds, which is different from what is found in the seeds of other origins. Studies also reported harmful water stress effects on the yield of *N. sativa* and quantitative yield of different components [33, 34]. Despite numerous studies on *N. sativa*, the volatile composition of black cumin cultivated in different regions of the world, especially in Bangladesh and India have not been explored. This study was undertaken to determine the compositional variation of volatile components of *N. sativa* seeds cultivated in Bangladesh and India.

## 2. Materials and methods

### 2.1. Plant materials: collection and storage of *N. sativa* seeds

The races of cultivated *Nigella sativa* species Bangladesh (Seed I) and India (Seed II). Seed I was used in this study and collected in October 2012. Seed I was purchased from a supermarket in Dhaka, and seed II (imported to Japan from India) was from a local market in Tokyo, Japan. After discarding the immature and broken seeds of *N. sativa*, the seeds (about 200 g) were cleaned under running tap water, rinsed with distilled water, and air-dried in an oven at 40 °C overnight. The dried matured seeds were then grounded into powder with a grinder (Panasonic, Japan, Model MJ-W176P), and the powder was kept frozen in an airtight container until analyzed (within one month). The plant material was identified and authenticated by compared with correctly identified herbarium specimens. A voucher specimen was retained for future reference.

### 2.2. Standard compounds for identification

The following compounds were purchased from commercial sources (Sigma-Aldrich, St. Louis, Missouri, USA):  $\beta$ -pinene,  $\alpha$ -terpinene,  $\gamma$ -terpinene, *p*-cymen,  $\alpha$ -terpinolene, acetic acid, bornyl acetate,  $\beta$ -caryophyllene, carvone, thymoquinone, cuminaldehyde, 2-tridecanone, anethole, 2-pentadecanone, nonanoic acid, decanoic acid, methyl hexadecanoate, methyl linoleate, vanillin, ethyl lactate, *d*-Limonene, linalool, 4-Terpineol,  $\alpha$ -terpineol, *p*-cymen-8-ol, thymol and piperitenone. Longifolene was a gift (Takasago Co. Ltd., Tokyo, Japan). They were of the highest purity available. All reagents and solvents were of analytical grade and were purchased from Wako Pure Chemical Industries, Osaka, Japan.

### 2.3. Extraction of the volatile compounds

Twenty-five grams powder of *Nigella sativa* L. seeds was soaked overnight twice in methanol (50 mL). After filtration, the supernatant was diluted with Milli-Q system (Millipore Corp., Saint-Quentin, France) purified water and adjusted to 10% methanol. The diluted solution was applied to a chromatography column packed with Porapak Q resin (porous polymer resin) (Waters, 50/80 mesh). After eliminating the water-soluble compounds with ultrapure water (200 mL), the adsorbed compounds were eluted with 300 mL mixture of pentane and diethyl ether (1:1). After desorption, 3  $\mu$ g of ethyl lactate was added as an internal standard (IS). To obtain the aroma concentrate (essential oil), the elute was dried in anhydrous sodium sulfate and the solvent was evaporated at 39.5 °C under atmospheric pressure. Each procedure was repeated twice. The oil percentage was then calculated and stored in an airtight amber container in a freezer at -20 °C for subsequent analyses. Just before injecting into the gas chromatograph, the extract was concentrated to 500  $\mu$ L with a nitrogen stream and the components were analyzed by using gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS) and GC-Olfactometry [35, 36].

### 2.4. Gas chromatography (GC)

A Hewlett-Packard (HP) 6890 Series II gas chromatograph (Hewlett-Packard Inc., Palo Alto, California, USA) equipped with a flame-ionization detector (FID) was employed for GC analysis. The GC was run at a flow rate of 1 mL/min and with a split ratio of 30:1 using helium as carrier gas. The column was 60 m  $\times$  0.25 mm i.d., 0.25  $\mu$ m film thickness, coated with DB-WAX (J & W Scientific Inc., Folsom, CA, USA) was used to separate the volatiles. The oven temperature was kept at 60 °C for 4 min initially and then increased at a rate of 2 °C/min to 200 °C. Injector and detector temperature were set at 200 °C and 220 °C, respectively. Each sample was analyzed in duplicate.

### 2.5. Gas chromatography-mass spectrometry (GC-MS)

A HP 5890 Series II gas chromatograph (Hewlett-Packard) equipped with an HP 5972 mass selective detector and Wiley275 library was used for the GC-MS analysis. The same GC conditions were used as described for the GC analysis above. We recorded electron impact (EI) spectra by maintaining the temperature of the ion source at 220 °C, and mass spectra were obtained from 30 to 400 mass units at 70 eV in a *m/z* range. Each procedure was repeated in duplicate.

