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Identification and Examination of Fungal species found in Fast Food samples collected from different restaurants in Erbil city, Iraq

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ABSTRACT

***Background*:** Foodborne illnesses remain a major global health issue, typically linked to bacteria, viruses, or parasites, though fungi also significantly contaminate and spoil food, posing serious hazards. Contamination can occur during processing, storage, or handling, especially with poor hygiene or temperature control. Some fungi produce mycotoxins, highly toxic compounds causing acute poisoning, immune suppression, cancer, and chronic diseases. Aspergillus flavus, an aflatoxin producer, is considered one of the most potent natural carcinogens known.

Subjective: To recognize and record the fungal species and assess which species are most frequently found in these establishments.

***Methodology*:** A total of 118 samples, including beef shawarma, chicken shawarma, falafel, potatoes, salad, and sauce, were obtained from five fast food restaurants between June and September 2025. The samples were cultured on PDA and SDA media for fungal isolation, and identification was performed through both macroscopic and microscopic examination using standard techniques.

***Results*:** Twelve fungal genera were identified, including *Absidia*, *Alternaria*, *Aspergillus*, *Bipolaris*, *Candida*, *Cunninghamella*, *Geotrichum*, *Penicillium*, *Rhizopus*, *Rhodotorula*, *Scytalidium*, and sterile mycelium. The recorded fungal counts were 285 in July, 121 in August, and 89 in September, with greater growth observed on PDA culture medium. *Aspergillus* was the most isolated genus.

***Conclusion*:** This study identified and mapped fungal species in fast food, creating a fungal calendar. The findings highlight major allergenic and pathogenic fungi, providing valuable information for consumers and supporting food safety assessments.

Keywords: Fungal food spoilage, Fast food restaurants, Fungal contamination

Introduction

Fungal contamination of food represents a significant worldwide concern, posing risks to both human and animal health. Molds and yeasts can spoil food and produce toxins that can trigger allergic reactions, disrupt the central nervous system, and harm internal organs[1].

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Mycology is a field of biology that deals with the study of fungi, encompassing their genetics, biochemical properties, practical applications, and possible hazards[2]. Fungi are found in almost every environment and hold significant ecological and medical importance. They contribute to the breakdown of organic materials, provide sources for antibiotics, and are used as food. Nevertheless, certain fungi are poisonous and capable of causing harm[3]. Mycology includes several specialized branches, one of which is medical mycology, the study of fungi responsible for diseases. Among the subfields, medical mycology is expanding the most rapidly[4].

