

First Report of *Penicillium Polonicum* Produce Ochratoxin A on Rice in Iraq

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ABSTRACT

This study aimed to isolate and identify fungal species associated with both locally produced and imported rice grains in Iraq, and to assess their ability to produce ochratoxin A (OTA). Twenty rice samples were collected from markets in Baghdad Governorate. The results revealed contamination with the following fungal genera: *Aspergillus* spp., *Penicillium* spp., *Cladosporium* sp., *Monascus* sp., *Trichothecium* sp., *Moniliella* sp., *Alternaria* sp., *Mucor* sp., *Curvularia* sp., *Fusarium* spp., and Yeast. *Aspergillus* spp. Showed the highest frequency (25%) in surface-sterilized samples, while *Penicillium* spp. was predominant (35%) in non-sterilized samples. Thin-layer chromatography (TLC) analysis demonstrated that 36 out of 45 fungal isolates possessed OTA-producing potential. Three isolates (PM11, PM18, and PM20) exhibited high OTA production. These isolates were molecularly identified as *Penicillium polonicum*, showing 99% sequence similarity with reference strains in the NCBI database. Quantitative analysis using high-performance liquid chromatography (HPLC) recorded high OTA concentrations produced by these isolates: 561.77 ppb, 518.95 ppb, and 495.9 ppb per kg, respectively. This study represents the first documented report of *P. polonicum* contamination and ochratoxin A production in locally and imported rice varieties in Iraq.

Key words: HPLC, OTA, Rice contamination, TLC.



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INTRODUCTION

Rice is the staple food for more than half of the world's population. It is classified as an important food grain that is grown and produced in large quantities. It ranks second after wheat in terms of production and cultivated area in the world (Fukagawa and Ziska, 2019). It is believed that the original homeland of rice is Southeast Asia. Rice cultivation has spread in India and China for more than 5000 years. In Iraq, rice cultivation has prevailed since 400 years BC. There are thousands of rice varieties around the world. Rice species are divided into one of two types, *Oryza sativa* and *Oryza glaberrima*, which are divided into two main groups: the India Group and the Japonica Group (Sweeney and McCouch, 2007). There are two main types of local Iraqi rice: Anber and Yassamine Cultivated in five provinces: Najaf, Diwaniyah, Muthanna, Dhi Qar and Babel

(Alhendi et al., 2019). The cultivated area is expected to reach 13,000 tons in 2023 (Directorate of Agricultural Statistics, 2023). Rice grains are contaminated with fungi and mycotoxins, whether in the field or storage, due to their high moisture content (Reedy et al., 2008) and unsuitable storage conditions (Ruadrew and Aidoo, 2013). This poses a threat to the lives of millions of people. Mycotoxins can be defined as chemostable, non-antigenic compounds that do not stimulate the immune system due to their low molecular weights ranging from 97-710 Daltons (Lee and Ryu, 2015). These toxins contaminate foods and grains such as corn, rice, oilseeds, dried fruits and peanuts, which are stored improperly (Kensler et al., 2011). Most of the fungi that produce mycotoxins and contaminate rice belong to the genera *Aspergillus*, *Penicillium* and *Fusarium* (Lee and Ryu, 2015). These fungi produce a group

of mycotoxins that contaminate rice grains, including aflatoxins, ochratoxins, fumonisins, zearalenone, trichothecenes and deoxynivalenol (Reedy et al., 2007). These mycotoxins present in food have acute or chronic health effects on humans and animals (Awuchi et al., 2022). Recent studies have shown that cancer, hepatotoxicity, nephrotoxicity, genetic mutations, embryonic changes and immunodeficiency are closely related to exposure to mycotoxins (Arce-Lopez et al., 2020). The aflatoxins and ochratoxin A are the predominant mycotoxins detected in rice grain. One of these dangerous mycotoxins is Ochratoxin A (OTA). These toxins are secondary metabolites that were first isolated and discovered in 1965 from the fungus *A. ochraceus* (Van der merwe et al., 1965). This fungus can produce OTA (Kensler et al., 2011). However, recent molecular studies indicate that *A. westerdijkiae* is the main species producing ochratoxin A (Frisvad and Samson, 2004). OTA has been classified as a probable human carcinogen (Class 2B) by the International Agency for Research on Cancer (IARC) (IARC, 1993). Moreover, a link between Balkan endemic nephropathy (BEN) and OTA toxins has recently been confirmed. This toxin is mainly produced by *Aspergillus* and *Penicillium* (El khoury and Atoui, 2010; Van der merwe et al., 1965). *Penicillium polonicum* is a psychrotolerant and xerophilic fungal species frequently implicated in the spoilage of various food commodities. It has been documented as a contaminant in multiple substrates, including onions, cereals, processed dried meats, peanuts, yam tubers, and citrus fruits (Duduk et al., 2014). *Penicillium polonicum* is a known producer of multiple mycotoxins with documented health risks, including citrinin, penicillic acid, nephrotoxic glycopeptides, verrucosidin (Frisvad and Samson, 2004), and ochratoxin A (Chen et al., 2011; Chen et al., 2013). These secondary metabolites demonstrate pronounced toxicological properties, notably nephrotoxicity, posing potential hazards to human and animal health upon exposure (Majeed et al., 2018).

