الجمهورية الجزائرية الديمقراطية الشعبية
People's Democratic Republic of Algeria
وزارة التعليم العالي والبحث العلمي
Ministry of Higher Education and Scientific Research

Constantine 1- Frères Mentouri University | **جامعة الاخوة منتوري قسنطينة**

Faculty of Natural and Life Sciences | **كلية علوم الطبيعية والحياة**

Department : Microbiology | **قسم:** ميكروبيولوجيا

A Thesis Presented in Partial Fulfilment of the Requirements for The Master's Degree

Field: Natural and Life Sciences

Sector: Biological Sciences

Specialty: Mycology and Fungal Biotechnology

Order No.:
Serial No.:

Evaluation of Microbiological Quality of some Commercial Black Seeds (*Nigella sativa* L.) in Constantine, Algeria: Implications for Food Safety

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Academic Year: 2024-2025

Abstracts

Abstract

The objective of this study was to assess the microbiological quality of commercial black seeds (*Nigella sativa* L.) available in Constantine, Algeria, with an emphasis on food safety implications. These seeds are widely recognized for their health and nutritional benefits with many applications in food, medicine, and cosmetics. Three *N. sativa* L. seed samples (BS1, BS2, and BS3) were purchased from different traditional herbal medicine stores and analyzed by using the culture media Plate Count Agar, Violet Red Bile Lactose Agar, Baird-Parker agar, and Sabouraud Dextrose Agar to enumerate total aerobic mesophilic bacteria, coliforms, staphylococci, and fungi, respectively. Microbiological studies were performed according to the International Organization for Standardization (ISO) methods. The results revealed significant variability in microbial contamination among samples. Total aerobic mesophilic bacterial counts ranged from 1.03×10^4 to 6.7×10^4 CFU/g, with BS1 exhibiting the highest load. This is presumably attributable to inadequate drying or substandard storage, aligning with other findings on Algerian herbs. Coliform counts were notably high, ranging from 2.2×10^4 to 2.5×10^5 CFU/g, substantially exceeding both Algerian (2×10^3 CFU/g) and international ($<10^3$ CFU/g) regulatory limits for dried herbs and spices. No coagulase-positive staphylococci (*Staphylococcus aureus*) were detected in any sample, suggesting minimal direct human contamination and possibly reflecting the antimicrobial properties of *N. sativa* constituents. Fungal counts were relatively low (1.0×10^2 to 5.6×10^2 CFU/g) and below national thresholds, although the presence of fungi still poses a potential risk for mycotoxin contamination. The observed differences in microbial quality among samples highlight the influence of handling and storage practices and underscore the need for standardized post-harvest protocols. These findings emphasize the importance of rigorous hygiene measures throughout the supply chain, regular microbiological monitoring, and consumer education to ensure the safety of edible seeds. Ensuring the microbiological safety of *N. sativa* seeds is essential for public health and consumer protection in Algeria.

Keywords: *Nigella sativa* L. seeds; Microbiological quality; Enumeration; Total bacteria; Coliforms; Staphylococci; Fungi.

Résumé

L'objectif de cette étude était d'évaluer la qualité microbiologique des graines de nigelle (Nigella sativa L.) disponibles à Constantine, en Algérie, en mettant l'accent sur leurs implications en matière de sécurité alimentaire. Ces graines sont largement reconnues pour leurs bienfaits pour la santé et leur nutrition, avec de nombreuses applications dans l'alimentation, la médecine et les cosmétiques. Trois échantillons de graines de N. sativa L. (BS1, BS2 et BS3) ont été achetés auprès de différentes herboristeries traditionnelles et analysés à l'aide des milieux de culture suivants : gélose pour comptage sur plaque, gélose au lactose et à la bile rouge violet, gélose de Baird-Parker et gélose dextrose de Sabouraud afin de dénombrer respectivement les bactéries mésophiles aérobies totales, les coliformes, les staphylocoques et les champignons. Les études microbiologiques ont été réalisées selon les méthodes de l'Organisation internationale de normalisation (ISO). Les résultats ont révélé une variabilité significative de la contamination microbienne entre les échantillons. Français Le nombre total de bactéries mésophiles aérobies variait de $1,03\times10^4$ à $6,7\times10^4$ UFC/g, BS1 présentant la charge la plus élevée. Ceci est probablement attribuable à un séchage inadéquat ou à un stockage non conforme aux normes, ce qui concorde avec d'autres résultats sur les herbes algériennes. Le nombre de coliformes était particulièrement élevé, allant de $2,2\times10^4$ à $2,5\times10^5$ UFC/g, dépassant largement les limites réglementaires algériennes (2×10^3 UFC/g) et internationales ($<10^3$ UFC/g) pour les herbes et épices séchées. Aucun staphylocoque à coagulase positive (Staphylococcus aureus) n'a été détecté dans aucun échantillon, ce qui suggère une contamination humaine directe minimale et reflète peut-être les propriétés antimicrobiennes des constituants de N. sativa. Les numérations fongiques étaient relativement faibles ($1,0 \times 10^2$ à $5,6 \times 10^2$ UFC/g) et inférieures aux seuils nationaux, bien que la présence de champignons présente toujours un risque potentiel de contamination par les mycotoxines. Les différences observées dans la qualité microbienne des échantillons soulignent l'influence des pratiques de manipulation et de stockage et soulignent la nécessité de protocoles post-récolte standardisés. Ces résultats soulignent l'importance de mesures d'hygiène rigoureuses tout au long de la chaîne d'approvisionnement, d'une surveillance microbiologique régulière et de l'éducation des consommateurs pour garantir la sécurité des graines comestibles. Garantir la sécurité microbiologique des graines de N. sativa est essentiel pour la santé publique et la protection des consommateurs en Algérie.

Mots-clés : Graines de *Nigella sativa* L.; Quality microbiologique; Dénombrement; Bactéries totales; Coliformes; Staphylocoques; Champignons.

الملخص

ان الهدف من هذه الدراسة هو تقييم الجودة الميكروبيولوجية لبذور الحبة السوداء (*Nigella sativa* L.) التجارية المتوفرة في قسنطينة، الجزائر، مع التركيز على آثارها على سلامة الغذاء. تُعرف هذه البذور على نطاق واسع بفوائدها الصحية والتغذوية، ولها تطبيقات عديدة في الأغذية والأدوية ومستحضرات التجميل. تم شراء ثلاث عينات من بذور الحبة السوداء (BS1 وBS2 وBS3) من متاجر مختلفة للأدوية العشبية التقليدية، وحُللت باستخدام أوساط الزراعة: أجار العد الطبقي، وأجار اللاكتوز الصفراوي الأحمر البنفسجي، وأجار بيرد-باركر، وأجار سكر العنب سابورو، وذلك لتعداد البكتيريا الهوائية المتوسطة، والقولونيات، والمكورات العنقودية، والفطريات، على التوالي. أُجريت الدراسات الميكروبيولوجية وفقًا لطرق المنظمة الدولية للمعايير (ISO). كشفت النتائج عن تباين كبير في التلوث الميكروبي بين العينات. تراوح إجمالي تعداد البكتيريا الهوائية متوسطة الحرارة بين 1.03×10^4 و6.7×10^4 وحدة تشكيل مستعمرة/غ، مع تسجيل أعلى حمل للبكتيريا BS1. ويُرجَّح أن هذا يُعزى إلى عدم كفاية التجفيف أو التخزين دون المستوى المطلوب، وهو ما يتوافق مع نتائج أخرى أجريت على الأعشاب الجزائرية. كان تعداد البكتيريا القولونية مرتفعًا بشكل ملحوظ، حيث تراوح بين 2.2×10^4 و2.5×10^5 وحدة تشكيل مستعمرة/غ، متجاوزًا بشكل كبير الحدود التنظيمية الجزائرية (2×10^3 وحدة تشكيل مستعمرة/غ) والدولية ($<10^3$ وحدة تشكيل مستعمرة/غ) للأعشاب والتوابل المجففة. لم يُكتشف أي بكتيريا عنقودية موجبة لإنزيم التخثر (*Staphylococcus aureus*) في أي عينة، مما يشير إلى تلوث بشري مباشر ضئيل، وربما يعكس الخصائص المضادة للميكروبات لمكونات *N. sativa*. كانت أعداد الفطريات منخفضة نسبيًا (من 1.0×10^2 إلى 5.6×10^2 وحدة تشكيل مستعمرة/غرام) وأقل من الحدود الوطنية، على الرغم من أن وجود الفطريات لا يزال يُشكل خطرًا محتملًا للتلوث بالسموم الفطرية. تُبرز الاختلافات الملحوظة في جودة الميكروبات بين العينات تأثير ممارسات المناولة والتخزين، وتُؤكد على الحاجة إلى بروتوكولات موحدة لما بعد الحصاد. تُؤكد هذه النتائج على أهمية تدابير النظافة الصارمة على طول سلسلة التوريد، والمراقبة الميكروبيولوجية المنتظمة، وتوعية المستهلك لضمان سلامة البذور الصالحة للأكل. يُعد ضمان السلامة الميكروبيولوجية لبذور ن. ساتيفا أمرًا أساسيًا للصحة العامة وحماية المستهلك في الجزائر.

الكلمات المفتاحية: بذور *Nigella sativa* L.؛ الجودة الميكروبيولوجية؛ العد؛ البكتيريا العامة؛ القولونيات؛ المكورات العنقودية؛ الفطريات.

Acknowledgements

Acknowledgements

Above all, we thank Almighty God for granting us the strength, courage, and perseverance, and for facilitating the means that allowed us to complete this modest work. We are grateful for the light He shed on our path toward success.

We extend our heartfelt thanks and sincere appreciation to our supervisor, **Dr. Kenza Fatima-Zohra Labbani**, Associate Professor at the Department of Natural Sciences, Teachers Training College *El Katiba Assia Djebar – Constantine*, University Town, Ali Mendjeli – Constantine, for her scientific guidance, unwavering dedication, valuable advice, and the trust she placed in us, which enabled us to carry out this research.

We also express our deepest gratitude to the members of the defense committee: **Dr. Hind Leghlimi**, from Constantine 1 – Frères Mentouri University, and **Dr. Djamila Benouchenne**, from the Higher National School of Biotechnology in Constantine (ENSB),
for kindly accepting to evaluate this work, and for their insightful comments and valuable suggestions that enriched and improved our study.

We sincerely thank our families. Without them, we may not have had the courage or energy to complete this work. They have always been our pillar of support through their prayers, patience, and unconditional encouragement at every step. They surrounded us with love and warmth, and were the guiding light in moments of exhaustion and doubt.

We would also like to express our deep appreciation to our dear friends for their continuous support, heartfelt encouragement, and reassuring words, which were a source of comfort during moments of uncertainty and fatigue.

Table of Contents

Table of Contents

List of Figures

	0.001). Different superscript letters (a, b, c) indicate statistically significant differences between groups according to Tukey's HSD post hoc test ($p < 0.05$).	
13	Mean fungal counts (CFU/g × 10^2 ± SD) in commercial *Nigella sativa* L. seed samples (BS1, BS2, and BS3) determined on SDA with chloramphenicol after incubation at 25°C for 5 days. Bars represent mean values with standard deviation (n = 3). All observed colony counts were below the ISO 21527-2:2008 recommended countable range (10–150 colonies per plate); therefore, values are presented as estimates rather than precise quantifications.	35

List of Tables

List of Abbreviations

List of Abbreviations

2hPG: 2-hour Postprandial blood Glucose

ANOVA: Analysis of Variance

CCPs: Critical Control Points

CFU: Colony Forming Unit

COVID-19: Coronavirus Disease 2019

Cr: Chromium

ELISA: Enzyme-Linked Immunosorbent Assay

FBS: Fasting Blood Glucose

FCR: Feed Conversion Ratio

FMEA: Failure Mode and Effects Analysis

FSMS: Food Safety Management Systems

FSMS: Food Safety Management Systems

GAPs: Good Agricultural Practices

GHP: Good Hygiene Practices

GMP: Good Manufacturing Practices

GSH: Gastric Mucin, Glutathione

HACCP: Hazard Analysis and Critical Control Point

HBA1C: Glycosylated Haemoglobin

HCV: Hepatitis C Virus

HDL-C: High-Density Lipoprotein Cholesterol

HIV: Human Immune Definciency Virus

HMG-CoA: 3-Hydroxy-3-Methylglutaryl-Coenzyme A

IBM SPSS 25: Statistical Package for Social Sciences

ICMSF: International Commission on Microbiological Specifications for Foods

IL-6: Interleukin-6

ISO: International Organization for Standardization

ISTA: International Seed Testing Association

ISTA: International Seed Testing Association

LDL-C: Low-Density Lipoprotein Cholesterol

LFD : Lateral Flow Devices

MDA: Malondialdehyde

MGT: Mean Germination Time

MPN: Most Probable Number

MRSA: Methicillin-Resistnt *Staphylococuss aureus*

MUFA: Monounsaturated Fatty Acids

MUG: 4-Methylumbelliferyl-D-glucuronide

NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cells

NK: Natural Killer

OECD: The Organisation for Economic Cooperation and Development

OECD: Organisation for Economic Cooperation and Development

ONPG: Ortho-Nitrophenyl-β-D-Galactopyranoside

P: Probability Value

PCA: Plate Count Agar

PCR: Polymerase Chain Reaction

PPE: Personal Protective Equipment

PUFA: Polyunsaturated Fatty Acid

qPCR: Quantitative PCR

RT-qPCR : Reverse Transcription Quantitative PCR

SARS-cov-2: Severe Acute Respiratory Syndrome Coronavirus 2

SD: Standard Deviations

SDA: Sabouraud Dextrose Agar

SFA: Saturated Fatty Acids

SGP: Speed of Germination Percentage

SOD: Superoxide Dismutase

SSOPs: Standard Operating Procedures for Sanitation

T4: Helper T lymphocytes ($CD4^+$)

T8: Cytotoxic T lymphocytes ($CD8^+$)

TC: Total cholesterol

TG: Triglycerides

TNO: Total nitric oxide

TQ: Thymoquinone

VRBL: Violet Red Bile Lactose

Introduction

Introduction

Nigella sativa L., commonly known as black cumin or nigella, is an annual herb belonging to the Ranunculaceae family. It is of profound medicinal and economic significance (John, 2025; Thabede and Shooto, 2021). Indigenous to Southern Europe, North Africa, and Southwest Asia, this plant has been cultivated for millennia and is integral to traditional medical systems such as Unani, Ayurveda, and Islamic medicine (Hannan et al., 2021; Kmail, 2023). Its seeds, historically documented in ancient Egyptian, Greek, and Middle Eastern texts, are revered for their therapeutic versatility, earning the epithet "remedy for all ailments except death" in Islamic tradition (Akbar, 2020; Kmail, 2023).

The seeds of *N. sativa* are distinguished by their unique phytochemical composition, which includes fixed and essential oils (notably thymoquinone, 21.1–48% of essential oil), proteins (20–27%), unsaturated fatty acids (linoleic and oleic acids, 50–58% and 20–28%, respectively), and a spectrum of bioactive compounds such as alkaloids, saponins, and sterols (Albakry et al., 2022; Hossain et al., 2024; Sharma & Longvah, 2021). These constituents underpin the seeds' antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, driving their incorporation into nutraceuticals, functional foods, and pharmaceutical formulations (Abdollahi et al., 2024; Wahab et al., 2023).

Despite their therapeutic value, the microbiological qualiy of *N. sativa* seeds remains a critical concern. Like other dried botanicals, the seeds are susceptible to contamination by aerobic mesophilic bacteria, coliforms (e.g., *Escherichia coli*), staphylococci, and mycotoxin-producing fungi (e.g., *Aspergillus,* and *Penicillium*) during cultivation, harvesting, processing, or storage (Anyogu et al., 2024; Bassam et al., 2018; Özçakmak et al., 2023). Studies have detected *Salmonella* spp., *E. coli*, and aflatoxins in commercial samples, highlighting risks of foodborne illness and toxin exposure (Bassam et al., 2018; Özçakmak et al., 2023).

Such contamination poses significant risks to consumer health and highlights the necessity for stringent quality control measures throughout the supply chain, including adherence to good agricultural and manufacturing practices, regular microbiological monitoring, and compliance with national and international food safety standards (Anyogu et al., 2024; Bassam et al., 2018).

In Algeria, *N. sativa* seeds are widely consumed through traditional herbal markets and are increasingly present in commercial products; however, there remains a notable paucity of data on their microbial quality, despite the existence of national regulations (Arrêté interministériel du 2 Moharram 1438 correspondant au 4 octobre 2016, Annexe I). This gap is particularly concerning

given the seeds' growing integration into functional foods and their widespread use in both culinary and medicinal contexts.

According to Algerian regulatory guidelines, strict microbiological thresholds are mandated for oilseeds and edible seeds, including *N. sativa*. For example, the maximum acceptable limits are ≤20 CFU/g for *E. coli*, ≤10^2 CFU/g for *Staphylococcus aureus* and molds, and the absence of *Salmonella* in 25 g of product, to ensure consumer safety (Arrêté interministériel, 2016). These standards are consistent with international recommendations, which emphasize the need for rigorous monitoring and quality control throughout the supply chain to minimize both primary and secondary sources of contamination (Bassam et al., 2018).

Consequently, comprehending the microbiological condition microbiological status of *N. sativa* seeds is essential for guaranteeing their safe utilization, especially in pharmaceutical and nutritional contexts. This study aims to assess the microbiological quality of selected commercially available N. sativa (black cumin) seeds in Constantine, Algeria, by evaluating the presence and levels of total aerobic mesophilic bacteria, coliforms, staphylococci, and fungi. This research addresses a significant knowledge gap regarding the safety and quality of black cumin seeds in the Algerian market, provides evidence-based insights for improving handling and storage practices, and contributes to the broader goal of safeguarding public health through enhanced food safety monitoring.

This thesis is organized as follows:

- Chapter One presents a comprehensive literature review, including an overview of *N. sativa* seeds, their nutritional and medicinal value, and current knowledge on their microbial quality.
- Chapter Two details the materials and methods employed in the microbiological assessment.
- Chapter Three reports and discusses the results, contextualizing the findings within national and international standards, and offers recommendations for improving the microbiological safety of *N. sativa* seeds in Algeria.

Chapter One

Literature Review

Part I. *Nigella sativa* L. (Black Seeds)

1. Overview of The Plant *Nigella sativa* L.

1.1. Scientific classification of *Nigella sativa* L.

Nigella sativa L. (*N. sativa* L.), commonly known as black cumin or nigella, is a short-lived annual plant belonging to the family Ranunculaceae, which includes about 2000 species of flowering plants (Kmail, 2023; Osman Yagoub, 2023; Pandey et al., 2024).

The *Nigella* genus comprises approximately 20 species, predominantly annuals with a short life cycle (Pandey et al., 2024). While the therapeutic potential of other species has been reported, *N. sativa* represents the most extensively cultivated species (Nyemb et al., 2022). The species *N. sativa* L. received its nomenclature from the Swedish botanist Carl Linnaeus in 1753 (Huchchannanavar et al., 2019). Table (1) shows the classification of *N. sativa* L.

Table 1. Scientific classification of *Nigella sativa* L. (El-Morsy et al., 2021).

Taxonomic rank	Classification
Domain	Eukarya
Kingdom	Plantae (Plants)
Sub-kingdom	Tracheobionta (Vascular plants)
Super division	Spermatophyta (Seed plants)
Phylum	Magnoliophyta (Flowering plants)
Class	Magnoliopsida (Dicotyledons)
Subclass	Magnoliidae
Order	Ranunculales
Family	Ranunculaceae
Genus	*Nigella*
Species	*N. sativa*
Binomial name	*N. sativa* L.

1.2. Botanical description of *N. sativa* L.

N. sativa L. is an annual flowering plant that typically reaches heights of 20-90 cm (Nyemb et al., 2022). It is a bisexual plant, distinguished by its narrow, linear-lanceolate, alternate grey-green leaves (Figure 1a, b) (Farag et al., 2024; Osman Yagoub, 2023; Pandey et al., 2024).

The flowers have five to ten petals (Figure 1c, d). They are renowned for their delicate appearance and range in color from white, yellow, pink, pale blue and pale purple, which enhances their ornamental appeal (Nyemb et al., 2022; Pandey et al., 2024).

The fruit is a large inflated green capsule comprising 3-7 joined follicles (Figure 1e), each containing approximately 20-60 black aromatic seeds (Nyemb et al., 2022; Pandey et al., 2024). These seeds contain up to 40% fixed oil and about 1.4% essential oil (Osman Ygoub, 2023).

