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Isolation and diagnosis of the fungi associated with maize seeds collected from local markets in Karbala, Iraq

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ABSTRACT
Results of isolating the fungi accompanying the maize grains stored in some warehouses, local markets and mills in Karbala Governorate, Iraq, showed that there are 11 genus of fungi including *Aspergillus* spp. (27.83%), *Penicillium* spp. (22.26%), *Fusarium* spp. (14.01%) *Mucor* spp. (5.76%), *Alternaria* spp. (4.80%), *Acremonium* spp. (2.69%) and yeast (6.14%). Seventy-three isolates of genus *Fusarium* were isolated from yellow corn grain samples stored in the traditional way, based on the phenotypic and microscopic characteristics of *Fusarium* spp. colonies and their reproductive structures, as well as by molecular methods. Seven species of fungi were recorded *F. verticillioides* (26.03%), *F. proliferatum* (20.55%), (16.44%) *F. equiseti*, F. oxysporium (13.7%), *F. sulawenes* (11%) and *F. thapsinum* (6.85%).

Keywords: Maize, Fungi, Zea mays, Fusarium, Market.
Article type: Research Article.

INTRODUCTION
Yellow corn, *Zea mays* is one of agriculture belonging to the Poaceae family. It is one of the important cereal crops in Iraq and the world. Its importance comes through its multiple uses as it enters into human food directly or indirectly, through its use as a main ingredient in the animal diet. In addition to various other industrial purposes, it is also the third largest crop in the world, which makes it attract the attention of many researchers. Its importance is also due to its high production capacity and its adaptation to different environmental conditions and the possibility of growing it for more than one season per year (Farnham *et al*. 2003; Shevchenko *et al.* 2021). Yellow corn is distinguished by containing 4% oil and 82% carbohydrates, so it is used as food for millions of people in the world. Since about 80% of calories or more, which people obtain at the global level, come from field crops, particularly grains, among which is the corn crop (Yongfeng & Jay-lin 2016). Animal feed components, including cereals and others containing them, are susceptible to infection by fungi, in addition to their production of fungal toxins. Usually, if the appropriate environmental conditions are available for the fungi production, they cause contamination of cereal crops, diets and food that leads to material losses and high costs every year. So that, the annual losses of the United States and Canada have amounted to billions USD as a result of contamination of agricultural crops and food with mycotoxins, in addition to annual losses of millions of dollars in the global poultry sector as a result of contamination of relationships with mycotoxins (Devegowda *et al*. 2005; Haider & Hussein 2022). The genus *Fusarium* is an economically important fungi including many pathogenic species to humans, plants and animals, as it is spread in various regions of the world, especially in the field of soy or in vegetable residue (Rheeder *et al*. 1990).
One of the most important genera responsible for a large number of plant diseases are *Fusarium* species. In several countries around the world, the occurrence of such fungi infects grains represents an issue (Hussain *et al*. 2018). Toxic fungal growth can affect grain quality and produce mycotoxins that should be monitored and tested during

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grain storage (Zhai *et al*. 2015; Sahi *et al.* 2022). Certain *Fusarium* species were found to be human food and feed pathogens (Placinta *et al*. 1999). Therefore, numerous *Fusarium* species are considered worldwide to be the most dangerous fungi (Bottalico & Perrone 2002).

MATERIALS AND METHODS

Sample Collection

A total of two hundred sixteen (216) yellow corn seed samples were collected from different area in Karbala Province, Iraq. The samples were bought from various locations of warehouse, mill and local markets. For each location, 3 replicates were taken from three different places. Each sample (100 g) was placed in a new paper bag, transferred immediately to the laboratory and stored in cool place at 4°C for fungal determination