MATERIALS AND METHODS

Sample collection: Rice samples were collected from Iraqi markets at a rate of 5 kg per sample. The samples included imported types (Ahlina, Abu Araba Gold, Al-Barari, Al-Kharafi, Al-Walima, Judy, Jihan, Joker, Daawat, Mahmoud, Mashkooor, Maharani, Basmati rice (ration card), Al-Awsi, Ali Pasha, King of Punjab, Super Pasha, Kobani). Among the local types, Amber (Al-Waha and Al-Khadhrawi) were selected. They were marked with the name of the sample, date and place of collection. All samples were stored in tightly sealed plastic bags. The samples were kept at room temperature in a dark and dry place until subsequent tests were conducted.

Culture media and solutions

Potato dextrose agar (PDA): According to manufacturer's drawings: 39 g PDA powder/L was autoclaved (121°C, 15 min) and plated (15-20 mL/plate). Liquid rice medium: 40 g of rice was ground, transferred to beaker and the volume was made up to 1000 ml by adding distilled water, then 2 g of peptone and 20 g of sucrose were added according to the method of Davis et al. (1966).

Standard Ochratoxin A: Standard Ochratoxin A (5mg, purity 98.0%) was prepared by Lab Instruments, Italy. Stored at -20°C until analysis.

Isolation and identification of fungi associated with rice grains: The fungi contaminating imported and local rice grains were detected by follow the following steps: Rice grains were washed with water and dried with filter paper. 25 gm of each rice grain sample was taken. It was superficially sterilized with sodium hypochlorite solution (2%) for 3 minutes (Pitt and Hocking, 2022). It was washed with distilled and sterile water 3 times and dried with filter paper. Also, 25 gm of each sample was washed with sterile water only and dried with filter paper without superficial sterilization of the grains. The grains were planted on the culture medium Potato dextrose agar (PDA) in Petri dishes with a diameter of 9 cm at a rate of 10 grains per dish, at a rate of 3 replicates for each type. The dishes were incubated for 5 days at a temperature of 25 + 2 °C. Fungi were purified by transferring one end of the fungal hyphae to

a dish containing PDA medium in another dish for purification and morphological and microscopic identification. Taxonomic keys were adopted (Ogorek et al., 2012; Pitt and Hocking, 2022). Then, the dishes were incubated at a temperature of $25 \pm 2^\circ\text{C}$ for 7 days. The frequency and appearance percentage of fungi accompanying the tested samples were recorded according to the following equations (Goveas et al., 2011):

Frequency percentage = $100 \times (\text{fungal isolates number}) / (\text{total isolates number})$.

Percentage of appearance = $100 \times (\text{number of samples in which the fungus appeared}) / (\text{number of total samples})$.

Growth fungal isolates in liquid rice medium to detect their ability to produce ochratoxin A (OTA): Flasks containing liquid rice medium (40 g of ground rice with 2 g of peptone and 20 g of sucrose added to a liter of distilled water) were inoculated with five discs of 0.7 cm diameter by cork porer from all 7-day-old fungal colonies known to produce OTA toxin, namely: *Penicillium* sp., *A. ochraceus*, *A. niger*, *A. terreus*, and incubated for 21 days at $25 \pm 2^\circ\text{C}$.

Extraction of Ochratoxin A from Liquid Rice Medium: After the incubation period was over, the liquid medium was filtered using filter paper. The filtrate was placed in a separating funnel and an equal volume of chloroform was added to the filtrate. The funnel was shaken for 30 seconds while monitoring the expulsion of the formed gases in order to separate the two layers to extraction OTA. The lower layer containing chloroform was taken, and the upper layer was ignored. Then, the filtrate was collected and passed through the Whatman No. 4 filter paper containing anhydrous sodium sulfate in order to remove water. For every 100 ml of the filtrate, 40 g of anhydrous sodium sulfate was added. The filtrate was collected in a glass bottle, measured according to its size, and stored in the freezer (-20°C) until further tests were performed (AOAC, 2005).

Detection of OTA toxin using Thin-Layer Chromatography (TLC) plates: TLC done according to Ventura *et al.*, (2005). The retradation factor (Rf) of the tested samples

and the standard poison was estimated according to the following equation:

$R_f = (\text{spot to origin point distance}) / (\text{front to origin point distance})$.

In light of the above, the isolate that produced the most OTA toxin was selected for subsequent tests (PCR and HPLC) and given the code name PM11.

Estimation of OTA toxin quantity for the most toxin-producing isolates using HPLC technique: High-Performance Liquid

Chromatography technique (HPLC): SYKAM model (Germany) was used to detect ochratoxin A. The mobile phase is acetonitrile: distilled water D.W: formic acid in volume ratios (50:47:3), flow rate at 1.0 ml/min, column C18 – ODS (25 cm x 4.6 mm) and fluorescent detector (Ex = 365nm, Em = 445 nm). The retention time is 4.0 min, and by comparing the retention time of the standard OTA toxin (at a concentration of 5ppb) injected into the device with the closest time that appeared in the sample, the toxin is diagnosed. The toxin concentration is also calculated according to the following equation: Toxin concentration in sample = (Standard Toxin Concentration X Sample Curve Area) / (Standard Toxin Curve Area) X (Number of Dilutions) / (Sample Volume or Weight). (Skarkova et al., 2013).

