



Diversity of fungal community associated with nuts collected from different markets in Basrah governorate

Saja aqab Al-Saadoun and Mustafa Abdul-Wahab Al-Dossary
University of Basrah, College of Science, Department of Ecology

*Corresponding author E-mail: mustafa.najem@uobasrah.edu.iq

Abstract

Nuts are considered a healthy food rich in beneficial fats, proteins, and vitamins, and they are widely consumed for their health benefits. However, nuts can be exposed to microbial contamination, including fungal contamination. This study aimed to evaluate the diversity of fungi associated with three common types of dried nuts available in local markets in Basrah, namely pistachios, cashews, and walnuts. The isolated and identified methods revealed 11 fungal genera, including 25 fungal species, with ascomycetous fungi in their asexual stage being the predominant. *Aspergillus niger* was identified as the dominant and most prevalent species, with a 100% occurrence rate in all nut samples, both surface-sterilized and non-sterilized. Ecological indices were used to analyze the structure of the fungal community in nut samples. The results showed that Shannon–Wiener index values were low, ranging from 0.707 to 1.625 in both sterilized and non-sterilized samples, which can be attributed to the dominance of *A. niger*. An inverse relationship was observed between the diversity index and the Berger–Parker dominance index, with values ranging from 0.549 to 0.8235 in both types of samples. In contrast, a positive relationship was recorded between the diversity index and the Evenness index, with values ranging from 0.3836 to 0.7058, which is used to assess the uniformity of individual distribution among different fungal species. The Margalef index, which reflects species richness, ranged from 0.948 to 3.538 in both sterilized and non-sterilized samples, with walnuts showing the highest prevalence and diversity of fungal species compared to the other nut types. These findings indicate that dried nuts available in Basrah markets may harbor fungal species that pose potential risks to public health, highlighting the need to implement effective programs for monitoring the quality and safety of these food products before they reach consumers.

Keywords: Fungal diversity, Diversity Indices, Nuts.

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1. Introduction

Nuts are widely consumed foods due to their high nutritional value and well-recognized health benefits. They provide an excellent source of proteins and beneficial unsaturated fats, along with essential vitamins, minerals, and antioxidants, which

play an important role in promoting human health and reducing the risk of various diseases, particularly cardiovascular conditions and certain chronic disorders. Moreover, nuts have significant economic importance, as they are regarded as agricultural crops with high commercial

value and are used in various food industries. However, their rich nutritional composition makes them a suitable environment for the growth of microorganisms, especially fungi, which may lead to quality deterioration and the formation of mycotoxins that pose a risk to consumer health (Sung *et al.*, 2021).

Microorganisms are among the most important contaminants affecting these foods, with fungi among the most harmful. This is due to their high ability to tolerate and adapt to living conditions in dry foods. Their presence on nuts poses a significant risk to human health. With the increasing global consumption of dried nuts, either directly or as raw materials used in many food industries, the risk of exposure to the harmful effects caused by fungi associated with these foods has increased, either through direct consumption of the fungi growing on them or through the mycotoxins that may be present (Stajich, 2017).

Fungi are capable of inhabiting almost all ecosystems and include both unicellular and multicellular organisms that may live freely, symbiotically, or parasitically. They are well known for their essential ecological roles, particularly as decomposers and symbionts, and have been used for centuries in various applications in the fields of food and medicine.

Given the low moisture content of nuts, they are generally considered highly resistant to contamination by microorganisms. However, fungi are still able to grow on them, as they are resilient organisms capable of surviving in extreme dry environments. The likelihood of fungal growth on dried nuts increases with rising moisture levels, particularly as a result of improper storage conditions (Van de Perre *et al.*, 2015).

When nuts are stored under unfavorable conditions, such as increased moisture and moderate temperatures, this promotes the growth and spread of fungi.

Fungal growth and proliferation in dried nuts can lead to economic losses due to product spoilage, rejection, and reduced consumption, in addition to health hazards to consumers resulting from the presence of fungi (Mateus *et al.*, 2021).

Various fungal genera are commonly isolated from nuts, with *Aspergillus* being one of the most frequently encountered. This genus belongs to the anamorphic fungi and is well known for its ability to withstand harsh environmental conditions, including dryness, high temperatures, and low moisture levels conditions that are typically present in nuts. *Aspergillus* includes numerous species capable of producing mycotoxins, which pose serious risks to human health and can also negatively impact the national economy. Among the most well-known species associated with nuts are *Aspergillus flavus* and *Aspergillus niger*, which are widely detected on different types of nuts (Visagie *et al.*, 2024).

This study aims to investigate the presence of fungi on three types of nuts, pistachios, cashews, and walnuts available in various local markets in Basrah Province, and fungal community on the nuts.

2. Materials and Methods

2.1: Collection of Samples

Samples of nuts were collected from various locations within Basrah Governorate. A total of 16 mixed nut samples were obtained from local commercial shops selling nuts. The collection period spanned from July 2024 to November 2024. The sampling procedure followed the method described by Ramadan *et al.* (2022). For each sample, 250 grams of nuts were randomly collected, placed in clean, dry paper bags, and labeled with the date and location of collection. The bags were then securely sealed and transported to the laboratory, where they were stored in a dry place until fungal isolation was performed.