### 2.6. Identification of the components

Peak components were identified according to Kovats GC retention indices [37] with authentic standard and comparing the mass spectra with those in the Wiley Library of MS spectra (Hewlett-Packard) patterns stored on the computer or with published literature. In case of unavailability of reference standard, the compounds were tentatively identified based on MS data only. We calculated the percent peak areas by dividing the peak area for a peak by the total peak area for the entire chromatogram on GC chromatogram with FID and expressed as a percentage [38].

### 2.7. Gas chromatography-Olfactometry (GC-O)

In split-less mode, with a column of 60 m  $\times$  0.53 mm i.d. (1  $\mu$ m film) coated with DB-WAX (J & W Scientific), the GC-O was performed. Same temperature conditions and instrument were employed as used for the GC analysis. Helium was used as carrier gas at a flow rate of 8.3 mL/min 1  $\mu$ L sample of concentrated volatile was injected into the column in split less mode and the carrier gas was passed through the FID detector and a glass sniffing port at 220 °C after splitting of 1:1 at the outlet of the column. Wet air was pumped into the sniffing port at 45 mL/min to retain the nose moist and remove the odorant quickly.

GC-Olfactometry sensory assessments were performed for all compounds with a panel of 9 females panelists aged 21–32 years. They had extensive training and more than three years' experience in GC/O using the method described previously [35, 36, 39]. The samples were served in a random order to each panelist. Each panelist selected the most appropriate terms for odor description, as reported by Shimoda et al. [40, 41]. A consensus-building discussion was held with the panelists to decide the final sensory descriptors, summarized in Table 1.

## 3. Results and discussion

To understand the implication of flavor of volatile components of *N. sativa* seeds essential oil and its application in food industry, it is vital to characterize the volatile components. Characterization of volatile components is also necessary to further probe its role in traditional medicine. In this study, we obtained 0.28 g (1.1%) and 0.06 g (0.24%) volatile flavor concentrates from 25 g *N. sativa* seeds from Bangladesh and Indian, respectively. We detected 38 and 41 volatile components and among these we identified 32 and 35 components from Bangladesh and Indian seeds respectively, which was 92.82% and 89.51% of the total constituents of volatile components. The typical gas chromatograms from

**Table 1.** Volatile components identified in methanol extracts of black cumin produced in Bangladesh and India.

Peak No <sup>a</sup>	t <sub>r</sub> <sup>b</sup> (min)	RI <sup>c</sup>	Compounds	Odor description	Peak area % <sup>d</sup>	
					Bangladesh <sup>e</sup>	India <sup>f</sup>
1	10.0	1032	$\alpha$ -thujene*	citrus, sweet, sour	12.40	13.93
2	13.0	1113	$\beta$ -pinene		2.41	2.96
3	13.5	1124	sabinene*		1.18	1.50
4	16.2	1182	$\alpha$ -terpinene		0.22	0.15
5	17.2	1201	<i>d</i> -limonene	citrus, sour	1.64	2.11
6	19.7	1249	$\gamma$ -terpinene	sweet, roasted	0.28	0.69
7	20.5	1264	unknown		0.79	1.20
8	21.4	1278	<i>p</i> -cymene	citrus, sour, gas-like	36.35	41.80
9	21.8	1285	$\alpha$ -terpinolene		0.05	0.04
10	23.1	1306	unknown	japanese cypress-like	4.92	7.46
11	29.9	1420	thujol*	metallic	0.08	0.22
12	30.7	1434	<i>p</i> -mentha-1,3,5,8-tetraene*	green	0.03	0.13
13	31.2	1442	acetic acid	vinegar, sour	0.03	0.50
14	31.4	1447	1,2-epoxy- <i>p</i> -menth-8-ene*	roasted bean	0.03	0.17
15	32.9	1471	$\alpha$ -longipinene*		0.14	0.20
16	35.4	1511	unknown		0.50	0.88
17	37.3	1545	linalool	sweet	0.03	0.09
18	37.8	1553	4,5-epoxy-1-isopropyl-4-methyl-1-cyclohexene*	floral, sweet milk	0.95	1.80
19	39.0	1573	longifolene	body-odor-like, unpleasant	0.91	0.61
20	39.4	1580	bornyl acetate	metallic	0.15	0.24
21	40.4	1596	$\beta$ -caryophyllene	earthy, herbaceous	0.06	0.12
22	40.7	1601	4-terpineol	minty	0.65	1.22
23	42.8	1639	3-thujen-2-one*	herbal, cress-like	0.02	0.06
24	46.2	1698	$\alpha$ -terpineol	herbaceous, floral	n.d.	0.14
25	48.0	1732	carvone	minty	0.09	0.28
26	49.1	1751	thymoquinone	clayey	29.77	10.27
27	50.7	1780	cuminaldehyde	sweet, floral	n.d.	0.50
28	52.1	1806	2-tridecanone		0.15	0.23
29	53.0	1824	anethole	minty	n.d.	0.14
30	53.9	1842	<i>p</i> -cymen-8-ol	floral, sweet	0.14	0.53
31	58.4	1929	piperitenone		n.d.	0.06
32	63.1	2025	2-pentadecanone*		0.16	n.d.
33	64.0	2045	unknown		0.12	0.09
34	66.7	2099	cuminol ( <i>p</i> -cymen-7-ol)*		n.d.	0.38
35	68.3	2135	nonanoic acid		0.10	n.d.
36	69.9	2170	thymol		0.07	0.09
37	71.4	2201	carvacrol	sweaty, rubber	2.85	3.65
38	71.9	2214	methyl hexadecanoate		0.08	0.48
39	73.0	2238	unknown		0.21	0.40
40	74.0	2262	decanoic acid	hay-like	0.04	0.10
41	75.8	2301	unknown		0.60	0.43
42	87.0	2490	methyl linoleate	breath-odor-like, unpleasant	1.33	4.02
43	93.5	2565	vanillin**	vanilla, sweet	0.43	0.10

