

Fungal contamination and Physicochemical Analysis of Algerian Dried Fig Varieties: Assessment of Aflatoxins and Ochratoxin A levels

Younes Arroul^{ID a,*}, Farid Boukhalfa^{ID a}, Mohand Teffane^{ID a}, Amar Riba^{ID b}, Amira Oufighou^{ID a},
Abdeslem Taibi^{ID a}, Ahcene Kadi^{ID a}, Javad Feizy^{ID c}, Fahimeh Mahmoodi^{ID c}, Sareh Hadjiabadi^c

^a University of Bejaia, Faculty of Natural and Life Sciences, Laboratory of Biochemistry, Biophysics, Biomathematics, and Scientometrics (L3BS), 06000 Bejaia, Algeria

^b Laboratory of Microbial Systems Biology, Kouba, Algeria, Department of Biology, M'hamed Bouguera University, Boumerdes, Algeria

^c Department of Food Safety and Quality Control, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran

Abstract

The fig (*Ficus carica* L.) is a nutritionally valuable fruit, rich in fiber, minerals, vitamins, and carbohydrates. In Algeria, dried figs are widely consumed. This study evaluated the physicochemical characteristics and fungal contamination of dried figs from two local varieties: "Thaamriouth" and "Abarkan". The methods used for fungal identification included direct plating on DG18 medium, purification of fungal isolates, and molecular identification of species using polymerase chain reaction (PCR). Contamination levels were quantified using high-performance liquid chromatography with a fluorescence detector (HPLC/FLD). Physicochemical analyses measured protein and carbohydrate content, water activity, pH, and titratable acidity to assess the suitability of dried figs as a micro-ecosystem for contamination by aflatoxigenic and ochratoxigenic fungi. Results showed that *Aspergillus* section *Nigrii* was the most frequently isolated fungal group, detected in 70% of samples. Less common isolates included *Mucorales* (15%), *Penicillium* (10 %), while the remaining 5% comprised *Aspergillus* section *Flavii*. The high carbohydrate content and acidic pH appeared to increase the figs' susceptibility to fungal contamination. HPLC/FLD analysis revealed no detectable levels of aflatoxins and ochratoxin A. PCR identification confirmed the presence of *Aspergillus niger* and *A. awamori*. Despite the absence of detectable aflatoxins and ochratoxin A in the fig samples, the potential risk to human health cannot be ruled out, particularly due to the presence of toxigenic fungal species. Therefore, proper agricultural and storage practices remain essential.

Keywords

Aflatoxins
Aspergillus
Dried figs
Nigrii
Ochratoxin A

Received: 22 February 2025

Revised: 30 April 2025

Accepted: 18 May 2025

Introduction

Mycotoxins are among the most significant contaminants in food and feed, due to their toxic effects on human and animal health (Cimbalo *et al.*, 2020). These secondary metabolites are produced by certain species of *Fusarium*, *Aspergillus* and *Penicillium* under favorable environmental conditions, such as high humidity and temperature (Ünusan, 2019). To day, over 400 mycotoxins have been identified. The most concerning mycotoxins from a public health and agro-economic perspective include aflatoxins, ochratoxin A, and Zearalenone, which are commonly produced during fungal growth in stored agricultural products (Köppen *et al.*, 2010). These compounds can cause a range of toxicological effects, including carcinogenic, nephrotoxic, immunosuppressive, and estrogenic effects, following the consumption of contaminated food and feed (Riba *et al.*, 2008)

The *Aspergillus* section *Nigri* includes several xerophilic fungal species, such as *A. niger*, *A. carbonarius*, *A. aculeatus*,

and *A. lacticoffeatus*, which are known to colonize dried fruits, including dried figs (*Ficus carica* L.), particularly when storage conditions are inadequate. This fruit is primarily found in tropical and subtropical countries due to the favorable conditions of high humidity, elevated temperatures in these regions. For example, Algeria is the third largest producer of figs after Turkey and Egypt. The climate of Algeria is mostly hot and dry, with some humid areas, that favour the development of fungal species and the production of different toxic substances (FAOSTAT, 2022; Tantaoui-Elaraki *et al.*, 2018).

To reduce these risks and ensure safe preservation, drying becomes a critical step in fig processing. In addition, the fresh fig is highly perishable at room temperature; therefore, drying the fruits is essential. Solar drying, a commonly used method for drying in food industry, aims to achieve physicochemical stability by reducing moisture content, thus enabling safe storage for an extended period. This process

* Corresponding author (younes.arroul@univ-bjaia.dz)

leads to the creation of new products with novel qualitative traits and notable nutritional and economic value (Mediani *et al.*, 2019). In contrast, the new methods of drying figs involve the use of high-temperature dryers or conventional dryers powered by fossil fuels or electricity, and these recent methods allow us to significantly control the parameters, which will lead us to reduce contamination levels. Dried fruits, resulting from these different drying techniques, are important components of traditional diets in many countries. In Algeria, for instance, dried figs, grapes (raisins), apricots, and plums (prunes) are commonly consumed, often blended into cakes and traditional dishes such as couscous (Benmeziane-Derradji, 2019).

Among dried fruits, figs are particularly valued for their nutritional qualities. Several varieties, such as 'Khfrak', 'Seyah', 'Rowno', 'Sigoto' and 'Matii' have been studied for their nutritional composition and potential health benefits (Padash *et al.*, 2016).