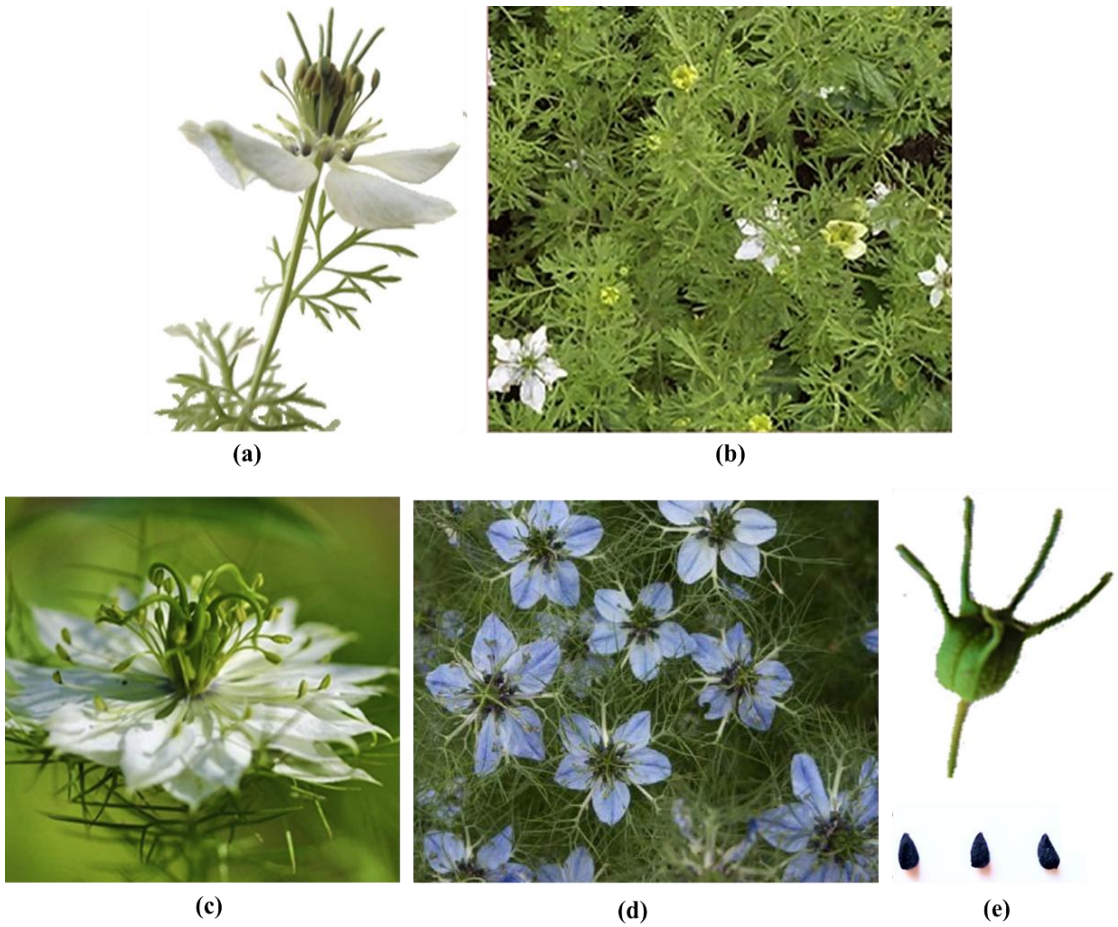

Figure 1. Morphological representation of various parts of *Nigella sativa* L. **(a, b):** *N. sativa* plant; **(c, d)**: *N. sativa* flowers; **(e):** *N. sativa* fruits and seeds (Ahmad et al., 2021; Benazzouz-Smail et al., 2023; Madjid, 2018).

The stem of the plant *N. sativa* is aerial, herbaceous, cylindrical and shiny green with a hollow interior (Figure 1). Its length ranges from 43.9 to 61.6 cm. The plant's stem is pubescent, featuring glandular hairs on its surface, while the upper portion is slender, upright, and occasionally glabrous. The root system is classified as taproots. They are robust, branched, elongated, and cylindrical. Their length varied between 10 and 14.66 cm (El-Morsy,2021; Pandey et al., 2024).

1.3. Geographical distribution and cultivation of the *N. sativa* plant

The plant *N. sativa* is native to Southern Europe, North Africa, and Southwest Asia (Nyemb et al., 2021; Singletary, 2022). It is presently widely cultivated in these regions as well as in many countries, including Egypt, Iran, Greece, Syria, Albania, Turkey, Saudi Arabia, Pakistan, and India (Hannan et al., 2021; Huchchannanavar et al., 2019; Nyemb et al., 2022; Singletary, 2022).

Despite its widespread cultivation, *N. sativa* faces several production challenges, including limited access to improved varieties, inadequate technical support, insufficient processing facilities, and underdeveloped markets, which limits its potential as a major crop (Osman Yagoub, 2023).

The cultivation of *N. sativa* involves several environmental and genetic factors that influence its growth, yield, and phytochemical composition, in addition to climate change that poses additional challenges. The plant thrives in sandy loam and loamy soils, often in the dry semiarid tropics where poor soil fertility, drought, and high temperatures are predominant (Osman Yagoub, 2023; Rahma, 2023).

The *N. sativa* plant is primarily cultivated for its seeds (Figure 2), which are used both as a culinary spice and for their medicinal properties. The seeds are particularly valued for their oil content and bioactive compounds, which contribute to their commercial and therapeutic importance (Dalli et al., 2021; Osman Yagoub, 2023).

Figure 2. Seeds of *Nigella sativa* L. (Thakur et al., 2025).

2. Seeds of *N. sativa* L. (Black Seeds)

2.1. Brief history

The historical utilization of *N. sativa* dates back to ancient times, as indicated by its discovery in the tomb of the Egyptian pharaoh Tutankhamun and ancient Turkey, about 1650 B.C. It was mentioned by ancient Greek physicians like Hippocrates and Dioscorides under the

name *Melanthion*, and was part of *Unani* medicine in India, where Muslim physicians practice it (Akbar, 2020). *N. sativa* is also mentioned in Islamic literature, where it is considered one of the most valuable medicinal plants. It is considered by Muslims to be one of the most effective forms of medical healing available, since the sayings of the Prophet Muhammad state that it is a remedy for all ailments. The esteemed physician Ibn Sina, also known as Avicenna, in his famous book "*The Encyclopedia of Medicine"*, stated: "*black seed functions as an agent for phlegm extraction, invigorates bodily energy, and aids in recovery following fatigue and lethargy*." (Ferizi et al., 2023). The seeds have been used in traditional medicine in Arab countries, Europe, and Iran for the treatment of various diseases (Akbar, 2020).

2.2. Traditional uses of the *N. sativa* (Black cumin) seeds

For millennia, the culinary and medicinal uses of black cumin (*N. sativa*) seeds have been employed by Arab nations, the Indian subcontinent, and Europe (Dalli et al., 2021; Nyemb et al., 2022).

Despite the pungentand bitter aroma of black cumin seeds, they are extensively been used as a spice in Middle Eastern and Indian cuisines,used to flavor vegetables, bread, curries, pickles, and pulses in their driedand roasted state. Black seeds are essential component of the phoron mix for Bengali panch ,which is popular in Bengali cuisine (Dhaybi et al., 2022; Nyemb et al.,2022).They are used as natural preservatives due to theirantimicrobial properties, which prolong the food product's shelflife (Perveen, 2019).

For more than two millennia, *N.sativa* has been a key component of Chinese, Islamic, Unani, Ayurvedic, Siddha,and Ayurvedic medicine (Ketenoglu et al., 2020; Kmail, 2023;Huchchannanavar et al., 2019). As "a remedy for allailments except death" (Kmail, 2023), it is highly esteemed in Islamic tradition and is used to treat a variety of ailments that affect the nervous,hepatic, respiratory, reproductive, digestive, cardiovascular, and respiratory systems (Hannan et al., 2021; Mariod and Tahir, 2023; Rashid et al., 2018;Singletary, 2022). Because of substances like thymoquinone and para-cymene, its seeds, oil, and extracts are prized fora variety of bioactivities, including antioxidant, anti-inflammatory, anticancer,antibacterial, antidiabetic, and more (Abdollahi et al., 2024).

2.3. Nutritional and chemical composition of *N. sativa* seeds (Black seeds)

Rich in nutrients and varied in chemical composition, *N. sativa* seeds are well known for their extensive application in food, medicine, and cosmetics (Albakry et al., 2022). The primary components of *N. sativa* seeds are fixed and essential oils, proteins, carbohydrates, crude fibre, and minerals (Kmail, 2023). The nutritional and chemical composition of *N. sativa* seeds is influenced

by factors, including genetic variation among seeds, crop-growing environmental conditions, soil types, and agricultural practices (Sharma, and Longvah, 2021).

2.3.1. Nutritional composition

2.3.1.1. Macronutrients

The seeds of *N. sativa* are rich in macronutrients, including proteins, lipids, and carbohydrates. Their protein content ranges from 20.3% to 26.7%, depending on genotype and growth conditions (Kabir et al., 2019; Sharma, and Longvah, 2021). The seeds also contain a considerable lipid content, ranging from around 35.17% to 45.4%, mostly composed of unsaturated fatty acids (Hossain et al., 2024; Kabir et al., 2019; Singletary, 2022). The total carbohydrate content varies from 25% to 35%, while the total dietary fibre ranges from 6.01 to 8.4% (Albakry et al., 2022; Hossain et al., 2024; Kabir et al., 2019; Sharma, and Longvah, 2021).

2.3.1.2. Amino acids

Amino acids are organic substances essential for life and serve as a significant supply of nitrogen. The seeds of *N. sativa* are generally regarded as a great source of amino acids (Albakry et al., 2022). Glutamic acid is the major amino acid present in *N. sativa* seeds (23.95%), succeeded by aspartic acid (9.32%), arginine (8.90%), glycine (6.11%), and leucine (6.0%) (Kabir et al., 2019). Similarly, Albakry et al. (2022) reported that glutamic acid in *N. sativa* seeds is the primary amino acid (4.10 g/100 g protein).

2.3.1.3. Minerals

The seeds of *N. sativa* are also a good source of minerals, with potassium as the most abundant element (1498.3 mg/100g), followed by phosphorus (481.5 mg/100g), sodium (44.8 mg/100g), calcium (366.7 mg/100g), magnesium (355.2 mg/100g), zinc (6.7 mg/100g), iron (42.6 mg/100g), manganese (3.1 mg/100g), and copper (1.5 mg/100g) (Albakry et al., 2022). In a comparable study, Iqbal et al. (2011), reported that the mineral composition of *N. sativa* seeds revealed that potassium (0.83%) was the main element, succeeded by phosphorus (0.57%), sodium (0.35%), calcium (9.13%), magnesium (10.2%), iron (0.26%), zinc and manganese (both 0.05% each), and copper (0.03%).

2.3.1.4. Vitamins

The seeds of *N. sativa* also contain water-soluble and fat-soluble vitamins. Among water-soluble vitamins, niacin (3.3-9.7), thiamin (1.3-1.8 mg/100g), pyridoxine (0.4-0.6 mg/100g), and folic acid (0.04-0.087 mg/100g) were identified in significant amounts, while a minimal quantity of ascorbic acid was also reported (Sharma, and Longvah, 2021). In similar research, Huchchannanavar et al. (2019), reported niacin, thiamin, and pyridoxine contents of 6 mg/100g, 1.5 mg/100g, and 0.7 mg/100g, respectively.

Among the fat-soluble vitamins, *N. sativa* seeds provide an abundant source of vitamin E comprising α-tocopherol, β-tocopherol, and γ-tocopherol, which are known to have potential antioxidant activity. The total tocopherol content of the *N. sativa* seeds is found between 9.15 mg/100 g and 27.92 mg/100 g. In addition to tocopherols, the seeds also include retinol, carotenoids (β-carotene), and vitamin D_2. Fat-soluble vitamins account for over 0.2% of total oil content, whereas all vitamins combined vary from 1 to 4% (Sharma, and Longvah, 2021).

2.3.1.5. Moisture and ash

The moisture content of *N. sativa* seeds is around 5.02 to 7.1% (Albakry et al., 2022; Hossain et al., 2024; Kabir et al., 2019). The total ash content of the seeds is between 3.02 and 7.4% (Albakry et al., 2022; Kabir et al., 2019; Sharma, and Longvah, 2021).

2.3.2. Essential and fixed oils

N. sativa seeds contain high levels of oil (34–39%) which is recognized as a medicinal food due to numerous health-promoting effects and therapeutic potential. The high oil content of *N. sativa* seeds makes them a perfect source of edible oil which contains numerous biologically active compounds and is responsible for several health benefits (Mezaheri et al., 2018; Parveen, 2019; Sharma and Longvah, 2021).

2.3.2.1. Essential oil (Volatile oil)

N. sativa seed contains essential (volatile) oil, which makes up about (0.4-2.5%) of the seed composition (Ciesielska-Figlon et al., 2022; Sharma, and Longvah, 2021). Thymoquinone (TQ) is identified as a chief bioactive compound with a total concentration of 21.1–48% of this oil (Albakry et al., 2022; Huchchannanavar et al. 2019; Kabir et al., 2019; Singletary, 2022).

The essential oil from *N. sativa* is also composed of β-thujene (16.02-17.22%) (Albakry et al., 2022; Gözcü and Akşit, 2023), while *p*-cymene, thymohydroquinone, and dihymoquinone contribute (7–15%) (Sharma, and Longvah, 2021; Zielinska et al., 2021), followed by carvacrol (6–12%) (Kabir et al., 2019; Sharma, and Longvah, 2021), sesquiterpene longifolene (1-8%) (Huchchannanavar et al. 2019), 4-terpineol (2-7%) (Sharma, and Longvah, 2021), T-anethol (1–4%) (Huchchannanavar et al. 2019; Sharma, and Longvah, 2021), and a sesquiterpene longifolene (1-8%) (Kabir et al., 2019), α-pinene (0-2.75%) (Degu et al.,2025), β-pinene (5.08%) (Albakry et al., 2022). These volatile compounds, confer seeds their aromatic properties and health benefits (Nyemb et al., 2022). The figure below shows the chemical structure of the major bioactive compounds in black seed essential oil.

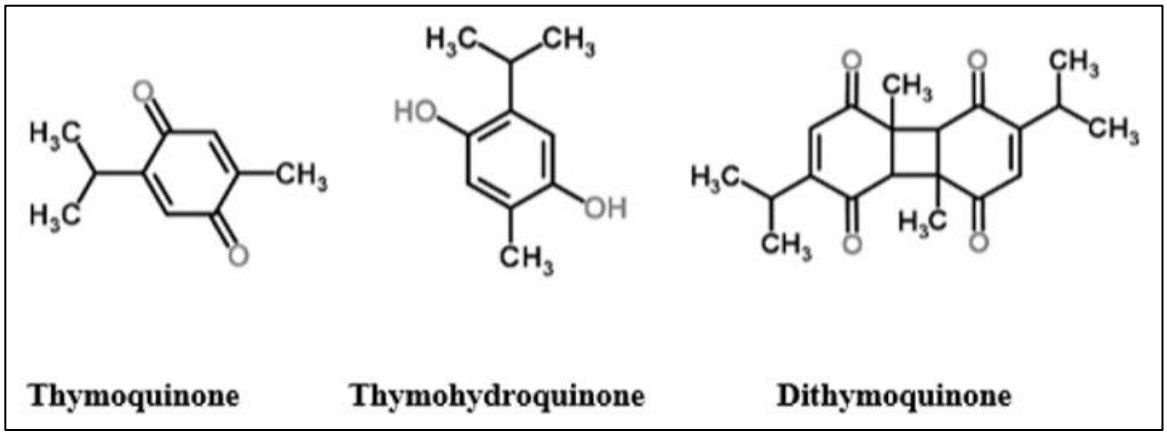

Figure 3. The chemical structure of certain significant active molecules of *Nigella sativa* L. seeds (Black seeds) (Kmail, 2023).

2.3.2.2. Fixed oil and fatty acids

N. sativa seed have a significant amount of fixed (stable) oil, about 32 to 40% of their composition. This oil contains the highest concentration of polyunsaturated fatty acid (PUFA) followed by monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA). It is distinguished by its high content of unsaturated fatty acids (UFAs) with approximately 85% of the overall fatty acid profile. linoleic acid is the predominant polyunsaturated fatty acid (PUFAs) at 50–58%, whereas oleic acid is the most abundant monounsaturated fatty acid (MUFA) at 20–28% (Sharma and Longvah, 2021). The fixed oil furthermore contains other UFAs in smaller amounts, such as arachidonic acid and eicosadienoic acid. Additionally, saturated fatty acids (SFAs) such as palmitic, stearic, and myristic acids are found (Sudhir et al., 2016).

2.3.3. Alkaloids

Two distinct kinds of alkaloids are found in *N. sativa seeds*: pyrazole alkaloids (also known as indazole ring-carrying alkaloids), such as nigellidine, nigellicine, and α-hederin, and isoquinoline alkaloids, such as nigellicimine and nigellicimine-N-oxide (Nyemb et al., 2022; Soltana et al., 2015).

2.3.4. Saponins and sterols

Saponins are secondary metabolites found in black cumin, characterized by structures that consist of steroids or aglycone triterpenes connected via a glycosidic bond to one or more oligosaccharides (Dalli et al., 2022). Saponins such as alpha-hederin are present, and known for their anticancer potential (Madjid, 2018). Sterols include beta-sitosterol and campesterol, contributing to health benefits (Matthäus and Özcan., 2011).

2.4. Biological properties of *N. sativa* seeds (Black seeds)

2.4.1. Antimicrobial activity

N. sativa seeds have shown significant antimicrobial effectiveness through multiple in many in vitro studies, exhibiting notable antibacterial, antiviral, antifungal, and antiparasitic properties (Hossain et al., 2021).

2.4.1.1. Antibacterial activity

The antibacterial properties of *N. sativa* have been extensively studied against various pathogenic bacteria (Abbas et al., 2024). Thymoquinone (TQ), a major bioactive component, demonstrates significant antibacterial activity, particularly against Gram-positive bacteria, with comparatively reduced efficacy against Gram-negative organisms (Alberts et al., 2024).

Studies have assessed the antibacterial effectiveness of *N. sativa* seeds against numerous bacterial species including *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus*, *Klebsiella pneumoniae*, *Candida albicans*, and *Candida glabrata* (Thakur et al., 2025).

A significant synergistic impact has been reported when antibiotics are administered alongside *N. sativa* against methicillin-resistant *S. aureus* (MRSA). This combination, especially with "Augmentin", demonstrated increased bactericidal efficacy against MRSA. Scanning electron microscopy demonstrated structural deformation of the bacterial cell membrane after this combination treatment (Dalli et al., 2021).

2.4.1.2. Antifungal activity

N. sativa seeds demonstrate superior inhibitory effects against *C. albicans* and *Madurella mycetomatis* compared to conventional antifungal drugs amphotericin-B and griseofulvin. Similarly, TQ effectively inhibits *Aspergillus niger*, *Fusarium solani*, and *Scopulariopsis brevicaulis*. Additionally, Islam (2016) reported that TQ, thymohydroquinone, and thymol possess antifungal efficacy against various clinical isolates including molds, yeasts, and dermatophytes.

Methanol and ethanol extracts from black cumin seeds have shown marked inhibition of *Aspergillus flavus*, *A. fumigatus*, *Issatchenkia orientalis*, *C. parapsilosis*, *C. albicans*, *C. tropicalis*, *Cryptococcus laurentii*, and *Cr. albidus*, with effectiveness above that of Amphotericin-B in comparative investigations (Abdallah, 2017).

2.4.1.3. Antiviral activity

N. sativa seeds have been shown in clinical trials to be effective in treating viral diseases including human immunodeficiency virus (HIV) and hepatitis C virus (HCV). Its antiviral property against the infectious laryngotracheitis virus was also demonstrated (Farmanli et al., 2020).

Moreover, TQ has demonstrated potential in the formulation of therapeutics for coronaviruses, particularly SARS-CoV-2, presumably due to its immune-modulating properties. *N. sativa* has demonstrated the ability to enhance natural killer (NK) cell activity and improve the ratio of helper T-cells (T4) to suppressor T-cells (T8). It has also shown the ability to reduce murine cytomegalovirus and HIV protease, while enhancing interferon-gamma production, so increasing both the amount and functioning of macrophages and CD4+ T cells (Islam, 2016).

2.4.1.4. Antiparasitic activity

Different research studies have shown the effectiveness of *N. sativa* seeds and TQ against various parasites, including adult worms, cercariae, miracidia, and *Schistosoma mansoni* parasites and their eggs. The antioxidative properties of the seeds inhibit glucose metabolism enzymes in adult worms, therefore weakening the parasites before elimination (Thakur et al., 2021). TQ has shown significant *in vitro* inhibition of various parasites, including *Theileria equi*, *Babesia caballi*, *B. bovis*, *B. bigemina*, and *B. divergens*. The combination of TQ with diminazene aceturate has demonstrated a notable impact on *Theileria*, *B. bovine*, and *B. equine* parasites (Dalli et al., 2021).

2.4.2. Antioxidant activity

The seeds of *N sativa* derive their health benefits primarily from their potent antioxidant properties, which have been extensively investigated in both in vitro and in vivo models (Hannan et al., 2021). Recent research has identified TQ, thymol, and carvacrol as the principal antioxidant compounds in *N. sativa* seeds (Salehi et al., 2021). These bioactive components function as free radical scavengers and increase the activity of endogenous antioxidant enzymes, including catalase, glutathione peroxidase, and glutathione-S-transferase (Huchchannanavar et al., 2019).