The use of fungi in food production has grown significantly in recent years, though many fungal-based foods are still relatively new to Western cultures. These foods rely on fungi during their preparation, such as in bread making, where yeast causes the dough to rise, creating a light texture, or in blue cheese, where specific fungi develop its distinct flavor and appearance. In contrast, Asian cultures have a long tradition of consuming diverse fungal-based foods. As global culinary curiosity increases, several of these traditional Asian foods are gaining popularity in Western regions, reflecting a broader acceptance of fungal-based cuisine [5]. The ability of fungi to cause disease appears to be incidental, as fungal pathogenicity except in certain dermatophytes is not vital for their survival or transmission. Key physiological barriers to fungal growth in the human body include temperature and redox potential. Since most fungi are mesophilic, they cannot grow well at 37°C. Moreover, being saprophytic, their enzymes function more efficiently on non-living matter than within the reduced conditions of living tissues. In addition, the human body has strong cellular defense systems that effectively limit fungal proliferation [6]. The primary basis of fungal pathogenicity lies in the organism's ability to adapt to host tissues and withstand the host's cellular defenses. In most cases, human fungal infections depend more on the host's immune condition and environmental exposure than on fungal traits. A few fungi can infect healthy individuals because of their unique enzymatic activity, thermal dimorphism, and capacity to evade or suppress cell-mediated immunity. Most other fungi are opportunistic, infecting individuals with weakened immune systems and possessing low intrinsic virulence despite being common in the environment. Opportunistic infections such as candidiasis, cryptococcosis, aspergillosis, and zygomycosis have increased significantly. Additionally, newly identified infections include hyalohyphomycosis and phaeohyphomycosis[7]. Fungi exhibit several virulence factors that enhance their pathogenicity. They can attach to host cells via cell wall glycoproteins and form protective capsules that resist phagocytosis. Some species, such as *Candida albicans*, produce GM-CSF to inhibit complement production and acquire iron from red blood cells. They secrete various enzymes, including keratinase, elastase, and collagenase, which damage host tissues, and may also release mycotoxins. Additionally, their thermal dimorphism, specialized enzymatic activity, and ability to suppress cell-mediated immune defenses contribute to their survival within the host [8]. The human body is protected from fungal infections through multiple defense mechanisms. Physical barriers, such as skin and mucous membranes, provide the first line of defense, aided by fatty acids and specific pH levels on these surfaces and in body fluids. Continuous renewal of epithelial cells and the protective activity of normal microbial flora further prevent fungal colonization. Chemical defenses, including bodily secretions and serum components, add another layer of protection. Additionally, natural effector cells like polymorphonuclear leukocytes and professional phagocytes, including monocytes and macrophages, play a crucial role in controlling fungal growth [9]. Several factors increase the risk of fungal infections in humans. Prolonged use of antibiotics and immunosuppressive medications can disrupt normal defenses, while underlying conditions such as HIV, cancer, or diabetes further compromise immunity. Age-related vulnerabilities, obesity, and substance or drug abuse also contribute to susceptibility. Medical interventions, including surgery, radiation therapy, organ or tissue transplantation, and the use of indwelling catheters, can facilitate

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infection [10]. Food spoilage is any change in a food's appearance, odor, or flavor that makes it undesirable or unfit for consumption. Such deterioration not only discourages consumers but also causes economic losses for producers and distributors due to reduced quality, quantity, and higher costs. Spoilage may also involve a decline in nutritional value, such as the loss of essential vitamins, rendering the food nutritionally inadequate. The microorganisms responsible for food spoilage include bacteria, fungi (molds and yeasts), viruses, and parasites [11]. Fungal spores can cause several health problems in humans, such as respiratory diseases and mycotoxicosis. Fungi are also a major contributor to food spoilage and are among the primary sources of foodborne illnesses [12]. Microbial spoilage is a significant issue for perishable foods such as fresh fruits, vegetables, meats, poultry, fish, baked products, milk, and juices. Because meat and dairy items are rich in nutrients and contain readily available carbohydrates, fats, and proteins, they provide ideal conditions for fungal growth and subsequent spoilage [13]. A wide variety of fungi, such as *Rhizopus*, *Alternaria*, *Penicillium*, *Aspergillus*, and *Botrytis* species, are responsible for food spoilage. Since fungi can grow in acidic and low-moisture conditions, items like fruits and bread are more prone to fungal contamination than bacterial spoilage. For example, *Aspergillus flavus* infects peanuts and various grains, producing aflatoxin, a highly carcinogenic toxin regulated by the Food and Drug Administration [14]. Fungal growth in food is affected by numerous factors, as well as the interactions that occur between microorganisms, which influence the type of spoilage and associated health risks. For clarity, these factors can be divided into three main categories: intrinsic, extrinsic, and implicit factors [15]. Intrinsic factors are inherent to the food itself and include nutrients, growth factors, antimicrobial compounds, water activity, pH, and oxidation–reduction potential. Each of these intrinsic factors can impact fungal growth, often acting together to either promote or inhibit it [16].

Extrinsic factors refer to environmental conditions, such as temperature, relative humidity, and the composition of surrounding gases, which also play a significant role in microbial development. Implicit factors involve the interactions among microorganisms, including specific growth patterns, synergism, antagonism, commensalism, and competition [17]. These interactions can change the physical and chemical properties of food, contributing to spoilage. The combined effect of intrinsic, extrinsic, and implicit factors determines the rate and nature of fungal growth. Nutrient availability and water activity directly influence which microorganisms can thrive [18]. Environmental conditions, including storage temperature and humidity, further shape microbial communities. Microbial interactions, such as competition or synergism, can enhance or suppress growth of specific fungal species [19].