To our knowledge, numerous studies have addressed mycotoxin contamination in various Algerian foodstuffs, including spices, wheat, nuts, and sesame seeds (Riba *et al.*, 2008; Azzoune *et al.*, 2016; Aït Mimoune *et al.*, 2016). Evaluating the mycological quality of Algerian dried figs is of particular importance, especially in the Bejaia region, which is renowned for its substantial production. This is notably true for the 'Thaamriouth' and 'Abarkan' varieties, which are produced in significant quantities and are recognized for their high nutritional value.

The aims of this study were to evaluate aflatoxins and ochratoxin A production by fungi isolated from Algerian dried figs varieties 'Thaamriouth' and 'Abarkan', to determine aflatoxins and ochratoxin A using the HPLC/FLD method, to perform molecular identification of fungal species belonging to the *Aspergillus* section *Nigri*, and to conduct physicochemical studies of these dried fruits.

Materials and methods

Collection of dried figs samples

The collection of samples is a critical step for the identification of fungal strains, including those capable of producing aflatoxins and ochratoxin A, since mycotoxins are often unevenly distributed within food matrices. One kilogram of dried fig samples representing the two local varieties, 'Thaamriouth' and 'Abarkan,' was collected from each of four distinct locations in the Bejaia region: Beni-Maouche (36°28'41" N, 5°38'18" E), Ait Nwal Mzada (36°32'32" N, 5°05'24" E), Ait Sidi Ali Barbacha (36°34'00" N, 4°58'00" E), and Adekar (36°41'33" N, 4°41'21" E). These areas were selected due to their notable fig production and biodiversity. The collected samples were intended for mycological analysis to assess the diversity and prevalence of fungal contaminants associated with dried figs.

Mycological analysis of dried figs samples by 'direct plating' method

Specific culture medium Dichloran (18%) Glycerol agar (DG18) was selected for the mycological analysis of dried figs, as recommended by Heperkan *et al.*, (2012). The epidermis of the dried figs was sterilized with 70% ethanol (v/v) to eliminate exogenous conidia. The figs were then cut into five

pieces and placed directly on the surface of the DG18 culture medium. The plates were incubated at 28°C for seven days. After incubation, the isolates obtained were purified by successive subculturing on the same DG18 culture medium pure strains were obtained.

Purification of the fungal isolates

The monospore culture method was used to obtain a pure culture from fungal conidia by spreading them on DG18 medium, which promotes the growth of ochratoxigenic and aflatoxigenic fungal species, specifically *Aspergillus niger* aggregates (Zhang *et al.*, 2024). One milliliter of the spore suspension was taken and introduced into a tube containing 9 mL of sterile distilled water. This process was repeated as many times as necessary to achieve the desired dilution. From the last two dilutions (10^{-3} , 10^{-4}), 1 mL was taken and spread on PDA medium, which served as the subculture medium. After 24 hours of incubation at 28°C, spores in the process of germination were identified and limited using a binocular magnifying glass. These spores were carefully collected, 3 to 4 conidia at a time, and transferred to new Petri dishes containing PDA medium. The dishes were then incubated at 28°C for 7 days.

According to Faheem *et al.*, (2023), each isolate was cultured in the center of Petri dishes containing PDA medium and then incubated at 28°C for 5 to 7 days. In the cases of contamination by other fungal strains, the purification process was performed by repeatedly subculturing discs from the cultures onto fresh PDA medium under the same incubation conditions until pure strains were obtained.

Morphological studies of fungal strains

The preliminary identification of fungal species was based on descriptions obtained from macro morphological and micromorphological studies. The macroscopic cultural criteria observed included mycelial appearance such as the color and texture of the mycelia, the production of diffusible pigments. The micromorphological characterization was performed through microscopic observation using a light microscope. This analysis focused on key morphological features, including the appearance of the septate mycelium, the arrangement of conidia on the conidiophores, and the presence or absence of vesicles. These characteristics were used as diagnostic criteria for the identification of fungal isolates at the genus level (Pitt and Hocking, 1997).

DNA extraction and 16S rDNA amplification.

The spores of isolates representative of *Aspergillus* section *Nigri*, previously identified using macro- and micromorphological studies, were collected from 10- days-old grown DG18 medium and inoculated into a 150 mL Potato Dextrose Broth (PDB) in 250 mL conical flasks. The fungi were incubated at 30 °C with shaking 120 rpm for 72 hours. Whatman filter paper was used to harvest the mycelia through filtration. Genomic DNA extraction was performed following the method described by Diniz *et al.*, (2023).

One gram of fresh mycelia was ground using a precooled mortar and pestle. Exactly 1.5 mL of lysis buffer (containing 20 g/L Cetyltrimethylammonium bromide (CTAB), 1.4 mol/L NaCl, 0.1 mol/L Tris, 0.02 mol/L Na₂EDTA, pH 8) was added

to the mycelial powder and incubated at 65°C for 40 min in a shaker. The suspension was then centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was transferred to a new tube, and 0.8 volumes of 3M sodium acetate and 0.5 volumes of cold isopropanol were added.

The solution was mixed thoroughly by inversion and incubated overnight in a freezer. The resulting solution was centrifuged at 12000 rpm for 5 min, and the pellets were washed with aqueous ethanol (70%v/v). Finally, the dried pellet was dissolved in 100µL TE buffer.

Genomic DNA was then amplified by polymerase chain reaction (PCR) using the universal primers for the International Transcript Spacers (ITS) regions. The primer sequences were as follows:

Forward primer: GCAAGTCTGGTGCCAGCAGCC

Reverse primer: CTTCCGTCAATTCCTTTAAG

PCR amplification was performed according to the following protocol: initial denaturation at 94°C for 5 min, followed by 36 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 7 minutes. The PCR products were visualized on 1.2% agarose gel and sequenced (Microsynth, Swiss).