n.d.: not detected.

\* Identified tentatively with only the mass spectrum.

\*\* Detected with only GC-Olfactometry.

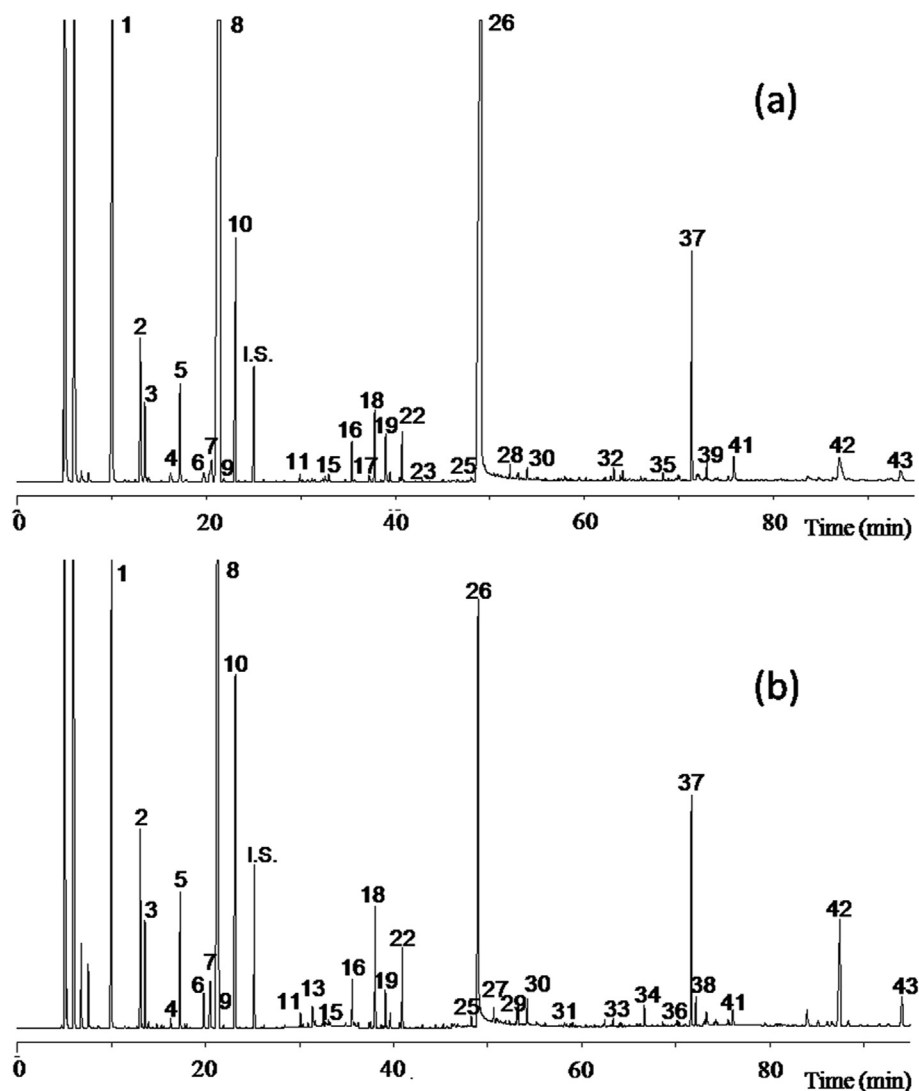
<sup>a</sup> Peak numbers correspond to those in Figure 1.<sup>b</sup> Retention times by GC when using DB-WAX.<sup>c</sup> Retention indices on DB-WAX.<sup>d</sup> Peak area was calculated according to the response of the FID detector.<sup>e</sup> Produced in Bangladesh.<sup>f</sup> Produced in India.

