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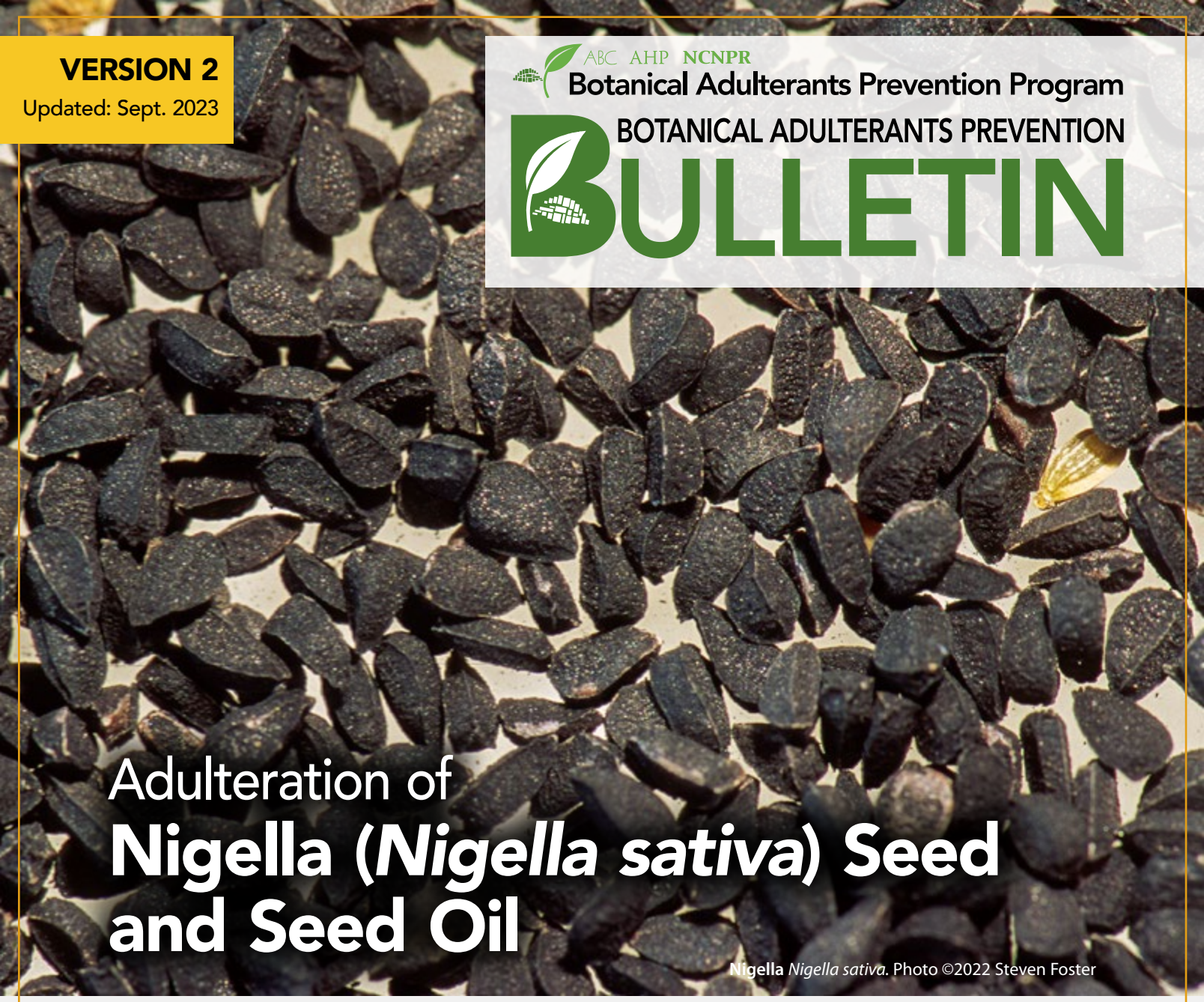

Adulteration of Nigella (*Nigella sativa*) Seed and Seed Oil

Nigella *Nigella sativa*. Photo ©2022 Steven Foster

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Goal: This bulletin aims to provide general information on the seed and seed oil of nigella (*Nigella sativa*) and summarizes the available information on adulteration, mislabeling, counterfeiting, and fraud in nigella raw material and its products. It also provides information on trade and market dynamics, laboratory methods for detecting adulteration, and economic and safety implications for the consumer and industry. It may be used as guidance for quality control personnel and members of the international phytomedicine and botanical supplement industries and the extended natural products community in general.

