

Detection of Fungi Associated with Some Spices in Local Market in Hilla City (Babylon)

Nawras Abid Al-abas¹, Abeer Fauzi Al-Rubaye², Hawraa Wahab Aziz²

¹MSc. Student, ²Assist. Prof., Department of Biology, College of Science for women, University of Babylon, Iraq

Abstract

The present study included the isolation and diagnosis of contaminated fungi of some spices sold in the local markets in Hilla city in Babylon Governorate (Iraq) and includes, Black pepper, White pepper, turmeric, mixed spices for meet, Pizza Spices, Beef Shawarma, Pickled Spices, Rosemary ground and others. Results were recorded 346 fungus isolates belonging to 9 different fungal group. The most predominant fungal genera encountered were *Aspergillus spp* (40.17%), *Penicillium spp* (25.14%), *Cladosporium spp* (8.67%) *Alternaria spp*, and *Fusarium spp*. Yeasts were also frequently recovered, but not identified. All fungi were isolated on P.D.A. The study focus on *aspergillus spp* diagnosis by using molecular method to diagnosed it by using sequence techniques. The highest appearance of *Aspergillus flavus* (41.7%) was recorded followed by *Aspergillus niger* (25.9%), *Aspergillus parasiticus* (12.9%).

Keywords: Spice, fungi, identification, P.D.A, Iraq.

Introduction

Spices were described as a natural compound or a combination of natural compounds obtained from seeds, fruits, flowers or trunks (skins, roots, leaves) of several crops of native or exotic origin, aromatic or with a powerful flavor, used in minute amounts and added to food preparing and processing worldwide to provide color, taste, smell or flavor^(3,11,16). Spices are a prominent component of traditional cooking activities and are essential component of millions of people's daily diets around the globe and the majority come from tropical countries and is used to flavoring, coloring or preserving food and beverages¹⁶.

Spices such as pepper, paprika, cumin, ginger, saffron and clove are widely used in the Middle East because they add excellent flavor to products and are

used as medicines because of their preservative and antioxidant properties are extremely important, They are mainly aromatic agents used in tiny quantities if correctly stored and have positive effects as well as antimicrobial characteristics^(4,5). High temperature, moisture and precipitation rates are prevalent in tropical climate. These climate parameters are appropriate for intense microbial development, particularly with regard to fungi. Spices are contaminated in large quantities upon import. Most spices are traded commercially It is in an equipped form, and the drying process is the most important process is used, spices are likely to be exposed to a broad spectrum of microbial contaminants Contamination is likely to happen during the pre- and post-harvest phases during storage, delivery and sale [and/or use]^(23,19). Although spices are present in tiny quantities in foods, they are acknowledged as significant carriers of microbial contamination primarily due to the circumstances under which they were cultivated, harvested and processed. Furthermore, due to possible negligence during sanitation or processing, foods containing spices are more likely to deteriorate and could also have damaging impacts with regard to health¹⁹.

It was observed that most spices contain large

Corresponding Author:

Nawras Abid Al-abas

MSc. Student, Department of Biology, College of Science for Women, University of Babylon, Iraq

e-mail: abeerbio15@gmail.com

numbers of molds and bacteria and small numbers of colon bacteria and yeasts. Although spices are present in foods in relatively small amounts. However, they are recognized as important carriers of microbial contaminants mainly due to different stages, The spice passes from planting and harvesting to processing, as well as due to possible neglect during discharge [Or sewage treatment]¹⁹ Food is contaminated with fungi and mycotoxins. and this very serious problems that threaten many developing countries, Especially those that lack the conditions of good food storage and are a source of great concern, which called on these countries to provide healthy food sources to achieve food security²¹. The Food and Agriculture Organization of the United Nations estimates that nearly 32% of the world's food and feed are at risk of contamination with mycotoxins²⁹.