Ochratoxigenic ability of isolates belonging to *Aspergillus* section *Nigrii*

Czapek Yeast Extract Agar (CYA) medium was used to detect ochratoxigenic strains according to Saito and Machida, (1999). The fluorescence coloring of colonies grown on CYA medium can be used to differentiate *Aspergillus niger* an ochratoxigenic specie, from *A. tubingensis*, which is a non-ochratoxigenic fungal specie.

Each mycelial plug from DG18 medium was placed on the center of a Petri dishes containing the test medium and incubated for 6-7 days at 28°C. The Petri dishes were visually examined for colony morphology and pigmentation. Subsequently, they were observed under a UV lamp at 365 nm in a complete darkness, where colonies exhibiting fluorescence with a blue hue were noted. The intensity of fluorescence was estimated subjectively to evaluate the ochratoxigenic potential of the 'black *Aspergilli*' strains.

Physicochemical analysis

Measurement of pH

According to Joseph and Raj, (2003), the pH of the sample was determined by adding 10mL of hot distilled water to 2g of each sample. The mixture was crushed, filtered, and allowed to cool. The pH value of the supernatant was measured directly using a pH meter.

Determination of the titratable acidity

The titratable acidity was determined using a sodium hydroxide solution (NaOH, 0.1 N) in the presence of phenolphthalein as an indicator, as described by Piga *et al.*, (2004). A 25 g of crushed dried figs was placed in a conical flask with 50 mL of distilled water. The mixture was heated in a water bath for 30 minutes. Afterward, 25 mL of the

filtrate was mixed with 0.3 mL of phenolphthalein and titrated with the 0.1 N NaOH solution until a persistent pink color was observed.

Determination of water activity

The water activity for dried fig samples was assessed following the method described by Piga *et al.*, (2004). The measurement was performed using a modern equipment (Novasina.) Five grams of dried figs were crushed and placed in glass crucibles and heated in an oven at 103 °C ± 2 °C for 24 hours. Afterward, the samples were placed in a desiccator to prevent rehydration. This process was repeated until a constant weight was achieved.

Determination of carbohydrate

1g test sample of powdered figs was mixed with 50 mL of distilled water and stirred for 45 min at room temperature. The mixture was filtered, and 200 µL of the filtrate was combined with 300 µL of 3,5-dinitrosalicylic acid (DNS) reagent. The mixture was then heated in a water bath at 100 °C for 5 min. After heating, 1.5 mL of distilled water was added, and the solution was left in the dark for 15 min. The absorbance was measured at a wavelength of 530 nm using a spectrophotometer, with glucose used as the standard (Miller 1959).

Protein determination

The total protein content of the fig sample extracts was determined according to Bradford (1976). For each 500 µL of extract, 2 mL of Bradford reagent was added. The mixture was homogenized and kept in the dark at room temperature for 20 minutes. The absorbance was then measured at 595 nm, with bovine serum albumin (BSA) used as the reference protein.

Mycotoxins analysis of dried figs collected from Bejaia with HPLC/FLD method

According to Norian *et al.*, (2015), HPLC analysis was performed using a Waters 1525 binary HPLC system connected to 2475 fluorescence detector, with data acquisition managed through Breeze software. Separation was achieved using a C₁₈ analytical column (150 × 4.6 mm, 5 µm). The samples were analyzed with a 20 µL injection volume, and the flowrate was maintained at 1.0 mL/min throughout the analysis. The excitation wavelength for aflatoxins was set at 365 nm, while the emission wavelength was 445 nm. For ochratoxin A (OTA), the excitation wavelength was 333 nm, and the emission wavelength was 443 nm. Linear isocratic elution chromatography was performed using the solvent system water: methanol: acetonitrile (6:2:2 v/v/v) at 40°C for aflatoxins, and water: acetonitrile: acetic acid (99:99:1 v/v/v) at 30 °C for ochratoxin A. For aflatoxins, online photochemical derivatization was carried out using a commercially available system (UVE LCTech GmbH, Dorfen, Germany) which was placed between the separation column and the fluorescence detector.

According to Heparkan *et al.*, 2012, the determination of aflatoxins involved mixing 50 g of fig sample with 40 mL of HPLC-grade water, 160 mL of HPLC-grade methanol, and 5 g

of sodium chloride. The mixture was then blended at high speed for 5 minutes using a Waring 8011S, blender (Torrington, CT, USA) to obtain a homogeneous sample. After mixing, the slurry was filtered through filter paper. Subsequently, 20 mL of the filtrate was diluted with 130 mL of phosphate-buffered saline (PBS) solution. The diluted solution was filtered through a glass microfiber filter, and 100 mL of the filtrate was passed through an immunoaffinity column. Aflatoxins were eluted from the column by gravity, using 2 mL of HPLC-grade methanol followed by 2 mL of HPLC-grade water. The eluate was collected into a glass vial at a flow rate of approximately 5 mL/min.