Definitions: *Nigella seed:* The seeds of the plant *N. sativa* are small, irregularly-shaped, and black seeds that are used whole or ground for various culinary and medicinal purposes.[1,2] In addition to the use of the whole seeds in foods, the major processed products from nigella seed are essential (volatile) oil and fatty (fixed)* oils.[3]

Nigella seed oil: Nigella seed oil (NSO) is a valuable edible oil obtained from crushed *N. sativa* seeds (usually between 30-38% of the seed).[4-6] It contains high concentrations of essential fatty acids, vitamins, minerals, phytosterols, thymoquinone (TQ), and the related compounds thymol and dithymoquinone.[7] Mechanical (screw-press) extraction is commonly used to obtain high-quality NSO having higher oxidative stability and lower nonhydratable phospholipids.[8]

Nigella seed essential oil: Nigella seeds contain 0.18-2.5% essential oil[5,9] that is yellowish-brown in color and has an unpleasant odor.[3] It can be obtained from the crushed seeds by steam distillation, hydrodistillation after Soxhlet extraction, or through hydrodistillation with a Clevenger apparatus.[10,11]

1. General Information

1.1 Common names: Nigella is the preferred standardized common name for the plant in the United States according to the second edition of the American Herbal Products Association's *Herbs of Commerce*.[12] Nigella is also known as black cumin,[13] black caraway,[1] black seed,[14] fennel flower,[1] Roman coriander,[13] and nutmeg flower.[14] Some of these common names may be confusing, since these names also refer to common spice plants, e.g., cumin (*Cuminum cyminum*), caraway (*Carum carvi*), fennel (*Foeniculum vulgare*), coriander (*Coriandrum sativum*) in the botanical family Apiaceae and nutmeg (*Myristica fragrans*) in the family Myristicaceae. Therefore, the name nigella is used throughout this document.

1.2 Common names in other languages[1,15]

Arabic: habat albaraka, habit assuda, kamun aswad, sanouz, shunez, sinouj

Chinese (Mandarin): hei zhong cao (黑種草)

Danish: zortkommen

Dutch: nigelle

French: nigella, poivrette

German: Schwarzkümmel, Nigella

Greek: melanthion (*μελάνθιον*), ninkéla (νιγκέλα)

Hindi: kalonji, kala jeera

Italian: nigella, grani neri

Japanese: nigera (ニゲラ)

Norwegian: svartkarve

Persian: siah daneh

Polish: czarnuszka

Portuguese: cominho-preto

Russian: chernushka (чернушка), chernyy tmin (черный тмин), kalindzhi (калинджи)

Spanish: neguilla, pasinara

Swedish: svartkummin

Turkish: çörek otu

1.3 Accepted Latin binomial: *Nigella sativa* L.

1.4 Synonyms: *Nigella cretica* Mill.[16]

1.5 Botanical family: Ranunculaceae

1.6 Distribution: The genus *Nigella* includes 26 species of annual herbaceous plants[17] that likely originated in the eastern Mediterranean, northeastern African, and southwestern Asian regions.[5] *Nigella sativa* is native to countries in southeastern and southern Europe (Bulgaria, Cyprus, and Romania) and the Near East (Azerbaijan, Georgia, Iran, Iraq, Turkey, and Turkmenistan). It is extensively cultivated from Morocco to northern India and Bangladesh, China, the Pacific Rim, East Africa, and Russia for use as a spice. *Nigella sativa* is also grown on a minor scale for medicinal purposes in North America, Europe, and Southeast Asia.[2,17]

1.7 Plant part and form: Whole or powdered seeds and seed oils of *N. sativa* are produced and sold according to current authorized uses in cosmetics, foods, and medicines around the world.[5] The global market of *N. sativa* is split among whole seed, powdered seed, seed oil, and capsules or gummies containing the oil.[18] There are many formulations made with NSO. It can be found in teas, cough syrups, wound salves, compresses, massage oils, and other products. Nigella seed honey, soap, shampoo, and creams are all available commercially. NSO has a spicy, peppery, and mildly bitter flavor; thus, it is sometimes

* Fatty oils, also known as fixed oils or oils, are non-volatile oils obtained from plants and animals that are usually composed of primarily triacylglycerols. Examples include, among many others, olive (*Olea europaea*, Oleaceae), peanut (*Arachis hypogaea*, Fabaceae) and sunflower (*Helianthus annuus*, Asteraceae) oils.

mixed with honey. Herbal teas also help make it more palatable.[14]

1.8 Key constituents and chemical markers: There are significant genetic variation and quantifiable differences in chemical composition among nigella seed chemotypes from different countries,[19] and several factors, including climatic conditions, location, harvesting and storage time, have notable effects on essential oil and fixed oil contents of nigella populations.[20,21] The main constituent of nigella seed is fixed oil (30-38%),[4-6,22] although lower and higher yields have been reported in a few cases.[23] It mostly consists of linoleic (50–60%), oleic (20–23.4%), palmitic (12.5%), dihomolinoleic (10%), and eicosadienoic (3%) acids, as well as arachidonic (0.01-0.4%), γ-linolenic (0.1-1%), stearic, and myristic acids, beta-sitosterol, sterol esters, and sterol glucosides, tocopherols (about 170 mg/kg) and some other minor lipid constituents.[5,6] Triacylglycerols (TAGs) are the major part of the saponifiable matter. Glyceryl trilinoleate (9.2-24.6%), glyceryl 1-oleate-2,3-dilinoleate (3.0-20.0%), glyceryl 1-palmitate-2,3-dilinoleate (12.4-18.5%), glyceryl 1-palmitate-2-oleate-3-linoleate (10.8-14.0%), glyceryl 1,2-dioleate-3-linoleate (9.1-9.6%) and glyceryl 1-stearate-2-oleate-3-linoleate (3.4%) are the principal TAGs of the NSO.[24-27]