Methodology

Samples collection: A total of 118 sample from 6 different foods (Falafel, Chicken Shaorma, Beef Meat Shaorma, Salad, Potato, Sauce) were obtained from five restaurants, collection time (10:30 AM, 12:00 PM) during the period June 2025- September 2025. The food samples were taken from restaurant in sterile plastic bags according to Cheesbrough 1984 Direct plate used for culturing the Individual yeast and Mold species. The samples transferred with sterile forceps into Petri dish contain sterilized SDA Sabouraud's dextrose agar and PDA Potato Dextrose Agar and the plates were incubated at 25°C for 5- 7 days.

Fungi Identification: Fungal isolates were subcultured onto sterilized plates for purification and subsequent identification. The developed fungal colonies were mounted on slides, stained with lactophenol cotton blue to visualize fungal and yeast structures, covered with a coverslip, and examined microscopically. Identification was carried out based on colony morphology and spore characteristics. Both macroscopic and microscopic examinations were performed on the fungal cultures. The physical features of the mycelia, including their color and texture, were recorded along with their microscopic characteristics [20,21]. Two types of fungal culture media utilized Potato Dextrose Agar (PDA) and Sabouraud's Dextrose

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Agar (SDA). Both were prepared following the manufacturer's guidelines: 39 g of PDA powder dissolved in 1 liter of distilled water for the PDA medium, and 62 g of SDA powder dissolved in 1 liter of distilled water for the SDA medium. The media were then sterilized and used for fungal cultivation.

Germ Tube Procedure: Add three drops of serum into a small test tube, then use a Pasteur pipette to touch a yeast colony and gently mix it into the serum, keeping the pipette in the tube if desired. Incubate the mixture at 37°C for 2 to 4 hours, ensuring the time is not exceeded. After incubation, place a drop of the mixture on a microscope slide, cover it with a coverslip, and examine under the microscope using the 40x objective lens. This test is particularly valuable for identifying white or cream-colored fungal colonies, as a positive outcome strongly indicates the presence of *Candida albicans*. [21,17]. Germ tubes are about half the width of the yeast cell and extend three to four times the length of the originating cell, remaining directly attached without any constriction at the point of attachment. A positive result is indicated by the presence of short lateral projections, while a negative result shows only yeast cells [22,23].

Result

In this study, a total of 495 fungal isolates were obtained from five restaurants in Erbil, including one in the city center and four in surrounding areas. The results indicated that the fast food served in these restaurants contained a variety of fungal groups, with multiple species present in the sampled foods. Twelve genera comprising 15 fungal species were identified (Table 1). Among the isolates, *Aspergillus niger* was the most common, with 83 isolates, representing 16.7% frequency and 84% occurrence. *Absidia corymbifera* followed with 78 isolates, a 15.7% frequency, and 50% occurrence, while *Alternaria alternata* had 63 isolates, a 12.7% frequency, and 30% occurrence in the samples. *Rhodotorula* sp. and *Candida* sp. were also frequently found, with 51 and 43 isolates respectively, showing frequencies of 10.3% and 8.6% and occurrences of 60% and 80% (Table 1, Figures 1–5). Overall, *Aspergillus niger*, *Absidia corymbifera*, *Alternaria alternata*, *Rhodotorula* sp., and *Candida* sp. were the predominant species among the fungi isolated from the food samples.