the DB-Wax column of Bangladesh and Indian samples are shown in Figure 1.

The percentage weights components according to their order of elution from the column is presented in Table 1. There was not a significant variation in the identity of volatile components of *N. sativa* mature seeds from Bangladesh and India, but the percentage composition

of each compound was different (Table 1), and many compounds were already identified as the aroma compounds of black cumin [42, 43, 44, 45].

It was not possible to identify six compounds from seeds of each origin by comparing with the existing spectra data in literature, fragmentation patterns of mass spectral, or the retention times and indices of



**Figure 1.** Gas chromatograms of essential oils of black cumin from Bangladesh (a) and from India (b). Compounds of the peak numbers are referred to Table 1. I.S. is ethyl lactate which is the internal standard added to Bangladeshi (250  $\mu\text{g/g}$  seed weight) and Indian (88  $\mu\text{g/g}$  seed weight) seeds, respectively.

the compounds. We could not identify a few compounds due to lack of reference standards. Identification of compounds was performed and reported only after comparing it with reference compounds and mass spectral data (Table 1). Five compounds detected in Indian seeds were not detected in Bangladeshi seeds and two compounds detected in Bangladeshi seeds were not detected in Indian seeds. This may be due to presence of components below the amount required for mass spectra detection. An unidentified compound with a unique odor was found both in Bangladesh and Indian seeds, contributing 4.92% and 7.46% of the volatile constituents respectively.

Of the total volatile compounds of Bangladeshi seeds, major (87.93%) portion comprise with *p*-cymene (36.35%), thymoquinone (29.77%),  $\alpha$ -thujene (12.40%), carvacrol (2.85%),  $\beta$ -pinene (2.41%), *d*-limonene (1.64%), methyl linoleate (1.33%) and sabinene (1.18%) (Table 1). On the other hand, *p*-cymene (41.80%),  $\alpha$ -thujene (13.93%), thymoquinone (10.27%), methyl linoleate (4.02%), carvacrol (3.65%),  $\beta$ -pinene (2.96%), *d*-limonene (2.11%), 4,5-epoxy-1-isopropyl-4-methyl-1-cyclohexene (1.80%), sabinene (1.50%) and 4-terpineol (1.22%) were the major representative constituents of Indian seeds (83.24%). In the seeds from both countries, *p*-cymene, thymoquinone and thujene were the major components. This result agrees with the results obtained for seeds cultivated in other countries such as Austria, Poland, Algeria, India, Iran, and Tunisia [4, 6, 7, 8, 43], with some variations in the quantitative compositions.

In the present study, *p*-cymene has been determined as the major components in both Bangladeshi and Indian seeds of *N. sativa*, which is consistent with findings from previous studies. However, there is a compositional variation of *p*-cymene that exists between different studies depending on the location of cultivation of seed [7, 8, 10]. Interestingly, some study reported thymoquinone as the key components of *N. sativa* essential oil [1, 9, 13]. In the present study, Bangladeshi *N. sativa* seeds contained almost 3-fold thymoquinone (29.77%) compared to Indian seeds (10.27%). Liu et al. [38] also reported a higher level of thymoquinone in seeds of Bangladesh compared to that of the Egyptian seed. This effect may be due to environmental conditions on the flowering as reported by D'Antuono et al. [46]. They reported that delay in sowing of seeds reduced the content of thymoquinone, while increasing the *p*-cymene and thymol content of *N. sativa* oil. Additionally, substantial compositional differences in *N. sativa* seeds have been reported for different plant origin [6, 8, 9, 30], seed maturity stages [30] and method of cultivation e.g. irrigation regime or type and amount of fertilizer used [31, 47, 48]. Thymoquinone has been reported as a promising therapeutic agent [13, 14, 15, 16, 17, 18].