Nigella *Nigella sativa*. Photo ©2023 Stefan Gafner

The seed also contains essential oil (0.18-2.5%), which is mainly composed of monoterpenes, including ρ-cymene, TQ, α-thujene, γ-terpinene, β-pinene, and carvacrol (6-12%), together with flavonol glycosides, proteins (20%), carbohydrates (32-37%), minerals, traces of saponins (hederagenin), nigellone (dithymoquinone), traces of isoquinoline alkaloids (nigellimine and nigellimine-*N*-oxide), indazole-type alkaloids (nigellicine, nigellidine, nigellidine-4-*O*-sulfite), and dolabellane-type diterpene alkaloids (nigellamines A1 to A5, B1, B2, and C). The myriad of therapeutic effects of NSO have been attributed to its key constituents and markers such as nigellicine, nigellidine, TQ, dithymoquinone, thymol, and carvacrol.[4-6] Dithymoquinone has been described as a dimer of thymoquinone that is present in the nigella essential oil and it was shown to be formed via photodimerization of TQ as a consequence of exposure to sunlight during separation and extraction processes.[28,29]

1.9 General use(s): Nigella is widely cultivated for its aromatic seeds, used as a flavoring, especially in Middle Eastern and Asian cuisines. They are commonly

sprinkled on bread, and used in lamb and poultry dishes, curries, yogurts, vegetables, salads, cottage cheese, and pickles.[1]

Whole or powdered nigella seeds are used to treat inflammations and respiratory conditions, as a carminative to ease bowel and digestive problems, for neurological disorders, and as a diuretic and diaphoretic agent. A tincture of the seed is taken for indigestion, loss of appetite, vomiting, dropsy, amenorrhea, dysmenorrhea, and treatment of skin eruptions. NSO is also used externally as a remedy for skin diseases, dry skin, dandruff, wounds, psoriasis, eczema, as an antiseptic, and internally to treat stomach problems, respiratory ailments, and allergies, as well as to improve circulation and the immune system.[14,30]

Additionally, powdered nigella seeds are ingested for worm infestations in Ayurvedic medicine.[5,31] Whole seeds are used for asthma, colic, flatulence, weakness of the stomach, headache and migraine, joint pain, lumbago, hemiplegia, Bell's palsy, jaundice, pityriasis, and leukoderma/vitiligo in the Unani system of traditional medicine.[5,32] It is also taken by lactating women to induce milk flow.[1] In Siddha medicine, dried seeds are used for the treatment of painful gastrointestinal disorders, jaundice, scalp eczema, scabies, and skin ulcers.[33]

Although the Ayurvedic, Unani, and Siddha Pharmacopoeias of India[31-33] provide basic information about nigella, there is neither an accepted monograph in the United States nor in the European Pharmacopoeia on nigella seed/seed oil that can be used to standardize nigella products. Nigella seed and its oil are classified as Generally Recognized as Safe (GRAS) for use as a spice, natural seasoning, or flavoring by the US Food and Drug Administration and are also accepted as a component of dietary supplement products in the United States.[34,35] In Canada, nigella seed and seed oil are regulated as medicinal ingredients of licensed natural health products.[36] The European Commission's Directorate-General for Health and Food Safety (formerly the Directorate-General for Health and Consumers) lists nigella seed extract[37] for perfuming and skin-conditioning functions, and the fixed oil expressed from the seeds of *N. sativa* for emollient, perfuming, and skin-conditioning functions in cosmetic products.[38] In Turkey, NSO soft capsules (900 mg) are available in pharmacies as traditional herbal medicinal product registered by the Turkish Ministry of Health.[39]

1.10 Historical use(s): Old civilizations considered nigella as part of their medicinal remedies as well as a spice and condiment. The oldest archaeological evidence of nigella, originating from Saqqara, Egypt, provides evidence that nigella was known and used in the Near East and Ancient Egypt since at least 2700 BCE.[40] Nigella seeds were found in the tomb of Egyptian pharaoh Tutankhamun, and the pharaohs' personal doctors are reported to have offered nigella seed as a digestive aid after large meals.[14] There is also evidence of cultivation and culinary and medicinal use in Mesopotamia from writings on cuneiform tablets of ancient Assyria from the third to first millennium BCE. In ancient Babylonia, the plant was used externally to treat swelling and bruises and internally to cure stomach problems.[41] Additionally, remains of nigella seeds mixed with honey or together with other seeds were found in many archeological sites such as Boyalı Höyük (Turkey),[42] Uluburun Shipwreck (Turkey),[43] Oedenburg (France),[44] and the Indo-