According to Herparkan *et al.*, 2012, the determination of ochratoxin A (OTA) was performed by mixing 25 g of fig sample with 40 mL of pure water, 60 mL of acetonitrile, and 0.3 g of sodium bicarbonate (NaHCO₃). The mixture was blended at high speed for 5 minutes using a Waring 8011S blender (Torrington, CT, USA) to achieve a homogeneous sample. After blending, the slurry was filtered, and 10 mL of the filtrate was mixed with 40 mL of phosphate-buffered saline (PBS) solution. The resulting solution was filtered through a glass microfiber filter (Whatman, Inc., Clifton, NJ, USA), and 40 mL of the filtrate was passed through an immunoaffinity column. OTA was eluted from the column by passing 1.5 mL of HPLC-grade methanol: acetic acid (98:2 v/v) followed by 1.5 mL of HPLC-grade water. The eluate was collected into a glass vial using gravity, at a flow rate of approximately 5 mL/min.

Results and discussion

Mycological analysis of fungal isolates from dried figs samples collected from Bejaia

According to the results of our mycological analysis presented in Figs. (1), (2), (3), and Table (1), the two studied varieties, 'Thaamriouth' and 'Abarkan,' originating from Bejaia, exhibited significant growth of filamentous fungal species. The macroscopic examination of the strains after 10 days of incubation at 28°C revealed powdery colonies with vibrant and diverse colors and production of diffusible pigments exactly on Potato Dextrose Agar medium "PDA" for *Aspergillus* section *Nigrii* (Figs. 1 and 2).



Fig 1. Cultural appearance of black fungal strains after 7 to 10 days of incubation at 28 °C on DG18 media.

Our analysis highlights the dominance of a fungal flora composed predominantly of strains exhibiting a characteristic black coloration, specific to microscopic fungal strains of the genus *Aspergillus* section *Nigri* (Figs. 1 and 2). This fungal group was detected in approximately 70% of the dried fig samples analyzed, while representatives of the *Flavi* section were detected at 05%. Additionally, other fungal flora, such as *Mucorales* strains with 15% and *Penicillium* isolates were

detected in 10 % (Table 1). Observations under a magnifying glass and optical microscope revealed the presence of siphoned (non-septate) mycelia, suspected to belong to *Mucorales* strains, based on the macro- and micromorphological characteristics described by Awad *et al.*, (2023). These findings suggest poor storage conditions, such as suboptimal temperature and humidity, which facilitate the growth of these species. Furthermore, the results confirm that *Mucorales* strains can proliferate under conditions similar to those favoring *Aspergillus* isolates, including those producing ochratoxin A.

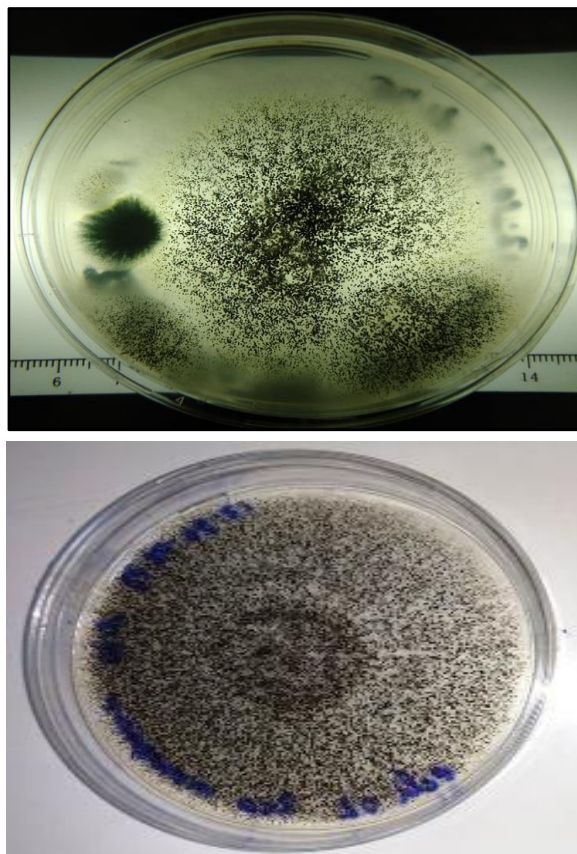


Fig 2. Macro morphological aspect of fungal strains proliferated after 7 to 10 days of incubation at 28°C on PDA and DG 18 media.

Table 1. Frequency presence of fungal isolates in dried fig samples

Fungal isolates	Frequency of presence in dried fig samples (%)
<i>Aspergillus</i> section <i>Nigrii</i>	70
<i>Aspergillus</i> section <i>Flavii</i>	05
<i>Mucorales</i> strains	15
<i>Penicillium</i> strains	10

On the other hand, the variety 'Abarkan' revealed the presence of aspergillate heads with long, septate conidiophores capped by aspergillate heads formed by two vesicles (biserial species). These characteristics closely align with those of *Aspergillus* section *Nigri* (Fig. 2). However, the morphological differentiation of species within the genus *Aspergillus* is challenging. While species identification based on morphological characteristics is possible, this method has notable disadvantages, including being time-consuming and not always straight forward (Rodrigues *et al.*, 2011).