Molecular identification of the most OTA Toxin producing isolates: The fungal isolates (PM11, PM18 and PM20) were grown for 1-3 days until growth appeared in the plate. A single colony was transferred to the PDA culture medium. Then, it was incubated at 25 ± 2 for 5-7 days. DNA was extracted and purified using the (Kit) of the Korean company Bioneer according to the manufacturer's instructions. This was followed by preparation of the polymerase chain reaction (PCR), electrophoresis and estimation of the molecular weight of the DNA. By targeting the ITS1, ITS4 region in 18S rRNA and using the following PCR primers sequences:

F: 5'-TCCGTAGGTGAACCTGCGG-3'

R: 5'-TCCTCCGCTTATTGATATGC-3'

prepared by the company Bioneer/Korea. The same company determined the sequence of nitrogenous bases. The nitrogenous base

sequences were analyzed by comparing them with the nitrogenous base sequences in the National Center for Biotechnology Information (NCBI) GenBank.

RESULTS AND DISCUSSION

Isolation and identification of fungi associated with rice grains: The results of isolation and identification of 20 type of rice grains collected from different areas of Baghdad city showed the presence of different genera of fungi, namely *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., *Monascus* sp., *Trichothecium* sp., *Moniliella* sp., *Alternaria* sp., *Mucor* sp., *Curvularia* sp., *Fusarium* spp. and Yeast. The results from surface-sterilized rice grains revealed that *Aspergillus* spp. was the most prevalent genus with a frequency of 25%, followed by *Penicillium* spp. (22%) and *Cladosporium* spp. (13%). Other genera occurred at lower frequencies: *Fusarium* spp. (8%), *Moniliella* sp. (3%), yeast (1%), and *Alternaria* sp., *Mucor* sp., and *Monascus* sp. (0.6% each). The remaining fungal genera showed variable frequency percentages (Figure 1). In non-sterilized grains, *Penicillium* spp. dominated (35%), surpassing *Aspergillus* spp. (28%) and *Cladosporium* spp. (16%). The other

contaminants included *Fusarium* spp. (10%), *Moniliella* sp. (3%), *Alternaria* sp. and *Mucor* sp. (2% each), *Curvularia* sp. (1%), and *Trichothecium* sp. (0.4%) (Figure 2). The morphological and microscopic identification methods employed in this study yielded results concordant with the approaches described by Pitt and Hocking (2022). Surface sterilization (e.g., ethanol or NaOCl treatment) effectively eradicates epiphytic fungal populations on grain surfaces, resulting in significantly lower microbial counts. In contrast, sterilized samples enable detection of endophytic fungal colonization that has penetrated the endosperm. Oh *et al.* (2007) reported that the most common fungi in stored rice are *Aspergillus* and *Penicillium*, as they tolerate storage conditions and relatively low relative humidity. This was confirmed by Pitt and Hocking, (2022) and Manna and Kim (2017) in that the species of these two genus are the most contaminating fungi of rice in storage. *Aspergillus* is the first contaminant, followed by *Penicillium* in second place. This explains the isolation of different species of these two genus in this study. These fungi may attack grains in the field and invade the seed embryo and other parts.

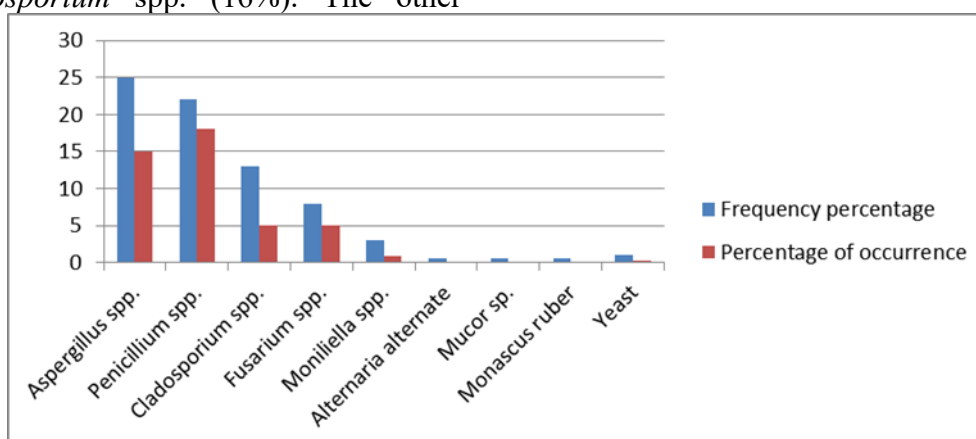


Figure 1. Percentage of frequency and appearance of fungi associated with sterilized rice grains

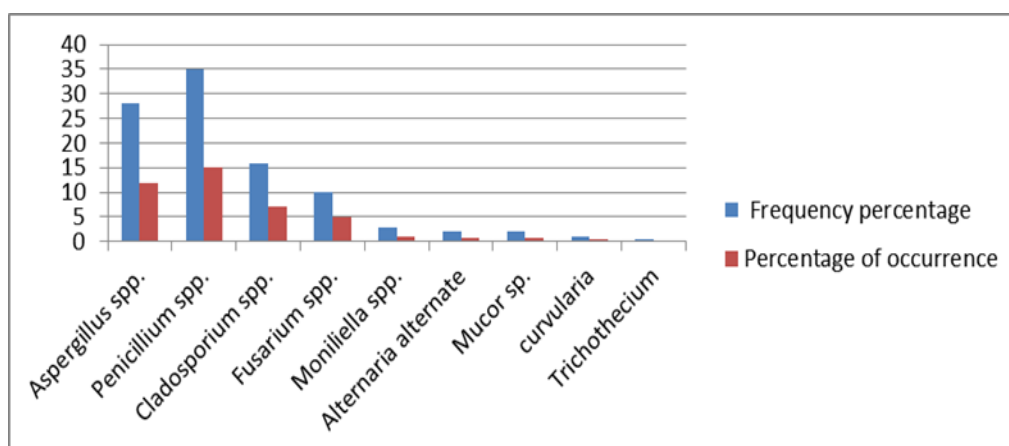


Figure 2. Percentage of frequency and appearance of fungi associated with non-surface sterilized rice grains