TQ, the primary bioactive constituent of *N. sativa*, has shown therapeutic potential in various pathological conditions, including diabetes, asthma, carcinogenesis, and encephalomyelitis. The antioxidant mechanisms of TQ involve both direct scavenging of reactive oxygen species and indirect enhancement of cellular antioxidant defense systems (Ahmad, M et al.,2020).

2.4.3. Anti-inflammatory activity

N. sativa seeds and their principal bioactive constituent, TQ, exhibit significant anti-inflammatory properties that effectively regulate inflammatory responses and prevent excessive inflammation (Hannan et al., 2021).

N. sativa seeds demonstrate therapeutic potential in alleviating symptoms and slowing the progression of various inflammatory disorders, including arthritis, diabetes, and inflammatory bowel disease, through the modulation of inflammatory pathways (Routh et al., 2024).

2.4.4. Antidiabetic activity

Research has shown that *N. sativa* seeds possess beneficial effects for individuals with insulin resistance syndrome and dyslipidemia (Islam et al., 2017). A 12-week investigation involving patients with type 2 diabetes revealed that adjunctive therapy with *N. sativa* seeds (26.7 mg/kg/day) diminished insulin resistance, glycosylated haemoglobin (HbA1c) levels, fasting blood glucose (FBS), and 2-hour postprandial blood glucose (2hPG), without adversely affecting renal or hepatic functions (Gholamnezhad et al., 2016).

Treatment with *N. sativa* seeds in streptozotocin-induced diabetic rats, significantly reduced fasting plasma glucose, serum malondialdehyde (MDA) levels, IL-6 concentrations, and immunoglobulins A, G, and M levels, while markedly enhancing the expression of endogenous antioxidant enzymes, including superoxide dismutase (SOD), glutathione-S-transferase, and catalase (Yimer et al., 2019). These findings suggest that *N. sativa* seeds represent a promising complementary therapy for managing diabetes and associated metabolic disorders.

2.4.5. Cardioprotective and Antihypertension activities

N. sativa seeds have demonstrated notable cardioprotective and antihypertensive properties. An oral supplement containing *N. sativa* grain extract showed hypotensive effects in individuals with mild hypertension over a two-month period. Treatment with 2.7 and 5.3 mg/kg/day of seed extract significantly decreased systolic and diastolic arterial pressure values compared to baseline. Additionally, *N. sativa* supplementation significantly reduced total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels (Gholamnezhad et al., 2016).

2.4.6. Anticancer activity

N. sativa seeds have shown significant anticancer potential through various mechanisms, including apoptosis induction, cell cycle arrest, and antioxidant activity. The principal bioactive component, TQ, inhibits cancer cell proliferation and induces programmed cell death in various organs, including blood, prostate, liver, lungs, kidneys, and skin. TQ also enhances immune function, providing additional protection against malignancies (Mehraj et al., 2022)

Research has shown that TQ inhibits cancer cell proliferation and induces apoptosis, and is effective against multidrug-resistant cancer cell lines, highlighting its potential in addressing treatment-resistant malignancies.

2.4.7. Anti-immunodulatory activity

N. sativa demonstrates complex immunomodulatory properties via its several bioactive constituents. Research indicates that while some *N. sativa* compounds can diminish B cell-mediated immunity *in vitro*, other constituents, including α-linolenic acid and estearidonic acid enhance T lymphocyte-mediated immune responses. *N. sativa* has shown the ability to positively modify the

helper-to-suppressor T cell ratio, enhance natural killer cell activity, and stimulate interleukin-3 release by T cells (Kooti et al., 2016).

Both *in vivo* and *in vitro* studies confirm *N. sativa* capacity to modulate immune responses across many pathological conditions. An important study revealed that TQ treatment in collagen-induced arthritic rats significantly decreased inflammation and improved immune function, indicating its therapeutic promise in inflammatory and autoimmune diseases (Balyan et al., 2022).

2.4.8. Anti-obesity and lipid-lowering effects

N. sativa supplements have demonstrated efficacy in reducing hunger and food consumption while enhancing energy expenditure, contributing to their anti-obesity effects. The key bioactive compounds, including TQ, thymol, lipase, and PUFAs, play significant roles in regulating lipid metabolism and genetic factors related with obesity. Studies have shown that *N. sativa* supplementation significantly reduces blood concentrations of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), while increasing high-density lipoprotein cholesterol (HDL-C) (Hosni et al., 2022).

Furthermore, extracts from *N. sativa* seeds exhibit anti-dyslipidemic properties by decreasing 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase expression and enhancing LDL receptor expression, contributing to reduced cholesterol levels (Derosa et al., 2024).

2.4.9. Impact on the gastrointestinal system

The seeds of *N. sativa* demonstrated significant gastroprotective properties through its various bioactive compounds, particularly TQ. It exhibited anti-ulcer activity in experimental gastric ulceration models (Cherrada et al., 2024). It reduced stomach acid secretion, pepsin activity, and oxidative stress indicators while increasing gastric mucin, glutathione (GSH), total nitric oxide (TNO), and superoxide dismutase (SOD) levels. These effects are linked to antioxidant and antisecretory processes, including prostaglandin-mediated pathways (Islam et al. 2017).

2.5. Applications of *N. sativa* seeds

2.5.1. Medicinal applications

N. sativa seeds are widely recognized for their medicinal properties in several cultures. For instance, in China, they are employed as remedies for insomnia, dizziness, tinnitus, anemia, and bronchial disorders. Similarly, in Algeria, they are used as hypoglycemic and hypotensive agents, while in Egypt, they are utilized for the treatment of jaundice (Wahab et al., 2023).

The therapeutic attributes of *N. sativa* are mostly attributed to TQ, which exhibits a wide range of therapeutic capabilities, including antibacterial, anti-inflammatory, antioxidative, hypolipidemic, hypoglycemic, and bronchodilatory effects. It is also effective in managing metabolic syndrome and autoimmune diseases (Wahab et al., 2023). Additionally, *N. sativa* seeds

and TQ have demonstrated efficacy in treating various chronic ailments, such as mental disorders, diabetes, infertility, inflammatory conditions, and infections caused by bacteria, fungi, parasites, and viruses. They are also utilized to treat cancer and COVID-19 (Yimer et al., 2019; Alberts et al., 2024; Wahab et al., 2023).

Moreover, *N. sativa* seeds have been successfully applied to treat bronchial asthma, coughs, bronchitis, fever, gastrointestinal issues like ulcers and dysentery, hypertension, neurodegenerative diseases such as epilepsy and Alzheimer's disease, allergies, obesity, skin conditions like eczema and jaundice, anorexia, rheumatism, immune dysfunctions, and additional health issues (El-Hack et al., 2016). These diverse therapeutic applications highlight the seed adaptability in human and veterinary medicine.

2.5.2. Cosmetic applications

The antioxidant properties of *N. sativa* seed oil render it a valuable anti-aging agent in cosmetics. It is also included in toothpaste and mouthwash formulations because of its efficacy against pathogens such as *Streptococcus* mutans, *Candida albicans*, and *Streptococcus mitis*. Clinical investigations indicated that hair oil containing *N. sativa* progressively reduces hair loss over time. Its antimicrobial and anti-inflammatory effects assist in the treatment of dermatological problems such as acne vulgaris and pigmentation issues (Abo-Atya et al., 2021; Eid et al., 2017).

N. sativa seeds have several advantages in both medicine and cosmetics; nonetheless, unpleasant consequences, including allergic responses or contact dermatitis, may occasionally arise from contaminants present in commercial black seed oil products (Alibre et al., 2015).

2.5.3. Food applications

For centuries, *N. sativa* seeds have been valued for their nutritional uses. They are frequently utilized as food preservatives and seasonings to enhance flavor in dishes such as cheese and baked goods. The seeds can be sprinkled on bread or incorporated into traditional sweet dishes consumed with honey or syrup. Furthermore, they can be consumed whole or processed to extract essential oils that serve as functional foods. *N. sativa* seeds and their essential oils are extensively utilized as nutraceuticals and flavouring (Bashir et al., 2021; Safvi et al., 2021).

2.5.4. Agricultural applications

Recent research has demonstrated that *N. sativa* seeds possess significant agricultural potential, particularly for plant growth enhancement. TQ in seed extract effectively reduces chromium (Cr) toxicity in plants (Ditta et al., 2021). Additionally, *N. sativa* seed extracts exhibit strong biopesticidal properties against pests such as *Aphelenchoides maculosa*, significantly

mitigating plant damage. These extracts show promise as effective and sustainable biopesticides for pest management in agricultural systems (Asgher et al., 2021).

2.5.5. Veterinary applications

Feed intake and efficiency are primary determinants of feed quality and animal growth performance. *N. sativa* seeds, when added to animal diets, can enhance feed conversion and growth performance. Multiple studies across various animal species have examined *N. sativa* effects on growth performance. Research has shown that adding 3.5% *N. sativa* seeds to camel rations significantly improves animal performance (Mohammed and Al-Suwaiegh, 2016).

In addition, numerous investigations have focused on N. sativa seed effects in broiler production. Incorporating pulverized *N. sativa* seed into broiler feed results in increased body weight. Specifically, 1% *N. sativa* seed supplementation improves average daily weight gain and feed conversion ratio (FCR) in broilers (Azeem et al., 2014).

While *N. sativa* seeds (black seeds) have been extensively studied for their medicinal, nutritional, and cosmetic applications, their microbial quality is another critical aspect that requires attention. Understanding the microbial quality of these seeds, including potential contamination, microbial diversity, and microbial load, is essential to ensure their safety and efficacy in various applications.

Part II. Microbial Quality of *N. sativa* L. seeds (Black Seeds)

1. Microbial Ecology of *N. sativa* Seeds

The seeds of *N. sativa* naturally harbor a diverse microbiome, including beneficial endophytic microorganisms. Endophytic fungi are defined as fungi that colonize healthy plant tissues for part or all of their life cycle without causing visible symptoms of disease (Shady et al., 2023). The term "endophyte" is derived from the Greek words "endon" (inner) and "phyton" (plant), reflecting their localization within plant tissues (Metwaly et al., 2019).

Some endophytic fungi associated with *N. sativa* seeds are known to produce bioactive secondary metabolites with activity against clinically significant pathogens, indicating their potential as sources for the development of broad-spectrum antibacterial agents from natural origins (Gopane et al., 2024). Studies have reported the isolation of various fungal genera from *N. sativa* seeds, such *Aspergillus*, *Alternaria*, *Penicillium* and *Fusarium,* highlighting the complex microbial ecology of these seeds (Metwaly et al., 2019; Shady et al., 2023).

In addition to fungi, several studies have identified endophytic bacteria in different parts of the *N. sativa* plant, such as roots, stems, and leaves, with these bacteria often exhibiting plant growth-promoting properties (Douka et al., 2024).

However, to date, there is no definitive evidence supporting the existence of true endophytic bacteria within *N. sativa* seeds. Reports of bacterial presence in these seeds primarily describe them as surface contaminants rather than internal, beneficial endophytes. Current research has focused predominantly on the isolation and characterization of endophytic fungi from *N. sativa* seeds, but not internal bacterial endophytes (Metwaly et al., 2019; Shady et al., 2023).

2. Microbial Contamination in Seeds

The microbial communities that live on and within the seeds are one of the main elements influencing seed quality (Tkalec et al., 2022). Seeds can be contaminated by a range of microorganisms, such as aerobic mesophilic bacteria, fungi (notably *Aspergillus*), coliforms, and staphylococci, which may be introduced during cultivation, harvesting, processing, or storage (Barar and Danyluk, 2018; EFSA Panel on Biological Hazards, 2011).

Despite the antimicrobial properties of *N. sativa* seed extracts and essential oils, which have demonstrated inhibitory activity against various pathogens, including *Staphylococcus aureus* and *Candida albicans* (Erdogrul et al.,2009), the risk of microbial contamination remains if seeds are not properly processed or stored. Therefore, regular microbiological assessment and adherence to

hygiene standards are essential to ensure the safety of *N. sativa* seeds for both culinary and medicinal uses (Ding et al., 2013; Mohammed et al., 2021; Usmani and Almoselhy, 2024**).**

3. Microbial Enumeration in Food Products

3.1. Methods of microbial enumeration

Microbial enumeration is critical for evaluating the safety and quality of food products, including seeds. Several established methods are commonly employed, which can be broadly categorized into classical and modern approaches

3.1.1. Classical methods

3.1.1.1. Viable plate counts

The plate count method, including the pour plate and spread plate techniques, is among the most widely used approaches for enumerating viable microorganisms in food samples (Figure 4). This technique involves homogenizing the food sample, performing serial dilutions in an appropriate diluent, and plating aliquots onto suitable agar media. After incubation for a specified period, visible colonies are counted, and results are expressed as colony-forming units per gram or milliliter (CFU/g or CFU/mL) (Basak et al., 2021; Tomasello et al, 2023). The results can be influenced by factors such as the natural food microbiota, incubation conditions, pH, and the presence of competing microorganisms (Basak et al., 2021).

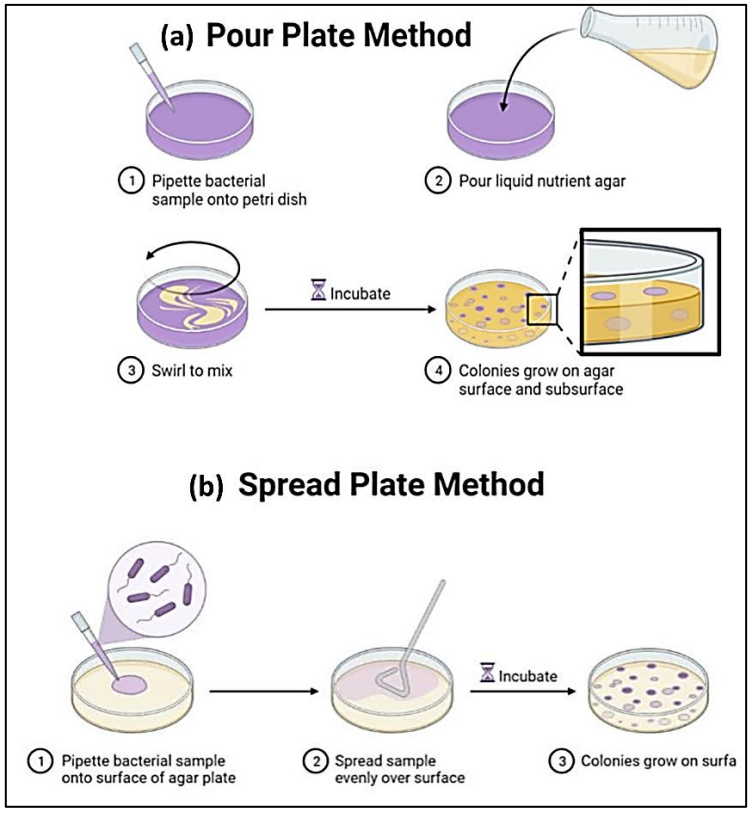

Figure 4. Viable plate counts (Classical methods): **(a)** The pour plate technique (Dahal, 2022a); **(b)** The spread plate method (Dahal, 2022b).

The choice of agar medium determines the specificity of the enumeration, allowing for the quantification of different microbial groups such as total mesophilic aerobic bacteria, fungi, coliforms, and staphylococci (Jaja and Muchenje, 2018; Abidi and Yamani, 2024). These methods provide reliable and reproducible results and are considered the gold standard for microbial enumeration in food microbiology (Basak et al., 2021).

3.1.1.2. Most Probable Number (MPN)

The Most Probable Number (MPN) method is a conventional methodology for identifying and quantifying coliform bacteria, including *E. coli*, and estimates microbial content by observable alterations in selective media. It relies on bacterial metabolism and is utilized through traditional or commercial variants such as Legiolert™. Although sensitive and appropriate for water testing, it can be laborious and resource-demanding (Ilsan et al., 2024).

specific microorganisms, particularly coliform bacteria, including *E. coli*. This method relies on detecting metabolic changes in selective liquid media following inoculation with serial dilutions of the sample. Commercial adaptations, such as Legiolert™, are also available. While the MPN method is sensitive and particularly suitable for water testing or samples with low microbial loads, it can be laborious and resource-demanding (Ilsan et al., 2024; ISO 4831:2006).

3.1.2. Modern methods

3.1.2.1. Enzymatic methods

Enzymatic enumeration techniques target specific enzymatic activities characteristic of certain bacteria, such as β-D-galactosidase and β-D-glucuronidase in coliforms and *E. coli*, respectively. These methods utilize chromogenic or fluorogenic substrates (e.g., ONPG for β-galactosidase and MUG for β-glucuronidase), which yield a colorimetric or fluorescent signal upon enzymatic hydrolysis. Commercial tests such as Colilert-18, Enterolert®, Colisure®, m-ColiBlue24®, Readycult®, Chromocult®, and E*Colite are widely used for rapid detection and enumeration (Manafi; 2000; Tambi et al., 2023).

3.1.2.2. Molecular methods

Molecular techniques, including polymerase chain reaction (PCR), quantitative PCR (qPCR), and reverse transcription qPCR (RT-qPCR), are utilized for the identification and quantification of bacteria, archaea, fungi, and other microbial communities. These methods amplify specific DNA sequences, enabling sensitive and specific detection. Additionally, transcriptomic (e.g., RT-qPCR), proteomic, and metabolomic approaches provide insights into gene expression, protein profiles, and metabolic activities of microorganisms (Perez et al., 2022).

3.1.2.3. Immunological methods

Immunological techniques, including Enzyme-Linked Immunosorbent Assay (ELISA) and Lateral Flow Devices (LFD), are widely used for the detection of bacteria, fungi, and viruses. These approaches rely on the specific affinity between microbial antigens and monoclonal or polyclonal antibodies, enabling precise and rapid identification of target microorganisms (Aladhadh, 2023).

The selection of a microbial enumeration method depends on the target organism, the characteristics of the food matrix, and the required sensitivity and specificity. While modern techniques offer valuable tools for rapid, sensitive, and targeted detection, plate count methods remain the cornerstone of routine food microbiological analysis due to their reliability and standardization (Basak et al., 2021; Hameed et al, 2023).

3.2. Key microbial groups in food microbiological enumeration

In microbiology, microbial enumeration typically focuses on specific groups of microorganisms that are indicators of food safety, spoilage, and quality. The selection of target microbial groups depends on the food product, its intended use, and relevant regulatory standards (Basak et al., 2021; International Commission on Microbiological Specifications for Foods (ICMSF), 2011).

For seeds such as *N. sativa*, the most relevant reference categories in Algerian regulations are oilseeds and, by extension, dried fruits and spices. Table (2) summarizes the principal microbial groups, their significance, and the Algerian regulatory limits for similar food categories (Arrêté interministériel, 2016).

Table 2. Microbiological Criteria for Oilseeds and Edible Seeds According to Algerian Food Regulations (Arrêté interministériel, 2016).

Microbial Group	Satisfactory (CFU/g)	Acceptable (CFU/g)	Unsatisfactory (CFU/g)	Reference Category
Total mesophilic aerobic bacteria	Not specified	Not specified	Not specified	Oilseeds/spices
Escherichia coli	≤2	3–20	>20	Oilseeds/spices
Staphylococcus aureus	$\leq 10^2$	10^3	$>10^3$	Oilseeds/spices
Molds	$\leq 10^2$	10^3	$>10^3$	Oilseeds/spices
Salmonella	Absence in 25g	-	Presence in 25g	Oilseeds/spices

3.2.1 Total mesophilic aerobic bacteria

These bacteria reflect the overall microbial load and serve as general indicators of hygiene, handling, and storage conditions. While no specific Algerian regulatory limit is set for oilseeds and spices, high counts are generally interpreted as poor sanitary conditions or spoilage (Belsali et al, 2023).

3.2.2 Coliforms

Coliforms, including *E. coli*, are used as indicators of fecal contamination and the potential presence of enteric pathogens (Figure 5). Their enumeration is critical for assessing the microbiological safety of food and water (ISO 4832:2006). For oilseeds and similar products, the satisfactory limit is ≤20 CFU/g, and results above 20 CFU/g are considered unsatisfactory, and the product is unsafe (Arrêté interministériel, 2016).