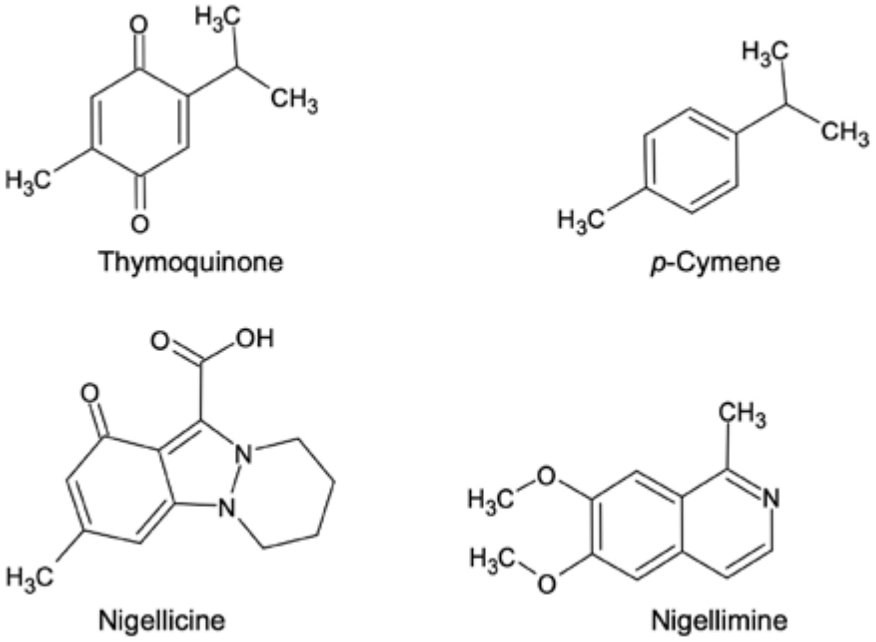


Figure 1. Representatives of secondary metabolites of nigella seed

Gangetic plains (India).[45] The health benefits of black cumin were well known and the seeds were used to fight illnesses and also to boost the health and wellbeing of sickly individuals.[46]

Nigella is referred by its Hebrew name *ketzah* in the Old Testament (Isaiah 28:27) where it is listed for its curative properties. Greek physicians Hippocrates and Galen described the use of *melanthion* (black cumin, section 1.2) to treat various maladies, including infections in the nose, while Dioscorides recorded its use as food and for curative purposes to treat headaches and toothache, to cure diseases of the eyes and skin, leprosy, to eliminate intestinal worms, to accelerate menstruation, to increase urine flow and milk flow, and to repel snakes.[41] The Prophet Muhammed reportedly recommended black cumin as a panacea to his associates in Arabia 1400 years ago. It remains one of the most famous medicinal herbs in the Muslim world.[47] The 10th century Persian physician Avicenna (Ibn Sina, also known as İbn-i Sina) refers to *N. sativa* seeds as able to "stimulate the body's energy and help recovery from fatigue and dispiritedness."[48]

2. Market

2.1 Importance in the trade: According to the American Botanical Council's annual Herb Market Report (T. Smith email to S. Gafner, June 19, 2018), the nigella dietary supplement sales in the United States were quite low in 2015 and 2016, but have exhibited substantial growth in subsequent years, especially in the natural channel (Table 1), which comprises natural health food and specialty stores such as co-ops, associations, independent stores, and large regional chains (excluding Whole Foods Market and Trader Joe's).[49,50] Herbal supplements containing nigella as a primary ingredient ranked 18th of the top-selling herbal dietary supplements list in this channel with a 21.6% increase in total sales from the previous year.[51]

According to Global Market Insights, the nigella seed oil market size exceeded US $6.46 million in the United States and US $17.63 million globally in 2020. It is estimated to register more than a 7.9% compound annual growth rate between 2021 and 2027. The demand for nigella seed oil in the United States is predicted to exceed 130 metric tons by 2025 for nutraceutical applications. The nigella seed oil global market is predicted to hit US $30 million by 2027.[52,53]

2.2 Supply sources: Historically, the major producers and exporters of cultivated nigella seed have been Egypt, Turkey, Syria, and India. Other important producers are Ethiopia, Pakistan, Saudi Arabia, and Bangladesh.[5,18] Certified organic nigella seeds come predominantly from farms in Egypt and, to a lesser extent, Turkey. The Turkish government website has nigella seed production data figures for the 10 years between 2012 and 2021, which indicate that Turkey produced about 6,435 metric tons of nigella seed in 2021 and the average Turkish nigella yield was 767 kg/ha in 2021.[5,54] According to the data of the Turkish Ministry of Agriculture and Forestry's *Nigella Report*, Turkey imported 2,414 tons of nigella seeds from Syria, 172 tons from India, 34 tons from the United States and 25 tons from Lebanon in 2019, and exported 370 tons to the United States, 49 tons to Germany and 118 tons from the Aegean Free Zone to unlisted countries in the same year.[55]

Although production of black cumin was influenced by several environmental, demographic, socioeconomic, and institutional factors, the most efficient production of nigella is reportedly in Ethiopia, with around 790 kg/ha. The annual production of nigella in Ethiopia from 2014 to 2015 was 18,000 metric tons. In local markets of Ethiopia, the demand is increasing for both oil and seeds of nigella. Nigella seed is one of the most important crops of Ethiopia that is traded to the international markets. Even though it is not possible to obtain up-to-date information, the majority of black cumin in Ethiopia's exports generally goes to the Arabian and other Muslim countries which accounted for 98% of total national exports in the year 2008.[18]

Table 1. Sales data for nigella seed dietary supplements in the United States from 2015 to 2020[49-51]