The superiority of *Aspergillus* and *Penicillium* fungi is due to their high enzymatic capacity and their great ability to grow in different environments (Leitao, 2009; Paulussen et al., 2017). The genus *Penicillium* is a storage fungus that thrives when the moisture content of grains is high during harvest and storage (Van der merwe et al., 1965). These conditions encouraged this genus to be the most contaminant of unsterilized rice grains. This is consistent with what Makun *et al.* (2007) reported that the genus *Penicillium* was the most contaminant of the rice samples that were studied. Species of this fungus contaminate food crops and fruits such as citrus fruits as well as foods (Taha et al., 2019). This could be because these fungi contain active compounds that have beneficial effects in competing with other fungi (Iqbal et al., 2016). Ali *et al.* (2018) reported that imported rice in Iraq contained unsafe fungal levels ($>1 \times 10^4$ CFU/g), which violates COSQC standards and puts consumer health at risk. Figure (3) showed that the surface-sterilized Ahlna (A) and Mahrani (L) rice samples were free of any fungi. However, fungal growth appeared in both types of non-sterilized rice. The explanation for this is that the fungi that grew in the absence of sterilization were external fungi (outside the rice grain) that stuck to the outer wall of the grain and were removed when the rice grains were superficially sterilized. Sterilized sodium hypochlorite does not affect fungi inside the grains (Figure 3). The non-sterilized local rice samples (Al-Waha/Anbar Al-Mashkhab M and Al-Khadhravi N) had the highest percentage

of fungal contamination compared to the imported types of rice, as the contamination percentages reached 100 and 77%, respectively (Figure 4). As for the superficially sterilized rice types, Mahmoud (J), Abu Araba (B), and Al-Khadhravi (N) rice outperformed, occupying the first places in the ranking of the most contaminated rice types with fungi. The contamination percentage reached 67, 43 and 40%, respectively (Figure 3). In the same context, the results showed that the rice types least contaminated with fungi were Jihan rice (G), Awsi rice (O), and Tawminiya rice (P), with contamination rates of (7, 10, 13)%, respectively, in the case of non-surface sterilized grains. However, after sterilization, the lead was given to Ahlna rice (A), Mahrani (L), Kharafi (D), and Tawminia rice (P) with infection rates of (0, 0, 10, 13)%, respectively. The results showed that the most fluorescent fungal isolates on the TLC plate and the most toxic by HPLC technique were from the rice variety (Al-Waha/Anbar Al-Mashkhab). It is the variety most contaminated with fungi and mycotoxins. This may be due to poor agricultural, marketing and storage practices. Rice grains contain a high moisture content that enables fungi to infect them in the field or storage (Reedy et al., 2008). Alwan and AL-Bakri (2014) found that stored Iraqi rice grains (Yasameen variety) were contaminated with the following fungi: *Aspergillus* spp., *Curvularia* sp., *Fusarium* sp., *Penicillium* spp., *Mucor* sp., *Nigrospora* sp. and *Rhizopus* sp.. In another study, Ali *et al.* (2018) were able to isolate several fungi contaminating rice

samples collected from Iraqi markets (imported and local) belonging to the genera *Aspergillus* spp., *pencilium* spp., *Fusarium*

spp., *Rhizopus* spp., *Alternaria* spp., and *Mucor* spp. .

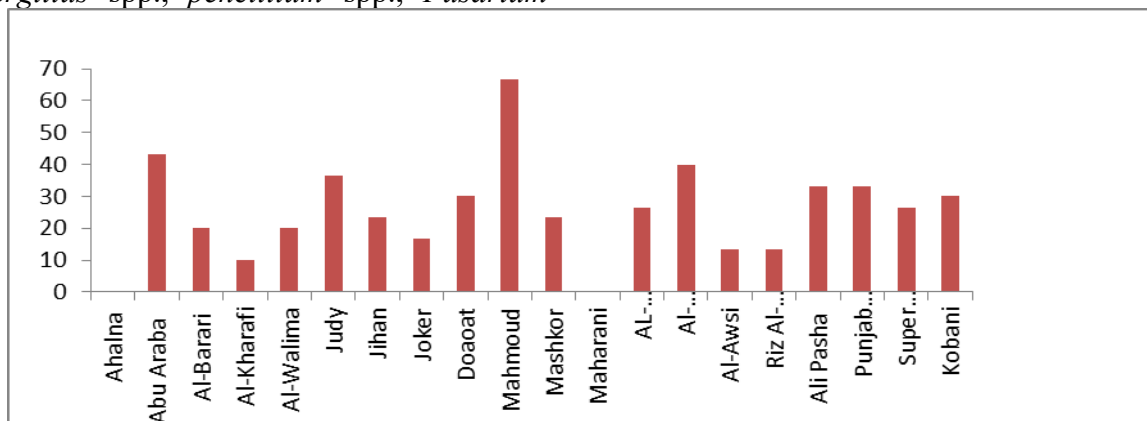


Figure 3. Percentages of contamination of surface-sterilized types of commercial rice (domestic and imported) with fungi