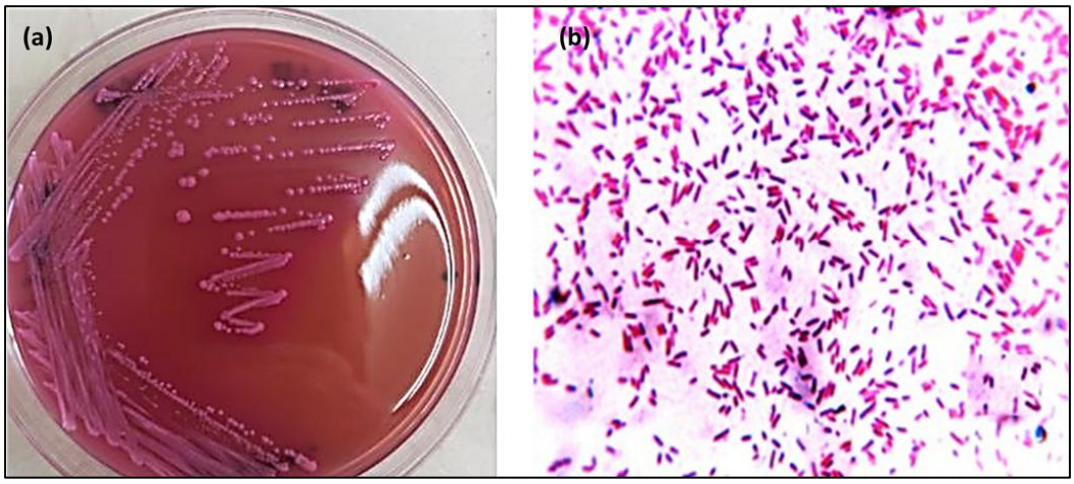

Figure 5. Culture of *Escherichia coli*: **(a)** Pure culture on MacConkey agar showing characteristic pink-red colonies (Abu-Sini et al., 2024); **(b)** Microscopic examination after Gram staining revealing pink-colored rod-shaped cells (Salam et al., 2017).

4.2.3 Staphylococci

Staphylococcus aureus is of particular concern due to its ability to produce heat-stable enterotoxins that can cause food poisoning (Figure 6). Enumeration of staphylococci is essential in foods prone to contamination by human handling (Zangerl and Asperger, 2003). For oilseeds and similar products, the satisfactory limit is $\leq 10^2$ CFU/g, with counts above 10^3 CFU/g deemed unsatisfactory, and the product is unsafe or of poor quality (Arrêté interministériel, 2016).

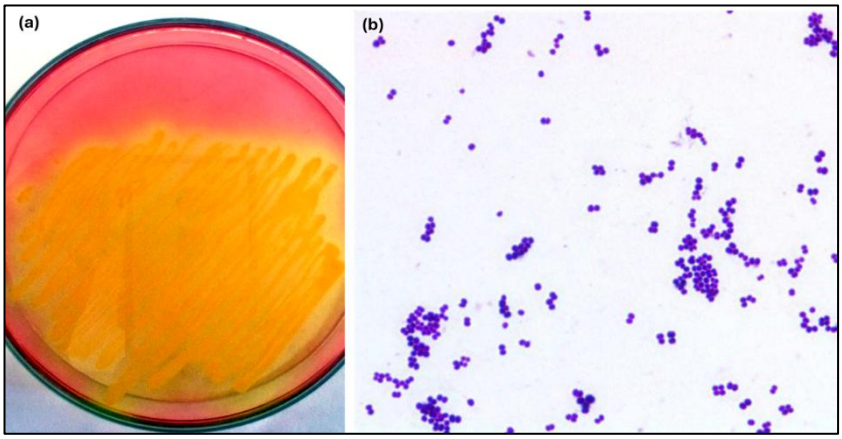

Figure 6. Culture of *Staphylococcus aureus*: **(a)** Pure culture on mannitol salt agar (Soliman et al., 2014); **(b)** Microscopic examination after Gram staining revealing purple-colored grape-like cluster (Jumaah et al., 2013).

3.2.4 Fungi (Yeasts and Molds)

Yeasts and molds are important spoilage organisms and, in some cases, mycotoxins producers (Figure 7). Their presence can affect the shelf life, safety, and sensory qualities of food products (Pitt and Hocking, 2009). For oilseeds and spices, the satisfactory limit is $\leq 10^2$ CFU/g, and results above 10^3 CFU/g are unsatisfactory (Arrêté interministériel, 2016).

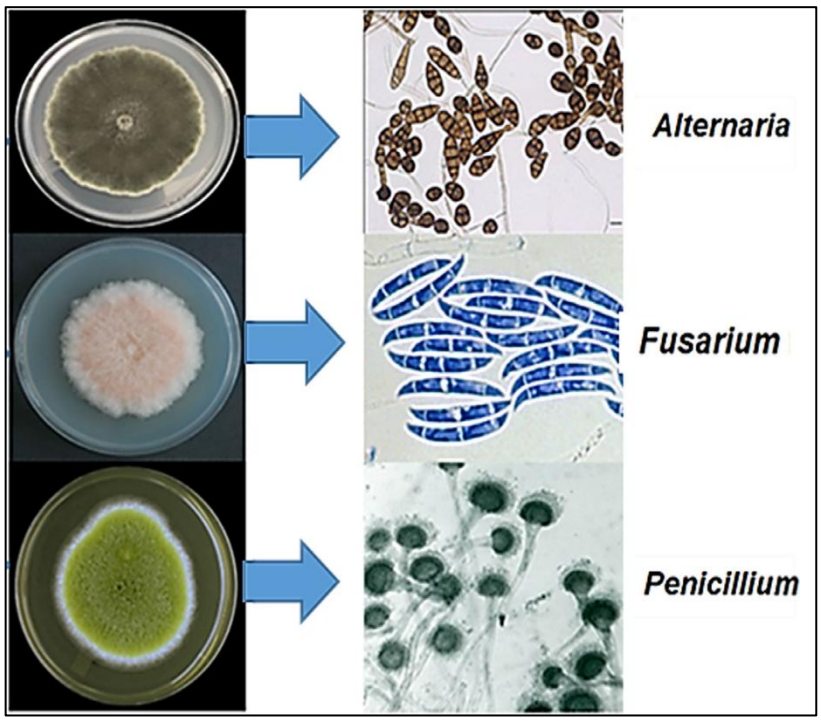

Figure 7. Morphological characteristics of three common fungal genera: *Alternaria, Fusarium*, and *Penicillium*. Left panel show representative colonies grown on agar plates; Right panel display microscopic features (Gopane et al., 2021).

3.2.5 Other pathogenic bacteria

Depending on the food matrix and risk assessment, enumeration or detection of specific pathogens such as *Salmonella*, *Listeria monocytogenes*, and *Campylobacter* may also be required to ensure food safety. For oilseeds and spices, *Salmonella* must be undetectable in 25g of product (Arrêté interministériel, 2016; ICMSF, 2011).

4. Factors Influencing the Microbial Quality of Seeds

The microbiological quality of seeds is significantly affected by multiple interrelated factors that function throughout the entire seed production process, from cultivation to storage. These factors can be categorized into pre-harvest conditions, harvest and post-harvest handling processes, and storage parameters, each influencing the final microbial load and seed viability.

4.1. Pre-harvest and production factors

The production environment and agronomic techniques employed during cultivation represent primary determinants of seed microbial quality. Environmental stresses, including extreme temperatures and biotic stresses, directly impact seed quality by fundamentally altering the composition, abundance, and structural organization of seed-associated microbiomes throughout both seed development and subsequent germination phases (Bishaw et al., 2009; Sun et al., 2023).

4.2. Harvest and post-harvest handling

Critical factors during the harvesting phase include timing of harvest, the stage of seed maturity, and moisture content at harvest. Suboptimal harvesting conditions can lead to increased microbial contamination and reduce the quality of seeds. Moreover, mechanical damage incurred during various post-harvest operations poses significant risks to seed integrity and microbial quality. Such damage can occur during multiple stages including harvesting, conveying, cleaning, treatment, and packaging processes, creating entry points for pathogenic microorganisms and compromising the seed's natural defense barriers (Bishaw et al., 2009; Sun et al., 2023).

4.3. Storage conditions and parameters

Storage conditions represent the most controllable factors affecting long-term seed microbial quality. High temperatures, elevated moisture content, and extended storage periods act synergistically to accelerate seed deterioration processes. Conversely, low relative humidity combined with moderate temperatures (such as 5°C) effectively preserves seed quality over extended periods (Alhamdan et al., 2011).

Moisture content emerges as the most crucial storage parameter, as it directly influences both respiratory processes and the development of rot-causing microorganisms. Optimal storage conditions have been established as temperatures between 20-25°C in sealed containers with moisture content maintained at 6% or below. Under these moderate conditions, seeds can be

preserved for significantly longer periods while maintaining acceptable quality standards. Low-temperature storage at 5°C demonstrates exceptional efficacy in maintaining seed germinability. However, it is important to note that while storage at 5°C preserves excellent germinability, Mean Germination Time (MGT) tends to increase and Speed of Germination Percentage (SGP) decreases over time, particularly when seeds are subsequently exposed to high-humidity and high-temperature conditions. Despite these temporal changes in germination kinetics, 5°C storage consistently maintains good overall germinability (Alhamdan et al., 2011).

5. Food Safety Regulations

Food safety involves the implementation of measures throughout the handling, processing, and distribution of food to prevent contamination that could cause foodborne illnesses (Janefer et al., 2024). Increasing global concerns about agrochemical hazards and consumer demand for safe, diverse foods have intensified food safety regulations. While some view these regulations as trade barriers, they also offer opportunities to improve food systems and promote sustainability. Understanding their function is crucial for formulating effective policies and safeguarding public health (Dou et al., 2015).

5.1. International standards for seeds

Seed quality assessment relies on standardized testing protocols measuring physical purity, genetic purity, germination, moisture content, and seed health, which are critical for trade and sowing (Kumar et al., 2022). International organizations such as the International Seed Testing Association (ISTA) and the Organisation for Economic Cooperation and Development (OECD) promote globally harmonized seed testing methodologies to ensure consistency, trust, and facilitate international seed trade (Matthews et al., 2012; Dadlani and Yadava, 2023). Despite progress, the seed industry requires further development of standardized health tests that are rigorously validated and reproducible to maintain credibility in global markets (Aveling, 2014).

5.2. Quality assurance and food safety management systems

The food industry is the largest global sector and faces ongoing challenges in ensuring food safety, defined as providing food that is safe, nutritious, and free from harmful substances (Dobrucka, 2020). Several internationally recognized systems and standards support food safety management:

5.2.1. International Organization for Standardization (ISO) Standards

The International Organization for Standardization (ISO) plays a crucial role in establishing global standards that promote consistency and accountability across various industries, including food and agriculture. ISO standards provide unified frameworks for quality management systems, enabling compatibility and trust between producers and consumers worldwide. By reducing

technical barriers and clarifying expectations, these standards facilitate international trade and support regulatory compliance, contributing to improved food safety, environmental management, and social responsibility (Heires, 2008).

The most prominent food safety ISO standards include:

a) ISO 22000: Food Safety Management Systems

ISO 22000 represents a comprehensive food safety management system that strategically combines the Hazard Analysis and Critical Control Points (HACCP) approach with ISO 9001 quality principles to develop safe food products. This integration provides a common language for international standards, ensures legal compliance, and facilitates seamless integration with other management systems (Mekimah and Sayad, 2020).

The ISO 22000:2005 standard was specifically released in 2005 with the primary objective of bridging the gap between HACCP and ISO 9001:2000 methodologies. To guarantee that the food supply chain remains free of vulnerabilities, the ISO 22000 series effectively combine the fundamental principles of the HACCP system with mandatory prerequisite programs, including Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) (Kök, 2009). The organization's commitment to preserving product quality is demonstrated through the implementation of ISO 22000:2018 Food Safety Management System, with adoption expected to significantly modify the established behaviors and practices of personnel within organizations (Purwanto et al., 2022).

ISO 22000 represents the first internationally accepted and auditable standard for Food Safety Management Systems (FSMS), encompassing every step of the food production chain, including manufacturing, processing, shipping, and retail distribution. The standard is currently implemented in over 167 countries worldwide, with primary adoption in the European Union, and significant implementation in China, India, Greece, Romania, Turkey, Russia, Spain, Egypt, Poland, and Ukraine (Bomba and Susol, 2020). The organization's safety management systems demonstrate its commitment to providing hazard-free food products, with these systems explicitly outlining the company's food safety objectives, which serve as a foundation for both local operations and global business expansion (Panghal et al., 2018).

b) ISO 9001: Quality Management Framework

ISO 9001 provides a comprehensive framework for quality management systems that can be effectively applied to food processing units, helping to ensure consistent product quality and enhanced customer satisfaction (Mekimah and Sayad, 2020).

5.2.2. HACCP system

HACCP methodology is a commonly used and recommended technique for food safety management. This system is designed to identify, evaluate, and control hazards significant for food safety. It employs a preventive approach to ensure that risks are avoided, eliminated, or reduced to acceptable levels before food reaches consumers. Food safety experts worldwide recognize HACCP as an effective system for preventing and managing foodborne diseases (Zaki and Tager, 2023).

The HACCP system consists of seven principles (Soliman and El-Makhzangy, 2019):

- Perform a hazard analysis to identify potential hazards associated with food production.
- Determine the Critical Control Points (CCPs) where control can be applied.
- Establish critical limits for each CCP.
- Define a monitoring system for CCPs.
- Establish corrective measures when monitoring indicates a CCP is not under control.
- Establish verification procedures to confirm the HACCP system is working effectively.
- Establish documentation and record-keeping procedures.

5.2.3. GMP

They include recommendations for staff hygiene to prevent contamination transfer from employees to food. These cover procedures such as hand washing, appropriate clothing, personal protective equipment (PPE), and training on safe food handling. GMP ensures employees don't inadvertently introduce contaminants into the food processing environment through stringent hygiene regulations. It also requires thorough documentation and record-keeping of all operations, from raw material procurement to production techniques and quality assurance inspections (Silva, 2023).

5.2.4. Failure Mode and Effects Analysis (FMEA)

It is a systematic risk assessment tool used to identify potential failures in production processes, evaluate their impact, and implement preventive measures to reduce hazards and improve product quality (Szczyrba and Dziuba, 2023).

5.2.5. Standard Operating Procedures for Sanitation (SSOPs)

They are facility-specific protocols designed to maintain hygienic conditions before and during food processing. They include documented and validated cleaning procedures to prevent product contamination (Lee et al., 2021).

Chapter Two

Material and Methods

1. Sample collection

Three samples of *N. sativa* seeds were purchased from three separate traditional herbal medicine stores in Constantine, Northeast Algeria (Figure 8). The samples were designated as BS1, BS2, and BS3 according to their respective stores of origin. Each seed sample was placed in a sterile bag and stored at ambient temperature (20-25°C) in a dark, dry location until processing and analysis.

Figure 8. A sample of *Nigella sativa* L. seeds (Black seeds) purchased from a traditional medicine store in Constantine.

2. Microbiological analysis

Microbiological analyses were conducted on the *N. sativa* seed samples (BS1, BS2, and BS3). Each sample was analyzed for total aerobic bacteria, staphylococci (*Staphylococcus spp.*), coliforms, and fungi.

2.1. Culture media and conditions

The microbial enumeration in *N. sativa* seed samples was performed using the following culture media (Abidi and Yamani, 2024; Leclercq et al., 2002):

- **Plate Count Agar (PCA) (See Appendix A for composition):** Is a medium utilized for the cultivation and enumeration of total aerobic mesophilic bacteria, thereby providing an assessment of overall bacterial contamination (Dogu-Baykut and Gunes, 2022; Somé et al., 2025).

- **Violet Red Bile Lactose (VRBL) Agar (Appendix A):** Utilized for the detection of coliforms, which serve as indicators of potential fecal contamination. *Escherichia coli* is a common species of coliform bacteria. VRBL agar is a standardized medium for the accurate enumeration of coliforms in a variety of food samples (Compaoré et al., 2021; Mahmoud and El-Sheikh, 2020).
- **Baird-Parker agar (Appendix A):** Selective medium for the enumeration of *Staphylococcus spp.*, specifically *S. aureus*, a foodborne pathogen capable of causing illness. The medium is selective because its composition inhibits the growth of non-staphylococcal organisms (Dong et al., 2024).
- **Sabouraud dextrose agar (SDA) supplemented with chloramphenicol (Appendix A):** Employed for the cultivation and enumeration of yeasts and molds. SDA is a standardized fungal culture medium that facilitates the growth of a wide range of fungi, including yeasts and molds. Chloramphenicol (0.1 g/L) inhibits bacterial growth while allowing fungal development. SDA is specifically effective for isolating fungi from food samples with water activity values ≤0.95, including black seeds (Berhanu, 2014).

2.2. Sample preparation and serial dilution

Ten grams (10 g) of each *N. sativa* seed sample (BS1, BS2, and BS3) were aseptically weighed and introduced into 90 mL of sterile peptone water (0.1%). The mixture was subjected to homogenization, after which serial decimal dilutions were performed to 10^{-5} using 0.1% sterile peptone water (Figure 9).

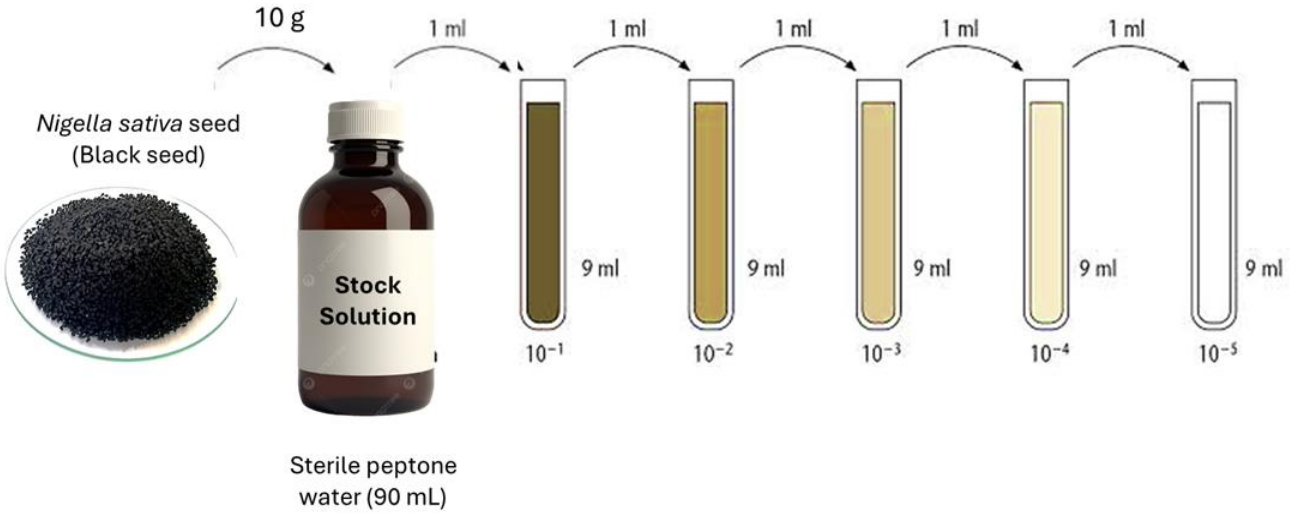

Figure 9. *Nigella sativa* seed (Black seed) preparation and serial dilution.

2.3. Enumeration method

2.3.1. Total mesophilic aerobic bacteria enumeration on PCA

The enumeration of total mesophilic aerobic bacteria was conducted with accordance with the International Organization of Standardization (ISO) 4833-2:2014 (ISO, 2014). For each sample of *N. sativa* seeds, aliquots (0.1 mL) from the appropriate decimal dilution were plated in triplicate onto PCA using the spread plate method. All plates were inverted and incubated at 30°C for a period of 72 hours (Atasever and Atasever, 2018; Atlabachew and Mamo, 2021; Loumani et al, 2020).

2.3.2. Coliform enumeration on VRBL agar

The coliform enumeration was performed following the standards and protocols of ISO 4832:2006 (ISO, 2006). For each dilution, 1 mL of the sample was transferred to sterile Petri dishes, subsequently supplemented by 15 mL of molten VRBL agar medium cooled to 44-45°C. The plates were gently swirled to guarantee thorough mixing of the sample with the medium and allowed to solidify. Following solidification, an additional 4 mL of molten VRBL agar was applied to create a double layer that prevented colony dispersion and generated semi-anaerobic conditions. The plates were incubated at 37°C for 24 to 48 hours in an inverted orientation (Banseka and Tume, 2024; Ehrampoush et al., 2017).

After incubation, the plates were assessed for distinctive presumptive coliform colonies, generally manifesting as purple or red-purple colonies (0.5 mm in diameter or larger) accompanied by or without surrounding purple haloes (Shrestha, 2024). Colonies exhibiting these characteristics were counted and classified as presumptive coliforms.

2.3. Staphylococci enumeration on Baird-Parker agar

The enumeration of staphylococci was conducted in accordance with the guidelines specified in ISO 6888-1:2021. From each generated decimal dilution, aliquots of 0.1 mL were spread in triplicate onto the surface of Baird-Parker agar plates and incubated in an inverted position at 37°C for 24–48 hours (Elahi, 2019; Ehrampoush et al., 2017).

The plates were examined for distinctive colonies of staphylococci, which typically manifest as black, shiny, convex colonies with regular margins (1.0–1.5 mm in diameter), surrounded by a clear zone of lipolysis (2–5 mm in width). Coagulase-positive *Staphylococcus aureus* can form extensive wide opaque zones of precipitate extending into the cleared medium after 48 hours of incubation. Colonies that meet these morphological criteria were classified as presumptive staphylococci and subjected to enumeration (Elahi, 2019; Morshdy et al., 2023; TM Media, 2019).