2.2: Culture Media for Fungal Isolation

Three culture media were used in this study for fungal isolation: Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA), which were prepared according to the manufacturer's instructions (HiMedia, India), and Rose Bengal Agar (RBA). The RBA medium was prepared using the following components (g/L): Peptone 5.0, Glucose 10.0, Potassium dihydrogen phosphate 1.0, Magnesium sulfate 0.5, Agar 15.0, and Rose Bengal 0.025, all dissolved in distilled water. Chloramphenicol was added to all media before sterilization as an antibiotic to inhibit bacterial growth.

2.3: Fungal Isolation from Nut Samples

Fungi were isolated from nut samples using the direct plating method, as described by Mirabile *et al.* (2021). From each sample, three types of nuts walnuts, pistachios, and cashews were selected for fungal isolation due to their nutritional importance and high consumption rates.

Each type of nut sample was processed using two methods:

1. Surface-Sterilized Method:

The surface of the nuts was sterilized by immersing them in 2% (v/v) sodium hypochlorite (NaOCl) solution in a sterile Erlenmeyer flask for 2 minutes. The nuts were then rinsed three times with sterile distilled water, with each rinse lasting 2 minutes, to remove any residual chemicals. For each nut type, five pieces were inoculated per plate, with two replicates prepared for each culture medium. The plates were incubated at 25 °C for two weeks.

2. Non-Sterilized Method:

The same plating procedure was applied, but the nut samples were not surface-sterilized. This portion was cultured directly without any prior treatment.

2.4: Phenotypic and Genetic Identification of Isolated Fungi

2.5: Study of the Fungal Community

2.5.1: Occurrence%

The isolated fungal species were initially examined using a dissecting light microscope, with observations recorded on colony characteristics, sporulation rates, and pigmentation. Subsequently, glass slide preparations were made from each colony, and a compound light microscope was used to study the morphological features of the fungi (Rivera *et al.*, 2012). Identification was performed based on the taxonomic keys provided by:

Raper and Fennell (1973), De Hoog and Guarro (1995), Watanabe (2002), Guarro *et al.* (2012), Rivera *et al.* (2012), Bensch *et al.* (2018), Tagele *et al.* (2018), Bandara *et al.* (2022), Lee *et al.* (2025).

For the genetic identification of isolated fungi, the pure colonies from each isolate were sub-cultured onto PDA medium and incubated for seven days. DNA extraction and PCR amplification were performed following the protocol of El-Gendi *et al.* (2021). The ribosomal DNA regions NL1–NL4 and Ben2f–Bt2a were amplified using the following primers: NL1 (F-5'-GCA TAT CAA TAA GCG GAG GAAA-3') and NL4 (R-5'-GGT CCG TGT TTC AAG ACG G-3'), consisting of 19 and 23 base pairs, respectively; and Ben2f (F-5'-TCC AGA CTG GTC AGT GTG TAA-3') and Bt2a (R-5'-ACC CTC AGT GTA GTG ACC CTT GGC-3'), consisting of 21 and 24 base pairs. The PCR program included an initial denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. The amplification produced distinct products ranging from approximately 400 to 850 bp. PCR products were purified for sequencing at Macrogen (Seoul, South Korea), and the resulting sequences were identified and aligned using the Basic Local Alignment Search Tool (BLAST).

The percentage of occurrence of fungal genera and species for fungi isolated during this study from different nuts species was

calculated according to the following equation:

$$\text{occurrence \%} = \frac{\text{Number of samples in which the genus or species appeared}}{\text{Total number of samples}} \times 100$$

2.5.2: Diversity indices

A set of indices was used to assess fungal biodiversity in the studied samples, as follows Sharma and Sumbali (2014):

1. Species richness

where:

D = Margalef's species richness index

S = total number of species in the sample

ln = natural logarithm

N = total number of individuals (fungal colonies) in the sample

The index ranges from $(0 - \infty)$ and was classified into the following categories:

$$H' = - \sum \frac{n}{N} \cdot \ln \frac{n}{N}$$

where:

H' = Shannon–Wiener diversity index,

n = number of individuals of a single species in the sample,

N = total number of individuals in the sample.

The values of the Shannon–Wiener diversity index range from 0 to 5, with higher values indicating greater species diversity. Based on the calculated values, species diversity was classified into the following categories:

- Very high diversity: 3.5 and above

To estimate fungal species richness, the following index was applied: Margalef (1968)

is expressed by the following equation:

$$D = \frac{S - 1}{\ln N}$$

- Low species richness: $R < 2.5$
- Medium species richness: $2.5 \leq R < 4$
- High species richness: $R \geq 4$

2. Species Diversity Index

Species diversity in the studied samples was assessed using the Shannon–Wiener diversity index (H') Shannon (1963) to evaluate overall biodiversity. The index was calculated using the following equation:

- High diversity: 3.0 – 3.49
- Moderate diversity: 2.5 – 2.99
- Low diversity: 2.0 – 2.49
- Very low diversity: 1.99 and below

3. Evenness Index

Species evenness in the studied samples was evaluated using Pielou's evenness index (J), Pielou (1975) which reflects the uniformity of species distribution within the community. The index was calculated according to the following equation:

$$J = \frac{H'}{S}$$

where:

J = Pielou's evenness index,

H' = Shannon–Wiener diversity index value,

S = total number of fungal species in the sample.

The values of Pielou's evenness index range from 0 to 1, with values closer to 1 indicating a more even distribution of species. Based on the obtained values, evenness was classified into the following categories:

where:

d = Berger–Parker dominance index,

n_{max} = number of individuals of the most dominant fungal species,

N = total number of individuals in the sample.