As shown in Table 1, monoterpenes were the main components in both Bangladeshi (88.36%) and Indian (79.96%) seeds, which is similar to the findings of others [6, 46, 49]. The representative monoterpenes of the total volatiles are monoterpene hydrocarbons, 54.53% vs 63.18%

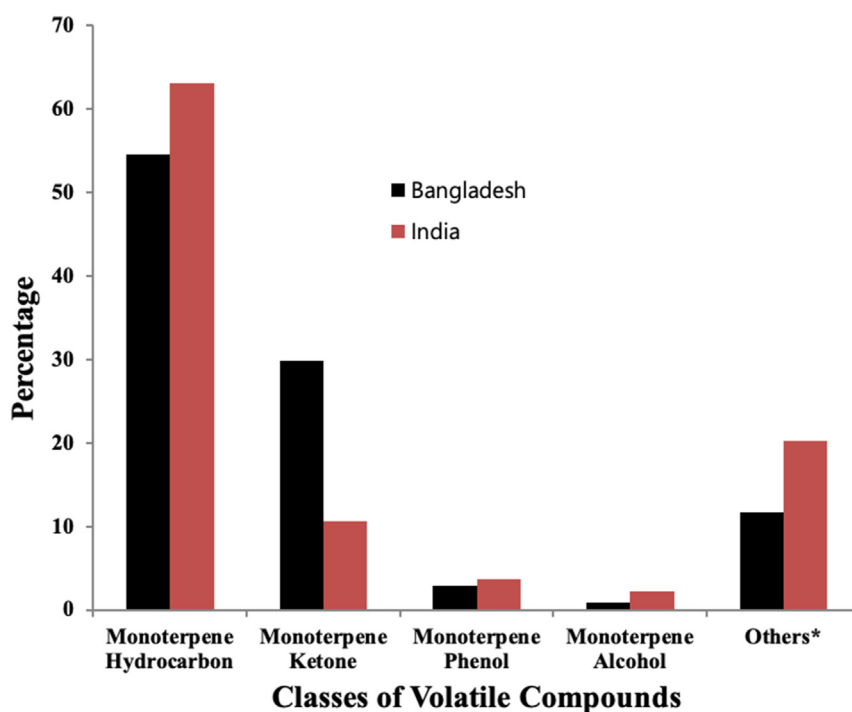


Figure 2. The percentage variations of volatile monoterpenes obtained from black cumin seeds of Bangladesh and Indian origins.

with main component *p*-cymene (36.35% vs 41.80%) followed by  $\alpha$ -thujene (12.40% vs 13.93%); monoterpene ketones, 29.86% and 10.61% with main component thymoquinone (29.77% vs 10.27%); monoterpene phenols, carvacrol and thymol with a percentage of 2.92% and 3.74%; and monoterpene alcohols, 4-terpineol and *p*-cymen-8-ol making only 0.9% and 2.2% respectively for Bangladesh and Indian seeds (Table 1). The sesquiterpene hydrocarbons represented only 0.97% and 0.73% of the essential oil respectively. Wais et al. [7] also reported that sesquiterpenes and their oxygenated derivatives constituted only a small fraction of the essential oil (0.7% and 0.3%, respectively) for seeds of *N. sativa* cultivated in Poland.

As shown in Figure 2, the content of terpene ketones was much higher in Bangladeshi seeds compared to Indian seeds, especially thymoquinone. On the other hand, Indian seeds contain higher amount of terpene hydrocarbons, mainly *p*-cymene than that of Bangladeshi seeds. The contribution of oxygenated terpenes in the total volatile constituents of *N. sativa* seeds was much higher (>15%) for Bangladesh seeds than Indian seeds. It has been reported [28] that oxygenated terpenes are unique and vital constituents for each essential oil and convey their distinct representative odor and/or taste. Bourgou et al. [50] also reported antioxidant activity and nitric oxide production inhibition ability of terpenoids isolated from the essential oil of Tunisian *Nigella sativa* L.

#### 4. Conclusion

Compositional analysis of cumin seeds from different sources is necessary to select the best variety for a wide range of purposes. Since the black cumin seeds cultivated and available in Bangladesh contained much higher (3-fold) thymoquinone, thus use of Bangladeshi seeds for medicinal purpose is a better option.

#### Declarations

##### Author contribution statement

Yearul Kabir: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yoko Akasaka-Hashimoto: Performed the experiments; Analyzed and interpreted the data.

Kikue Kubota, Michio Komai: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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##### Competing interest statement

The authors declare no conflict of interest.

##### Additional information

No additional information is available for this paper.