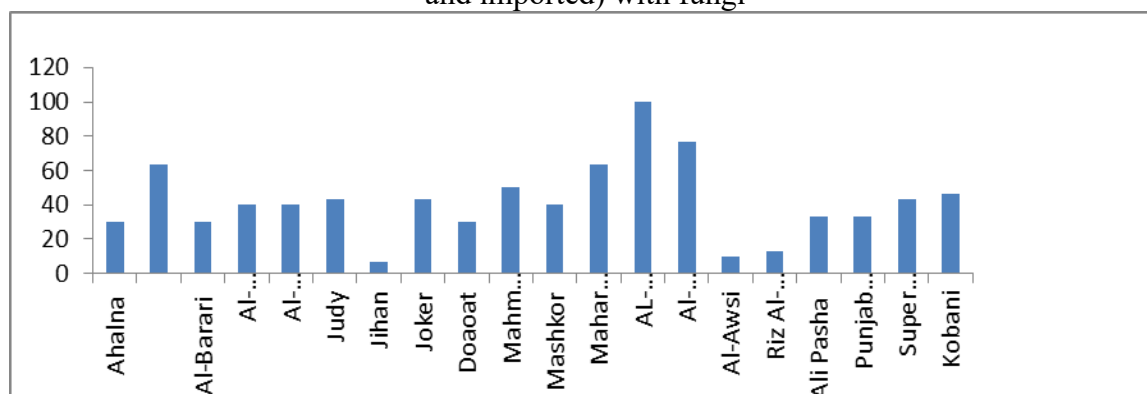


Figure 4. Percentages of contamination of non-sterilized types of commercial rice (domestic and imported) with fungi

Detection of ochratoxin A in Iraqi amber rice (Al-Waha and Al-Khudrawi): High-performance liquid chromatography (HPLC) analysis revealed that amber rice grains (Al-Waha and Al-Khadrawi cultivars) were contaminated with ochratoxin A (OTA), with

concentrations reaching 66.5 ppb and 51.6 ppb, respectively (Figure 5). These levels exceed those reported by Iqbal et al. (2016) for white rice, which had OTA levels ranging from 8.50 to 24.9 ppb.

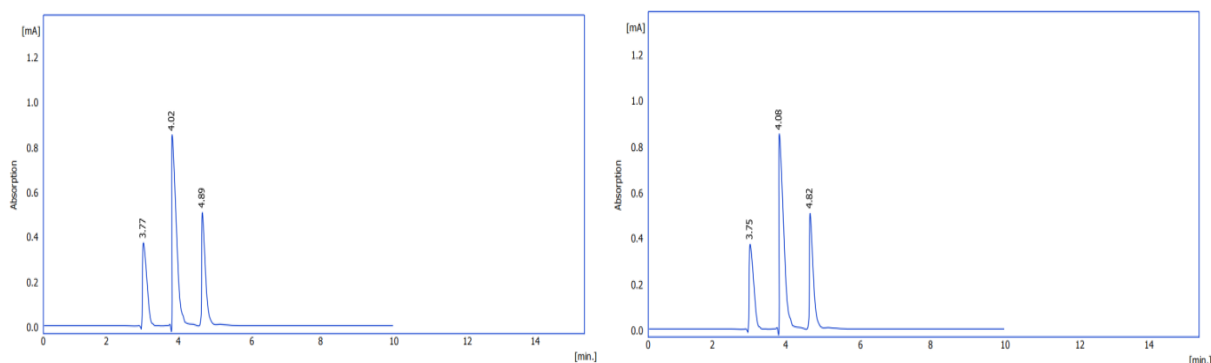


Figure 5. Amber rice: a- Al-Waha, b- Al-Khadrawi

Testing the ability of different isolates to produce OTA toxin on liquid rice medium using the Thin Layer Chromatography (TLC) technique: The results of the detection of

OTA toxin using thin-sheet chromatography (TLC) for fungal isolates associated with rice grains and grown on liquid rice medium showed the appearance of blue-green

fluorescent spots on TLC plates when examined under ultraviolet light (360 nm). All isolates were producers of OTA toxin except for *A. niger* and *A. carbonarius* isolates, which were not producers (Table 1). The isolates producing the toxin varied in toxin concentrations from one isolate to another, and the isolate that produced the most toxin from the rice type (Al-Waha/Anbar Al-Mashkhab)

belonged to *Penicillium polonicum*. It was given the code name PM11 and was identified based on a visual examination of the spot size and intensity of its fluorescence on the TLC plate when exposed to ultraviolet light. This poison glows blue. Then, this sample was selected along with the most luminescent samples for quantitative analysis of ochratoxin A using HPLC.

Table 1. Assessment of OTA-producing capacity of fungal isolates grown in rice liquid medium using TLC analysis.