3. Results and discussion

3.1: Fungal isolation and identification

In total, 11 fungal genera were isolated from the nut samples examined, whether with surface sterilization or without, in addition to sterile mycelia (Table 1). The genus *Aspergillus* showed the highest occurrence rate reaching 100 % in non-sterilized samples

- Balanced: 0.8 – 1.0
- Semi-balanced: 0.5 – 0.8
- Unbalanced: 0 – 0.49

4. Dominance Index

Species dominance in the studied samples was assessed using the Berger–Parker dominance index (d), Berger and Parker (1970) to determine the extent to which a single fungal species dominates the community. The index was calculated using the following equation:

$$d = \frac{n_{max}}{N}$$

and 94% in surface-sterilized samples. The genus *Penicillium* recorded the second highest occurrence, reaching 69% in non-sterilized samples and 56% in surface-sterilized samples. The high prevalence of these genera are generally attributed to their ability to withstand and adapt to harsh conditions, and their tolerance to a wide range of temperatures. Also, they produce numerous small-sized reproductive units that enable their rapid dissemination in the environment (Arastehfar *et al.*, 2021).

Table 1: The isolated fungal genera with their percentage of occurrence

No.	Fungal genera	No. of samples in which the genera appeared		Occurrence %	
		Surface- sterilized	Non-sterilized	Surface- sterilized	Non-sterilized
1	<i>Alternaria</i>	1	2	6	13
2	<i>Aspergillus</i>	15	16	94	100
3	<i>Cladosporium</i>		1		6
4	<i>Curvularia</i>		1		6
5	<i>Emericella</i>	1	1	6	6
6	<i>Fusarium</i>	1	1	6	6

7	<i>Mucor</i>		2		13
8	<i>Neosartorya</i>		1		6
9	<i>Penicillium</i>	9	11	56	69
10	<i>Rhizopus</i>	8	3	50	19
11	Sterile mycelia	3	1	19	6
12	<i>Trichoderma</i>	1	1	6	6

In the study of Nooralden and Mohammed (2022) four fungal genera were isolated from nut samples collected from local markets and the two genera *Penicillium* and *Aspergillus* were also isolated in high percentage.

A total of 25 fungal species were isolated and identified by phenotypic and

phylogenetic during this study (Table 2). The results showed that most of the fungal species isolated in the present study belonged to the ascomycota in their asexual state (anamorphic fungi), with an occurrence rate of 84%, representing 21 species.

Table 2: isolated fungal species with their percentage of occurrence

No.	Fungal species	No. of samples in which the species appeared		Occurrence%	
		Surface-sterilized	Non-sterilized	Surface-sterilized	Non-sterilized
1	<i>Alternaria alternata</i>	1	1	6	6
2	<i>Alternaria heterospora</i>		1		6
3	<i>Aspergillus allahabadii</i>		1		6
4	<i>Aspergillus amstelodami</i>	2	1	13	6
5	<i>Aspergillus costaricensis</i>	1	1	6	6
6	<i>Aspergillus flavus</i>	4	4	25	25
7	<i>Aspergillus niger</i>	16	16	100	100
8	<i>Aspergillus pseudoglaucus</i>		1		6
9	<i>Aspergillus sp.</i>	2	1	13	6
10	<i>Aspergillus terreus</i>	1	1	6	6
11	<i>Aspergillus versicolor</i>		1		6
12	<i>Aspergillus fumigatus</i>		2		13

13	<i>Cladosporium angulosum</i>		1		6
14	<i>Curvularia senegalensis</i>		1		6
15	<i>Emericella nidulans</i>	1	1	6	6
16	<i>Fusarium equiseti</i>	1	1	6	6
17	<i>Mucor hemialis</i>		2		13
18	<i>Neosartorya hirastukae</i>		1		6
19	<i>Penicillium chrysogenum</i>		1		6
20	<i>Penicillium crustosum</i>	1	5	6	31
21	<i>Penicillium mallochii</i>	2	1	13	6
22	<i>Penicillium sajarovii</i>	1	1	6	6
23	<i>Penicillium rubens</i>	5	6	31	38
24	<i>Rhizopus stolonifer</i>	8	3	50	31
25	<i>Trichoderma harzianum</i>	1	1	6	6

Aspergillus niger recorded the highest occurrence rate (100%) in both sterilized and non-sterilized methods. (Fig. 1). This fungus owns a complex enzymatic system that allows it to degrade a varied range of compounds, comprising those

that are resistant to decomposition. Moreover, this species along with *Aspergillus flavus* and *Aspergillus amstelodami* are considered xerophilic fungi adept will to survive in harsh environmental conditions.



Fig. 1: Some fungi isolated from pistachios on PDA medium

3.2: Diversity indices

The structure of the fungal community during the present study was studied using the following ecological indices as follows:

3.2.1: Shannon–Wiener Index

The results of the Shannon–Wiener diversity index for the fungal community in non-sterilized samples showed a value of 0.9532 in pistachio. In cashew, the index value increased and reached 1.1, while walnut recorded the highest value at 1.534 (Figure. 2).

In sterilized samples, the index value was 0.707 in pistachio and increased to 1.191 in walnut, whereas the highest value was recorded in cashew (1.625) (Figure .3).