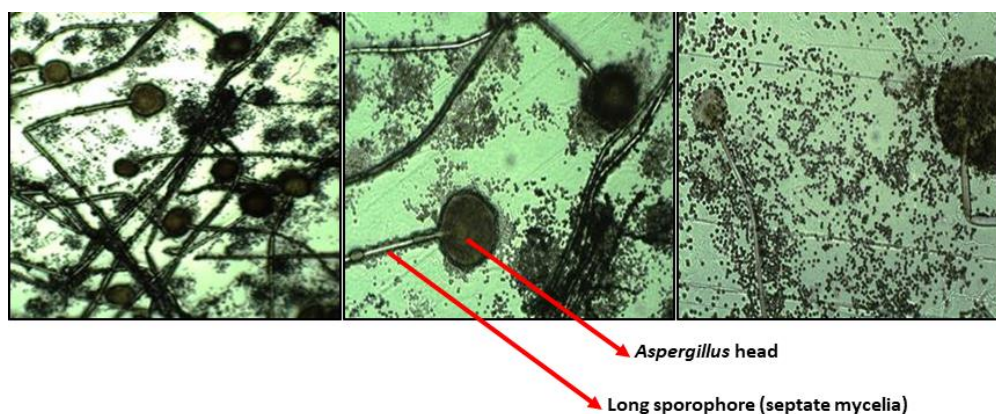


Fig 3. Micromorphological aspect of fungal strains under optical microscope (G*40) with the appearance of a long mycelium and an aspergillus head.

In addition, the ochratoxigenic species of the genus *Aspergillus* belonging to the section *Nigri* are closely related fungi, and a combination of specific characteristics has been suggested for their identification (Rodrigues et al., 2011). Plates incubated under optimal conditions at 28°C for 5 to 7 days exhibited impure cultures characterized by significant fungal growth, presenting as stems with dark-colored heads (Fig. 2). To obtain homogeneous colonies purified of blackish colonies, a series of successive subcultures was performed using the direct method with a platinum loop. The observation of a fresh smear on a slide under a light microscope equipped with a camera at magnification of 10x and 40x revealed the presence of black *Aspergillus* heads, a dark conidiophore, siphoned hyphae, and black exogenous spores. Based on its morphological and cultural characteristics, the fungal strain is suspected to belong to the *Aspergillus niger* aggregate (Fig. 3), (Awad et al., 2023). Significant fungal growth was observed after incubation at optimal temperatures of 25°C and 28°C for 7 days. Visual inspection of the plates showed heterogeneous morphological characteristics of the fungal strains, necessitating purification by subculturing (Fig. 1). After purification and observation of black colonies suspected to be *Aspergillus* section *Nigri*, further examination under an optical microscope at magnifications of 10x and 40x revealed the presence of mycelia with long conidiophores, capped by dark heads formed by two vesicles (Fig. 3).

According to Awad et al., (2023), these characteristics closely resemble of *Aspergillus niger* aggregates (commonly referred to as the *Aspergillus niger* group). During the progression of the fungal culture, a variation in coloration of the colonies was observed, transitioning from white to yellow and eventually to black. This progression suggests immature *Aspergillus* colonies that mature after a specific incubation period, aligning with the macro- and micromorphological characteristics of this genus as described by Awad et al., (2023) (Fig. 2). The persistence of the black coloration across the entire surface of the plate was indicative of *Aspergillus niger*, a storage-associated fungal species frequently isolated from dried fruits. This species is known to contribute to post-harvest contamination and is a significant producer of ochratoxin A (OTA), as reported by Medina et al. (2021).

As a result, the dominance of this genus in the fungal flora contaminating the analyzed samples was anticipated, based on studies reported by Riba et al., (2013). Fungal identification was further refined using morphological

identification keys described by Pitt and Hocking (1997). Macroscopic characteristics, including colony color, texture, and growth rate, were first assessed. This was followed by microscopic examination of features such as the presence of septate mycelia, the organization of spores on conidiophores, and the presence or absence of vesicles as key criteria for distinguishing among fungal genera and species (Pitt and Hocking, 1997).

Detection of black *Aspergillus* species using PCR method

A single band of approximately 600 bp was observed for six isolates (Fig. 4). A Similarity search revealed that isolates AY1:27-582, AY3:30-579 and AY5:44-562 showed maximum similarity with *A. niger* while isolates AY2:35-580, AY4:45-564 and AY6:52-583 showed maximum similarity with *A. awamori*. These isolates were submitted to GenBank with the accession number AY1:27-582, AY2:35-580, AY3:30-579, AY4:45-564, AY5:44-562 and AY6:52-583. Using FLA1 /FLA2 primers, the ITS1-5.8S-ITS2 region of the ribosomal DNA was successfully amplified in 06 isolates, which were presumptive representatives of *Aspergillus niger* or *A. awamori* confirming their identity as either *A. niger* or *A. awamori*. Descriptive taxonomic keys were employed as the initial fungal isolation criteria, aiding in the selection of presumptive *Aspergillus* section *Nigrii* isolates. Colony growth initially appeared as white mycelium that expanded radially to cover the entire surface of the media. Upon sporulation, the white colony turned a dark black color due to conidia formation, progressing outward from the center. This observation aligns with the protocol developed by González-Salgado et al., (2008). The primers were designed to target a highly variable region of the International Transcript Spacer (ITS) and specifically amplified the *A. niger* target sequence. Furthermore, the results obtained from our molecular identification were consistent with those reported by Robert et al., (2020).

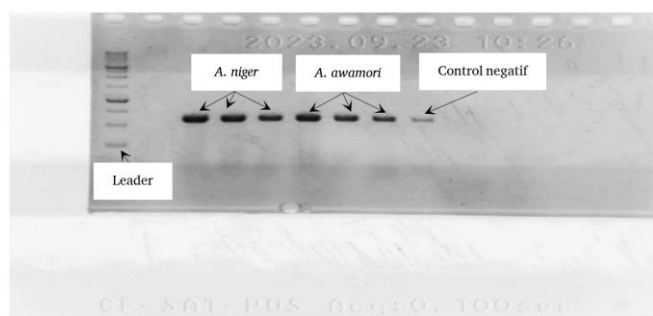


Fig. 4. A gel image showing 600 pb markers of ITS1-5.8-ITS2 region of *Aspergillus* section *Nigri* isolates from dried figs amplified using FLA 1/FLA2, while *M* is a 100 pb DNA molecular ladder. *A. niger*: *Aspergillus niger*, *A. awamori*; *Aspergillus awamori*.