Mycotoxins have significant public health effects. It causes kidney poisoning, immunosuppression, fetal teratogenicity and congenital malformations. These toxins are capable of causing severe acute effects and chronic in human and animal ranging from disorder of the central nervous system, heart, blood vessels, pulmonary systems and death^(9,15). Among the most important toxins are aflatoxins, which are secondary carcinogenic metabolites produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*⁽²⁸⁾ Exposure to aflatoxins is known to produce various diseases like carcinogenic, mutagenic, teratogenic, tremorigenic and hemorrhagic. It also cause damage in the central nervous system, liver or kidneys and effect of male reproductive toxic effects².

Material and Method

Sample Collection: A total of twenty two dried ground samples (Black pepper, White pepper, turmeric, cumin, mixed spices for chicken, mixed spices for meat, Pizza Spices, Rosemary ground, Pickled Spices, Biryani spices, Ginger Spices, cloves, dried Mint, dried Lime, saffron ground, Fenugreek, grill spices, cinnamon ground, Maggi beef, Maggi chicken, Maggi vegetables, Noodles spices) were bought from various locations of local markets Hilla city, depicting distinct kinds of spices. These spice products were selected based on their market accessibility and popular use. Spice samples were generally discovered outside, stored in or on the bare floor in metal or plastic containers, wooden boxes or gunny bags. For each spice 3 replicates were taken from three different places. Each sample (100 g) was put in a new paper bag and transferred immediately to the laboratory and stored in cool place at 4°C for fungal

determination.

Culture of fungal isolate on Potato dextrose agar PDA: Prepare the food medium according to the manufacturer's instructions to dissolve 43 g of the medium in 1 liter of distilled water, then sterilize the sterilizer (autoclave) at 121° C for 15 minutes, then cool the medium and add the antibiotic chloramphenicol at a concentration of 1.2 mg. The fungal samples were cultured on potato dextrose agar (PDA) with three replications for each medium followed by incubated at 25 C° for 7 days, after this period the fungi were investigated²⁶

Identification of the fungal genera:

The fungal isolates were transferred to sterilized plates for purification and identification. Identification of different fungi was done with help of slides prepared by direct mount from the culture. The examined under microscope and identified on the basis of their colony morphology and spore characteristics^(12,27)

Recorded of Results: After incubation and identification percentages of isolated fungi, infection (contamination) in each sample were calculated according to the formula

$$\text{Percentage of appearance} = \frac{\text{Number of isolate that appeared in the same type}}{\text{Total number of samples}} \times 100$$

$$\text{Percentage of frequency} = \frac{\text{Number of isolates per species}}{\text{Total number of isolates of all species}} \times 100$$

Results and Discussion

Three hundred forty six isolates represent 22 species. The results of this study noticed that all samples of spices were infected with fungi Table (1), the fungi belong to 9 fungal genus, the *Aspergillus spp* was (40.17%) followed by *Penicillium spp.* (25.14%) and the *Cladosporium spp.* (8.67%). The results of this study agree with²⁴ were isolated and identified 17 genera. *Aspergillus spp*, *Penicillium spp* and *Rhizopus spp* were the most common genera, *Alternaria* emerged in 40% of samples and *Eurotium spp*, *Fennellia spp* and *Fusarium spp* were detected as moderate contaminating agent where, they were isolated from 26.7% to 33.3% of samples. As well as the results of the present study indicated to occurrence of yeast in some of spice samples but not identified and this result corresponds to what is reached by³¹ When he studied contaminated