Isolation and diagnosis of fungi with yellow corn grains

To isolate the *Fusarium* fungus, 100 corn seeds were taken randomly from each of the collected samples. It was sterilized using 2% sodium chlorate for 2 min and then washed with sterile distilled water twice to remove traces of sterile material and dried with sterile filter paper. It was transferred with sterile forceps to 9-cm petri dishes containing 20 mL pre-prepared PDA (with chloramphenicol; 50 mg L^{-1}) to prevent bacterial growth (Hoching & Pitt 1997). Isolated fungi were then diagnosed based on the taxonomic keys of both Nelson *et al*. (1983) and Seifert (1996). After incubation and identification, the rate (%) of frequency and appearance of isolated fungi were calculated according to the following equations:

$$Appearance\ (\%) = \frac{Number\ of\ isolate\ that\ appeared\ in\ the\ same\ type}{Total\ number\ of\ samples} \times 100$$

$$Frequency\ (\%) = \frac{Number\ of\ isolates\ per\ species}{Total\ number\ of\ isolates\ of\ all\ species} \times 100$$

Morphological identification of *Fusarium* fungi

The isolated fungi were identified according to colony morphology and microscopic examination. Fungal colonies were transferred on to PDA slants for species identification and were identified in the Plant Pathology Department, National Research Centre, Iraq according to Barnett & Hunter (1972) and Leslie & Summerell (2006).

RESULTS AND DISSCUTION

Isolation and diagnosis of fungi associated with yellow corn seeds

Five hundred twenty one (521) Fungal isolates were identified depending on cultural and morphological features isolated from two handed sixteen (216) yellow corn seed samples (table 1). Eleven (11) genus of fungi were isolated *Fusarium* spp. (14.01 %), *Aspergillus* spp. (27.83%), *Penicillium* spp. (22.26%), *Mucor* spp. (5.76%), *Alternaria* spp. (4.80%), *Acremonium* spp. (2.69%) and yeasts (6.14%; Table 1). The results in Fig. 4 depicts the fungal rate (%) of appearance associated with those from different sample collection sites (stores, mills and local markets), including *Fusarium* spp. (33.80%), *Aspergillus* spp. (67.13%), *Penicillium* spp. (53.70%), white sterile fungi (23%) and yeasts appeared in a percentage reached to 14.81%. The results showed that the fungus *Aspergillus* spp. came first as a contamination of corn grains, followed by the *Penicillium* spp. and *Fusarium* spp. came at the third rank (Table 1 and Fig. 1).

These results coincide with many studies in terms of the fact that the fungal species that are included in this study are in the typical stores, monitored in different countries of the world (Proctor *et al*. 1995). Abeer (2016) diagnosed the fungi accompanying the maize seed taken from regions famous for cultivating the maize crop in Babylon Governorate, Iraq and the species were reproductive to different genera of *Alternata. Aspergillus flavus, A niger, A. oryzae, A. prasiticus, A. terreus, Fusarium graminearum. F. solani, F. verticillioides, Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizoctonia solani, Rhizopus* sp. and *Trichoderma harzianum*. The aforementioned author reported that *Fusarium* infection rate was 45%. Intessar (2019) detected *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Alternaria* spp., *Rhizopus* spp., *Rhizoctonia* spp. and *Mucor* spp. were the fungi associated with corn seeds in Babylon Provence, Iraq. The *Fusarium* spp. recorded the highest occurrence of 56.98% fallowed by *Aspergillus* spp. (27.85%). Theodora *et al*. (2018) reported the percentage of occurrence of filamentous fungi in maize destined for human consumption in South Africa. The incidence of different genera isolated in their study revealed the predominance of *Fusarium* (82%), *Penicillium* (63%), and *Aspergillus* species (33%) compared to other genera. In a study performed by Abdel- Sater *et al*. (2017) a total of 19 species belonging to 8 genera were collected from maize grains. *Aspergillus*, *Eurotium*, *Fusarium* and *Rhizopus* were the most common genera, of those, *A. flavus, A. niger, E. rubrum, E. repens, Rhizopus stolonifer* and *F. verticillioides* were the most encountered. *Aspergillus* and *Penicillium* spp., however, usually co-infected with *Fusarium* spp. (Bush *et al*. 2004). El-Shanshoury *et al*. (2014) found that *A. flavus, A. niger, Penicillium* spp. and *Fusarium* spp. were the most common fungi in samples of cereal grains (maize and wheat) and peanut collected from Central Delta Province, Egypt. A similar trend was reported for stored sorghum grains in Kenya (Kange *et al*. 2015). In Nigeria, Abdulsalaam *and* Shenge (2011) recorded *Aspergillus, Fusarium, Rhizoctonia* and *Curvularia* species as the most common in washed sorghum grains. In a study conducted by Ismail *et al*. (2012), *Aspergillus* and *Eurotium* were isolated in high incidences from cereal baby foods locally produced in Uganda.