2.4. Fungal count on SDA

The enumeration of fungi was performed following the standards of ISO 21527-2:2008, with modification utilizing the SDA supplemented with chloramphenicol (0.1 g/L) as the solid medium.

Aliquots of 0.1 mL from suitable dilutions were spread-plated in triplicate onto SDA plates. The inoculated plates were incubated at an inverted position at 25°C for 5 days (Elshafie et al.,2022).

Following the incubation period, the fungal colonies were classified based on their macroscopic morphological characteristics:

- Molds: Colonies manifested filamentous with aerial hyphae and a fuzzy or cotton-like texture.
- Yeasts: Colonies exhibited a smooth, creamy or glossy appearance with a moist texture and the absence of aerial hyphae.

This differentiation enabled preliminary discrimination between yeasts and molds prior to colony enumeration.

3. Colony enumeration

Following the incubation period, the number of colonies developed on each plate was assessed utilizing a colony counter (Figure 10).

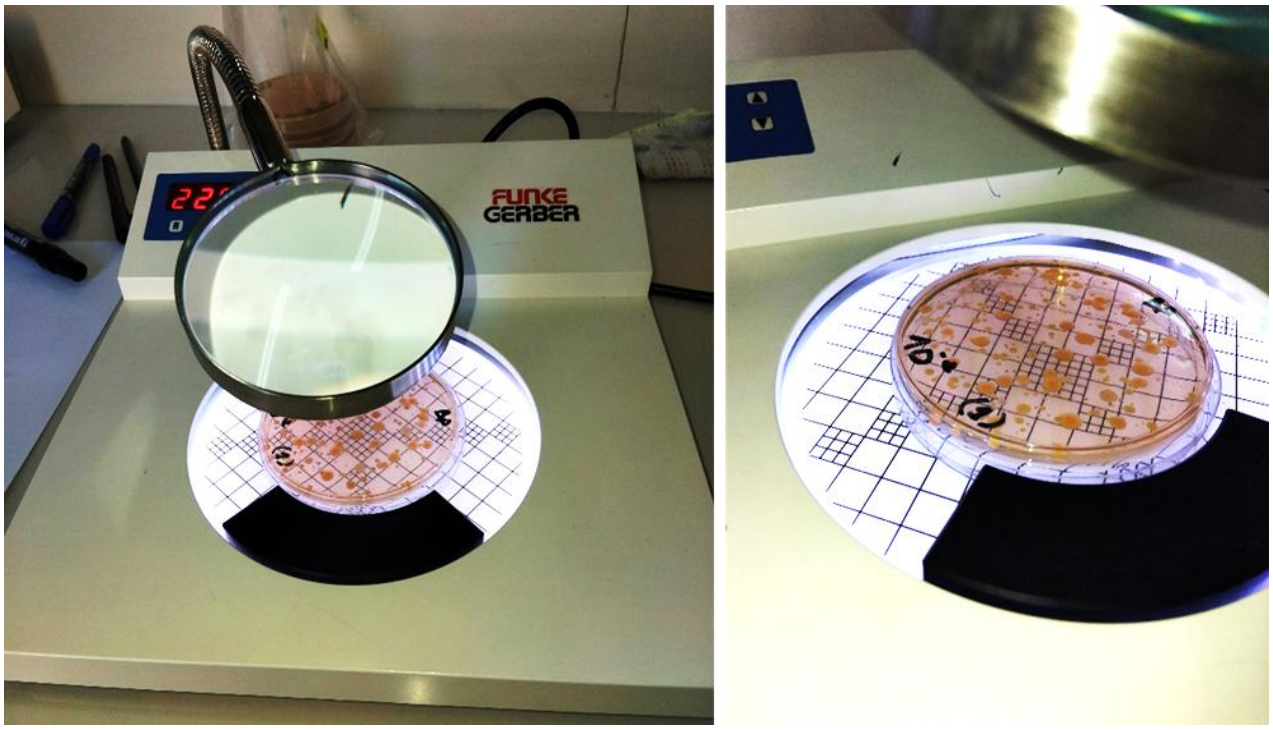

Figure 10. Images diplaying the colony counter utilized for the enumeration of colonies on various media.

The plates demonstrating suitable colony countable limits for each medium were evaluated as specified in ISO standards (Table 3). However, colony counts beneath the recommended countable range were also recorded and utilized for the calculation of microbial load but were designated as estimated values rather than quantitative results in accordance with ISO standards.

These values were included as they reflect the actual microbial contamination levels detected in the *N. sativa* seed samples.

Table 3. Acceptable colony countable ranges for various microbial groups as per ISO standards.

Microbial group	Colony countable range (ISO standards)
Total mesophilic aerobic bacteria	25 – 250 colonies (ISO 4833-2:2013)
Coliforms	10 - 150 colonies (ISO 4832:2006)
Staphylococcus spp.	15 - 150 colonies (ISO 6888-1:2021)
Fungi: Molds and Yeasts	10 – 150 colonies (ISO 21527-2:2008)

The microbial counts in the seed samples were determined by averaging triplicate analyses for each sample. Colony counts were recorded and subsequently converted to colony-forming units per gram (CFU/g), in accordance with standard microbiological protocols. The following formula was utilized (Houchmandzadeh and Ballet., 2023; Martini et al., 2024):

$$\mathbf{CFU/g} = \left(\frac{\mathbf{Mean\ number\ of\ colonies}}{\mathbf{Plated\ volume\ in\ mL}}\right) \times \mathbf{Dilution\ factor}$$

Standard deviations (SD) were calculated to represent the variability between replicates. The results were expressed as the mean colony-forming units (CFU) per gram (g) ± standard deviation (SD) of triplicate counts.

4. Statistical analysis (SPSS analysis)

Microbial count results were expressed as mean ± standard deviation (SD) based on triplicate determinations. Statistical analysis was conducted using SPSS software (version 25.0, IBM). One-way analysis of variance (ANOVA) was performed to evaluate significant differences between seed samples. Statistical significance was set at $p < 0.05$.

Chapter Three

Results and Discussion

Part I. Results

The quantification of total aerobic mesophilic bacteria, coliforms, *Staphylococcus* spp., and fungi was performed on three samples (BS1, BS2, and BS3) of *N. sativa* seeds (Black seeds), sourced from three distinct retail stores. The enumeration was conducted utilizing four different culture media to quantify the various microbial groups.

1. Enumeration of Total Aerobic Mesophilic Bacteria on PCA in *N. sativa* Seed

Total aerobic mesophilic bacterial enumeration was conducted on three commercial black seed (*N. sativa* L.) samples (BS1, BS2, and BS3) obtained from different retail outlets in Constantine, Algeria. The analysis was performed following ISO 4833-2:2013 standards (ISO, 2013) using Plate Count Agar (PCA) medium. Plates were incubated under aerobic conditions at 30°C for 72 hours, and each sample was analyzed in triplicate using the spread plate method.

The results revealed the presence of total aerobic mesophilic bacteria in all three *N. sativa* seed samples, with bacterial counts ranging from 1.03×10^4 to 6.7×10^5 CFU/g (Table 4). Sample BS1 exhibited the highest level of aerobic bacterial contamination with a count of $6.7\times10^4 \pm 1.3\times10^4$ CFU/g, followed by BS3 with $2.73\times10^4 \pm 1.1\times10^3$ CFU/g, while BS2 recorded the lowest count at $1.03\times10^4 \pm 1.6\times10^3$ CFU/g. These findings indicate variations in the microbial load among the different commercial black seed samples analyzed.

Table 4. The total aerobic mesophilic bacteria count in *Nigella sativa* seed (Black seed) samples (BS1, BS2, and BS3) from three commercial sources. The mean ± SD is given with n = 3.

Seed sample	Mean colony	Mean CFU/g ± SD
BS1	67	$6.7\times10^4 \pm 1.3\times10^4$
BS2	103	$1.03\times10^4 \pm 1.6\times10^3$
BS3	273	$2.73\times10^4 \pm 1.1\times10^3$

The results of the one-way ANOVA performed in SPSS to compare total aerobic mesophilic bacterial counts among the three *N. sativa* seed samples (BS1, BS2, and BS3) showed a statistically significant difference in mean bacterial counts between the commercial seed samples ($p = 0.0024$) (Appendix B).

Post hoc Tukey tests were conducted to identify specific differences between in total aerobic mesophilic bacterial counts among the three *N. sativa* seed samples. The results indicated that BS1 had significantly higher bacterial counts than both BS2 ($p = 0.001$) and BS3 ($p=0.014$). Additionally, BS3 showed significantly higher contamination levels than BS2 ($p = 0.043$).

These findings demonstrate significant variability in microbiological quality across the three commercial seed samples, with BS1 presenting the highest bacterial load ($6.7\times10^4 \pm 1.3\times10^4$ CFU/g). These results are visually represented in Figure (11), where superscript letters (a, b, and c) denote statistically distinct groups.

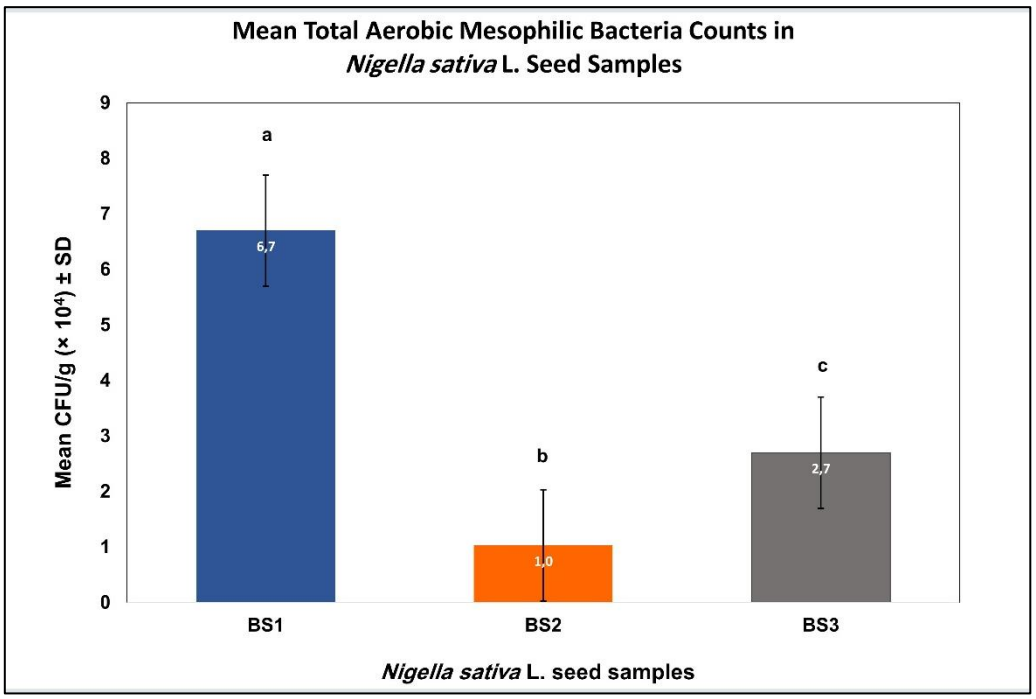

Figure 11. Mean total aerobic mesophilic bacterial counts (CFU/g × 10^4 ± SD) in commercial *Nigella sativa* L. seed samples (BS1, BS2, BS3). Bars represent CFU/g ± standard deviation (n = 3). Statistical analysis by one-way ANOVA revealed significant differences between all groups ($p < 0.001$). Different superscript letters (a, b, c) indicate statistically significant differences between groups according to Tukey HSD post hoc tests ($p < 0.05$).

2. Enumeration of Coliforms on VRBL Agar

Coliform enumeration was conducted according to ISO 4832:2006 methodology using Violet Red Bile Lactose (VRBL) agar plates incubated at 37°C for 24–48 hours. Colony counts within the acceptable range of 10–150 colonies per plate were enumerated (ISO, 2006). The results revealed varying levels of presumptive coliform contamination among the three *N. sativa* seed samples (Table 5).

The finding showed that BS1 had the highest coliform count at $2.5 \times 10^5 \pm 4.5 \times 10^4$ CFU/g, followed by BS3 with $1.5 \times 10^5 \pm 9.7 \times 10^3$ CFU/g, while BS2 exhibited the lowest count at $2.2 \times 10^4 \pm 2.6 \times 10^4$ CFU/g. These results indicate detectable levels of coliforms in all tested samples, with notable variation in contamination levels among the different commercial seeds.

Table 5. Coliform enumeration of *Salvia hispanica* seed samples from three commercial sources (Mean ± SD, n = 3). The coliform enumeration in *Nigella sativa* seed (Black seed) samples (BS1, BS2, and BS3) from three commercial sources. The mean ± SD is given with n = 3.

Seed sample	Mean colony	Mean CFU/g ± SD
BS1	247	$2.5 \times 10^5 \pm 4.5 \times 10^4$
BS2	22	$2.2 \times 10^4 \pm 2.6 \times 10^3$
BS3	147	$1.5 \times 10^5 \pm 9.7 \times 10^3$

A one-way ANOVA was conducted to compare coliform counts among the three commercial *N. sativa* seed samples. The analysis revealed statistically significant differences between seed samples ($p = 0.0016$), indicating that coliform contamination levels varied significantly among the different commercial sources. Post-hoc Tukey HSD tests indicated that all pairwise comparisons were statistically significant ($p < 0.001$), with BS1 exhibiting the highest coliform contamination ($2.5 \times 10^5 \pm 4.50 \times 10^4$ CFU/g), followed by BS3 ($1.47 \times 10^5 \pm 9.7 \times 10^3$ CFU/g), and BS2 showing the lowest levels ($2.2 \times 10^4 \pm 2.6 \times 10^3$ CFU/g). These results are visually represented in Figure (12), where superscript letters (a, b, and c) denote statistically distinct groups.

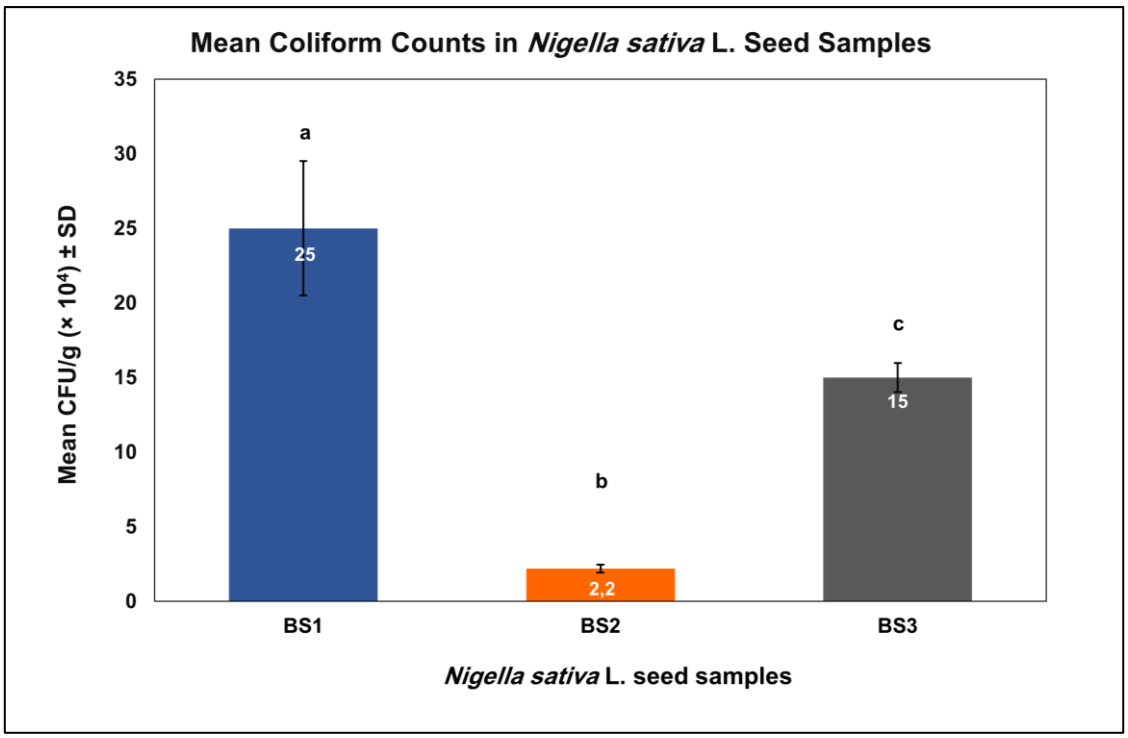

Figure 12. Mean coliform counts (CFU/g × 10^5 ± SD) in commercial *Nigella sativa* L. seed samples (BS1, BS2, BS3) as determined on Violet Red Bile Lactose (VRBL) agar after incubation at 37°C for 24–48 hours. Bars represent means ± standard deviation (n = 3). One-way ANOVA revealed significant differences among groups ($p < 0.001$). Different superscript letters (a, b, c) indicate statistically significant differences between groups according to Tukey's HSD post hoc test ($p < 0.05$).

3. Enumeration of Staphylococci on Baird-Parker Agar

Examination of black seed (*N. sativa* L.) samples (BS1, BS2, and BS3) using ISO 6888-1:2021 methodology revealed no characteristic *S. aureus* colonies on Baird-Parker agar plates after 48 hours incubation at 37°C. Typical *S. aureus* colonies (black, shiny, convex, 1.0–1.5 mm diameter with distinct lipolytic zones) were absent from all samples.

Instead, plates exhibited non-characteristic colony morphologies with pale coloration (white, cream, or yellow), irregular edges, and absence of lipolytic activity or opaque precipitation zones. Due to the lack of presumptive *S. aureus* morphology, no enumeration was performed. Coagulase-positive staphylococci were not detected in any sample, consistent with the method's detection limit (<10 CFU/g).

4. Fungal Enumeration on SDA

Fungal counts were determined for the three *N. sativa* L. seed samples (BS1, BS2, and BS3) following ISO 21527-2:2008 standards (ISO, 2008). The plates were incubated aerobically at 25°C for 5 days, as specified in the standard. Each sample was analyzed in triplicate using the spread plate method. The results are presented in Table (6).

Table 6. Fungal enumeration of *Nigella sativa* L. seed samples from three commercial sources (Mean ± SD, n = 3).

Seed sample	Mean colony	Mean CFU/g ± SD
BS1	1	$1.0 \times 10^2 \pm 0$
BS2	6	$5.6 \times 10^2 \pm 3.0 \times 10^2$
BS3	3	$3.3 \times 10^2 \pm 1.1 \times 10^2$

The findings indicated varying levels of fungal contamination among the samples. Specifically, BS1 exhibited a fungal count of $1.0 \times 10^2 \pm 0$ CFU/g, BS2 showed the highest count at $5.6 \times 10^2 \pm 3.0 \times 10^2$ CFU/g, and BS3 recorded $3.3 \times 10^2 \pm 1.1 \times 10^2$ CFU/g. These values represent the means and standard deviations from triplicate analyses for each sample (Figure 13).

It should be noted that all observed colony counts were below the ISO-recommended countable range of 10–150 colonies per plate (ISO, 2008). Consequently, these values should be regarded as estimates rather than precise quantifications. Statistical analyses, such as ANOVA, were not conducted due to the low colony counts, but the descriptive results provide an overview of fungal contamination levels in the analyzed commercial black seed samples.