This index was used to understand the species composition of the fungal community associated with nuts and to compare diversity among different types of nuts and their relationship with dominance. The results showed low index values due to the high dominance of individuals belonging to

Aspergillus niger (Figures 4,5). Diversity indices are inversely related to dominance, which reflects the harsh nature of the nut environment, as it is not suitable for the growth and reproduction of all fungal species due to low moisture content and limited nutrients, with a clear dominance of tolerant and competitive species (Wu *et al.*, 2018).

The lowest index values were recorded in pistachio samples, both sterilized and non-sterilized, due to the high dominance of *A. niger* compared to other species (Kim *et al.*, 2017).

In contrast, higher index values were observed with reduced dominance of *A. niger* in walnut samples under non-sterilized conditions and in cashew samples under sterilized conditions, indicating a decrease in the numerical abundance of this species and an increase in the abundance of individuals belonging to other species (Sivakaame *et al.*, 2025).

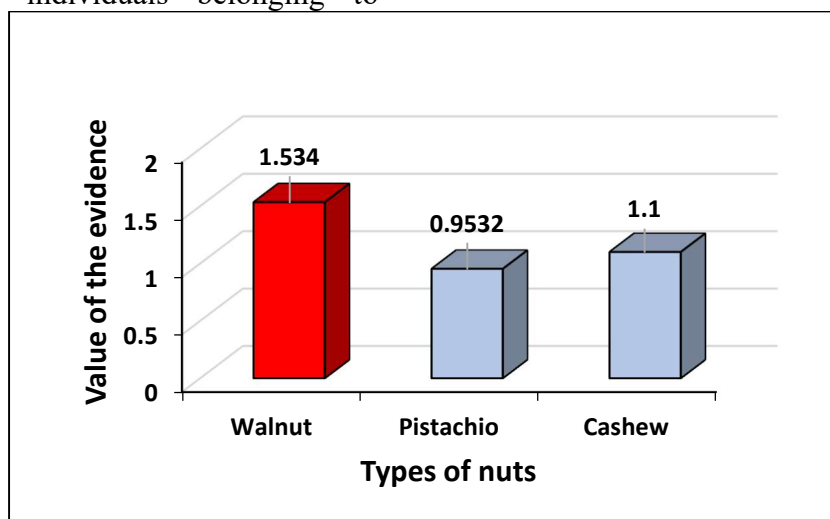


Figure .2: Shannon–Wiener index values in non-sterilized samples

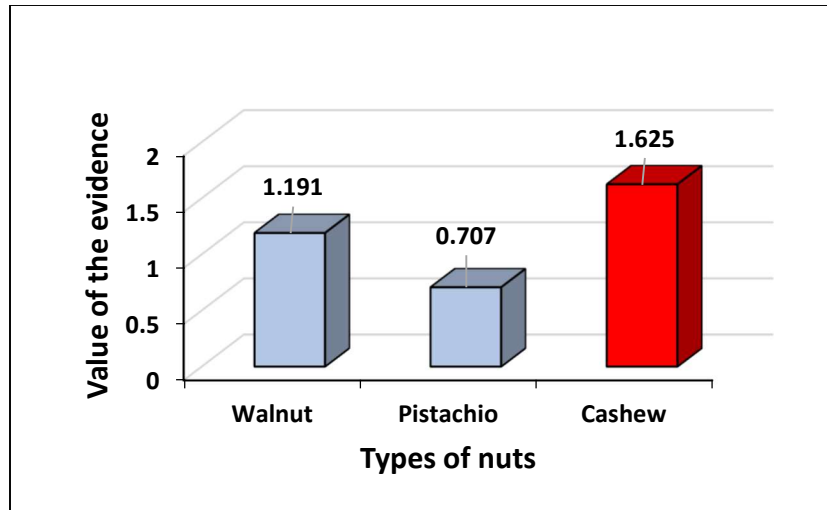


Figure .3: Shannon–Wiener index values in sterilized samples

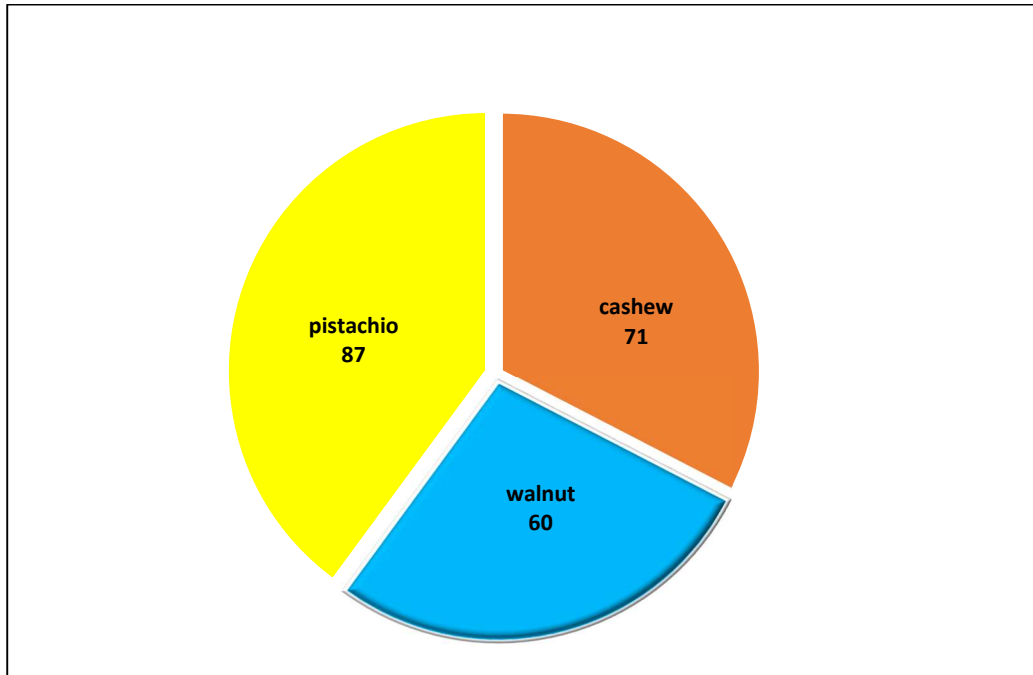


Figure .4: Number of *Aspergillus niger* colonies in nuts using the non-sterilized method