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##### References

- [1] S. Padhye, S. Banerjee, A. Ahmad, R. Mohammad, F.H. Sarkar, From here to eternity – the secret of Pharaohs: therapeutic potential of black cumin seeds and beyond, *Cancer Ther.* 6 (b) (2008) 495–510.
- [2] Z. Tarakci, K. Ekici, O. Sagdic, E. Kucukoner, The effect of black cumin on ripening of Tulum cheese, *Arch. Lebensmittelhyg.* 56 (2005) 135–139.
- [3] M.F.R. Hassanien, A.M.A. Assiri, A.M. Alzohairy, H.F. Oraby, Health-promoting value and food applications of black cumin essential oil: an overview, *J. Food Sci. Technol.* 52 (10) (2015) 6136–6142.
- [4] M.F. Ramadan, Nutritional value, functional properties and nutraceutical applications of black cumin (*Nigella sativa* L.): an overview, *Int. J. Food Sci. Technol.* 42 (10) (2007) 1208–1218.
- [5] P. Paarakh, *Nigella sativa* Linn.- A comprehensive review, *Indian J. Natl. Prod. Res.* 1 (4) (2010) 409–429.

- [6] H. Rchid, R. Nmila, J.M. Bessiere, Y. Sauvaire, M. Chokairi, Volatile components of *Nigella damascene* L. and *Nigella sativa* L. seeds, *J. Essent. Oil Res.* 16 (6) (2004) 585–587.
- [7] A. Wajs, R. Bonikowski, D. Kalembe, Composition of essential oil from seeds of *Nigella sativa* L. cultivated in Poland, *Flavour Fragrance J.* 23 (2) (2008) 126–132.
- [8] I. Hamrouni-Sellami, M.E. Kchouk, B. Marzouk, Lipid and aroma composition of black cumin (*Nigella sativa* L.) seeds from Tunisia, *J. Food Biochem.* 32 (3) (2008) 335–352.
- [9] N. Ahmad, R. Ahmad, A. Al-layly, H. Al-shawi, A. Al-ali, M. Amir, et al., Ultra-high-performance liquid chromatography-based identification and quantification of thymoquinone in *Nigella sativa* extract from different geographical regions, *Phcog. Mag.* 14 (57) (2018) 471–480.
- [10] A. Piras, A. Rosa, B. Marongiu, S. Porcedda, D. Falconieri, M.A. Dessi, et al., Chemical composition and in vitro bioactivity of the volatile and fixed oils of *Nigella sativa* L. extracted by supercritical carbon dioxide, *Ind. Crop. Prod.* 46 (2013) 317–323.
- [11] Z. Solati, B.S. Baharin, H. Bagheri, Antioxidant property, thymoquinone content and chemical characteristics of different extracts from *Nigella sativa* L. seeds, *J. Am. Oil Chem. Soc.* 91 (2) (2014) 295–300.
- [12] N.K. Mohammed, M.Y. Abd Manap, C.P. Tan, B.J. Muhiuddin, A.M. Alhelli, A.S. Meor Hussin, et al., The effects of different extraction methods on antioxidant properties, chemical composition, and thermal behavior of black seed (*Nigella sativa* L.) oil, *Evid. Based Complement. Alt. Med.* 2016 (2016) 6273817.
- [13] R.M. Shrivastavai, R.C. Agrawal, Z. Parveen, A review on therapeutic applications of *Nigella Sativa*, *J. Chem. Sci.* 1 (2011) 241–248.
- [14] S.N. Goyal, C.P. Prajapati, P.R. Gore, C.R. Patil, U.B. Mahajan, C. Sharma, et al., Therapeutic potential and pharmaceutical development of thymoquinone: a multitargeted molecule of natural origin, *Front. Pharmacol.* 8 (2017) 656.
- [15] A. Ahmad, A. Husain, M. Mujeeb, S.A. Khan, A.K. Najmi, N.A. Siddique, et al., A review on therapeutic potential of *Nigella sativa*: a miracle herb, *Asian Pac. J. Trop. Biomed.* 3 (5) (2013) 337–352.
- [16] M. Alemi, F. Sabouni, F. Sanjarian, K. Haghbeen, S. Ansari, Anti-inflammatory effect of seeds and callus of *Nigella sativa* L. Extracts on mix glial cells with regard to their thymoquinone content, *AAPS PharmSciTech* 14 (1) (2013) 160–167.
- [17] C.C. Woo, A.P. Kumar, G. Sethi, K.H. Tan, Thymoquinone: potential cure for inflammatory disorders and cancer, *Biochem. Pharmacol.* 83 (4) (2012) 443–451.