Penicillium spp is one of the important fungi and accompaniment to the maize grains and leads to damage to the crop and its poor quality in addition to its release of many toxic metabolic compounds (Agarwal & Sinclair 1997). *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. remaine linked to yellow corn grains and what helps their growth and development is the availability of appropriate conditions like availability of moisture, storage temperature and the rate of exposure to insects before and during storage (Elham *et al*. 2015; Moshiur *et al*. 2016). Similar observations were reported by Mohammed *et al*. (2015); The most common genera isolated were *Aspergillus, Penicillium* and *Fusarium*. Among the *Aspergillus* spp*., A. flavus, A. parasiticus, A. niger* and *A. ochraceus* were identified. Binyam & Girma (2016) recorded highest frequency of *Aspergillus* spp. (40.4%) at farms preserved *Zea* seed. In the case of Ethiopia, in various grains, *Aspergillus* and *Fusarium* were frequently isolated from stored maize seeds in addition to *Penicillium* spp. Omaima *et al*. (2018) examined the fungi associated with corn, rice, barley and wheat, finding different genus of fungi including *Aspergillus*, *Alternaria, Fusarium, Penicillium* and *Rhizopus*. The rate (%) of *Fusarium* spp. included: wheat (11.81%), white corn (11.81%), feed corn (10.31%), yellow corn (3.15%), barley (17.91%) and rice (20.00%). The variations in the proportions of the presence of these fungi can be explained by the difference in the physiological maturity of the cultivated corn varieties, the degree of infection with the diseases and the insects, the timing of the harvest and post-harvest conditions such as drying, sorting, transporting, providing the appropriate conditions during storage and cleanliness of stores in each governorate, since most of these fungi are spread by air. It is found in many places that natural environment affects yellow corn when appropriate environmental conditions are available, especially humidity and temperature.

The *Fusarium* spp. affects corn grains during the growth of the crop in the field and can continue its activity in the post-harvest and storage stages (El-Shabrawi 2007; EL-Sheikh *et al*. 2009; Sally *et al*. 2015). Moisture content of the grain is a critical factor for fungal growth on the grain, leading to quality loss. Moisture contents of sorghum grain samples were much higher in sorghum than in maize. Moisture content is one of several factors known to influence fungal development and secondary metabolite production in agricultural products. It was reported that, the fungi require moisture content ranging between 13 and 18% to invade cereal starchy grains (Moubasher *et al*. 1972). In most regions of the world, *Fusarium* spp. especially the two types (*F. verticilloides* and *F. proliferatum*) are natural contaminants for corn seeds and their products (Bailly *et al*. 2005). Some studies showed that there are other types of fungi accompanying the maize grains, however, their presence was in small proportions, in agreement with many authors (Munkvold *et al*. 1997b; Niaz & Dawar 2009). Noteworthy, the contamination of corn grains with *Aspergillus* spp., *Penicillium* spp. *Fusarium* spp. and *Cladosporium* spp. were high in varying proportions between the three sites (Warehouse, Mill and Local markets. So that, *Fusarium* spp. infections were 45.20%, 28.77% and 26.03% respectively, while those of *Aspergillus* spp. were 38.62%, 31.72% and 29.66% respectively.