Year	Mass channel rank	Mass channel $	Natural channel rank	Natural channel $
2020	> 40	n/a	18	6,468,066
2019	> 40	n/a	18	5,436,146
2018	> 40	n/a	19	5,839,472
2017	110	77,797	23	4,675,514
2016	146	16,399	54	1,661,227
2015	156	4,253	82	836,532

n/a: not available

One of the net importers of the seeds of black cumin is Australia. It is estimated that Australia imported about 70.3 tons of nigella from India with a unit price of AU $4000 to AU $10,000 per ton in 2016.[53]

2.3 Market dynamics: Market prices for cultivated *N. sativa* seed from the two main countries of origin, Egypt and India, have generally been stable. The international trade price of Indian nigella seeds in late 2016 ranged from US $2,258 to US $2,750 per ton, according to the limited literature available, while the value of Egyptian nigella seeds is US $2,900–$3,000 per ton. Certified organic nigella seed comes predominantly from farms in Egypt and, to a lesser extent, Turkey.[5] It attracts a higher market price compared to conventionally grown nigella seeds. Nigella seed and seed oil prices increased significantly during the COVID-19 pandemic and as a consequence of drought in the Middle East. In the middle of 2022, the price of conventionally grown nigella seed from Turkey was US $3,000 per ton and the seed oil prices were between US $13 to $23 per kg (M. Büyükhelvacıgil [Zade Global] and Halis Ertaş [Talya] email to N. Orhan, April 10, 2022), while the price for cold-pressed organic nigella seed oil from India was US $35–$45 per kg (B. Darji [Verdure] email to S. Gafner, April 13, 2022). According to Menevseoglu,[56] the costs for NSO are about 25–30 times higher than for other vegetable oils such as sunflower (*Helianthus annuus*, Asteraceae), soybean (*Glycine max*, Fabaceae), corn (*Zea mays*, Poaceae), and canola (*Brassica napus* and other *Brassica* spp.,Brassicaceae) oil.

3. Adulteration

3.1 Known adulterants: The whole seed is subject to adulteration by the seeds of other *Nigella* spp. (*Nigella damascena*)[5] or with seeds similar in size and color such as black sesame (*Sesamum radiatum* Thonn. ex Hornem., Pedaliaceae), onion (*Allium cepa* L., Liliaceae), Mexican prickly poppy (*Argemone mexicana* L., Papaveraceae), *Corchorus* spp. (Malvaceae), and *Clitoria guianensis* (Fabaceae) seeds.[57-60] Additionally, grass seed coated with charcoal dust, cumin or fennel seeds covered with marble dust, and peanut butter powder or shell are listed as adulterants of cumin and nigella seeds.[61]

Nigella seed oil is a target for adulteration with lower-cost oils such as palm (*Elaeis guineensis*, Arecaceae) oil,[62] or refined corn (*Zea mays*, Poaceae), sunflower (*Helianthus annuus*, Asteraceae), soybean (*Glycine max*, Fabaceae), grape (*Vitis vinifera*, Vitaceae) seed, canola (*Brassica napus*, Brassicaceae), or walnut (*Juglans regia*, Juglandaceae) oil.[7,56,63]

3.2 Sources of information supporting adulteration: Historically, nigella seed was frequently confused with the toxic- and black-seeded corn cockle (*Agrostemma githago*, Caryophyllaceae), which grows in the same habitat.[40] This is explained by both plants having seeds of the same size and color despite otherwise strikingly different morphological traits. Heiss et al.[40] suggest that reports of nigella toxicity in historical texts are likely due to confusion between the two plants. This unintentional type of adulteration no longer appears to be an issue.

Nigella seed oil adulteration has become a concern because NSO has numerous health benefits and a relatively high price. Although several adulterants of the nigella seed and nigella seed oil are mentioned in various articles,[7,58-60,62-65] there are only a few studies confirming adulteration in commercial products.[56,57,66,67]

Sudhir et al.[57] analyzed nigella seed samples collected from open markets of India, Pakistan, Saudi Arabia, Egypt, Turkey, Syria, Tunisia, and Oman. Two of ten commercial samples were found to be different based on morphological and microscopic studies, and the *rbcL* sequences of the distinct seeds were identified as *Allium cepa* (Amaryllidaceae) and *Clitoria guianensis* (Fabaceae) by comparison with sequences available in public databases using the BLASTn application.[57] In a study from 2022, five of 40 commercial NSO products, purchased online and from various local markets in Turkey, were found to be adulterated according to results from chromatographic and spectroscopic analysis. Canola or soybean oils were suspected to be used as adulterants.[56] Additionally, an investigation into the composition of 20 commercial NSO products resulted in two samples for which none of the selected fatty acids could be determined, likely due to adulteration according to the authors.[68] Finally, the analysis of fatty acids in five NSOs from Hyderabad, Pakistan, showed uncharacteristic profiles in two samples due to dilution with other vegetable oils.[66] On the other hand, the analysis of two commercial NSO samples originating from Ethiopia and India did not provide evidence of adulteration.[67]