fungi for some dried and ground spice samples which indicated *Aspergillus spp*, *Penicillium spp*, *Rhizopus spp*, *Cladosporium spp* and *Trichoderma spp* were the most predominant fungal genera encountered were Yeasts were also frequently recovered. When he studied contaminated fungi for some Relative occurrence values of taxa disclosed ranged between 36.4% for *A. flavus* and 0.6% for *A. parasiticus* and *Absidia spp*.¹⁴ identified fungi in spices like *Acremonium spp*, *Alternaria spp*, *Aspergillus aculeatus*, *A. flavus*, *A. niger*, *A. fumigatus*, *A. terreus*, *Cheatomium spp*, *Choanephora spp*, *Cephalosporium spp*, *Drechslera spp*, *Colletotrichum spp*, *Curvularia spp*, *Fusarium spp*, *Penicillium spp*, *Phoma spp*, *Stemyphylium spp* and *Stachybotrys spp*.¹⁷.¹⁰ show that the predominant mycoflora obtained was distributed in 10 genera. The genus *Aspergillus spp* was the most dominant genus recovered (179 isolates) followed by *Penicillium spp* (44 isolates).

The prevalence of fungi associated with some spices sample appeared in table (2) *Aspergillus spp* was (70.20%) followed by *Penicillium spp* (43.94%) and *Cladosporium spp* (15.15%). In study done by³¹ exhibit the genus *Aspergillus spp* is the most predominant genus encountered, with four species, followed by *Penicillium spp* with two species and all the remaining fungi with one species and the highest percentage relative density was shown by *A. flavus* (36.39), followed by *A. niger* (20.60) and *R. stolonifer* (14.58). The lowest density of occurrence was indicated by *A. parasiticus* and *Absidia corymbifera* (0.60).

²⁴ Record in their research that the *Aspergillus spp* was represented by 10 species and *Penicillium spp* was represented by 7 species, however, *Rhizopus spp* was represented by only 1 species. *A. niger* polluted 93.3% of spices samples and *A. flavus* polluted 60% of them, were the common *Penicillium spp* where, they isolated from 53.3% and 46.7%. of the samples, respectively and *R. stolonifer* contaminated 66.7% of spices. *Aspergillus spp*. and *Penicillium* genera were more frequently detected than other genera of fungi. *Aspergillus niger*. was found in all examined spices samples except Black pill, Caraway, sesame, Black pepper and Cumin while, *Penicillium spp*. were dominant in all samples except Coriander, Ginger, and Caraway¹⁴.

Table (3) showed that the dominant fungal species (percentages of appearance) were *Aspergillus spp* *Penicillium spp*, appeared in all samples of spices such as pepper, paprika, cumin, ginger, saffron and clove

Cladosporium spp, Alternaria spp, Fusarium spp, Mucor spp Rhizopus:¹⁸ isolated 81 species belonging to 38 genera were from different 34 spices in Egypt Where was the *Aspergillus spp* the most prevalent (25) species. This difference in the numbers of isolated species and fungi may be due To the different numbers and types of spices studied in addition to that spices are likely to be vulnerable to the group a wide range of microbial contaminants during the pre- and post-harvest phase, as well as contamination is likely to occur during [Operations of storage, distribution, sale and/or use.⁷ found the *Aspergillus* and *Penicillium spp*. the main components of cardamom, cinnamon, fennel, coriander, cumin, black cumin and white pepper.

The present study recorded the highest appearance of *A. flavus* and *A. niger* in all kinds of spices, And other species of *Aspergillus* appeared in different proportions *A. minisclerotigenes* *A. parvisclerotigenus* *A. oryzae* *A. parasiticus* (table 4) and fig (2).

²² recorded that the most species isolated from different spices were *Aspergillus ruber*, *Aspergillus chevalieri*, *Aspergillus montevidensis*, *Aspergillus pseudoglaucus* and *Aspergillus penicillioides* were the most frequent *Aspergillus* as well, the mycotoxigenic species were *Aspergillus flavus* and *Aspergillus niger*. In other study, *Aspergillus alutaceus*, *Aspergillus fumigatus*, *Chaetomium globosum*, *A. montevidensis*, *A. chevalieri* and *P. chrysogenum* were isolated^{1, 25} isolated 25 species of *Aspergillus* on anise and fennel fruits, of which *A. niger*, *A. flavus*, *A. ochraceus* and *A. flavus var. columnaris* were prevalent in both spices