No	Type of Fungus	presence of the Toxin	No	Type of Fungus	presence of the Toxin
1	<i>A. carbonarius</i>	-	24	<i>Penicillium</i> sp.	+
2	<i>A. niger</i>	-	25	<i>Penicillium</i> sp.	++
3	<i>A. niger</i>	-	26	<i>Penicillium</i> sp.	+
4	<i>A. carbonarius</i>	-	27	<i>Penicillium</i> sp.	+
5	<i>A. carbonarius</i>	-	28	<i>Penicillium</i> sp.	+
6	<i>A. carbonarius</i>	-	29	<i>Penicillium</i> sp.	+
7	<i>A. carbonarius</i>	-	30	<i>Penicillium</i> sp.	++
8	<i>A. niger</i>	-	31	<i>Penicillium</i> sp.	+
9	<i>A. niger</i>	-	32	<i>Penicillium</i> sp.	+
10	<i>Penicillium</i> sp.	+	33	<i>Penicillium</i> sp.	+
11	<i>Penicillium</i> sp.	++++	34	<i>Penicillium</i> sp.	++
12	<i>Penicillium</i> sp.	+++	35	<i>Penicillium</i> sp.	++
13	<i>Penicillium</i> sp.	++	36	<i>Penicillium</i> sp.	+
14	<i>Penicillium</i> sp.	+	37	<i>Penicillium</i> sp.	++
15	<i>Penicillium</i> sp.	++	38	<i>Penicillium</i> sp.	+
16	<i>Penicillium</i> sp.	++	39	<i>Penicillium</i> sp.	+
17	<i>Penicillium</i> sp.	+	40	<i>A. terreus</i>	++
18	<i>Penicillium</i> sp.	++++	41	<i>A. terreus</i>	+
19	<i>Penicillium</i> sp.	+	42	<i>A. terreus</i>	+
20	<i>Penicillium</i> sp.	++++	43	<i>A. terreus</i>	+
21	<i>Penicillium</i> sp.	+	44	<i>A. terreus</i>	+
22	<i>A. ochraceus</i>	+	45	<i>A. terreus</i>	+
23	<i>Penicillium</i> sp.	+			

- Non-toxic , + Very weak production of Toxin, ++ Weak production of Toxin , +++ Average production of Toxin, ++++ High toxin production, +++++ The most toxin-producing isolate

TLC technology is widely used in the qualitative detection of mycotoxins. Previous studies have indicated that a small percentage of *A. niger* isolates were OTA producers. Al-Khafaji (2017) found that 7 from 20 *A. niger* isolates isolated from feed were OTA producers. As for *A. carbonarius* isolates, they were not OTA producers. This may be due to one of three reasons: (a) The gene responsible

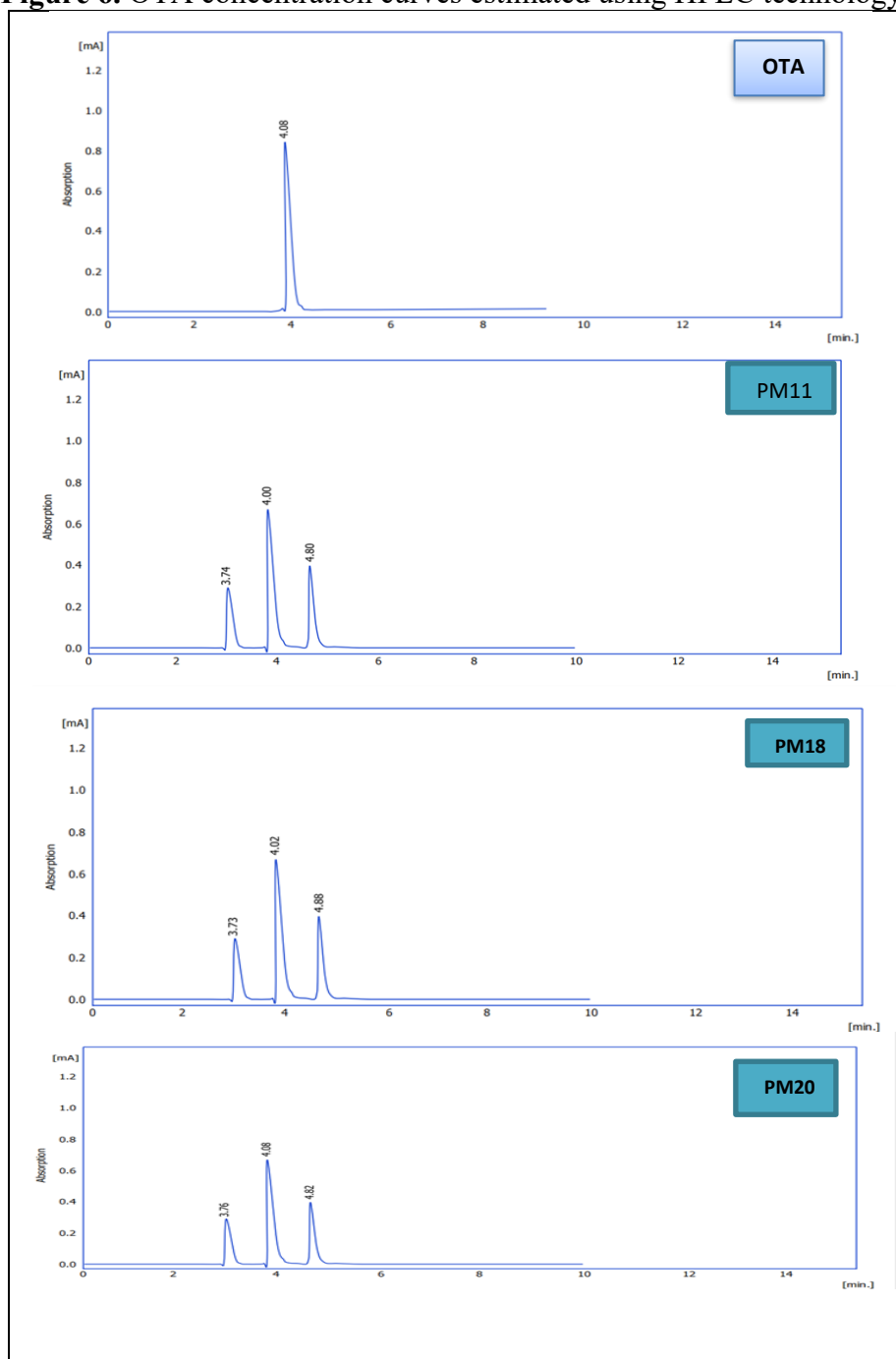
for OTA production is absent in these isolated strains of the two fungi. This was confirmed by Al-Khafaji (2017) that only 35% of *A. niger* isolates contained the PKS gene responsible for the production of OTA toxin. (b): It may be due to the TLC technique, which is characterized by being insensitive to very low concentrations (Singh and Mehta, 2020). (c): Or it may be due to the environmental conditions that inhibited the production of this toxin. The fungus *A. carbonarius* produces OTA optimally at a cooler temperature: 15 °C and a water activity of 0.98 aw - 0.97 or 20 °C