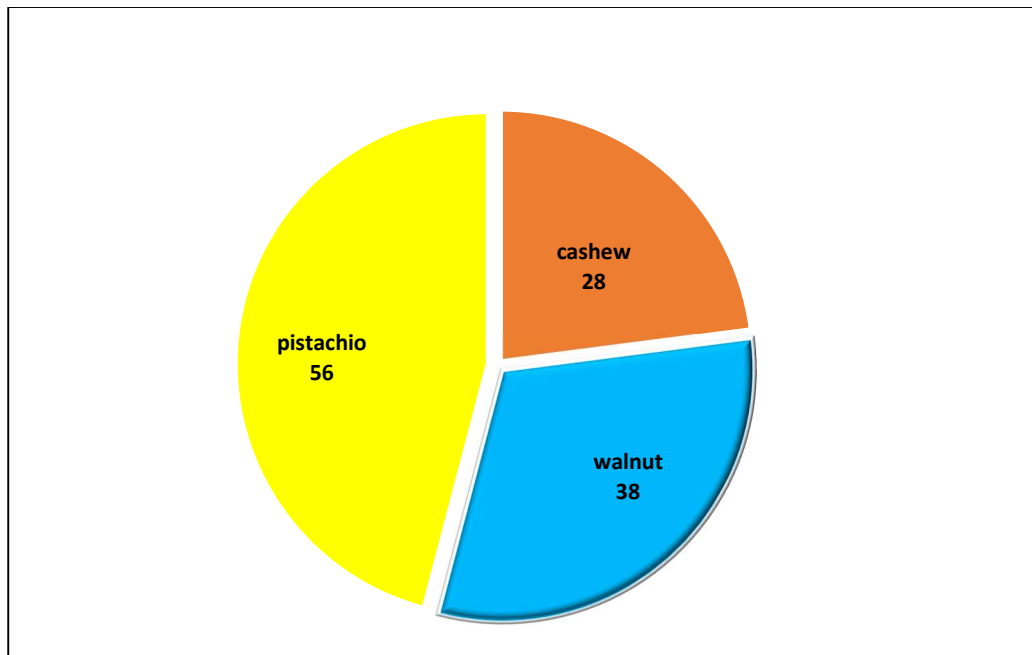


Figure .5: Number of *Aspergillus niger* colonies in nuts using the sterilized method

3.2.2: Evenness Index

The highest Evenness index value in non-sterilized samples was recorded in walnut (0.5415), followed by cashew (0.4776), while the lowest value was observed in pistachio (0.3836) (Figure .6).

In sterilized samples, cashew recorded the highest Evenness value (0.7058), which decreased in walnut (0.5171) and further declined in pistachio (0.4393) (Figure .7).

The Evenness index is used to measure the distribution of individuals among species present in the studied environment. There is a positive relationship between the Evenness index and the Shannon diversity index, where an increase in one index is accompanied by an increase in the other (Kim *et al.*, 2017).

The results indicate that the distribution of individuals was nearly even in walnut in non-sterilized samples, whereas a clear uneven distribution was observed in pistachio and cashew. This pattern is attributed to the distribution and dominance of *Aspergillus niger* individuals in walnut, pistachio, and cashew samples (Sivakaame *et al.*, 2025).

In sterilized samples, the Evenness index values indicated a near-even distribution of individuals in both cashew and walnut, while pistachio continued to show uneven distribution. This may be attributed to the use of sample sterilization, which significantly reduced the number of growing colonies and contributed to a more balanced distribution of individuals, thereby increasing the Evenness index values (Kim *et al.*, 2017).