Table (1): The number and frequency of fungal species isolated from spices

No.	Fungi	Percentage of Frequency	
1	<i>Aspergillus spp</i>	139	40.17
2	<i>Penicillium spp.</i>	87	25.14
3	<i>Cladosporium spp.</i>	30	8.67
4	<i>Alternaria spp</i>	26	7.51
5	<i>Fusarium spp</i>	7	2.02
6	<i>Mucor spp</i>	8	2.31
7	<i>Rhizopus spp.</i>	6	1.73
8	White sterile fungi	16	4.62
9	Yeasts	27	7.80
Total		346	

Table (2): The percentages of fungi isolates appearance associated with spices

No.	Fungi	Percentage of prevalence	
1	Aspergillus spp	139	70.20
2	Penicillium spp.	87	43.94
3	Cladosporium spp.	30	15.15
4	Alternaria spp	26	13.13
5	Fusarium spp	7	3.53
6	Mucor spp	8	4.04
7	Rhizopus spp.	6	3.03
8	White sterile fungi	16	8.08
9	Yeasts	27	13.63

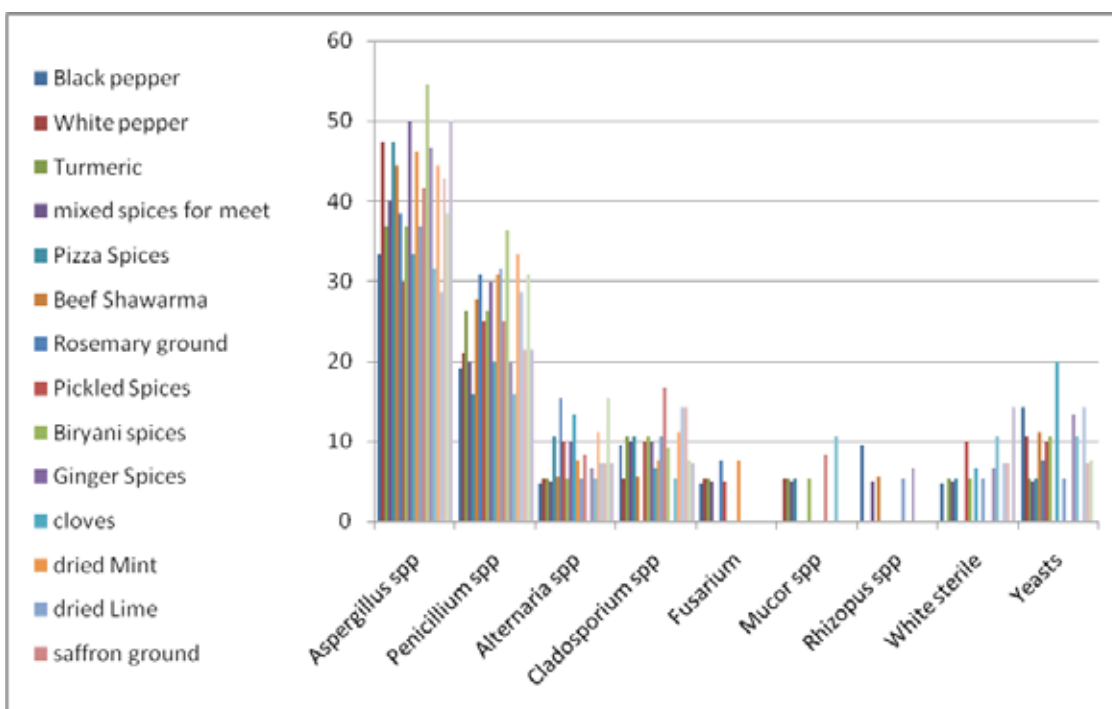


Fig (1): The percentages of appearance of fungi isolated from each samples of spices