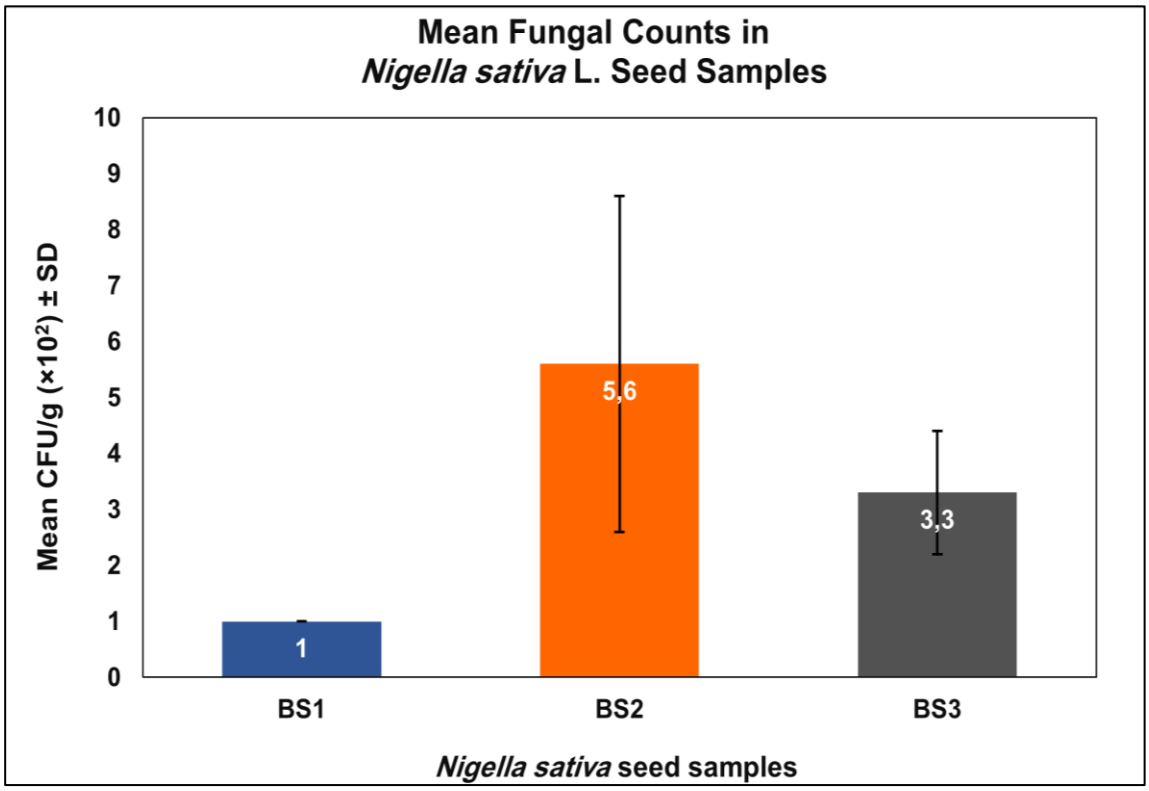

Figure 13. Mean fungal counts (CFU/g × 10^2 ± SD) in commercial *Nigella sativa* L. seed samples (BS1, BS2, and BS3) determined on SDA with chloramphenicol after incubation at 25°C for 5 days. Bars represent mean values with standard deviation (n = 3). All observed colony counts were below the ISO 21527-2:2008 recommended countable range (10–150 colonies per plate); therefore, values are presented as estimates rather than precise quantifications.

Part II. Discussion

This study provides a comprehensive assessment of the microbiological quality of some commercial *N. sativa* (black seed) samples (BS1, BS2, BS3) available in Constantine, Algeria. The results highlight significant variability in microbial contamination levels, reflecting differences in handling and storage conditions. The findings are discussed here in the context of international standards and relevant scientific literature.

1. Total Aerobic Mesophilic Bacteria Enumeration

The total aerobic mesophilic bacterial counts in the three *N. sativa* samples (BS1, BS2, BS3) ranged from 1.03×10^4 to 6.7×10^4 CFU/g, with BS1 showing the highest load. These values fall within the Algerian regulatory limits for dried herbs and spices (between 10^4 and 10^5 CFU/g). However, the highest count approaches the upper threshold for unsatisfactory quality, suggesting that certain retail samples may be at risk of exceeding acceptable limits in the event of hygiene deficiencies (Arrêté interministériel, 2016). These results are consistent with previous findings on Algerian herbs, indicating that inadequate drying practices and suboptimal storage conditions frequently result in increased bacterial loads (Cicero et al., 2022). The observed variability between samples likely reflects differences in hygiene standards during processing, as similarly reported for *N. sativa* seeds in Saudi Arabia (Al-Jassir, 1992).

It is noteworthy that the bacterial count in BS2 (1.03×10^4 CFU/g) was well within the EU Commission Regulation (EC) No. 2073/2005 limit for dried herbs ($<10^5$ CFU/g), while BS1 approached this threshold, underscoring the necessity for rigorous quality control in local markets (Cicero et al., 2022). The microbial loads determined in this study are higher than those reported for Indian and Ethiopian black cumin seeds, which exhibited total plate counts of less than 10 CFU/g (Thilakarathna and Nawarathna, 2018) but remain lower than those recorded for certain Saudi Arabian samples, where counts reached up to 7×10^7 CFU/g (Al-Jassir, 1992). Such variability in bacterial contamination is likely attributable to differences in post-harvest handling, storage conditions, and environmental factors (Al-Jassir, 1992; Cicero et al., 2022).

Overall, these findings highlight the importance of standardized post-harvest protocols, including optimized drying and storage, to prevent bacterial proliferation and ensure compliance with food safety standards (Cicero et al., 2022; Thilakarathna and Nawarathna, 2018).

2. Coliform Enumeration

Coliform counts varied significantly among *N. sativa* seed samples ($p = 0.0016$), ranging from 2.2×10^4 to 2.5×10^5 CFU/g. BS1 exhibited the highest contamination (2.5×10^5 CFU/g), followed by

BS3 (1.5× 10^5 CFU/g), while BS2 showed the lowest levels (2.2×1 0^4 CFU/g). These values substantially exceed the Algerian regulatory limits for dried herbs (10^2 - 10^3 CFU/g) (Arrêté interministériel, 2016) and international safety standards (<10^3 CFU/g) (Cicero et al., 2022). Elevated coliform levels indicate potential fecal contamination from unhygienic harvesting, processing, or storage practices (Mashat et al., 2018).

While *E. coli* was not isolated in this study, its presence in regional *N. sativa* markets has been documented (Mashat et al., 2018), underscoring the need for vigilance. These findings align with global reports of coliform contamination in edible seeds, including a UK study detecting *E. coli* in 9% of samples, with 1.5% exceeding 100 CFU/g (Silva et al., 2022).

The significant inter-sample variability ($p = 0.0016$) revealed in this study highlights inconsistent adherence to hygiene practices among different commercial sources, emphasizing the need for standardized post-harvest practices to mitigate contamination risks.

3. Staphylococci Enumeration

No coagulase-positive staphylococci (*S. aureus*) colonies were detected in any of the *N. sativa* seed samples, with counts below the detection limit of 10 CFU/g. This result is favorable and aligns with Algerian standards (10^2 - 10^3 CFU/g) (Arrêté interministériel, 2016) as well as international reports, where *S. aureus* is rarely isolated from properly handled dried seeds and spices (Silva et al., 2022). These findings are also consistent with previous studies on *N. sativa* seeds from Saudi Arabia and other regions, which also reported negligible staphylococcal contamination (Al-Jassir, 1992; Thilakarathna and Nawarathna, 2018). The absence of *S. aureus* may be attributed to the antimicrobial properties of *N. sativa* seed constituents such as TQ (Özçakmak et al., 2023).

Collectively, these findings suggest minimal direct human contamination and effective control of this pathogen in the commercial distribution of *N. sativa* seeds.

4. Fungi Enumeration

Fungal counts ranged from 1.0×10^2 to 5.6×10^2 CFU/g, with all values below the Algerian threshold for molds in dried fruits and oilseeds (10^2 – 10^3 CFU/g) (Arrêté interministériel, 2016). However, all observed colony counts were below the ISO (2008) recommended limit of countable range (15 – 150 colonies). Due to the low colony numbers, these values should be interpreted as estimates rather than precise quantifications (ISO, 2008).

The relatively low fungal loads observed are reassuring and likely reflect favorable storage conditions, particularly low seed moisture content (approximately 4.64%), which is known to inhibit fungal proliferation when water activity falls below 0.75 (Al-Jassir, 1992; Cicero et al., 2022).

Nevertheless, the detection of fungi in seeds remains a concern due to the potential for mycotoxin production. Studies have reported the presence of genera such as *Aspergillus*, *Penicillium*, *Alternaria*, and *Cladosporium* in black seeds and other medicinal herbs, some of which are known mycotoxin producers (Gopane et al., 2021; Mashat et al., 2018). Although aflatoxins were not detected in recent analyses of cold-pressed *N. sativa* oil, yeast and mold concentrations in some commercial products have exceeded local regulatory limits, highlighting the importance of ongoing monitoring (Özçakmak et al., 2023).

Overall, while fungal contamination in the present study was minimal, ensuring proper storage conditions and regular microbiological assessment remains essential to prevent the growth of mycotoxigenic fungi and to safeguard food safety (Cicero et al., 2022; Mashat et al., 2018).

5. Food Safety Implications

The results of this study are consistent with international literature, which demonstrates that edible seeds, including *N. sativa*, are susceptible to microbial contamination at multiple points in the production and supply chain (Cicero et al., 2022; Mashat et al., 2018; Silva et al., 2022). Although seeds are considered low water activity foods and generally have good microbiological stability, outbreaks of foodborne illness linked to seeds, such as *Salmonella* in sesame and chia, have been reported, highlighting the ongoing risk even in these matrices (Silva et al., 2022). The persistence of both bacteria and fungi in low water activity environments underscores the need for robust hygiene measures throughout the supply chain (Cicero et al., 2022; Mashat et al., 2018; Silva et al., 2022).

Critical control points include pre-harvest conditions (soil and irrigation water quality), harvest and post-harvest handling (equipment, containers, and transport), and storage (humidity and temperature control) (Cicero et al., 2022; Mashat et al., 2018; Silva et al., 2022). Inadequate drying and improper storage can lead to increased microbial loads and elevate the risk of spoilage or mycotoxin contamination, as observed in the significant variability in microbial counts among commercial *N. sativa* samples in this study. While thermal treatments such as roasting can reduce microbial contamination, these processes must be managed to preserve the nutritional and sensory qualities of the seeds (Silva et al., 2022).

6. Recommendations and Perspectives

Based on the findings of this study, the following recommendations and perspectives are proposed:

- Ensure that importers, distributors, and retailers source *N. sativa* seeds from suppliers who comply with Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs).
- Implement regular microbiological monitoring and risk assessment, with particular attention to coliforms, *Escherichia coli* and mycotoxin-producing fungi.

- Establish training and education programs for workers in the seed supply chain to reinforce the importance of hygiene and safe handling.
- Evaluate the effectiveness of interventions such as decontamination treatments and monitor emerging microbial hazards in edible seeds through further research.
- Integrate traceability systems and digital supply chain monitoring to increase transparency and enable prompt responses to contamination incidents.
- Maintain ongoing surveillance to assess the impact of climate change and evolving agricultural practices on the contamination risk profile of seeds and spices.
- Promote consumer education on proper storage and handling of seeds at home to reduce risks of microbial growth and mycotoxin formation, especially in warm or humid climates.
- Encourage Algerian research institutions to develop and validate rapid, cost-effective detection methods for pathogens and mycotoxins suited to local conditions.

In addition, future perspectives are needed:

- ✓ Expand future studies to include a wider range of retail locations across Constantine and eastern region of Algeria for a more comprehensive assessment of the microbiological quality of *N. sativa* seeds.
- ✓ Conduct detailed identification and characterization of isolated microorganisms using molecular and biochemical methods.
- ✓ Monitor antimicrobial resistance profiles of bacterial isolates in imported seeds.
- ✓ Include chemical analyses (e.g., heavy metals, mycotoxins) in future safety assessments.
- ✓ Investigate the impact of storage duration, conditions, and seasonal factors on microbial contamination.

In summary, ensuring the microbiological and chemical safety of edible seeds like *N. sativa* requires a comprehensive, preventive approach that integrates robust hygiene practices, regular monitoring, regulatory oversight, and ongoing research across the entire supply chain, from field to consumer.

Conclusion

Conclusion

This study offers a comprehensive evaluation of the microbiological quality of commercial *N. sativa* (black seed) samples available in Constantine, Algeria, with a focus on key microbial indicators: total aerobic mesophilic bacteria, coliforms, staphylococci, and fungi. The results revealed significant variability in microbial contamination among the three samples analyzed (BS1, BS2, and BS3), underscoring the influence of differing handling and storage practices across retail sources.

Total aerobic mesophilic bacterial counts ranged from 1.03×10^4 to 6.7×10^4 CFU/g, with all values falling within Algerian and international regulatory limits for dried herbs and spices. However, the highest count approached the upper threshold for unsatisfactory quality, highlighting the necessity for rigorous quality control to prevent potential exceedance of acceptable levels in the event of inadequate hygiene. Coliform contamination exhibited even greater variability, with counts ranging from 2.2×10^4 to 2.5×10^5 CFU/g. Notably, these values substantially exceeded both Algerian and international safety standards, indicating lapses in hygiene during harvesting, processing, or storage and emphasizing the need for standardized post-harvest practices.

No coagulase-positive staphylococci (*S. aureus*) were detected in any sample, a favorable finding that aligns with both local and international standards and suggests effective control of this pathogen in the commercial distribution of *N. sativa* seeds. Fungal contamination was minimal, with counts ranging from 1.0×10^2 to 5.6×10^2 CFU/g, all below regulatory thresholds. While these low levels are reassuring, the presence of fungi in edible seeds remains a concern due to the potential for mycotoxin production, reinforcing the importance of ongoing surveillance and proper storage conditions.

The observed inter-sample variability in microbial loads highlights the importance of implementing standardized hygiene protocols throughout the supply chain. To enhance the microbiological safety of N. sativa seeds marketed in Algeria, this study recommends the adoption of (GAPs) and GMPs, regular microbiological monitoring, comprehensive training for supply chain personnel, and the integration of traceability systems to enable rapid response to contamination incidents. Additionally, expanding future research to include a broader range of retail sources, detailed microbial identification, and monitoring of antimicrobial resistance and chemical contaminants will provide a more robust assessment of seed safety.

Limitations of this study include the restricted geographic scope and the focus on selected microbial indicators, which may not fully capture the broader contamination risks present in the national market.

Future research should address these limitations by conducting more extensive sampling and incorporating seasonal and environmental variables into risk assessments.

In summary, ensuring the microbiological and chemical safety of edible seeds like *N. sativa* requires a preventive, holistic approach that integrates robust hygiene practices, regular monitoring, regulatory oversight, and ongoing research across the entire supply chain. Continued vigilance and the implementation of evidence-based interventions are essential to safeguard consumer health and maintain the nutritional and functional value of *N. sativa* seeds in the Algerian market.

References

References

Abbas, M., Gururani, M.A., Ali, A., Bajwa, S., Hassan, R., Batool, S.W., Imam, M., Wei, D., 2024. Antimicrobial properties and therapeutic potential of bioactive compounds in *Nigella sativa*: A review. Molecules 29(20), 4914. Retrieved from https://doi.org/10.3390/molecules29204914

Abdallah, E.M., 2017. Black seed (*Nigella sativa*) as antimicrobial drug: a mini-review. Novel Approaches in Drug Designing & Development 3(2), 1-5. Retrieved from https://doi.org/10.19080/NAPDD.2017.03.555603

Abdollahi, A., Karimi, A., Sadeghi, A., 2024. The effects of black cumin (*Nigella sativa* L.) seed on performance, intestinal morphology, ileum microbial count, and nutrient digestibility of broilers. Journal of Central European Agriculture 25(4), 932–944. Retrieved from https://hrcak.srce.hr/324744

Abidi, S. M., Yamani, M. I., 2024. Microbiological and chemical profiles of retail falafel sandwich in Jordan. African Journal of Food, Agriculture, Nutrition and Development 24(1), 25221–25239. Retrieved from https://doi.org/10.18697/ajfand.126.23930

Abo-Atya, D. M., El-Mallah, M. F., El-Seedi, H. R., Farag, M. A., 2021. Novel prospective of *N. sativa* essential oil analysis, culinary and medicinal uses. Black cumin (*Nigella sativa*) seeds: Chemistry, technology, functionality, and applications 97-129. Retrieved from https://doi.org/10.1007/978-3-030-48798-0_9

Abu-Sini, M.K., Maharmah, R.A., Abulebdah, D.H., Al-Sabi, M.N.S., 2023. Isolation and Identification of Coliform Bacteria and Multidrug-Resistant Escherichia colifrom Water Intended for Drug Compounding in Community Pharmacies in Jordan. Healthcare 11(3), 299. Retrieved from https://doi.org/10.3390/healthcare11030299

Ahmad, M.F., Ahmad, F.A., Ashraf, S.A., Saad, H.H., Wahab, S., Khan, M.I., Ali, M., Mohan, S., Hakeem, K.R., Athar, M.T., 2021. An updated knowledge of Black seed (*Nigella sativa* Linn.): Review of phytochemical constituents and pharmacological properties. Journal of Herbal Medicine Valor 25, 100404. Retrieved from https://doi.org/10.1016/j.hermed.2020.100404

Akbar, S., Akbar, S., 2020. *Nigella sativa* L. (Ranunculaceae) (Syns.: N. indica Roxb. Ex Flem.; N. truncata Viv.). In: S. Akbar, ed., Handbook of 200 Medicinal Plants: A Comprehensive Review of Their Traditional Medical Uses and Scientific Justifications, Cham: Springer, pp. 1287–1312. Retrieved from https://link.springer.com/chapter/10.1007/978-3-030-16807-0_135

Aladhadh, M., 2023. A review of modern methods for the detection of foodborne pathogens. Microorganisms 11(5), 1111. Retrieved from https://doi.org/10.3390/microorganisms11051111

References

Albarky, Z., Karrar, E., Ahmed, I. A. M., Oz, E., Proestos, C., El Sheikha, A. F., Oz, F., Wu, G., Wang, X., 2022.Nutritional composition and volatile compounds of black cumin (*Nigella sativa* L.) seed, fatty acid composition and tocopherols, polyphenols, and antioxidant activity of its essential oil. *Horticulturae 8*(575). Retrieved from https://doi.org/10.3390/horticulturae8070575

Alberts, A., Moldoveanu, E. T., Niculescu, A. G., Grumezescu, A.M., 2024. *Nigella sativa*: A comprehensive review of its therapeutic potential, pharmacological properties, and clinical applications. International Journal of Molecular Sciences 25(24), 13410. Retrieved from https://doi.org/10.3390/ijms252413410

Alhamdan, A. M., Alsadon, A. A., Khalil, S. O., Wahb-Allah, M. A., El Nagar, M., Ibrahim, A. A., 2011. Influence of storage conditions on seed quality and longevity of four vegetable crops. American-Eurasian Journal of Agricultural & Environmental Sciences 11(3), 353–359.

Ali, S. R., Hussain, S. T., Ahsan, S. A., Shehzadi, N., Ashfaq, M., Bano, S., Akhlaq, M., Umrani, J. H., 2022.Black Seeds (*Nigella Sativa* L.) – kalonji: A brief review of its anti-cancer and anti-tumour qualities. Hamdard Medicus 65(1), 25–29.

Aljabre, S. H., Alakloby, O. M., & Randhawa, M. A., 2015.Dermatological effects of *Nigella sativa*. Journal of dermatology & dermatologic surgery 19(2), 92-98. Retrieved from http://dx.doi.org/10.1016/j.jdds.2015.04.002

Al-Jassir, M. S., 1992. Chemical composition and microflora of black cumin (*Nigella sativa* L.) seeds growing in Saudi Arabia. Food Chemistry 45(4), 239–242. Retrieved from https://doi.org/10.1016/0308-8146(92)90153-S

Asgher, S., & Avasthi, S., 2021. Comparative study of field efficacy of Curcuma longa and *Nigella sativa* against Apheliona maculosa on Glycine max. International Journal of Creative Research Thoughts (IJCRT) 9(8), 63–67. Retrieved from https://www.ijcrt.org/papers/IJCRT2108011.pdf

Aveling, T. A., 2014. Global standards in seed health testing. In: Global perspectives on the health of seeds and plant propagation material, pp. 17-28.

Azeem, T., Zaib-Ur-Rehman, Umar, S., Asif, M., Arif, M., & Rahman, A., 2014. Effect of *Nigella sativa* on poultry health and production: A review. Science Letters 2(2), 76–82.

Balyan, P., Akhter, J., Kumar, P., & Ali, A., 2022. Traditional and modern usage of Nigella sativa L. (Black cumin). Annals of Phytomedicine 11(2), 255–265 Retrieved from https://doi.org/10.54085/ap.2022.11.2.28

Banseka, Y. J., Tume, S. J. P., 2024. Coliform bacteria contamination of water resources and implications on public health in Fako division, South West Region, Cameroon. Advances in Environmental and Engineering Research 5(2), 1-13.

References

Basak, S., Shetty, P. H., 2021. Conventional microbial counting and identification techniques. In: Techniques to measure food safety and quality: microbial, chemical, and sensory, pp. 69–89

Bashir, O., Jan, N., Gani, G., Naik, H.R., Hussain, S.Z., Reshi, M. and Amin, T., 2021. Food applications of Nigella sativa seeds. In: Ramadan, M.F., ed. Black cumin (Nigella sativa) seeds: Chemistry, technology, functionality, and applications. Food Bioactive Ingredients. Springer Nature Switzerland, pp.191–192. Retrieved from https://doi.org/10.1007/978-3-030-48798-0_13

Belasli, A., Herrera, M., Ariño, A. and Djenane, D., 2023. Occurrence and exposure assessment of major mycotoxins in foodstuffs from Algeria. Toxins 15(7), 449. Retrieved from https://doi.org/10.3390/toxins15070449

Benazzouz-Smail, L., Achat, S., Brahmi, F., Bachir-Bey, M., Arab, R., Lorenzo, J.M., Benbouriche, A., Boudiab, K., Hauchard, D., Boulekbache, L., Madani, K., 2023. Biological properties, phenolic profile, and botanical aspect of Nigella sativa L. and Nigella damascena L. seeds: A comparative study. Molecules 28(2), 571. Retrieved from https://doi.org/10.3390/molecules28020571

Berhanu, A., 2014. Microbial profile of Tella and the role of gesho (Rhamnus prinoides) as bittering and antimicrobial agent in traditional Tella (beer) production. International Food Research Journal 21(1), 357–365. Retrieved from http://www.ifrj.upm.edu.my

Bishaw, Z., Makkawi, M. and Niane, A.A., 2009. Seed quality and alternative seed delivery systems. In: Yadav, S.S., McNeil, D.L. and Stevenson, P.C., eds. Lentil: An ancient crop for modern times. CABI, pp.350–367. https://doi.org/10.1079/9781845934873.0350

Brar, P.K. Danyluk, M.D., 2018. Nuts and grains: Microbiology and preharvest contamination risks. Microbiology Spectrum 6(2), 1–12. Retrieved from https://doi.org/10.1128/microbiolspec.PFS-0028-2018.