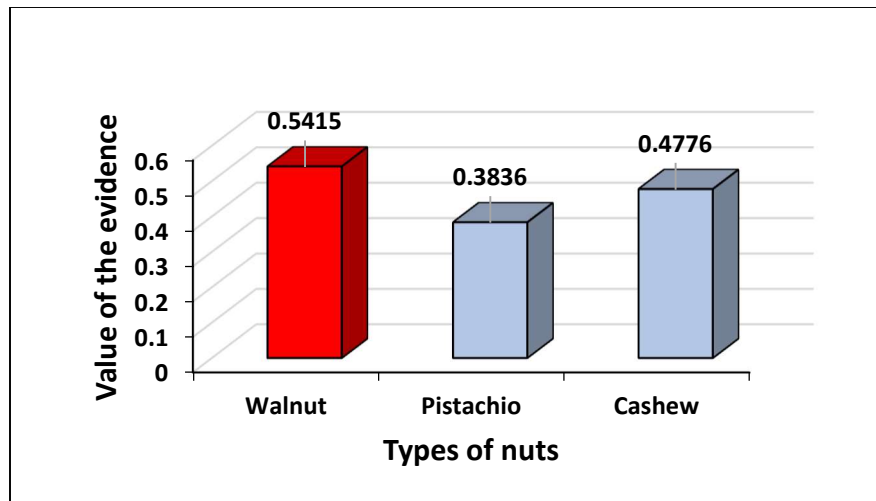


Figure .6: Evenness index values in non-sterilized samples

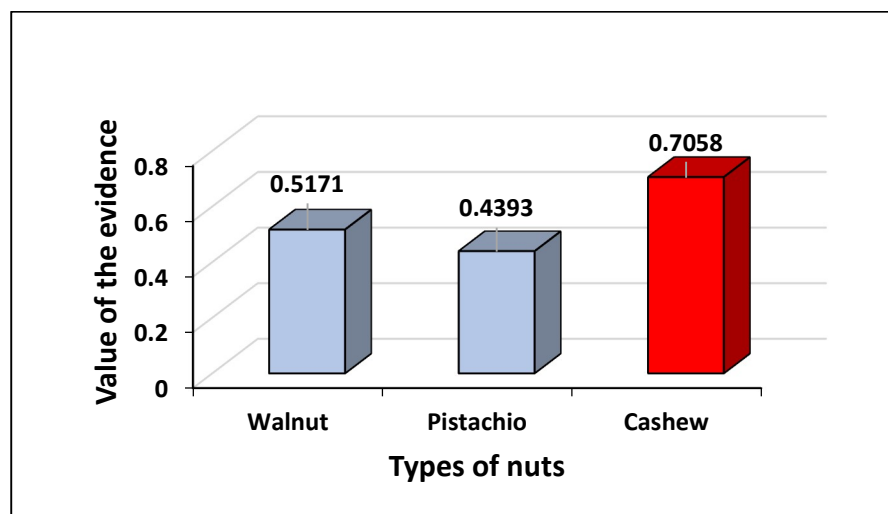


Figure .7: Evenness index values in sterilized samples

3.2.3: Berger–Parker Dominance Index

The lowest Berger–Parker dominance index value in non-sterilized samples was recorded in walnut (0.6522), followed by an increase in cashew (0.7474), while the highest dominance value was observed in pistachio (0.8056) (Figure .8).

In sterilized samples, the lowest index value was recorded in cashew (0.549), which increased to 0.7451 in walnut, whereas pistachio showed the highest dominance value (0.8235) (Figure .9).

The results indicate a clear dominance of a single species, *Aspergillus niger*, over the

other fungal species. This species recorded the highest frequency and occurrence throughout the study and is considered highly tolerant to harsh and extreme environmental conditions (Ma *et al.*, 2023).

The Berger–Parker index reflects the relative importance of the most abundant and dominant species in terms of individual numbers and shows an inverse relationship with the Shannon diversity index, whereby an increase in one index is accompanied by a decrease in the other (Norros *et al.*, 2015). This pattern was clearly observed in non-

sterilized pistachio samples, which recorded the highest Berger–Parker index value due to the dominance of *A. niger*, which accounted for 87 colonies. In contrast, the Shannon index recorded its lowest value in non-

sterilized pistachio samples. Similar trends were observed in the remaining samples, which demonstrated an absolute dominance of *A. niger* over the other studied species.