- [18] F. Vaillancourt, P. Silva, Q. Shi, H. Fahmi, J.C. Fernandes, M. Benderdour, Elucidation of molecular mechanisms underlying the protective effects of thymoquinone against rheumatoid arthritis, *J. Cell. Biochem.* 112 (1) (2011) 107–117.
- [19] F. Isik, A.T. Tunalı, A. Yarat, Z. Genc, R. Pisirciler, E. Caliskan-Ak, et al., Protective effects of black cumin (*Nigella sativa*) oil on TNBS-induced experimental colitis in rats, *Dig. Dis. Sci.* 56 (2011) 721–730.
- [20] K. Chaieb, B. Kouidhi, H. Jrah, K. Mahdouani, A. Bakhrouf, Antibacterial activity of Thymoquinone, an active principle of *Nigella sativa* and its potency to prevent bacterial biofilm formation, *BMC Compl. Alternative Med.* 11 (2011) 29.
- [21] A.G.M. Mostofa, M.K. Hossain, D. Basak, M.S. Bin Sayeed, Thymoquinone as a potential adjuvant therapy for cancer treatment: evidence from preclinical studies, *Front. Pharmacol.* 8 (2017) 295.
- [22] M.A. Randhawa, M.S. Alghamdi, Anticancer activity of *Nigella sativa* (black seed) – a review, *Am. J. Chin. Med.* 39 (6) (2011) 1075–1091.
- [23] S. Banerjee, S. Padhye, A. Azmi, Z. Wang, P.A. Philip, O. Kucuk, et al., Review on molecular and therapeutic potential of thymoquinone in cancer, *Nutr. Cancer* 62 (7) (2010) 938–946.
- [24] T. Yi, S.G. Cho, Z. Yi, X. Pang, M. Rodriguez, Y. Wang, et al., Thymoquinone inhibits tumor angiogenesis and tumor growth through suppressing AKT and extracellular signal-regulated kinase signaling pathways, *Mol. Cancer Therapeut.* 7 (7) (2008) 1789–1796.
- [25] M.H. Boskabady, N. Mohsenpoor, L. Takaloo, Antiasthmatic effect of *Nigella sativa* in airways of asthmatic patients, *Phytomedicine* 17 (10) (2010) 707–713.
- [26] R. Velagapudi, A. El-Bakoush, I. Lepiazar, F. Ogunrinade, O.A. Olajide, AMPK and SIRT1 activation contribute to inhibition of neuroinflammation by thymoquinone in BV2 microglia, *Mol. Cell. Biochem.* 435 (1–2) (2017) 149–162.
- [27] E.C. Gökce, R. Kahveci, A. Gökce, B. Cemil, N. Aksoy, M.F. Sargon, et al., Neuroprotective effects of thymoquinone against spinal cord ischemia-reperfusion injury by attenuation of inflammation, oxidative stress, and apoptosis, *J. Neurosurg. Spine* 24 (6) (2016) 949–959.
- [28] M.K. Sahak, N. Kabir, G. Abbas, S. Draman, N.H. Hashim, D.S. Hasan Adli, et al., The role of *Nigella sativa* and its active constituents in learning and memory, *Evid Based Complement Alternat Med* 2016 (2016) 6075679.
- [29] I. Tekeoglu, A. Dogan, L. Demiralp, Effect of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models, *Phytother. Res.* 20 (10) (2006) 869–871.
- [30] I. Botnick, W. Xue, E. Bar, M. Ibdah, A. Schwartz, D.M. Joel, et al., Distribution of primary and specialized metabolites in *Nigella sativa* seeds, a spice with vast traditional and historical uses, *Molecules* 17 (9) (2012) 10159–10177.
- [31] M. Ashraf, Q. Ali, Z. Iqbal, Effect of nitrogen application rate on the content and composition of oil, essential oil and minerals in black cumin (*Nigella sativa* L.) seeds, *J. Sci. Food Agric.* 86 (6) (2006) 871–876.
- [32] S. Bourgou, R. Ksouri, I. Skandrani, L. Chekir-Ghedira, B. Marzouk, Antioxidant and antimutagenic activities of the essential oil and methanol extract from Tunisia *Nigella sativa*, *Ital. J. Food Sci.* 20 (2) (2008) 191–202.
- [33] N. Karimi-Yeganeh, H. Zeinali, The effect of water stress and phosphorous fertilizer on some quantitative characteristics and yield of *Nigella sativa* L., in: *The Proceedings of 11th Iranian Crop Science Congress, Environmental Science Research Institute. Shahid Beheshti University, Tehran, 2010.*