Mycotoxins analysis in dried figs collected from Bejaia with HPLC/FLD method

The co-occurrence of ochratoxin A and aflatoxins was investigated by HPLC/FLD in dried figs samples. The differences in aflatoxins and ochratoxin A levels in dried figs samples between areas were determined by Heperkan *et al.*, (2012). The results as chromatograms of HPLC/FLD were presented in the Figs. (5) and (6) that showed the absorbance in function of time (min) the four peaks in the Fig. (5) presented the four types of Aflatoxins standards such as Aflatoxin B2 with retention time at 7.093 min, Aflatoxin G2 at 8.503 min, Aflatoxin G1 at 9.55 min and the aflatoxin B1 at 11.687 min. In comparison with the results of our studies of dried fig samples that showed Aflatoxin B2 at 7.433, Aflatoxin G1 at 8.991 min, Aflatoxin G2 at 10.153 min and the aflatoxin B1 at 12.528 min but for the ochratoxin A present in dried fig samples as showed in Fig. (6) was detected

at 6.499 min. In our study dried figs samples were collected from Bejaia in the season of autumn with humid and high temperature as climate conditions. This is the first study of contamination of two varieties of dried figs 'Thaamriouth' and 'Abarkan' by aflatoxins and ochratoxin A as a bad condition of storage and conservation. According to these results the contamination of dried figs samples by both of Aflatoxins and ochratoxin A, in comparison with the European regulations (European commission 2023). None of the analyzed dried fig samples contained detectable levels of aflatoxins or ochratoxin A. The absence of these mycotoxins may be attributed to the variable quality of the sampled figs collected from the Bejaia region. Some of the samples belonged to lower nutritional quality categories typically intended for animal feed, which makes them less favorable for fungal contamination. The relatively low contamination levels observed suggest a limited exposure to these toxins in the current sampling. However, even with low detected levels, the potential health risk associated with long-term consumption cannot be entirely ruled out, particularly for vulnerable populations. Therefore, continued monitoring remains important to ensure food safety (Trucksess and Scott., 2008).

In this context, understanding the natural occurrence and contributing factors of ochratoxin A (OTA) contamination in dried fruits becomes essential. The natural occurrence of ochratoxin A (OTA) in dried fruits, including figs, has been widely documented in the literature. Several surveys have also reported the presence of both aflatoxins and ochratoxin A in dried figs. Contamination typically begins during the drying process, particularly when figs are left to sun-dry on the tree or on the ground.