Table 2 depicts the frequency (%) of isolated fungi depending on the sample collection sites. According to Table 3, the appearance of fungal isolates depending on the sample collection sites (72 sample of each location), the percentage of *Fusarium* spp. in warehouse, mill and local markets were 45.20%, 29.17% and 26.39%; while those of *Aspergillus* spp. were 77.78%, 68.06% and 56.72%; those of *Penicillium* spp. were 54.17%, 51.39% and 55.56%; and finally, those of *Cladosporium* spp. were 23.61%, 18.06% and 13.88% respectively. Tesfaye & Dawit (1998) also identified four *Fusarium* species associated with maize grain in Ethiopia. The major genera commonly encountered on maize grain in tropical regions were *Fusarium, Aspergillus* and *Penicillium* (Orsi *et* *al*. 2000). Theodora *et al*. (2018) reported that contamination of maize samples from both small scale and commercial farms by *Fusarium* spp. with the most common being *F. verticilloides, Fusarium graminearum*, and *F. proliferatum* might be explained by the late harvesting method used in rural areas. The results of this study were in agreement with Girma *et al*. (2009), who reported that in Ethiopia, various grain mold fungi including *Fusarium*, *Penicillium*, *Aspergillus*, and *Nigropora* spp. have been detected on maize samples collected from Hawassa, Areka, Billito, Shallo and Arsi-Negele. Binyam (2016) recorded highest frequency of *Aspregilu*s spp. (40.4%) at farms preserved seed with surface disinfected kernels, followed by 32% on Melco verity which was non-surface disinfected kernels, as recorded on agar plate test (Fig. 2).

The highest frequency of *Fusarium* spp. and *Penicillium* spp. were 28% and 18% respectively. The development of these fungi can be influenced by moisture content of the product (Gtorni *et al*. 2009), temperature, storage time, degree of fungal contamination prior to storage, insect. In addition, mite activity facilitates fungi dissemination (Suleiman & Omafe 2013). During storage, several kinds of fungi can remain associated with corn seeds either causing their deterioration or simply remain viable to infect germinating seedling. The fungi genera typically found in stored grains are *Aspergillus, Penicillium, Fusarium* and some xerophytic species, several of them with capabilities of producing toxins (Castellari *et al*. 2010). *Aspergillus* and *Penicillium* spp. are the major fungal genera commonly encountered in maize-producing regions, however, usually co-infect with *Fusarium* spp. (Bush *et al.* 2004). Theodora *et al*. (2018) identified different genera isolated from corn revealed the predominance of *Fusarium* (82%), *Penicillium* (63%), and *Aspergillus* species (33%). The dominance of *Fusarium* may be due to the fact that maize was incorrectly dried. Insufficient drying and precarious condition of storage could promote *Fusarium* growth, since *Fusarium* genera need water for growth (Harrigan & MeCance 1988). Most *Fusarium* spp. are plant pathogens and invade plant tissues and developing seeds in the fields (Pitt & Hocking, 1997), however, some are able to persist in harvested and stored grain and grow in storage when moisture content becomes favorable (Mills 1989).

This may explain their presence in these analyzed samples from both areas by late harvesting and improper storage. Fig. 2 illustrates that the frequency of *Fusarium* spp. depends on sample collection site, so that, in Al-Sharia store, Al-Hosseinieh store and Al-Jamalia store were 13.70%, 19.18% and 12.33% respectively. In mill, the frequency of *Fusarium* spp. in Al-Ali Mills, Aoun Mill sand and Karbala Technical Mills were 8.22%, 9.59% and 11.00% respectively. In City Center Markets it was 26.03% and in all sites the *Fusarium* found at different rates. Girma (2009) reported that the populations of all the fungi were higher in samples collected from farmers' stores than in the samples collected from research and seed multiplication stores.

The development of these fungi can be affected by moisture content of the product (Gtorni *et al*. 2009), temperature, storage time and degree of fungal contamination prior to storage. Insect and mite activity facilitates fungi dissemination (Suleiman & Omafe 2013). During storage, several kinds of fungi can remain associated with corn seeds either causing their deterioration or simply remain viable to infect germinating seedling. The fungi genera typically found in stored grains are *Aspergillus*, *Penicillium*, *Fusarium* and some xerophytic species, several of them with capabilities of producing toxins (Castellari *et al*. 2010).