and aw 0.98-0.99 Although *A. carbonarius* is more commonly known to produce OTA than *A. niger* (Abarca et al.,2003), it was not a producer of OTA under the same conditions.

Determination of the most toxin-producing isolate using HPLC: The results of the examination of samples containing ochratoxin A using HPLC for the three most toxin-producing isolates (Figure 6) showed that the amount of ochratoxin A was 561.77 ppb, 518.95 ppb, 495.9 ppb per Kg for isolates

PM11, PM18 and PM20 respectively. Isolate PM11 was selected as the most toxin-producing isolate for subsequent tests (Figure 7). Fungal isolates of the same species vary in the amount of OTA toxin produced according to genetic factors related to the gene responsible for synthesizing this toxin, which carries all the necessary information to produce a certain amount and type of toxin (Pitt and Hocking, 2022).

Figure 6. OTA concentration curves estimated using HPLC technology



This technology (HPLC) is also sensitive to measuring small concentrations of mycotoxins. It is one of the common techniques for detecting the amount of OTA toxins in foods and grains (Frisvad and Samson, 2004; Lee and Rya, 2015). *polonicum* produces a group of toxins are: nephrotoxicglycopeps and verrucosidin, which are found in foods. It also produces penicillic acid, which is not found in food (Frisvad and Samson, 2004), and ochratoxin A (Chen et al., 2011, 2013). And toxins Roquefortine, mycophenolic, and Patulin (Oztarkoglu-Budak and Ozturk, 2023). At the same time, Mantle and Miljkovic (1998) stated that *P. polonicum* does not produce OTA but produces toxins called nephrotoxins. They are considered a possible cause of kidney atrophy in endemic nephropathy in the Balkans. These toxins can lead to loss of the tubular epithelium through programmed cell death. This occurs gradually and imperceptibly over many years in humans. Studies that addressed the cause of the spread and outbreak of endemic nephropathy in the Balkans have confirmed the relationship of this disease to mould-contaminating food. *P. polonicum* (formerly known as *P. aurantiogriseum*) is common in the Balkans. This hypothesis was supported by an experiment in which mice were fed wheat contaminated with an extract of this fungus for five days (Mantle et al., 1998). Upon dissection of the mice on the sixth day, significant histopathological changes were observed in the epithelium of the renal tubules, including mitotic figures, nucleolar hypertrophy, apoptotic bodies, and accumulation of renal DNA adducts (Miljkovic et al., 2003). The presence of DNA adducts may hinder the replication process, which contributes to the occurrence of mutations (Koike et al., 2018). The sources of mutations may be external, internal, or environmental factors. It may also be a result of anti-cancer chemotherapy (Buniya et al., 2018).

Morphological and microscopic characterization: Colony Characteristics: Blue-green with moderate radial growth and sparse central sporulation. Reverse: Cream-coloured. Conidiophores: Terverticillate

branching pattern with septate stipes displaying smooth to rough-walled textures. Conidia: Globose to subglobose, and borne on distinct supporting columns. Phialides: Flask-shaped to ampulliform, arranged in clusters of 5–8 phialides per metula. These results are consistent with Duduk *et al.* (2014)

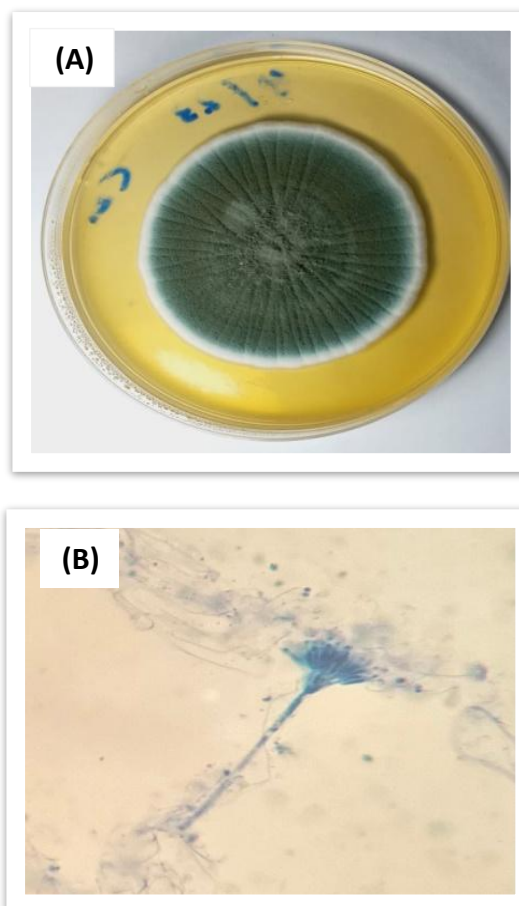


Figure 7. Morphological traits of *P. polonicum* (A) growing of colony on PDA at 25°C for 7 days. (B) Conidia, conidiophores and phialides with a magnification of 40x