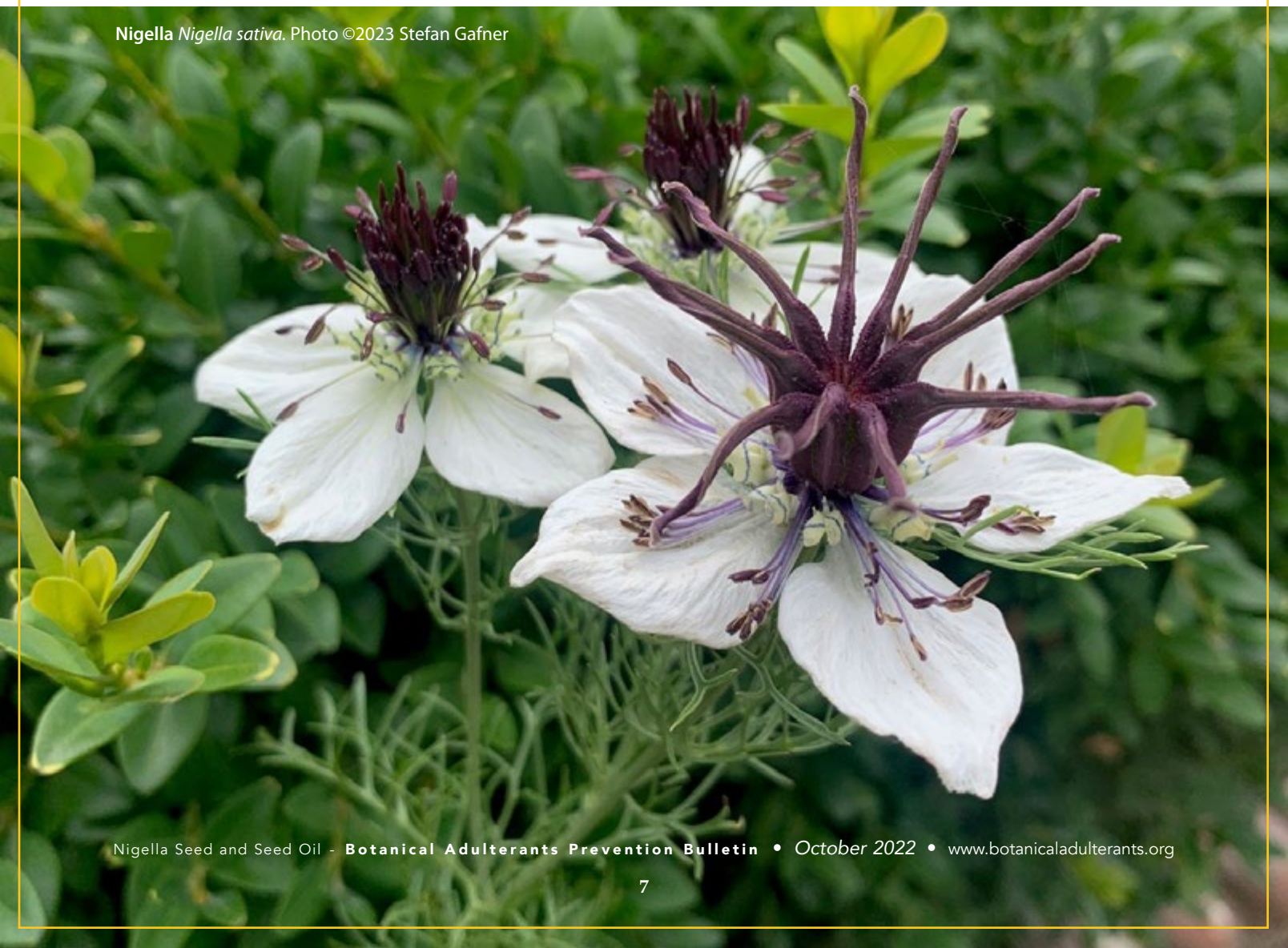
3.3 Accidental or intentional adulteration: The seeds of *N. sativa*, *N. damascena,* and *N. arvensis* are used in folk medicine and as a spice in Turkey and many farmers plant the seeds of *N. damascena* in the same fields with *N. sativa* thereby exposing nigella to the risk of mistakenly harvesting the seeds of the wrong species.[5,20] On the other hand, intentional adulteration and substitution by morphologically similar seeds such as *Sesamum indicum*, *Allium cepa*, *Argemone mexicana*, *Corchorus* spp., and *Clito-*

ria guianensis may be encountered in commercially available nigella seed.[57-60] Because NSO now has a relatively high commodity value, and its price per liter is almost 25-30 times higher than known vegetable oils such as sunflower, soybean, corn, and canola oil, the relative ease of diluting NSO with these oils and the challenges of detecting it, adulteration of NSO has become a concern.[56] Such adulteration is clearly economically motivated.