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Table1: Fungal Species isolated during the study

No.	Fungal Species isolated during the study	Number of isolates	Occurrence %	Frequency %
1	*Absidia corymbifera*	78	50	15.7
2	*Alternaria alternata*	63	30	12.7
3	*Aspergillus flavus*	8	16	1.6
4	*Aspergillus fumigatus*	17	28	3.4
5	*Aspergillus niger*	83	84	16.7
6	*Bipolaris oryzae*	1	4	0.2
7	*Candida albicans*	31	52	6.2
8	*Candida* sp.	43	80	8.6
9	*Cunninghamella sp.*	41	76	8.2
10	*Geotrichum candidum*	22	32	4.4
11	*Penicillium* sp.	29	35	5.8
12	*Rhizopus stolonifer*	3	4	0.6
13	*Rhodotorula* sp.	51	60	10.3
14	*Scytalidium hyalinum*	2	8	0.4
15	*Sterile mycelium*	23	31	4.6
	Total	495		

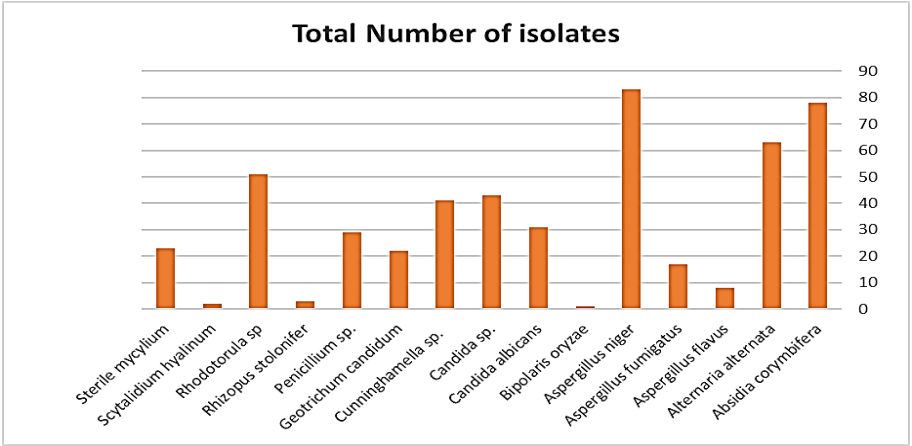


Figure 1. Total Number of Isolates of fast-food Fungal Species at Erbil City

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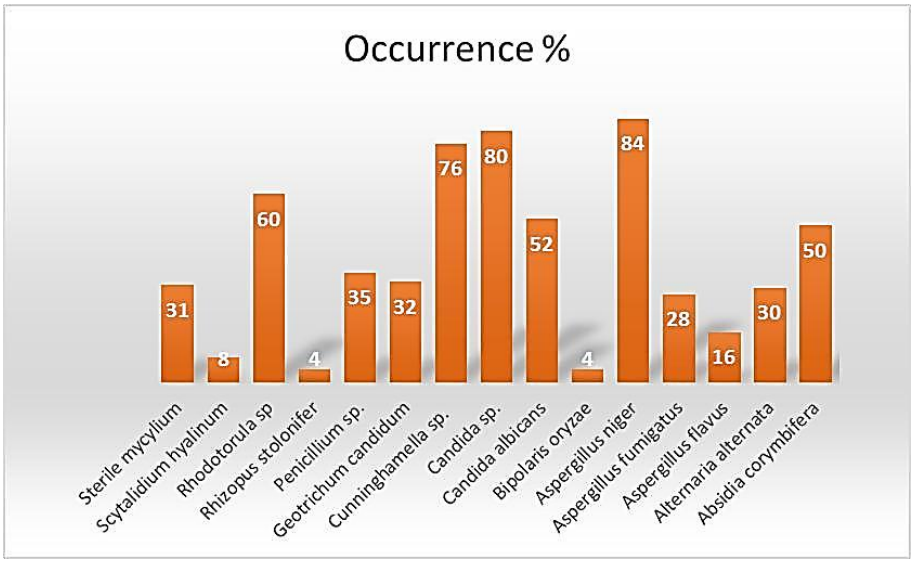