Table 1. The number and frequency of fungal species isolated from corn samples in different regions of Karbala.

NO.	Fungi	No.	Percentage of frequency
1	*Fusarium* spp.	73	14.01
2	*Aspergillus* spp.	145	27.83
3	*Penicillium* spp.	116	22.26
4	*Cladosporium* spp.	40	7.68bb
5	*Mucor* spp.	30	5.76
6	*Alternaria* spp.	25	4.80
7	*Acremonium* spp.	14	2.69
8	*Rhizopus* spp.	13	2.50
9	*Humicola* spp.	10	1.92
10	White sterile fungi	23	4.41
11	Yeasts	32	6.14
Total		521	

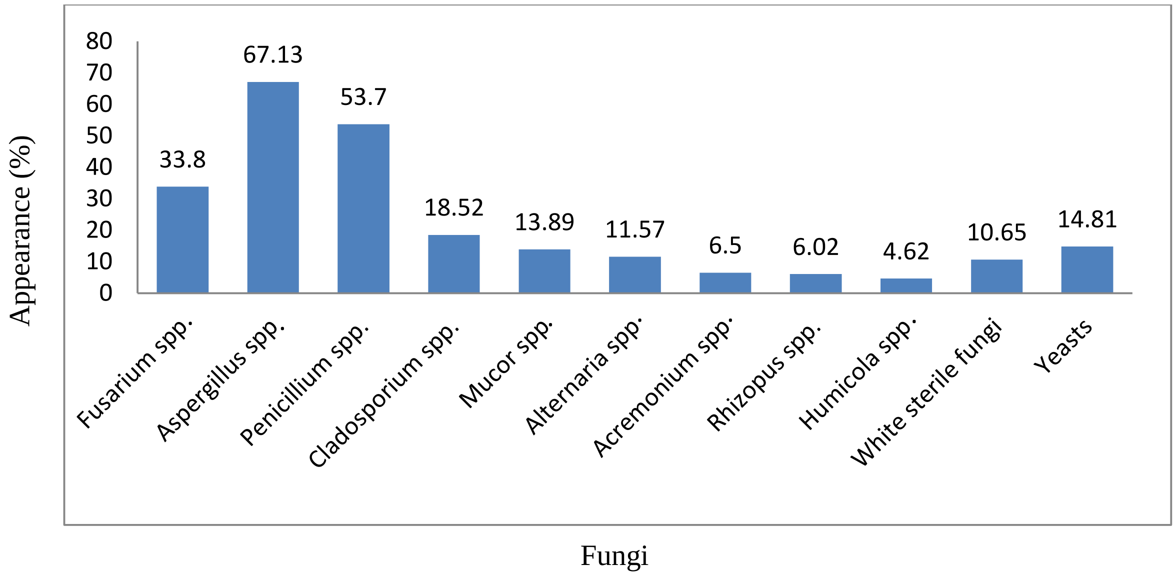

Fig 1. The percentages of fungi isolates appearance associated with corn samples from different sample collection in site Karbala (216 samples).

Table 2. The frequency of isolated fungi depending on sample collection site.

No.	Fungal isolates	Warehouse		Mill		Local markets		Total
		No.	%	No.	%	No.	%	
1	*Fusarium* spp.	33	45.20	21	28.77	19	26.03	73
2	*Aspergillus* spp.	56	38.62	46	31.72	43	29.66	145
3	*Penicillium* spp.	39	33.62	37	31.90	40	33.62	116
4	*Cladosporium* spp.	17	42.50	13	32.50	10	25.00	40
5	*Mucor* spp.	6	20	9	30	15	50	30
6	*Alternaria* spp.	9	36	7	28	9	36	25
7	*Acremonium* spp.	6	42.86	5	35.71	3	21.43	14
8	*Rhizopus* spp.	5	38.46	3	23.08	5	38.46	13
9	*Humicola* spp.	3	30	3	30	4	40	10
10	White sterile fungi	11	47.83	8	34.78	4	17.39	23
11	Yeasts	14	43.75	10	31.25	8	25	32
	Total	196	37.62	167	32.05	158	30.33	521

Table 3. The appearance (%) of isolated fungi depending on sample collection site (72 samples of each location).