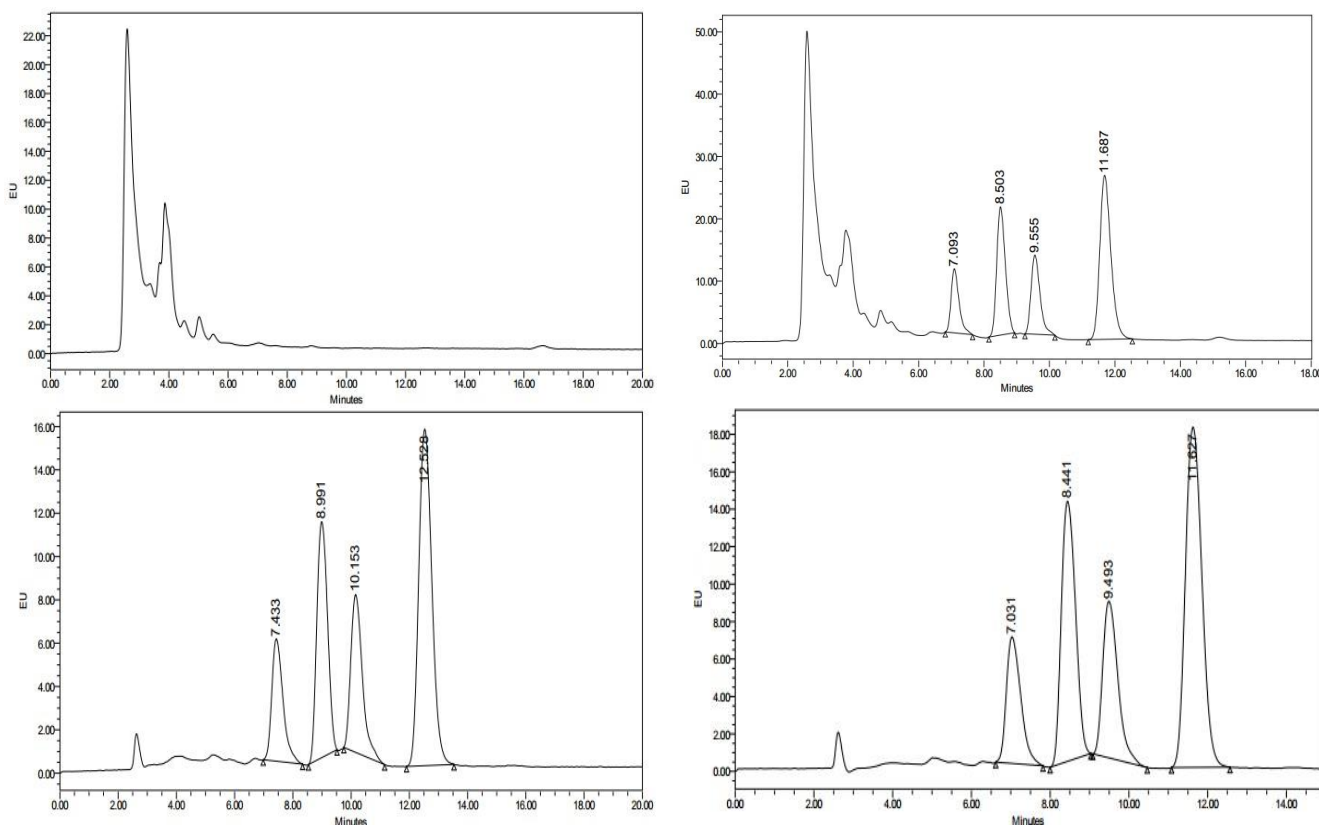


Fig. 5. Aflatoxins assay chromatograms of dried figs samples collected from Bejaia in test solution containing aflatoxin B1, B2, G1 and G2 standard. E.U: Absorbance Unit.

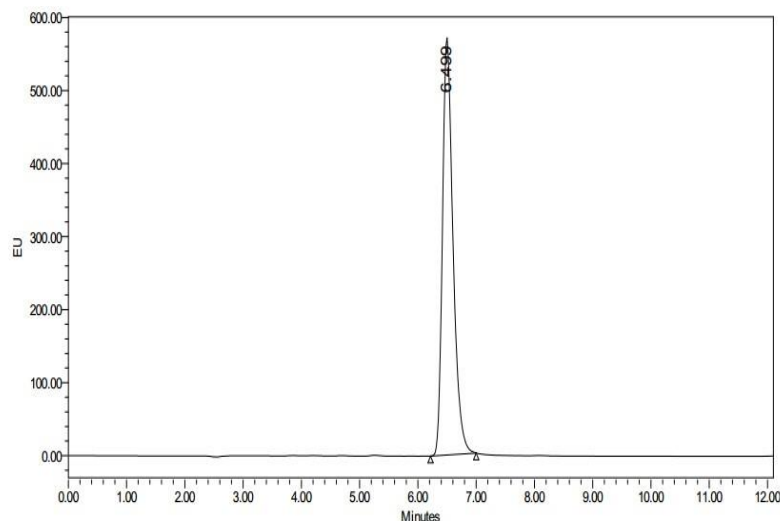


Fig. 6. Ochratoxin A essay chromatogram of dried figs samples collected from Bejaia in test solution containing ochratoxin A standard. E.U: Absorbance Unit.

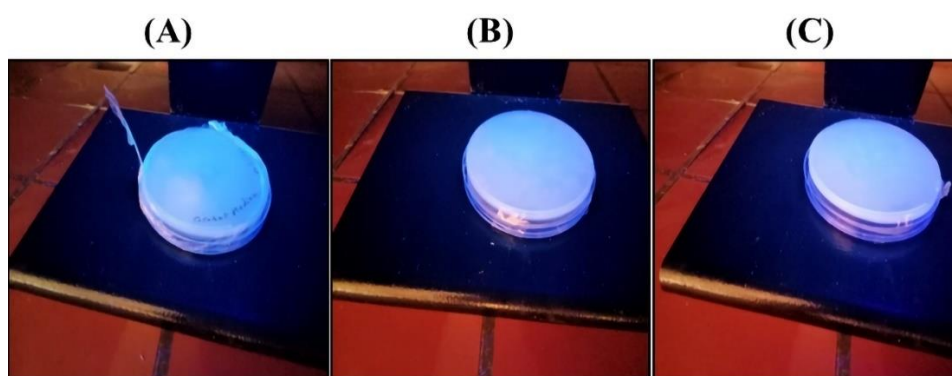


Fig. 7. Rapid detection of ochratoxigenic abilities of isolates belonging to *Aspergillus section Nigrii* under U.V lamp (365nm) with blue fluorescence, A) High toxicogenic potential, B) Middle toxicogenic potential, and C) Weak toxicogenic potential.

These conditions, especially when combined with high humidity and elevated temperatures, create a favorable environment for the growth of toxigenic fungi such as *Aspergillus* and *Penicillium* species. Improper handling, prolonged drying periods, and exposure to soil and insects further increase the risk of fungal growth and subsequent mycotoxin production. As such, both pre-harvest and post-harvest practices play a critical role in determining the final mycotoxin content in dried figs (Trucksess and Scott, 2008). Similar concerns extend to other dried fruits, as aflatoxin B1 has also been detected in dried apricots and prunes (Ozer *et al.*, 2012). In recognition of these health risks, the European Commission has set maximum allowable limits for these toxins in dried fruits: Levels of 2 $\mu\text{g}/\text{kg}$ for OTA in dried apricots, grapes, and figs, and 2 $\mu\text{g}/\text{kg}$ for aflatoxin B1 (with a combined limit of 4 $\mu\text{g}/\text{kg}$ for total aflatoxins B1, B2, G1, and G2) (European commission, 2023).

In contrast to other studies, the absence of detectable mycotoxins in our samples, despite evidence of fungal contamination, may be due to favorable handling and drying conditions that limited toxin production by the fungi present.