Molecular diagnosis of the most toxin-producing isolate using PCR: The results of molecular diagnosis of the isolates after electrophoresis on a 1% agarose gel for the extracted DNA of the isolates (PM11, PM18 and PM20) showed the presence of a band with a molecular weight of 590 bp (Figure 8). The results of the analysis of the nitrogenous base sequences of the isolates (PM11, PM18 and PM20) showed that they belong to the fungus *P. polonicum*. They showed 99% identity with the global isolates in the NCBI GenBank. The nucleotide sequences of the isolates were deposited under the accession

numbers (LC843909) for isolate PM11, (LC842015) for isolate PM18 and (LC842014) for isolate PM20. The ITS region located within the 18S rRNA gene is used to distinguish between different fungal species and strains with high accuracy (Marciano et al., 2005), as it remains highly conserved due

to evolutionary constraints. This is the first record of *P. polonicum* producing (OTA) in Iraqi rice grains. It was first recorded in Iraq in wheat grains without confirmation of OTA production by Abd Oun (2023). The world record of *P. polonicum* producing OTA was by (Chen et al., 2013).

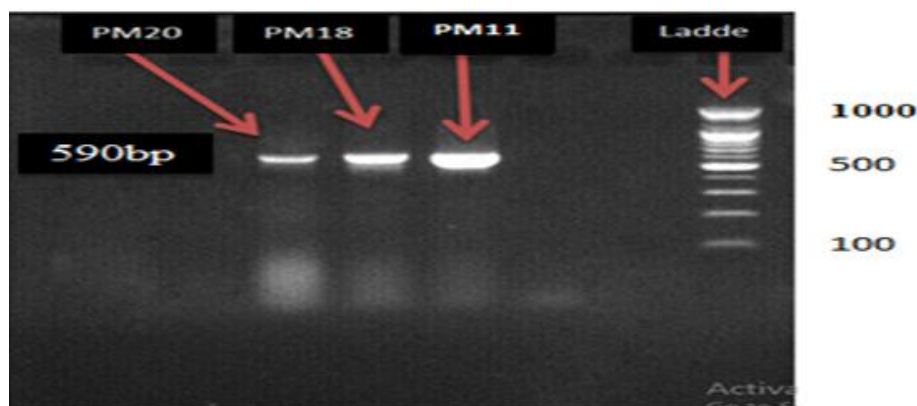


Figure 8. The result of electrophoresis on an agarose gel for DNA replication for the three fungal isolates (PM11, PM18, PM20)

CONCLUSION

Rice grains were contaminated with field and storage fungi. Local rice varieties were the most contaminated. This may be due to poor agricultural, marketing and storage practices, especially since its harvest season is in mid-November, which is characterized by low temperatures, rainfall and high humidity. This encouraged the invasion of rice by *Penicillium* species. Despite the contamination of rice grains with fungi, not all of them were contaminated with OTA.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. AdAuthor/s signature on Ethical Approval Statement.

Ethical Clearance and Animal welfare

Funds:

AUTHOR'S CONTRIBUTION STATEMENT

All authors contributed equally to the preparation of all parts and sections of this

manuscript, including the introduction, methodology, results, discussion, and conclusions. All authors have read and approved the final version of the manuscript.

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التسجيل الاول للفطر *PENICILLIUM POLONICUM* المنتج لسم الاوكراتوكسين A على حبوب الرز في العراق

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المستخلص

هدفت الدراسة الحالية الى عزل وتشخيص الفطريات المرافقة لحبوب الرز العراقي والمستورد وتقييم قدرتها في انتاج الاوكراتوكسين A. جمعت (20) عينة من حبوب الرز من أسواق محافظة بغداد. وقد أظهرت النتائج تلوث الحبوب بالأجناس التالية: *Trichothecium* sp.، *Monascus* sp.، *Cladosporium* sp.، *Penicillium* spp.، *Aspergillus* spp.، *Alternaria* sp.، *Moniliella* sp.، *Mucor* sp.، *Curvularia* sp.، *Fusarium* spp.، yeast. حيث كانت انواع جنس *Aspergillus* spp. هي الاعلى ترددا 25% في العينات المعقمة سطحيا، اما جنس *Penicillium* spp. فهو الاكثر ترددا في العينات غير المعقمة بنسبة 35%. كشف تحليل TLC ان (36) عزلة فطرية من أصل (45) قادرة على إنتاج سم الاوكراتوكسين A، مع تفوق ثلاثة عزلات هي: PM11، PM18 و PM20 تعود لفطر *Penicillium polonicum* والتي شخصت جزيئيا بنسبة تطابق 99% مع العزلات في NCBI. سجّلت هذه العزلات تراكيز عالية من الاوكراتوكسين A بـ (HPLC) بلغت (ppb 561.77، ppb 518.95، ppb 495.9) لكل كغم على التوالي. مما يمثل اول تسجيل لـ *P. polonicum* كملوث ومنتج للاوكراتوكسين A في اصناف الرز المحلية والمستوردة في العراق.

الكلمات المفتاحية: HPLC، OTA، تلوث الرز، TLC.