3.4 Frequency of occurrence: There is no comprehensive published study on the frequency of nigella seed oil adulteration, but information from publications albeit with small sample numbers indicates that adulteration is an issue. In the most extensive investigation to date, Menevseoglu determined that five of 40 commercial NSO samples tested (12.5%) were suspected of adulteration with different vegetable oils.[56] Other studies found adulteration rates of 10% and 40%, respectively.[66,68] On the other hand, one study on two Ethiopian NSO samples did not provide evidence of adulteration.[67]

The extent of seed adulteration is not known. The available data from 10 commercial nigella seed samples from open markets in Asia and North Africa, suggesting 20% adulteration, may not be reflective of the global nigella seed market.[57]

3.5 Possible safety/therapeutic issues: Even though nigella seed and seed oil have been safely used around the world for centuries, the European Food Safety Authority (EFSA) listed *N. damascena* and *N. sativa* seeds in the "EFSA Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic, or other substances of concern"[69] and in the "Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements" due to the occurrence of alkaloids in the plant.[70] According a French order (June 24, 2014), the use of *N. sativa* seed and its oil in food supplements is authorized but, due to the presence of isoquinoline alkaloids, requires a warning on the label to avoid its use in children, adolescents, pregnant or breastfeeding women.[71] Data on the alkaloid concentrations in



Nigella *Nigella sativa*. Photo ©2023 Stefan Gafner

Nigella damascena. Photo ©2022 Steven Foster

NSO are lacking, but according to Karl-Werner Quirin (Flavex Naturextrakte GmbH, Rehlingen, Germany), the alkaloids are left in the pomace when extracting nigella seeds with supercritical CO_2 (email to S. Gafner, June 3, 2022). A review of available safety data suggests that NSO has a relatively good safety profile.[72-81]

On the other hand, adulteration of *N. sativa* seeds with *Garidella nigellastrum* (syn. *N. garidella,* Apiaceae) can cause poisoning,[14] and substitution with *N. arvensis* and *N. damascena* affects the bioactivity of the seed oil since they do not contain TQ, one of the primary active compounds of NSO.[82,83] Adulteration of nigella seed with other similar seeds may be harmful to health since *Agrostemma githago*[84] and *Argemone mexicana*[85] seeds are known to be highly toxic and *Sesamum indicum* seed can sometimes be allergenic, especially in children.[86] Additionally, adulteration with lower-cost oils dilutes the NSO and causes a decrease or loss in its expected beneficial effect on health.

3.6 Analytical methods to detect adulteration: The botanical identity of nigella seeds may be confirmed based on organoleptic and macroanatomical characteristics. The identity of powdered material may be determined using a combination of organoleptic, microscopic, and chemical techniques provided in a number of sources that include pharmacopoeial monographs.[1,2,32,33,40,60,83,87]

Physiochemical properties of NSO were studied to indicate its quality in many studies. Refractive index, color, specific gravity, free fatty acids, peroxide value,

iodine value, UV absorption (232 nm and 270 nm) to measure conjugated dienes and trienes, saponification value, and iodine value were investigated to determine the quality and the purity of the oils.[8,13,24,88-90] Limits of these specifications are also included in the draft monograph of NSO in the *Turkish Pharmacopoeia*.[91] NSOs that do not meet the specifications suggest low quality or adulteration.

The Siddha Pharmacopoeia of India specifies the use of thin-layer chromatography (TLC) for the authentication of alcohol extracts of nigella seeds,[33] and the high-performance thin-layer chromatography (HPTLC) method of the USP for fixed oils (USP41-NF36, fixed oil method <202>) can be used for nigella seed oil analysis. Different TLC[91] and HPTLC methods[92] have been developed for the identification of nigella seed and seed oil by the HPTLC Association (TQ in seed and seed oil), *Hong Kong Chinese Materia Medica Standards*† (seed),[93] and *Thai Herbal Pharmacopoeia* (seed).[94] Several additional HPTLC methods have been proposed by various authors for the standardization of nigella seed and seed oils, and to determine their TQ content.[95-98] The fingerprints obtained from these TLC/HPTLC analyses of nigella seed and seed oils can be used as a reference for identification, quality control and to differentiate the seeds/seed oils from their adulterants.

Vibrational spectroscopy techniques (near-infrared [NIR], mid-infrared [MIR], Raman) are rapid, sensitive, and non-destructive methods to provide information about the analyzed sample and can be combined with chemometrics to detect NSO adulteration.[56] Fourier transform infrared spectroscopy (FTIR) coupled with multivariate calibration using partial less squares (PLS) regression has been used for quantifying the levels of grape seed oil,[63] corn, soybean,[64] walnut, and sunflower oils[65] in NSO samples. A combination of attenuated total reflection (ATR)-FTIR and synchronous fluorescence (SyF) spectroscopy with multivariate data analysis was used to analyze eight different commercial brands of cold-pressed NSO, 36 refined oils, and 96 oil blends. Fluorescence spectroscopy was found to be more selective than FTIR to classify pure NSO and other vegetable oils such as sunflower, soybean, hazelnut, cottonseed, grape seed, and olive oils.[7] In a recent study, NSO adulteration with vegetable oils was assessed using handheld FT-NIR, portable FT-MIR, and Raman spectrometers. Portable vibrational spectroscopic units, combined with chemometrics, provided a rapid (~10 s), non-invasive, and reliable determination of black cumin oil adulterated with vegetable oils.[56] Raman spectroscopy was also used to compare lab-produced cold-pressed oil samples of nigella, almond, and walnut with their commercial cold-pressed equivalents and to determine the adulteration of these oils with corn and sunflower oils.[21]