Cherrada, N., Chemsa, A.E., Gheraissa, N., Laib, I., Gueboudji, Z., El-Shazly, M., Zaater, A., Abid, A., Sweilam, S.H., Bin Emran, T., Nani, S., Benamor, B., Amara, D.G., Atoki, A.V., Messaoudi, M., 2024. Gastroprotective efficacy of North African medicinal plants: A review on their therapeutic potential for peptic ulcers. Food Science & Nutrition 12(11), 8793–8824. Retrieved from https://doi.org/10.1002/fsn3.4536

Cicero, N., Gervasi, T., Durazzo, A., Lucarini, M., Macrì, A., Nava, V., Giarratana, F., Tardugno, R., Vadalà, R., Santini, A., 2022. Mineral and Microbiological Analysis of Spices and Aromatic Herbs. Foods 11(4), 548. Retrieved from https://doi.org/10.3390/foods11040548

Ciesielska-Figlon, K., Daca, A., Kokotkiewicz, A., Łuczkiewicz, M., Zabiegała, B., Witkowski, J. M., & Lisowska, K. A., 2022.The influence of Nigella sativa essential oil on proliferation, activation, and apoptosis of human T lymphocytes in vitro. Biomedicine & Pharmacotherapy Valor 153, 113349. Retrieved from https://doi.org/10.1016/j.biopha.2022.113349

References

Compaoré, M. K. A., Bazie, B. S. R., Nikiema, M. E. M., Dakené, V. M., Dembélé, R., Kpoda, D. S., Kabré, E., Barro, N., 2021. Assessment of the sanitary quality of ready to eat sesame, a low moisture street food from Burkina Faso. BMC microbiology 21(1), 207. Retrieved from https://doi.org/10.1186/s12866-021-02269-0

Dadlani, M. and Yadava, D.K., 2023. Seed science and technology: biology, production, quality. Springer Nature, pp.430.

Dahal, P., 2022. Pour Plate Method – Definition, Principle, Procedure, Uses. Microbe Notes. URL https://microbenotes.com/pour-plate-method/ (Accessed 15 Jun. 2025).

Dahal, P., 2022. Spread Plate Method – Definition, Principle, Procedure, Uses. Microbe Notes. URL https://microbenotes.com/spread-plate-method-definition-principle-procedure-uses/ (Accessed 15 Jun. 2025).

Dalli, M., Bekkouch, O., Azizi, S.E., Azghar, A., Gseyra, N., Kim, B., 2022. Nigella sativa L. phytochemistry and pharmacological activities: A review (2019–2021). Biomolecules 12(1), 20. Retrieved from https://doi.org/10.3390/biom12010020

Degu, B., Tesfaye, B., Abebe, W., & Assefa, K., 2025.Diversity of Ethiopian black cumin (*Nigella sativa* L.) based on compositions of essential oil. Biochemistry Research International 2025, Article ID 2065593. Retrieved from https://doi.org/10.1155/2025/2065593

Derosa, G., D'Angelo, A., Maffioli, P., Cucinella, L., Nappi, R.E., 2024. The use of Nigella sativa in cardiometabolic diseases. Biomedicines 12(2), 405. Retrieved from https://doi.org/10.3390/biomedicines12020405

Ding, H., Fu, T.-J. Smith, M.A., 2013. Microbial Contamination in Sprouts: How Effective is Seed Disinfection Treatment Journal of Food Science 78(4), 495–501. Retrieved from https://doi.org/10.1111/1750-3841.12044

Ditta, H. M. A., Aziz, A., Hussain, M. K., Mehboob, N., Hussain, M., Farooq, S., Azhar, M. F., 2021. Exogenous application of black cumin (Nigella sativa) seed extract improves maize growth under chromium stress. Environmental Science and Pollution Research 28(9), 11349–11359. Retrieved from https://doi.org/10.1007/s11356-021-12434-5

Dobrucka, R., 2020. Metal nanoparticles in nanosensors for food quality assurance. LogForum 16(2), 271-278.

Dogu-Baykut, E., Gunes, G., 2022. Effect of ultraviolet (UV-C) light and gaseous ozone on microbial and color qualities of whole black pepper seeds (Piper nigrum L.). Carpathian Journal of Food Science and Technology 14(2), 122–131. Retrieved from https://chimie-biologie.ubm.ro/carpathian_journal/Papers_14(2)/CJFST14(2)10.pdf

References

Dong, T., Jiao, C., Qiu, Y. & Han, C., 2024. Comparison of different media for the quantitative detection of Staphylococcus aureus. Advances in Food Science and Human Nutrition 6(1). 1–6. Retrieved from https://www.clausiuspress.com/assets/default/article/2024/02/05/article_1707145560.pdf

Dou, L., Yanagishima, K., Li, X., Li, P., & Nakagawa, M., 2015. Food safety regulation and its implication on Chinese vegetable exports. Food Policy Valor 57, 128–134. Retrieved from https://doi.org/10.1016/j.foodpol.2015.09.007

Douka, D., Spantidos, T.-N., Tsalgatidou P.C., Katinakis P., Venieraki, A., 2024. Whole-genome profiling of endophytic strain B.L.Ns.14 from *Nigella sativa* reveals potential for agricultural bioenhancement. Microorganisms 12(12), 2604. Retrieved from https://doi.org/10.3390/microorganisms12122604

EFSA Panel on Biological Hazards (BIOHAZ)., 2011. Scientific opinion on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds. EFSA Journal 9(11), 2424. Retrieved from https://doi.org/10.2903/j.efsa.2011.2424.

Ehrampoush, M. H., Mazloomi, S. M., Zarei, S., Hemmati, F., Ghaneian, M. T., Hajimohammadi, B., Dehghan, A., 2017. Microbiological quality of sausage during slicing at food retail stores in Shiraz, Iran. International Journal of Nutrition Sciences 2(1), 21-26.

Eid, A. M., Elmarzugi, N. A., Abu Ayyash, L. M., Sawafta, M. N., Daana, H. I., 2017.A Review on the Cosmeceutical and External Applications of *Nigella sativa*. Journal of tropical medicine 2017 1, 7092514. Retrieved from https://doi.org/10.1155/2017/7092514

El Dhaybi, M., Mesutoğlu, D., Biçer, D.R., 2023. Medical and therapeutic effects of black seed oil on the science and health life. Phoenix Medical Journal 5(1), 6–9. Retrieved from https://doi.org/10.38175/phnx.1134490

Elahi, S., 2019. Enterotoxin production and growth of *Staphylococcus aureus* under various environmental conditions. The United Graduate School of Veterinary Sciences, Gifu University (Tokyo University of Agriculture and Technology).

El-Hack, M. E. A., Alagawany, M., Farag, M. R., Karthik, K., Dhama, K., 2016. Nutritional, healthical and therapeutic efficacy of black cumin (Nigella sativa) in animals, poultry and humans. International Journal of Pharmacology 12(3), 232-248. Retrieved from https://doi.org/10.3923/ijp.2016.232.248

El-Morsy, M.H. and Osman, H.E.-S., 2021. Morphological characters of *Nigella sativa*. In: M.F. Ramadan, ed., Black cumin (*Nigella sativa*) seeds: Chemistry, technology, functionality, and applications. Cham: Springer, pp.23–42. Retrieved from https://doi.org/10.1007/978-3-030-48798-0_3

References

Elshafie, S. S., Elshafie, H. S., El Bayomi, R. M., Camele, I., Morshdy, A. E. M. A., 2022. Evaluation of the antimicrobial activity of four plant essential oils against some food and phytopathogens isolated from processed meat products in Egypt. Foods 11(8), 1159. Retrieved from https://doi.org/10.3390/foods11081159

Erdogrul, Ö., Ciftci, E., Bozdogan, H., Toroglu, S., 2009. Antimicrobial activity of black cumin seeds (Nigella sativa L.). Asian Journal of Chemistry 21(1), 467–470.

Farag, N. E. H., Ahmedani, E. I., Sheriff, M. M., Alsubaie, A. S., Alkhamis, Z. A., Almatari, S. S., AlSobhi, L. A., Alrefai, A. K., Saeed, E. E., Abdalla, L. O., Alharbi, B. S., Obaid, A. A. A., Elbahaie, M. A., Alghamdi, Y. A. M., & Hazazi, O. A. H., 2024. Assessment of phytochemical properties of *Nigella sativa* seeds essential oil and its potential antimicrobial activity against Staphylococcus aureus in the context of safety profiles and therapeutic applications in neurosurgical interventions. Uttar Pradesh Journal of Zoology 45(17), 301–317. Retrieved from https://doi.org/10.56557/upjoz/2024/v45i17/4373

Farmanli, O., Bastug, S., Donmez, O.F., Gokdemir, C., Uckun, I., Pamuk, I., Jafarov, A., (2020). A traditional medicine, *Nigella sativa* can be effective on novel coronavirus (SARS-CoV-2) and pulmonary diseases (Hypothesis). SOJ Pharmaceutical Sciences 7(1), 1–5. Retrieved from https://doi.org/10.15226/2374-6866/7/1/001100

Ferizi, R., Ramadan, M.F., Maxhuni, Q., 2023. Black seeds (*Nigella sativa*) medical application and pharmaceutical perspectives. Journal of Pharmacy and Bioallied Sciences 15(2), 63–67. Retrieved from https://doi.org/10.4103/jpbs.jpbs_364_22

Gholamnezhad, Z., Havakhah, S., & Boskabady, M. H., 2016. Preclinical and clinical effects of Nigella sativa and its constituent, thymoquinone : A review. Journal of Ethnopharmacology Valor 190, 372–386. Retrieved from https://doi.org/10.1016/j.jep.2016.06.061

Gopane, B., Tchatchouang, C. K., Regnier, T., Ateba, C. N., Manganyi, M. C., 2021. Community diversity and stress tolerance of culturable endophytic fungi from black seed (Nigella sativa L.). South African Journal of Botan Valor 137, 272-277. Retrieved from https://doi.org/10.1016/j.sajb.2020.10.026

Gopane, B., Tchatchouang, C. K., Regnier, T., Ateba, C. N., & Manganyi, M. C., 2021. Community diversity and stress tolerance of culturable endophytic fungi from black seed (Nigella sativa L.). South African Journal of Botany Valor 137, 272-277.

Gopane, B., Tchatchouang, C.-D. K., Regnier, T., Ateba, C. N., Manganyi, M., 2024. Bioactivity and enzymatic properties of culturable endophytic fungi associated with black seeds (Nigella sativa L.). Journal of Applied Pharmaceutical Science 14(7), 102–110. Retrieved from https://doi.org/10.7324/JAPS.2024.160668

References

Gözcü, S., & Akşit, Z., 2023. Chemical composition and antibacterial activity of three volatile oils extracted from Nigella sativa L. seeds. Black Sea Journal of Health Science 6(4), 662–666. Retrieved from https://doi.org/10.19127/bshealthscience.1318520

Hameed, S., Xie, L., Ying, Y., (2018). Conventional and emerging detection techniques for pathogenic bacteria in food science : A review. Trends in Food Science & Technology, Valor 81, 61–73. Retrieved from https://doi.org/10.1016/j.tifs.2018.05.020

Hannan, M. A., Rahman, M. A., Sohag, A. A. M., Uddin, M. J., Dash, R., Sikder, M. H., Rahman, M. S., Timalsina, B., Munni, Y. A., Sarker, P. P., Alam, M., Mohibbullah, M., Haque, M. N., Jahan, I., Hossain, M. T., Afrin, T., Rahman, M. M., Tahjib-Ul-Arif, M., Mitra, S., Oktaviani, D. F., … Kim, B., 2021. Black Cumin (*Nigella sativa* L.): A Comprehensive Review on Phytochemistry, Health Benefits, Molecular Pharmacology, and Safety. Nutrients 13(6), 1784. Retrieved from https://doi.org/10.3390/nu13061784

Heires, M., 2008. The International Organization for Standardization (ISO). New Political Economy 13(3), 357–367. Retrieved from https://doi.org/10.1080/13563460802302693

Hosni, R., Haffez, H., Elkordy, H., 2023. Common applications of black cumin seed (Nigella sativa) oil in folk medicine. Journal of Advanced Pharmacy Research 7(1), 1–14. DOI: 10.21608/aprh.2022.166251.1196

Hossain, M. S., Rahman, M. Z., Mozumder, S. N., Rahman, J., 2024.Nutritional composition, fatty acids, bioactive compounds, and antioxidant activity of *Nigella sativa* seed grown in Bangladesh. *Discover Food 4*, Article 52. Retrieved from https://doi.org/10.1007/s44187-024-00102-x

Hossain, M.S., Sharfaraz, A., Dutta, A., Ahsan, A., Masud, M.A., Ahmed, I.A., Goh, B.H., Urbi, Z., Sarker, M.M.R., Ming, L.C., 2021. A review of ethnobotany, phytochemistry, antimicrobial pharmacology and toxicology of Nigella sativa L. Biomedicine & Pharmacotherapy Valor 143, 112182. Retrieved from https://doi.org/10.1016/j.biopha.2021.112182

Houchmandzadeh, B., Ballet, P., 2023. A novel procedure for CFU plating and counting. Journal of Microbiological Methods Valor 206, 106693. Retrieved from https://doi.org/10.1016/j.mimet.2023.106693

Huchchannavar, S., Yogesh, L. N., Prashant, S. M., 2019.The black seed *Nigella sativa*: A wonder seed. International Journal of Chemical Studies 7(3), 1320–1324.

Ibrahim, A. R., Zaki, M. M., & Tager, A. G., 2023. Analyzing the food safety management programs (HACCP /ISO 22000) implemented in hotels. International Journal of Tourism and Hospitality Management 6(1), 168–181.

Ilsan, N.A., Nurfajriah, S., Inggraini, M., Krishanti, N.P.R.A., Yunita, M., Sipriyadi, R., Anindita, R., Huang, T.-W., 2024. Application of miniaturized most probable number method for bacterial

detection in water samples: detection of multi-drug-resistant Ralstonia insidiosa in drinking water. Journal of Water and Health 22(9), 1618–1627. Retrieved from https://doi.org/10.2166/wh.2024.086

International Commission on Microbiological Specifications for Foods (ICMSF)., 2011. Microorganisms in foods 8: Use of data for assessing process control and product acceptance, Vol 8. Springer Science & Business Media.

International Organization for Standardization (ISO), 2006. ISO 4832:2006. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony-count technique.

International Organization for Standardization (ISO), 2008. ISO 21527-2:2008. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds - Part 2: Colony count technique in products with water activity less than or equal to 0,95.

International Organization for Standardization (ISO), 2013. ISO 4833-2:2013 Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 2: Colony count at 30 °C by the surface plating technique.

International Organization for Standardization (ISO), 2021. ISO 6888-1:2021. Microbiology of the food chain - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Method using Baird-Parker agar medium.

International Organization for Standardization (ISO), 2006. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms – Colony-count technique. ISO 4832 : 2006. Geneva, Switzerland: ISO.

Iqbal, M. S., Ghafoor, A., Inamullah, Abbas, F. M., Qureshi, A. S., Ahmad, H., 2011. Study of nutritional characteristics, mineral nutrients and agro-biodiversity in black cumin (Nigella sativa L.) genotypes from Pakistan. African Journal of Biotechnology 10(66), 14757–14766. Retrieved from https://doi.org/10.5897/AJB10.2219

Islam, M. T., 2016. Biological activities and therapeutic promises of Nigella sativa L. International Journal of Pharma Sciences and Scientific Research 2(6), 237–252. Retrieved from https://doi.org/10.25141/2471-6782-2016-6-0237

Islam, M.T., Guha, B., Hosen, S., Riaz, T.A., Shahadat, S., Sousa, L.R., Santos, J.V.O., Júnior, J.S.S., Torres de Lima, R.M., Braga, A.L., dos Reis, A.C., de Alencar, M.V.O.B., Cavalcante, A.A.C.M. 2017. Nigellalogy: A review on *Nigella sativa*. MOJ Bioequivalence & Bioavailability 3(6), 167–181. Retrieved from https://doi.org/10.15406/mojbb.2017.03.00056

Jaja, I.F., Green, E., Muchenje, V., 2018. Aerobic mesophilic, coliform, *Escherichia coli*, and *Staphylococcus aureus* counts of raw meat from the formal and informal meat sectors in South

References

Africa. International Journal of Environmental Research and Public Health 15(4), 819. Retrieved from https://doi.org/10.3390/ijerph15040819

Janefer, C., Kumar, D., 2024. Role of Consumer in Food Safety Regulation. Kristu Jayanti Journal of Management Sciences (KJMS), pp. 25-35.

Jumaah, N., Joshi, S.R. Sandai, D., 2014. Prevalence of bacterial contamination when using a diversion pouch during blood collection: a single center study in Malaysia. The Malaysian Journal of Medical Sciences (MJMS) 21(3), pp. 47–52.

Kabir, Y., Shirakawa, H., Komai, M., 2019.Nutritional composition of the indigenous cultivar of black cumin seeds from Bangladesh. *Progress in Nutrition 21* (Supplement 1), 428–434. Retrieved from https://doi.org/10.23751/pn.v21i1-S.6496

Ketenoglu, O., Kiralan, S.S., Kiralan, M., Ozkan, G., Ramadan, M.F., 2020. Cold pressed black cumin (*Nigella sativa* L.) seed oil. In: Cold pressed oils, Academic Press, pp. 53–64. Retrieved from https://doi.org/10.1016/B978-0-12-818188-1.00006-2

Kmail, A., 2023.The benefits of *Nigella sativa* for skin diseases and heal skin injuries: An overview of phytochemicals and pharmacological properties. Biomedical Journal of Scientific & Technical Research 54(1). Retrieved from https://doi.org/10.26717/BJSTR.2023.54.008505

Kmail, A., Said, O., Saad, B., 2023. How thymoquinone from *Nigella sativa* accelerates wound healing through multiple mechanisms and targets. Current Issues in Molecular Biology Valor 45, 9039–9059. Retrieved from https://doi.org/10.3390/cimb45110567

Kök, M. S., 2009. Application of food safety management systems (ISO 22000/HACCP) in the Turkish poultry industry: A comparison based on enterprise size. Journal of Food Protection 72(10), 2221–2225.

Kooti, W., Hasanzadeh-Noohi, Z., Sharafi-Ahvazi, N., Asadi-Samani, M., Ashtary-Larky, D., 2016. Phytochemistry, pharmacology, and therapeutic uses of black seed (Nigella sativa). Chinese Journal of Natural Medicines 14(10), 732–745. Retrieved from https://doi.org/10.1016/S1875-5364(16)30088-7

Kumar, V., & Sridhar, K., 2022. Seed standards and seed testing protocols for forage legume seeds.

Leclercq, R., 2002, Résistance des staphylocoques aux antibiotiques. Annales françaises d'anesthésie et de réanimation 21(5), 375–383. Retrieved from https://doi.org/10.1016/S0750-7658(02)00622-6

Lee, J. C., Daraba, A., Voldozova, C., Bazen, G., Enahany, H. A., Vournazos, T., 2021. Implementation of Food Safety Management Systems along with Other Management Tools (HACCP, EMEA, Ishikawa, Pareto): The Case Study of Listeria Monocytogenes and Correlation with Microbiological Criteria. Foods 10(10), 2301.

References

Mahmoud, H. M., El-Sheikh, M. M., 2020. Microbiological quality of organic wheat grains and sprouts. Plant Archives 20(2), 8319–8324.