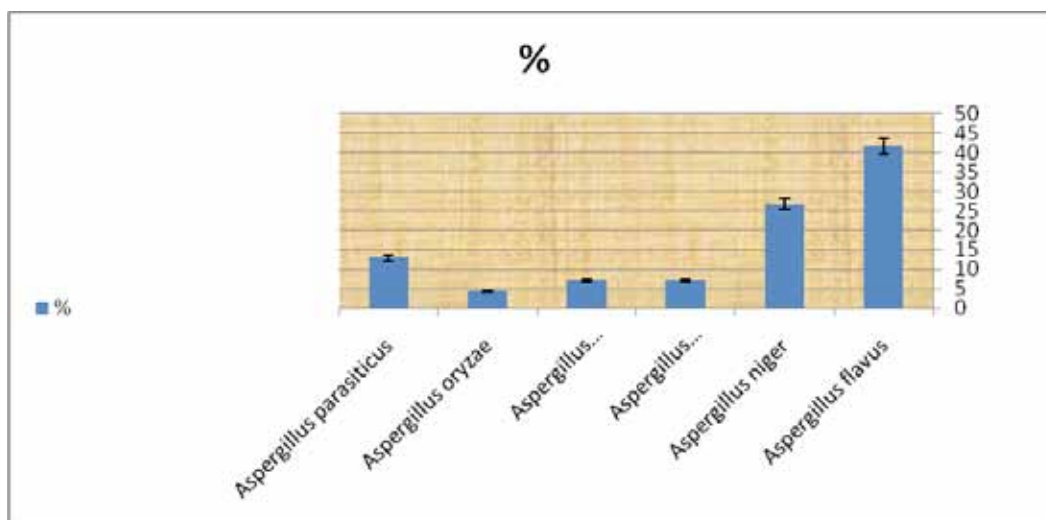


Fig (2): The percentages of appearance Aspergillus spp isolated from each samples of spices

Table (3): The percentages of appearance of Aspergillus spp isolated from each samples of spices

No.	Samples	A.flavus	A. niger	A.minisclerotigenes	A.parvisclerotigenus	A.oryzae	A.parasiticus
1	Black pepper	42.86%	0	14.29%	14.29%	0	28.57%
2	White pepper	33.33%	22.22%	11.11	11.11%	0	22.22%
3	Turmeric	42.86%	28.57%	0	0	14.29%	14.29%
4	mixed spices for meet	37.5%	25%	12.5	12.5%	0	12.5%
5	Pizza Spices	44.44%	22.22%	11.11	11.11%	11.11%	0
6	Beef Shawarma	37.50%	25%	12.50	12.50%	0	12.50%
7	Rosemary ground	40%	20%	0	0	20%	20%
8	Pickled Spices	50%	33.33%	0	0	0	16.66%
9	Biryani spices	42.85%	28.57%	0	0	14.28%	14.28%
10	Ginger Spices	40%	20%	20%	20%	0	0
11	Cloves	60	20	0	0	0	20%
12	dried Mint	33.33%	33.33%	0	0	16.66%	16.66%
13	dried Lime	42.86%	28.57%	14.29%	14.29%	0	0
14	saffron ground	40%	40%	0	0	0	20%
15	Fenugreek	33.33%	33.33%	0	0	0	33.33%
16	grill spices for meet	42.85%	28.57%	14.28%	14.28%	0	0
17	Cumin	33.33%	33.33%			16.66	16.66%
18	cinnamon ground	50%	50%	0	0	0	0
19	Maggi beef	75%	0	0	0	0	25%
20	Maggi chicken	16.66%	33.33%	16.66%	16.66%	0	16.66%
21	Maggi vegetables	60%	40%	0	0	0	0
22	Noodles spices	42.85%	14.28%	14.28%	14.28%	0	14.28%
Total (139)		58	36	10	10	6	18
%		41.7%	25.9%	7.2%	7.2%	4.3%	12.9%

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Biology, Iraq and all experiments were carried out in accordance with approved guidelines.

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