Figure 2. Fungal Species Occurrence which isolated during the study

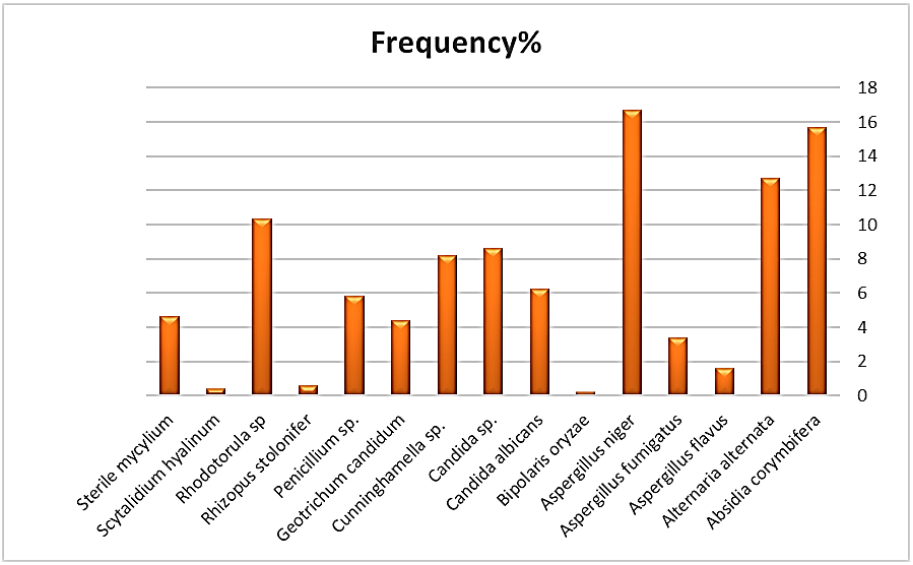


Figure 3. Fungal Species Frequency which isolated during the study

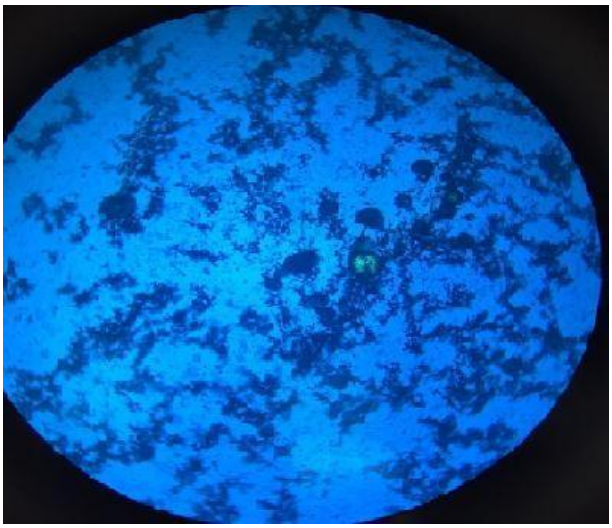
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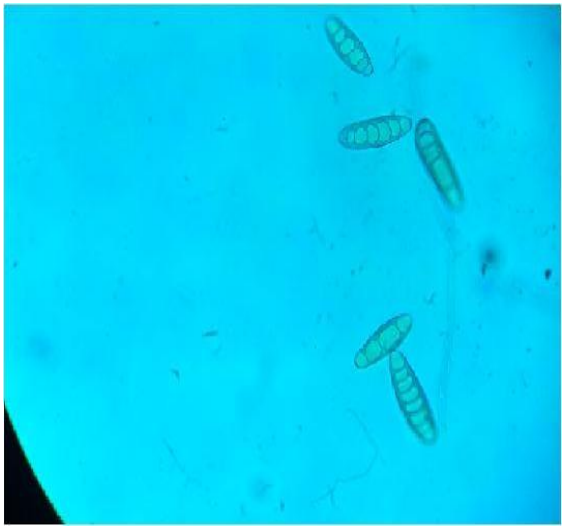
Figure 4. Selected restaurants were included in the study, and samples were examined macroscopically and microscopically