- [34] M. Bannayan, F. Nadjafib, M. Azizia, L. Tabrizia, M. Rastgoo, Yield and seed quality of *Plantago ovate* and *Nigella sativa* under different irrigation treatments, *Ind. Crops Prod. Amst.* 27 (1) (2008) 11–16.
- [35] X. Wang, D. Wang, J. Li, C. Ye, K. Kubota, Aroma characteristics of cocoa tea (*Camellia pilophylla* Change), *Biosci. Biotechnol. Biochem.* 74 (5) (2010) 90752–1–90752–8.
- [36] Y. Ito, A. Sugimoto, T. Kakuda, K. Kubota, Identification of potent odorants in Chinese jasmine green tea scented with flowers of *Jasminum sambac*, *J. Agric. Food Chem.* 50 (2002) 4878–4884.
- [37] E. Kováts, Gas chromatographic characterization of organic substances in the retention index system, in: J.C. Giddings, R.A. Keller (Eds.), *Advances in Chromatography*, Marcel Dekker, New York, 1965, pp. 229–247.
- [38] X. Liu, A.M.A. El-Aty, S.-K. Chod, A. Yanga, J.-H. Park, J.-H. Shim, Characterization of secondary volatile profiles in *Nigella sativa* seeds from two different origins using accelerated solvent extraction and gas chromatography-mass spectrometry, *Biomed. Chromatogr.* 26 (2012) 1157–1162.
- [39] C.M. Delahunty, G. Eyres, J.P. Dufour, Gas chromatography-olfactometry, *J. Separ. Sci.* 29 (2006) 2107–2125.
- [40] M. Shimoda, H. Sasaki, Y. Doi, W. Kameda, Y. Osajima, Characterization of abstract terms for odor-description of food products, *Nippon. Shokuhin Kogyo Gakkaishi* 36 (1989) 7–16.
- [41] M. Shimoda, Y. Tsukamoto, H. Ito, Y. Doi, W. Kameda, Y. Osajima, Preparation and evaluation of odor reference solution, *Nippon. Shokuhin Kogyo Gakkaishi* 38 (1991) 371–376.
- [42] S. Abera, B. Hirko, Chemical composition of essential oils of released black cumin varieties grown in Ethiopia, *Chem. Mater. Res.* 12 (2) (2020) 9–14.
- [43] K.H. Ghahramanloo, B. Kamalidehghan, H.A. Javar, R.T. Widodo, K. Majidzadeh, M.I. Noordin, Comparative analysis of essential oil composition of Iranian and Indian *Nigella sativa* L. extracted using supercritical fluid extraction and solvent extraction, *Drug Des. Dev. Ther.* 11 (2017) 2221–2226.
- [44] M.Y. Hadi, G.J. Mohammed, I.H. Hameed, Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography-mass spectrometry, *J. Pharmacogn. Phytotherapy* 8 (2) (2016) 8–24.
- [45] A.K. Khalid, M.R. Shedeed, GC-MS analyses of black cumin essential oil produces with sodium chloride, *Int. Food Res. J.* 23 (2) (2016) 832–836.
- [46] L.F. D’Antuono, A. Morreti, A.F.S. Lovato, Seed yield, yield components, oil content and essential oil content and composition of *Nigella sativa* L. and *Nigella damascena* L., *Ind. Crop. Prod.* 15 (1) (2002) 59–69.
- [47] F.S. Mozaffari, M. Ghorbanli, A. Babai, M.F. Sapher, The effect of water stress on the seed oil of *Nigella sativa* L., *J. Essent. Oil Res.* 12 (1) (2000) 36–38.
- [48] M. Tunçturk, R. Tunçturk, B. Yıldırım, The effects of varying phosphorus doses on yield and some yield components of Black Cumin (*Nigella Sativa* L.), *Adv. Environ. Biol.* 5 (2) (2011) 371–374.
- [49] F. Benkaci-Ali, A. Baaliouamer, B.Y. Meklati, F. Chemat, Chemical composition of seed essential oils from Algerian *Nigella sativa* extracted by microwave and hydrodistillation, *Flavour Fragrance J.* 22 (2007) 148–153.
- [50] S. Bourgou, A. Pichette, S. Lavoie, B. Marzouk, J. Legault, Terpenoids isolated from Tunisian *Nigella sativa* L. essential oil with antioxidant activity and the ability to inhibit nitric oxide production, *Flavour Fragrance J.* 27 (1) (2012) 69–74.