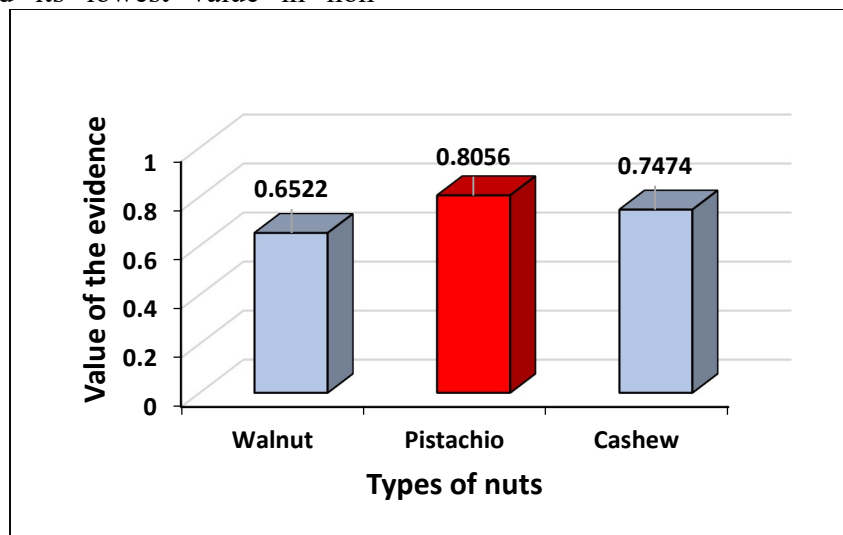


Figure .8: Berger–Parker dominance index values in non-sterilized samples

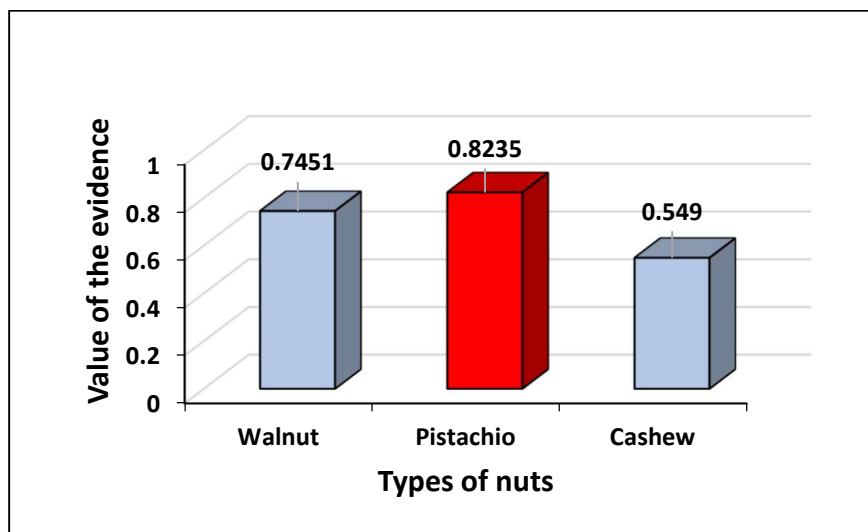


Figure .9: Berger–Parker dominance index values in sterilized samples

3.2.4: Margalef Index

The results showed that the highest Margalef index value, used to measure species richness within the fungal community, was recorded in walnut at 3.538 in non-sterilized samples (Figure .10), with a total of 17 fungal species identified (Figure.11). This index value indicates

moderate species richness, which may be attributed to the high fat and protein content of walnuts that serve as nutrient sources for fungi. In addition, the cracked and irregular surface of walnuts provides a more favorable habitat for fungal growth (Zhou *et al.*, 2022).

In pistachio, the Margalef index value was 2.349, while cashew recorded a lower value of 1.976, indicating low species richness.

In sterilized samples, both walnut and cashew recorded an index value of 2.289, whereas pistachio showed a further decline, recording a value of 0.948 (Figure 12 ,13).

Overall, the Margalef index values indicated low species richness in the studied samples, which can be attributed to the nature of the studied environment. In addition, sterilization contributed to a further reduction in fungal diversity (Ghosh *et al.*, 2012).

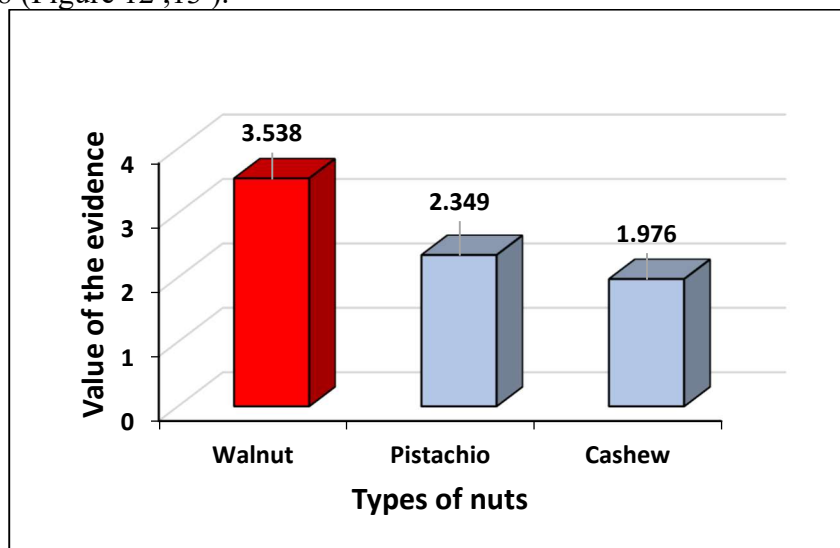


Figure .10: Margalef index values in non-sterilized samples

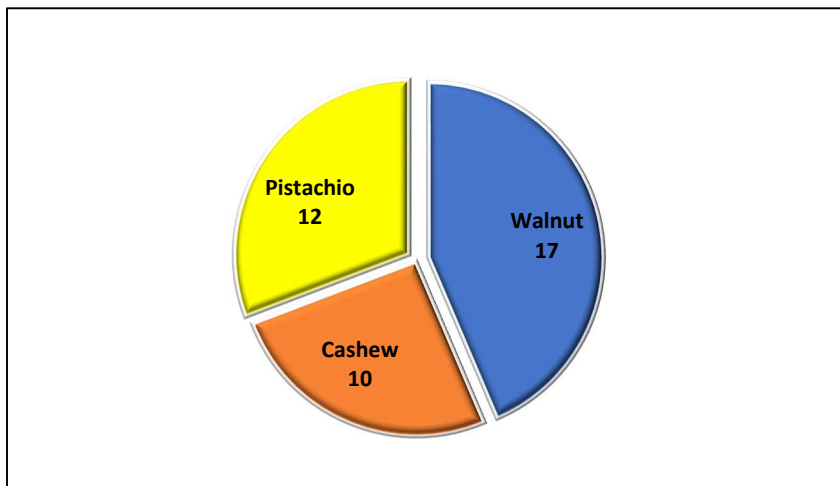


Figure .11: Fungal species richness on different nut types in non-sterilized samples