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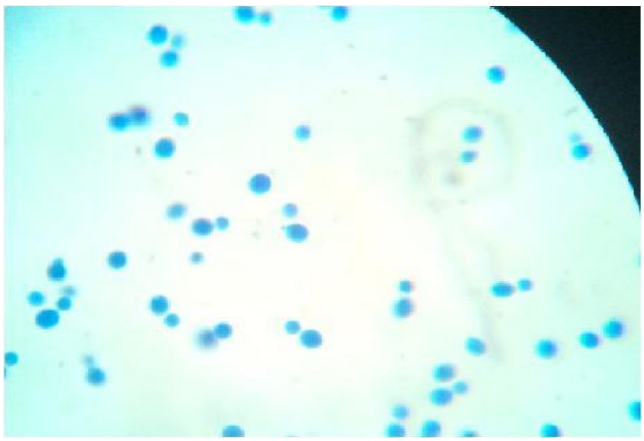
Microscopic Slides



Aspergillus niger



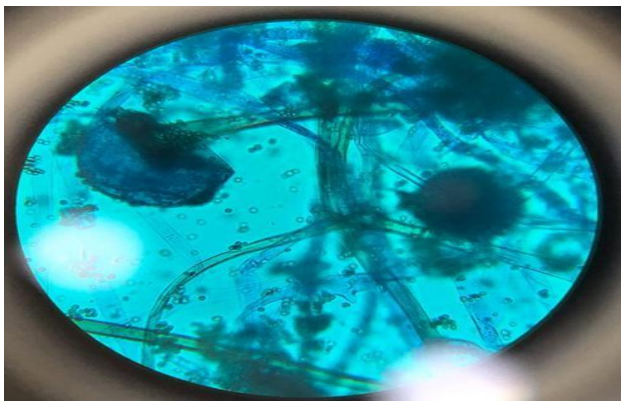
Bipolaris oryzae



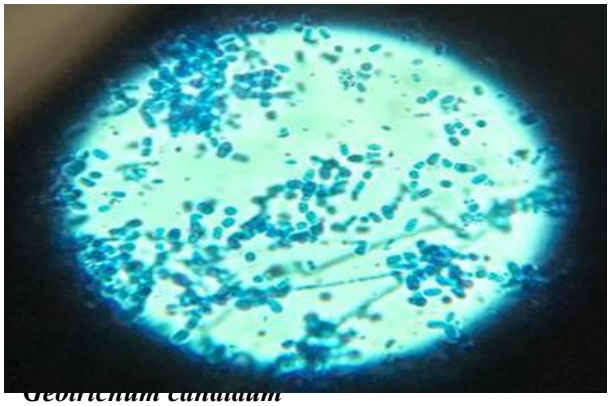
Candida albicans



Candida sp.



***Cunninghamella* sp.**



Geotrichum candidum

Figure 5.

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Discussion

Evaluating food quality is essential because it directly affects human health, the food industry, and the prevalence of certain diseases. In recent years, there has been increasing research attention on fungi in fast food due to their potential health risks [11, 24, 25]. This study surveyed culturable fungi from fast food samples collected from five restaurants in Erbil, Iraq, and found that most of the isolated fungal species are cosmopolitan and widely distributed globally. Fungal isolation from fast food samples obtained from five different restaurants led to the identification of fifteen species across twelve genera (Table 1), aligning with the results reported by Easa, S. M. H. (2011) [15]. The restaurants included in this study were selected due to the higher risk of exposure for people in these areas to pathogenic fungi and yeasts, which can be transmitted through contaminated food, soil, the hands of workers, or direct person-to-person contact.

The total fungal colonies (isolates) observed were 285 in July, 121 in August, and 89 in September. This fluctuation is likely due to higher temperatures in July compared to the following months. Increased temperatures, combined with inadequate food handling and storage, promote the growth of fungal colonies, especially in July. Species from the genera *Alternaria*, *Aspergillus*, *Candida*, and *Penicillium* have also been frequently reported in other environments, including outdoor settings, and have been documented in various locations throughout the Middle East [20, 26].