No.	Fungal isolates	Warehouse		Mill		Local markets		Total
		No.	%	No.	%	No.	%	
1	*Fusarium* spp.	33	45.20	21	29.17	19	26.39	73
2	*Aspergillus* spp.	56	77.78	46	68.06	43	56.72	145
3	*Penicillium* spp.	39	54.17	37	51.39	40	55.56	116
4	*Cladosporium* spp.	17	23.61	13	18.06	10	13.88	40
5	*Mucor* spp.	6	8.33	9	12.50	15	20.83	30
6	*Alternaria* spp.	9	12.50	7	9.72	9	12.50	25
7	*Acremonium* spp.	6	8.33	5	6.94	3	4.16	14
8	*Rhizopus* spp.	5	6.94	3	4.16	5	6.94	13
9	*Humicola* spp.	3	4.17	3	4.16	4	5.55	10
10	White sterile fungi	11	15.28	8	11.11	4	5.55	23
11	Yeasts	14	19.44	10	13.88	8	11.11	32
	Total	196	37.62	167	32.05	158	30.33	521

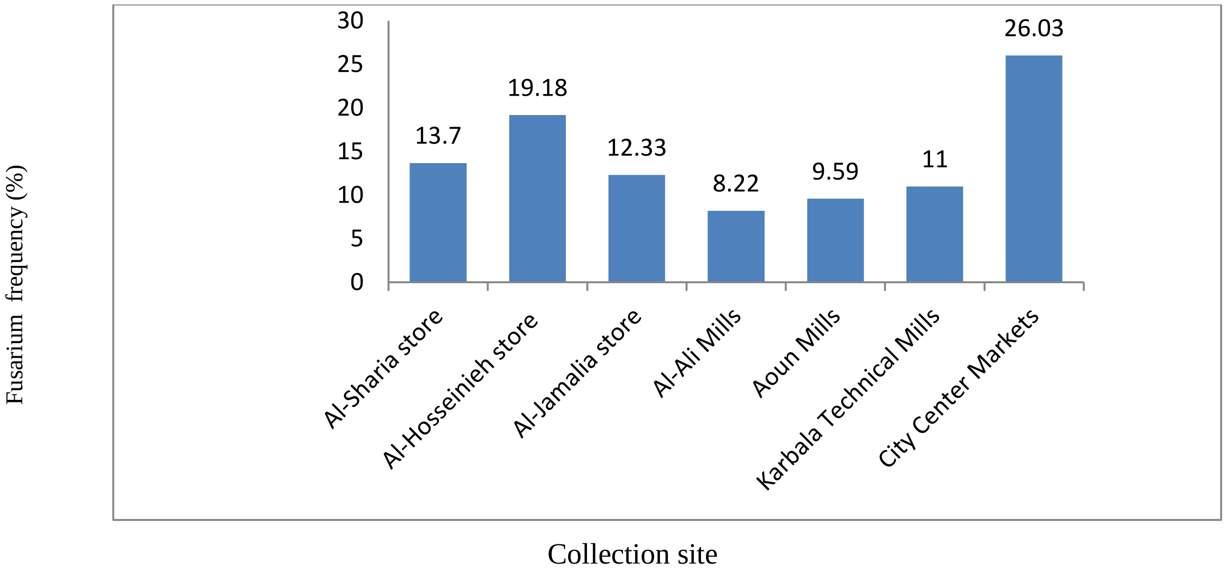

Fig. 2. The frequency of *Fusarium* spp. depending on sample collection site.

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