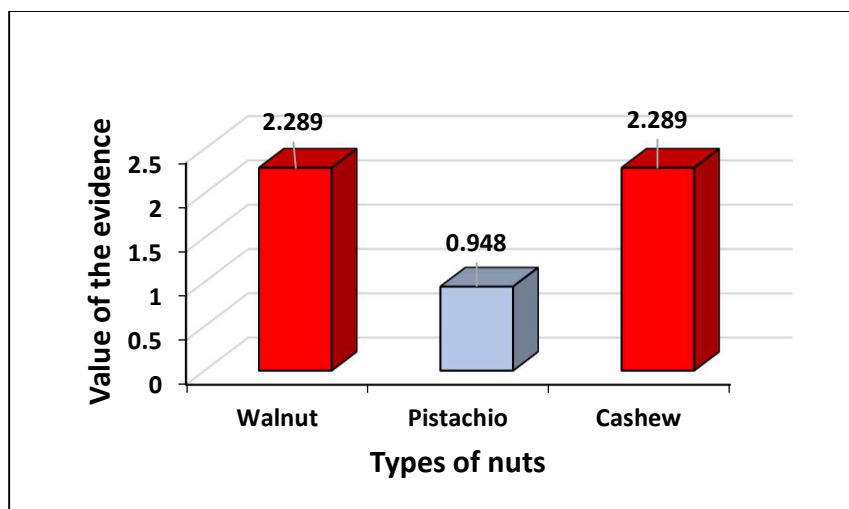


Figure .12: Margalef index values in sterilized samples

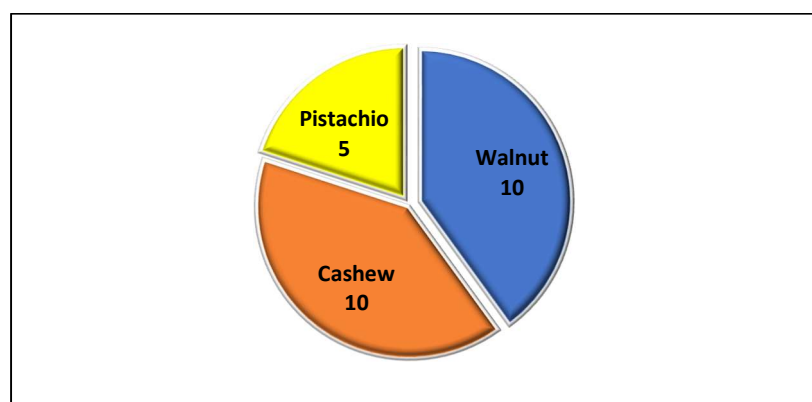


Figure .13: Fungal species richness on different nut types in sterilized samples

4. Conclusion

Fungal contamination of nuts negatively affects their nutritional and commercial value and represents a source of health and economic risks to the community. The results of this study showed a higher incidence of fungi in non-sterilized samples, with a clear dominance of the genus *Aspergillus*. The prevalence of this genus can be attributed to its ability to produce mycotoxins, in addition

to its tolerance to harsh environmental conditions, which explains its superiority and dominance among the isolated fungal genera. Ecological indices were used to analyze the structure of the fungal community in nut samples, and the results indicated low and uneven diversity. The findings also revealed the dominance and prevalence of the fungal species *Aspergillus niger* over the other identified species.

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تنوع مجتمعات الفطريات المرتبط بالمكسرات المجمعة من أسواق مختلفة في محافظة البصرة

سجى عكاب السعدون، مصطفى عبد الوهاب الدوسري
جامعة البصرة، كلية العلوم، قسم البيئة

المستخلص

تعتبر المكسرات غذاء صحي وغني بالدهون الصحية والبروتينات والفيتامينات وهي من الاغذية المستهلكة لفوائدها الصحية ولكن يمكن للمكسرات ان تتعرض للتلوث الميكروبي ومنها التلوث الفطري تهدف هذه الدراسة إلى تقييم تنوع الفطريات المرتبطة بثلاثة أنواع شائعة من المكسرات الجافة المعروضة في الأسواق المحلية في البصرة، وهي الفستق والكاجو والجوز. كشفت طرق العزل والتحديد عن 11 جنساً فطرياً، تضم 25 نوعاً، حيث كانت الفطريات الزقية في طورها اللاجنسي هي السائدة. وكان النوع *Aspergillus niger* هو النوع المهيمن والسائد بنسبة ظهور 100% في كل المكسرات المعقمة وغير معقمة سطحياً، استُخدمت الأدلة البيئية لتحليل تركيب المجتمع الفطري في عينات المكسرات حيث أظهرت النتائج أن قيم دليل Shannon–Wiener كانت منخفضة، إذ تراوحت بين 0.707–1.625 في كل من العينات المعقمة وغير المعقمة، ويُعزى ذلك إلى سيادة النوع *Aspergillus niger*. كما بينت النتائج وجود علاقة عكسية بين دليل التباين ودليل السيادة Berger–Parker حيث تراوحت قيمته بين 0.549–0.8235 في العينات المعقمة وغير المعقمة في المقابل سُجلت علاقة طردية بين دليل التباين ودليل Evenness إذ بلغت قيمه 0.3836–0.7058 في كلا نوعي العينات، والذي يُستخدم لتقييم مدى انتظام توزيع الأفراد بين الأنواع المختلفة. أما دليل Margalef الذي يعكس غنى الأنواع فقد تراوحت قيمه بين 0.948–3.538 في العينات المعقمة وغير المعقمة، وسجل الجوز أعلى انتشار للأنواع الفطرية مقارنة ببقية العينات وتشير هذه النتائج إلى أن المكسرات الجافة في أسواق البصرة قد تحتوي على أنواع فطرية قد تشكل خطراً على الصحة العامة وهو ما يستدعي تطبيق برامج فعّالة لمراقبة جودة وسلامة هذه الأغذية قبل وصولها إلى المستهلكين.

الكلمات المفتاحية: التنوع الفطريات، ادلة التنوع، المكسرات.