The results of this study are aligned with previous research on fungal growth across different media. More fungal colonies were observed on Potato Dextrose Agar (PDA) than on Sabouraud's Dextrose Agar (SDA), even though both media contained antibiotics to suppress bacterial growth. This difference may be because PDA serves as a general-purpose medium for cultivating molds and yeasts, whereas SDA was initially designed for isolating fungi related to skin infections, making PDA somewhat more suitable for mold growth [27].

Likewise, this study demonstrated that these fungi are prevalent in fast food samples collected from various restaurants in Erbil city. The most dominant species among the isolates were *Aspergillus niger*, *Absidia corymbifera*, *Alternaria alternata*, *Rhodotorula* sp., *Cunninghamella* sp., and *Candida* sp. These results are consistent with a previous study conducted in Samawah Governorate, Iraq, which reported similar fungal species in fast food restaurants [21].

Earlier studies on food-associated fungi have reported a significant occurrence of imperfect fungi, which is consistent with the results of the present study. Many of the isolated fungi with potential pathogenicity to humans also belong to this group, including the most prevalent species identified in this research: *Alternaria alternata*, *Aspergillus niger*, and *Aspergillus fumigatus* [22].

Some findings of this study align with those reported by Paterson & Lima (2017) [28] and Ramadan & Mohammed (2022) [20]. However, our research revealed a greater variety of fungal species, including *Alternaria alternata*, *Aspergillus niger*, *Candida* sp., *Penicillium* sp., *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Scytalidium hyalinum*, *Bipolaris oryzae*, and *Aspergillus flavus*. These fungi were isolated from food samples collected from restaurants in Erbil city and have been previously linked to allergic reactions. The identified species are xerophilic, xerotolerant, and psychrophilic, and they possess the ability to produce various enzymes [29], which are likely to explain their widespread presence across almost all tested food samples.

Alternaria alternata, isolated from food prepared in fast food restaurants, is considered toxic. This saprophytic hyphomycete produces harmful metabolites, including tenuazonic acid. It is found worldwide and is commonly isolated from plants, food products, soil, and textiles. The fungus has been linked to

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hypersensitivity pneumonitis (often called woodworkers' lungs) and type I immediate hypersensitivity reactions (IgE-mediated), such as extrinsic asthma, which is especially prevalent among individuals with atopic conditions [25].

In this study, *Aspergillus* species were detected more frequently than other fungi, aligning with previous research indicating that *Aspergillus* is the most isolated genus responsible for food spoilage in samples from various fast-food items [15, 28].

This study is notable for documenting culturable fungi isolated from fast food restaurants in Erbil city, a region with limited previous research on this topic. The observed fungal growth may be linked to multiple factors, including workers not wearing proper fast-food uniforms, insufficient cleaning of equipment and surrounding areas, and food preparation in open spaces near sidewalks and traffic. Other contributing factors may include using the same cutting board for both vegetables and meats, covering broken knife handles with plastic, and using expired condiments such as sauces, mayonnaise, amba, and ketchup.

Conclusions

This study was carried out in five fast food restaurants in Erbil City and successfully identified the concentration and distribution of various fungal species in food samples. The collected data enabled the development of a fungal species calendar, providing useful information on major allergenic and pathogenic fungi for individuals who frequently visit these establishments. A total of 495 fungal isolates were obtained, with the highest number recorded in July (285 Iso.), followed by August (121 Iso.), and the lowest in September (89 Iso.). The most identified fungi included *Aspergillus niger*, *Absidia corymbifera*, *Alternaria alternata*, *Rhodotorula* sp., *Candida* sp., *Cunninghamella* sp., *Candida albicans*, and *Penicillium* sp. The findings suggest a possible association between the fungal species detected in fast food and recurrent cases of foodborne illness among frequent consumers.

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