Majid, A., 2018. The chemical constituents and pharmacological effects of *Nigella sativa*: A review. Journal of Bioscience and Applied Research 4(4), 389–400. Retrieved from https://doi.org/10.21608/jbaar.2018.151793

Manafi, M., 2000. New developments in chromogenic and fluorogenic culture media. International Journal of Food Microbiology 60(2–3), pp. 205–218. https://doi.org/10.1016/S0168-1605(00)00312-3

Mariod, A.A., Tahir, H.E., 2023. The history of medicinal use of black cumin seed and its position in various cultures. In: Biochemistry, Nutrition, and Therapeutics of Black Cumin Seed, Academic Press, pp. 7–10. Retrieved from https://doi.org/10.1016/B978-0-323-90788-0.00004-4

Martini, K. M., Boddu, S. S., Nemenman, I., Vega, N. 2024. Maximum likelihood estimators for colony-forming units. Microbiology Spectrum 12(9), 03946-23. Retrieved from https://doi.org/10.1128/spectrum.03946-23

Mashat, B. H., Fathi, S. M., Elkhayat, E. S., 2018. Microbial assessment of some medicinal herbs sold in Makkah, Saudi Arabia. Int. J. Chem. Tech. Res 11(02), 326-333. Retrieved from http://dx.doi.org/10.20902/IJCTR.2018.110239

Matthäus, B. and Özcan, M.M., 2011. Fatty acids, tocopherol, and sterol contents of some *Nigella* species seed oil. Czech Journal of Food Sciences 29(2), 145–150

Matthews, S., Noli, E., Demir, I., Khajeh-Hosseini, M., Wagner, M. H., 2012. Evaluation of seed quality: from physiology to international standardization. Seed Science Research 22(S1), 69-73. Retrieved from http://journals.cambridge.org/abstract_S0960258511000365

Mehraj, T., Elkanayati, R.M., Farooq, I. and Mir, T.M., 2022. A review of *Nigella sativa* and its active principles as anticancer agents. In: Black Seeds (*Nigella sativa*). Elsevier Inc., Chapter 4, pp.91–92. https://doi.org/10.1016/B978-0-12-824360-3.00012-3

Mekimah, S., Sayad, N., 2020. The Impact of Food Safety Management System ISO 22000 on Customer Satisfaction and Loyalty: Case Study of Coca-Cola Company in Algeria. Economics and Business 34(2), 246-255.

Metwaly, A. M., Ashour, M. A., Khan, S., Ma, G., Kadry, H. A., El-Hela, A. A., Mohammad, A. I., Cutler, S. J., Ross, S. A., 2019. Comparative biological evaluation of four endophytic fungi isolated from Nigella sativa seeds. Al-Azhar Journal of Pharmaceutical Sciences 59(1), 123–136. Retrieved from https://doi.org/10.21608/ajps.2019.64111

Ministère du Commerce, Ministère de l'Industrie et des Mines, Ministère de l'Agriculture, du Développement Rural et de la Pêche, Ministère des Ressources en Eau et de l'Environnement, et

References

Ministère de la Santé, de la Population et de la Réforme Hospitalière. 2016. Arrêté interministériel du 2 Moharram 1438 correspondant au 4 octobre 2016 fixant les critères microbiologiques des denrées alimentaires. Journal Officiel de la République Algérienne Démocratique et Populaire, 39, 8 Chaoual 1438/2 juillet 2017.

Mohammed, A. E. A., Al-Suwaiegh, S. B., 2016.Effects of Nigella sativa on mammals' health and production. Advances in Animal and Veterinary Sciences 4(12), 630–636. Retrieved from https://doi.org/10.14737/journal.aavs/2016/4.12.630.636

Mohammed, T., Krishnan, R., Arunima, S.H. and Kumar, G.S., 2021. *Nigella sativa*: Properties, processing and food applications.The Pharma Innovation Journal 10(4), 173–184. Retrieved from https://www.thepharmajournal.com/

Morshdy, A.E.M.A., Nagaty, E.A., Abdallah, K.M.E., Darwish, W.S., Alsayeqh, A.F. Mahmoud, A.F.A., 2023. Microbial status of the retailed meat products in Sharkia Governorate, Egypt. Journal of Advanced Veterinary Research 13(10), 2173–2177.

Nyemb, J.N., Shaheen, H., Wasef, L., Nyamota, R., Segueni, N., Batiha, G.E.S., 2022. Black cumin: A review of its pharmacological effects and its main active constituent. Pharmacognosy Reviews 16(32), 107–125. Retrieved from https://doi.org/10.5530/phrev.2022.16.16

Özçakmak, S., AlMatar, M., Var, I., & Tekin, A., 2023. Investigation on the Microbiological Quality and Fatty Acid Methyl Esters Composition of Commercially Available Cold-pressed Nigella sativa L. Oil. Current Analytical Chemistry 19(7), 561-569.

Pandey, R., Pandey, B., Bhargava, A., 2024. Morphological and anatomical studies in *Nigella sativa* L. (Ranunculaceae). Journal of Applied Horticulture 26(2), 159-164. https://doi.org/10.37855/jah.2024.v26i02.30

Panghal, A., Chhikara, N., Sindhu, N., & Jaglan, S., 2018. Role of food safety management systems in safe food production: A review. Journal of Food Safety 38(2), 12464. Retrieved from https://doi.org/10.1111/jfs.12464

Perez, C., Lors, C., Erable, B., 2022. Methodological approaches for the structural, chemical, and microbial analysis of microbial biofilms developed on the surface of cementitious materials: Overview and future prospects. International Biodeterioration & Biodegradation Valor 175, 105485. Retrieved from https://doi.org/10.1016/j.ibiod.2022.105485

Perveen, R., 2019. Therapeutic effects of black cumin (*Nigella sativa*): A systematic review. Progress in Nutrition, 21(Supplement 1) 40–49. Retrieved from https://doi.org/10.23751/pn.v21i1-S.5796

Pitt, J.I. and Hocking, A.D., 2009. *Fungi and food spoilage*. 3rd ed. Springer, New York.

References

Purwanto, A., Fahmi, K., Syahril, I., Irwansyah, Y., Cahyono, Y., Firmansyah, A., 2022 The correlation analysis of ISO 22000: 2018 food safety management system on the family companies performance. UNPRI Journal of Science and Technology 1(1), 10–11.

Rahma, A.E., 2023. Effects of climate, soil type, irrigation and selected agricultural practices on yield and seeds quality bioactive compounds of black cumin. In Biochemistry, Nutrition, and Therapeutics of Black Cumin Seed. Academic Press, pp.11–18

Rashid, S., Zafar, M., Ahmad, M., Lone, F.A., Shaheen, S., Sultana, S., Shinwari, M.I., 2018. Microscopic investigations and pharmacognostic techniques used for the standardization of herbal drug Nigella sativa L. Microscopy Research and Technique 81(12), 1443–1450. Retrieved from https://doi.org/10.1002/jemt.23110

Routh, D., Mondal, P., Chatterjee, I., Sarkar, B., Sarkar, S., Kayal, A., 2024. Checking the antimicrobial activity of the seed extract of Nigella sativa extract. International Journal of Pharmaceutical Sciences 2(7), 1161–1176. Retrieved from http://dx.doi.org/10.5281/zenodo.12746381

Safvi, A. F., & Yousuf, O., 2021. Application of Black Cumin (*Nigella sativa*) Seed as Functional Food. Agrospheres: e-Newsletter 2(5), 1–4.

Salehi, B., Quispe, C., Imran, M., Ul-Haq I., Živković J., Abu-Reidah I.M., Sen S., Acharya, K., Taheri, Y., Azadi, H., Contreras, M.M., Segura-Carretero, A., Mnayer, D., Sethi, G., Martorell, M., Razis, A.F.A., Sunusi, U., Kamal, R.M., Suleria, H.A.R., Sharifi-Rad, J., 2021. Nigella plants – Traditional uses, bioactive phytoconstituents, preclinical and clinical studies. Frontiers in Pharmacology Valor 12, 625386. Retrieved from https://doi.org/10.3389/fphar.2021.625386

Sallam, A., Yonis, A. and Garamoun, S., 2017. Some studies on immunoglobulin A, complement 3 and nitric oxide on the level of Escherichia coli enteritis in broiler with pathological changes in Bohera Province. Animal Health Research Journal 5(4B), 184–195

Shady, N. H., Sobhy, S. K., Mostafa, Y. A., El-Kashef, H. A., El-Hawary, S. S., El-Shazly, M. (2023). Phytochemical analysis and anti-infective potential of fungal endophytes isolated from Nigella sativa seeds. BMC Microbiology Valor 23, 343. Retrieved from https://doi.org/10.1186/s12866-023-03085-4

Sharma, P., & Longvah, T., 2021.Nigella (*Nigella sativa*) seed. In B. Tanwar & A. Goyal (Eds.), Oilseeds: Health attributes and food applications. Springer. pp. 331–346 https://doi.org/10.1007/978-981-15-4194-0_13

Shrestha, P., 2024. VRBL Agar (Violet Red Bile Lactose Agar). Microbe Notes. URL https://microbenotes.com/vrbl-agar/ (Accessed 15 Jun. 2025).

References

Silva, D., Nunes, P., Melo, J., Quintas, C., 2022. Microbial quality of edible seeds commercially available in southern Portugal. AIMS microbiology 8(1), 42. Doi: 10.3934/microbiol.2022004

Silva, M., 2023. The role of Good Manufacturing Practices (GMP) in food safety. Journal of Food Microbiology 7(4), 154. Retrieved from https://www.alliedacademies.org/journal-food-microbiology/

Singletary, K.W., 2022. Black seeds: Potential health benefits. Nutrition Today 57(6), 348–366. https://doi.org/10.1097/NT.0000000000000580

Soliman, M. A. M., El-Makhzangy, A., 2019. Application of HACCP system, based on ISO 22000: 2005 methodology for producing strawberry concentrates. Journal of Product & Development 24(1), 15–35.

Soliman, M. K., Ellakany, H. F., Gaafar, A. Y., Elbialy, A. K., Zaki, M. S., Younes, A. M., 2014. Epidemiology and antimicrobial activity of methicillin-resistant Staphylococcus aureus (MRSA) isolated from Nile tilapia (Oreochromis niloticus) during an outbreak in Egypt. Life Sci. J 11(10), 1245-1252.

Somé, T., Ousman, A. H., Tapsoba, F., Ali, H. M., Erbi, D., Ahmat, B. A., Gamougam, K., Ouchar, O., Zongo, C., Tidjani, A., Savadogo, A., 2025. Isolation, Identification and Antibiotic Resistance Profile of Bacterial Strains Isolated from 'Charmout', a Dried Meat Sold in Chad. European Journal of Nutrition & Food Safety 17(3), 45–55. Retrieved from https://journalejnfs.com/index.php/EJNFS/article/view/1678

Sudhir, S. P., Deshmukh, V. O., Verma, H. N., 2016. Nigella sativa seed, a novel beauty care ingredient: A review. International Journal of Pharmaceutical Sciences and Research 7(8), 3185–3196. Retrieved from https://doi.org/10.13040/IJPSR.0975-8232.7(8).3185-96

Sun, Z., Adeleke, B. S., Shi, Y., Li, C., 2023. The seed microbiomes of staple food crops. Microbial Biotechnology 16(12), 2236–2249. Retrieved from https://doi.org/10.1111/1751-7915.14352

Szczyrba, A., Dziuba, S., 2023. Good manufacturing practices for quality and safety management in the food industry. Materials Research Proceedings Valor 34, 288–297. Retrieved from https://doi.org/10.21741/9781644902691-34

Tambi, A., Brighu, U., Gupta, A.B., 2023. Methods for detection and enumeration of coliforms in drinking water: a review. Water Supply 23(10), 4047–4058. Retrieved from https://doi.org/10.2166/ws.2023.247

Thakur, A., Pathania, K., Sharma, L., Deepika, 2025. Exploring the potential of Nigella sativa seeds: A modern perspective on an ancient remedy. Journal of Advances in Medicine and Medical Research 37(1), 6–16. Retrieved from https://doi.org/10.9734/jammr/2025/v37i15691

References

Thakur, S., Kaurav, H., Chaudhary, G., (2021). *Nigella sativa* (Kalonji): A black seed of miracle. International Journal of Research and Review 8(4), 342–357. Retrieved from https://doi.org/10.52403/ijrr.20210441

Thilakarathna, R. C. N., Navaratne, S. B., 2018. Comparison of physico-chemical and microbial properties of Indian and Ethiopian origin black cumin (Nigella sativa) seeds. International Journal of Food Science and Nutrition 3(4), 30–31.

TM Media (2019) TM 636 – Agar Medium O (Baird Parker Agar Base) (as per USP/EP/BP): Product Data Sheet. A-902A, RIICO Industrial Area, Phase III, Bhiwadi-301019: TM Media. Revision: 08 Nov., 2019

Tomasello, F., Valero, A., Serraino, A., Possas, A., 2023. Methods of inoculation and quantification for collecting data on microbial responses in foods. In: Alvarenga, V.O. (ed.) Basic Protocols in Predictive Food Microbiology, Springer US, New York, NY, pp. 31–46. Retrieved from https://doi.org/10.1007/978-1-0716-3413-4_2

Usmani, A. and Almoselhy, R.I.M., 2024. Current trends in Nigella sativa L. (Black seed) from traditional to modern medicine with advances in extraction, formulation, quality control, regulatory status, and pharmacology. International Journal of Pharmaceutical Chemistry and Analysis 11(1), 11–23. Retrieved from https://www.ijpca.org/

Wahab, S., Alsayari, A., 2023. Potential pharmacological applications of Nigella sativa and its constituents against chronic inflammatory diseases: Progress and future opportunities. Plants Valor 12, 3829. Retrieved from https://doi.org/10.3390/plants12223829

Website references

Yagoub, S.O., 2023. Black cumin: Morphology, physiology, growth, and agricultural yield. In: Biochemistry, Nutrition, and Therapeutics of Black Cumin Seed. Elsevier, pp. 19–25. Retrieved from https://doi.org/10.1016/B978-0-323-90788-0.00016-0

Yagoub, S.O., 2023. Introduction, agronomy, varieties, and production of black cumin seed, In: M.A. Mohamed, ed. Biochemistry, nutrition, and therapeutics of black cumin seed. 1st ed. Cambridge, MA: Academic Press, pp.1–6. Retrieved from https://www.sciencedirect.com/science/article/abs/pii/B9780323907880000111?via%3Dihub

Yimer, E. M., Tuem, K. B., Karim, A., Ur-Rehman, N., & Anwar, F., 2019. Nigella sativa L. (Black Cumin): A promising natural remedy for wide range of illnesses. Evidence-Based Complementary and Alternative Medicine 2019, Article ID 1528635. Retrieved from https://doi.org/10.1155/2019/1528635

References

Zangerl, P., & Asperger, H., 2003. Media used in the detection and enumeration of Staphylococcus aureus. In: Progress in Industrial Microbiology Vol. 37. Amsterdam : Elsevier, pp. 91–110.

Zielińska, M., Dereń, K., Polak-Szczybyło, E., & Stępień, A. E., 2021.The role of bioactive compounds of Nigella sativa in rheumatoid arthritis therapy—Current reports. Nutrients 13(10), 3369. Retrieved from https://doi.org/10.3390/nu13103369.

Appendices

Appendix A

Culture Media Composition

A.1. Composition of Plate Count Agar (PCA) Medium for Total Aerobic Mesophilic Bacteria Enumeration

Component	Concentration (g/L)
Peptone	5.0
Yeast extract	2.5
Glucose (Dextrose)	1.0
Agar	15.0
Final pH	7.0 ± 0.2

A.2. Violet Red Bile Lactose (VRBL) Agar for Coliform Enumeration

Component	Concentration (g/L)
Peptone	7.0
Yeast extract	3.0
Lactose	10.0
Bile salts	1.5
Sodium chloride	5.0
Cristal violet	0.002
Agar	15.0
Final pH	7.4 ± 0.2

A.3. Baird-Parker Agar for Staphylococci Enumeration

Component	Concentration (g/L)
Peptone	10
Yeast extract	1.0
Beef extract	5.0
Sodium pyruvate	10
Lithium chloride	5.0
Glycine	12.0
Agar	20
Final pH	7.0 ± 0.2

A.4. Sabouraud Dextrose Agar (SDA) with Chloramphenicol

Component	Concentration (g/L)
Peptone	10.0
Glucose (Dextrose)	40
Agar	15.0
Chloramphenicol	0.1
Final pH	5.6 ± 0.2

Appendix B

Statistical Analysis Tables

B.1. One-way ANOVA for total aerobic bacterial counts (log CFU/g) among *Nigella sativa* seed (Black seed) samples (BS1, BS2, and BS3)

Source of variation	Sum of squares	df	Mean square	F-value	Sig
Between groups	2.72×10^{11}	2	1.36×10^{11}	17.27	0.0024
Within groups	4.74×10^{10}	6	7.90×10^{9}		
Total	3.20×10^{11}	8			

Post-hoc Analysis (Tukey's HSD Test)

Sample source		Mean Difference (I-J)	Standard Error	Sig.	95% Confidence Interval	
					Lower bound	Upper bound
BS1	**BS2**	5.67×10^{4}*	1.25×10^{4}	0.001	3.21×10^{4}	8.13×10^{4}
	BS3	3.97×10^{4}*	1.25×10^{4}	0.014	1.51×10^{4}	6.34×10^{4}
BS2	**BS1**	-5.67×10^{4}*	1.25×10^{4}	0.001	-8.13×10^{4}	-3.21×10^{4}
	BS3	-1.70×10^{4}*	1.25×10^{4}	0.043	-4.16×10^{4}	7.56×10^{3}
BS3	**BS1**	-3.97×10^{4}*	1.25×10^{4}	0.014	-6.34×10^{4}	-1.51×10^{4}
	BS2	1.70×10^{4}*	1.25×10^{4}	0.043	-7.56×10^{3}	4.16×10^{4}

*The mean difference is significant at the 0.05 level.

B.2. One-way ANOVA for coliform counts (log CFU/g) among *Nigella sativa* seed (Black seed) (BS1, BS2, and BS3)

Source of Variation	Sum of squares	df	Mean square	F-value	Sig
Between Groups	7.64×10^{10}	2	3.82×10^{10}	100.04	<0.001
Within Groups	2.29×10^{9}	6	3.82×10^{8}		
Total	7.87×10^{10}	8			

Post-hoc Analysis (Tukey's HSD Test)

Sample source		Mean Difference (I-J)	Standard Error	Sig.	95% Confidence Interval	
					Lower bound	Upper bound
BS1	**BS2**	2.28×10^5*	1.09×10^4	<0.001	1.95×10^5	2.61×10^5
	BS3	1.00×10^5*	1.09×10^4	<0.001	6.73×10^4	1.33×10^5
BS2	**BS1**	-2.28×10^5*	1.09×10^4	<0.001	-2.61×10^5	-1.95×10^5
	BS3	-1.28×10^5*	1.09×10^4	<0.001	-1.61×10^5	-9.55×10^4
BS3	**BS1**	-1.00×10^5*	1.09×10^4	<0.001	-1.33×10^5	-6.73×10^4
	BS2	1.28×10^5*	1.09×10^4	<0.001	9.55×10^4	1.61×10^5

*The mean difference is significant at the 0.05 level.

A Thesis Presented in Partial Fulfilment of the Requirements for The Master's Degree in Mycology and Fungal Biotechnology

Submitted by: Oumaima Hachelaf and Amani Kacemi

Title: Evaluation of Microbiological Quality of some Commercial Black Seeds (*Nigella sativa* L.) in Constantine, Algeria: Implications for Food Safety

Abstract

The objective of this study was to assess the microbiological quality of commercial black seeds (*Nigella sativa* L.) available in Constantine, Algeria, with an emphasis on food safety implications. These seeds are widely recognized for their health and nutritional benefits with many applications in food, medicine, and cosmetics.

Three *N. sativa* L. seed samples (BS1, BS2, and BS3) were purchased from different traditional herbal medicine stores and analyzed by using the culture media Plate Count Agar, Violet Red Bile Lactose Agar, Baird-Parker agar, and Sabouraud Dextrose Agar to enumerate total aerobic mesophilic bacteria, coliforms, staphylococci, and fungi, respectively. Microbiological studies were performed according to the International Organization for Standardization (ISO) methods.

The results revealed significant variability in microbial contamination among samples. Total aerobic mesophilic bacterial counts ranged from 1.03×10^4 to 6.7×10^4 CFU/g, with BS1 exhibiting the highest load. This is presumably attributable to inadequate drying or substandard storage, aligning with other findings on Algerian herbs. Coliform counts were notably high, ranging from 2.2×10^4 to 2.5×10^5 CFU/g, substantially exceeding both Algerian (2×10^3 CFU/g) and international ($<10^3$ CFU/g) regulatory limits for dried herbs and spices. No coagulase-positive staphylococci (Staphylococcus aureus) were detected in any sample, suggesting minimal direct human contamination and possibly reflecting the antimicrobial properties of *N. sativa* constituents. Fungal counts were relatively low (1.0×10^2 to 5.6×10^2 CFU/g) and below national thresholds, although the presence of fungi still poses a potential risk for mycotoxin contamination.

The observed differences in microbial quality among samples highlight the influence of handling and storage practices and underscore the need for standardized post-harvest protocols. These findings emphasize the importance of rigorous hygiene measures throughout the supply chain, regular microbiological monitoring, and consumer education to ensure the safety of edible seeds. Ensuring the microbiological safety of *N. sativa* seeds is essential for public health and consumer protection in Algeria.

Keywords: *Nigella sativa* L. seeds; Microbiological quality; Enumeration; Total bacteria; Coliforms; Staphylococci; Fungi.

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