High performance liquid chromatography (HPLC) is used for the quantification of TQ in NSO samples.[6,24,99] There is a significant variation in TQ content of NSOs that may be due to the chemotype, source of the seeds, the method of extraction, storage conditions, and the age of the oils. The TQ content of commercially sold, cold-pressed NSO samples (n = 10) and cold pressed NSO capsules (n = 10) from Turkey were determined to be between 0.05-0.619% and 0.037-2.023%, respectively.[6] Similarly, TQ contents were found to be between 0.348-0.873% in commercially sold cold-pressed NSO samples (n = 6) from the United States.[99] Solati et al.[89] compared the TQ content in NSOs from Iran by HPLC and the amounts were found to be 0.106% and 0.407% in oils obtained from Soxhlet and supercritical carbon dioxide extraction techniques, respectively. HPLC was also used to evaluate the chemical profile of the nigella seeds. Kaempferol was identified only in the *N. sativa* seed extract (6.06 ± 0.02 µg/g dry weight [dw] seed), while quercetin (14.35 ± 0.02 µg/g dw seed), and hyperoside (1.08 ± 0.01 µg/g dw seed) were detected only in *N. damascena* seeds.[100]

Khan et al.[87] performed an ultra high-performance liquid chromatography (UHPLC)-mass spectrometer (-MS) based fingerprinting analysis for the chemical profiling of nigella seeds and its adulterants *Allium cepa*, and *A. tuberosum*. Allicin, allyl ionone, propiin, and methiin were identified in the potential adulterants as chemical markers in *Allium* spp. that can be used to distinguish these seeds from nigella.[87]

Gas chromatography (GC) coupled with flame ionization (GC-FID) and/or a MS detector is the preferred method for NSO and essential oil analysis. Some of the published studies focused on the differentiation of various *Nigella* species, such as *N. arvensis*[82] and *N. damascena*,[83,101] while others targeted the detection of NSO adulteration by vegetable oils (e.g., palm,[62] grape seed,[63] corn, soybean,[64] sunflower, walnut[65] and canola oils).[56] GC can be used for both fingerprinting of the lipid constituents and quantification of fatty acids,[21,22,24,56,63-65,83,102] sterols,[22,24,62] and thymoquinone[11,91,103] in NSO samples to verify authenticity, although care needs to be taken when measuring thymoquinone by GC due to stability issues (K.W. Quirin email to S. Gafner, May 31, 2022). There are only a few studies that investigate the constituents, quality, and adulteration of cold-pressed NSO[21,24,103]

† The monograph by the *Hong Kong Chinese Materia Medica Standards* uses the scientific name *Nigella glandulifera*, which is considered an infraspecies of *N. sativa*.

or commercial products that contain NSO.[21,56,66,68,103]

Essential oil of *N. damascena*, contains a blue-fluorescing protoalkaloid called damascenine (nigelline) which is absent in *N. sativa*. *Nigella damascena* and *N. arvensis* do not contain thymoquinone, whereas this substance is characteristic of *N. sativa*.[5,82,83] Essential oils of *N. damascena* and *N. orientalis* contain predominantly sesquiterpenes, such as β-elemene, germacrene A, and α-selinene, while essential oils from *N. sativa* and *N. arvensis* seem to be similar in the concentrations of monoterpenes. *Nigella arvensis* essential oil contains a higher amount of alkanes, alkenes, and related aldehydes, in particular *n*-undecane, and GC is mainly used to examine these differences.[82,101]

According to the results of GC-MS analysis of Deme et al.,[62] sitosterol, campesterol and stigmasterol are the dominant sterols of NSO, and lupeol, lanosterol, and olean-12-en-3-one are specific to NSO. Cholesterol and 24-nor-22,23-methylenecholest-5-en-3β-ol, which are present in palm oil, can be used to trace adulteration of NSO by palm oil.[62]

DNA barcoding and other genetic methods have been used to identify whole nigella seeds and their adulterants: *Allium cepa* seed, *Clitoria guianensis* seed, and *Corchorus* spp. seed.[57,58] According to the results, certain DNA genomic regions, especially the *rbcL* locus can be used for *N. sativa* authentication and distinction from its potential adulterants.

4. Conclusions

Nigella seed oil (NSO) is a popular ingredient in many parts of the world with numerous purported health benefits and uses. High prices in the market and the availability of low-cost edible oils such as sunflower, grape seed, and walnut oil have increased the risk of adulteration. Multiple analytical methods (TLC, IR, GC, etc.) to authenticate nigella seeds and NSO have been published and a few pharmacopeial monographs exist which provide basic methods of identification and quality assessment. It is obvious that there is a need for more comprehensive *N. sativa* seed and seed oil monographs that will establish quality standards to provide better tools to reduce the adulteration of nigella seed, NSO, and their products in the culinary and dietary supplement market. Companies and individuals involved in the purchase, trade, or quality control of nigella seed oil ingredients should be aware of the quality requirements and of existing adulteration issues, and take the necessary precautionary measures to avoid purchasing, selling, or manufacturing products made with adulterated nigella seed oil materials.

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REVISION SUMMARY

Version # , Author,	Date Revised	Section Revised	List of Changes
Version 1, N. Orhan	N/A	N/A	None
Version 2, N. Orhan	Sept 22, 2023	Photos on page 3 and 7	Changed out photos inaccurately labeled as *Nigella sativa*