Ochratoxigenic capacity of isolates belonging to *Aspergillus section Nigrii*

The results of rapid detection of ochratoxigenic abilities of isolates belonging to *Aspergillus section Nigrii* under U.V lamp

(365nm) with blue fluorescence are presented in Fig. (7). With (A) the strain producer with high ochratoxigenic potential, (B) the middle strain in the production of ochratoxin A and the (C) is the weak strain in production. After 7, 10 days of incubation at 28°C, an important growth of *Aspergillus section Nigrii* on Czapek Yeast extract Agar (CYA) medium is observed. The aim to detect ochratoxigenic abilities of these fungal strains, the colour of fluorescence blue green is used as a reference. To distinguish between ochratoxigenic and non ochratoxigenic fungal species, for ochratoxigenic strains blue green colour was observed and for no-ochratoxigenic strains absence of colour, these results are the same as those obtained by Saito and Machida,(1999).

Physicochemical studies of dried figs

Determination of Hydroxide Potential (pH) and Titratable Acidity

The results of pH and titratable acidity measurements are presented in Table (2). Both parameters are closely related and are critical factors influencing the growth of microorganisms within the fruit, as well as determining the optimal harvest time and ripeness (Chimi *et al.*, 2005).

pH measures the concentration of hydrogen ions in the fruit, with lower pH values indicating higher acidity. In our dried fig samples, pH values ranged from 4.38 to 4.76, indicating a slightly acidic environment. Such as acidity

inhibits the growth of most bacterial species but can favor the development of fungal strains (Doukani and Tabak, 2015). This observation is consistent with previous findings on dried figs (Villalobos et al., 2019, Lachtar et al., 2022) and with the pH values reported by Askari et al. (2012) (pH 4.9,5.4) which ranged from 4.9 to 5.4, taking into account the slight differences observed. These variations are likely due to factors such as geographic origin, climatic conditions, and the ripening stage at harvest (Brzonkalik et al., 2012).

Complementing the pH results, titratable acidity provides a measure of the total concentration of organic acids in the fruit. Our samples had an average titratable acidity of 0.50%, higher than the values reported by Askari et al. (2012) (0.26,0.38%) but lower than those found by Bachir Bey et al. (2017) (1.92%). This organic acidity, mainly due to compounds such as citric acid (up to 3% of dry weight) (Askari et al., 2012), contributes to the slightly acidic pH observed. Variations in acidity can be attributed to genotypic differences, harvesting time, climatic conditions, and ecological factors affecting fig trees (Simsek and Yildirim, 2010; Doukani and Tabak, 2015).

Thus, the pH and titratable acidity together provide complementary information about the chemical environment of the figs, which in turn affects microbial stability and overall fruit quality.

Moisture and water content

The results of moisture and water content were presented in Table (2). According to Askari et al., (2012), the totality of water contained in fresh figs could not be eliminated by the drying process, this is due to the fact that the fruits were composed of free water and bound water, the latter remains attached to hydroxyl, carbonyl and amino groups of the compounds of simple sugars, complex sugars...etc. Our results included in the range from 21.13 to 22.10%, in agreement with those presented by Bachir Bey et al., (2017) and to those of Benemechermene et al., (2023). According to Askari et al., (2012) the international standard of dry fig, which states that this moisture content should not be higher than 30%. This could be explained by geographical and climatic variations as well as the harvesting period, variety and type of irrigation that varies the moisture content as explained

Benemechermene et al., (2023). In their study, characteristics such as plant age, vegetative cycle period and even genetic factors can influence moisture contamination. According to Doukani and Tabak (2015), moisture levels are very favorable for the growth of ochratoxigenic strains belonging to *Aspergillus* section *Nigrii*. Samples that recorded significant growth at high rates were those exposed or found at moisture levels reflected by their results (between 16% and 20%) (Doukani and Tabak, 2015).

Carbohydrate determination

The Carbohydrate results were presented in Table (2). Our evaluation revealed an average level of 54.51% and 79.35% for carbohydrate in 'Thaamriouth' and 'Abarkan' varieties, respectively. Khairuddin et al., (2017) reported the value of sugars at 57.36%, and Eshak and Arafa (2018) who concluded that the sun-dried fig samples recorded a value of (76.90%). Carbohydrates form the main part of our diet and are provided mainly by fruits (Matsuzawa et al.,2015).The concentration of carbohydrates in fruits is of great interest, because of their influence on organoleptic properties and is a character for evaluating ripening, it also depends on the conditions of the stability and preservation of fruits (Chen et al.,2022). Dried figs contain significant amounts of carbohydrates, a 100 g serving of this fruit provides 63.87 g of carbohydrates including 47.92 g of sugars 24.79 g of glucose, 22.93 g of fructose, 5.07 g of starch, 0.13 g of galactose and 0.07 g of Saccharose according to the study by Lai et al., (2012).

Protein determination

The results of proteins levels were presented in Table (2). The dried fig protein resulted in a value of 3.84% for 'Thaamriouth' variety and 6.17% for 'Abarkan' one. The previous studies of Chauhan et al., (2015), reported that dried figs contain only 3.01% protein, while Eshak and Arafa (2018) noted in his research that sun-dried samples recorded protein content with a value of 7.45%. Compared to these studies, our results clearly fall within the reported range but tend to be lower than the maximum values observed.

Table 2. Physicochemical properties of dried figs "Thaamriouth" and "Aberkan" varieties

Varieties	Acidity (%)	Water activity	Brix (%)	Dry extract (%)	Humidity (%)	Carbohydrates (%)	Protein level (%)	Ph
Thaamriouth	0.50 ± 0.09 ^a	0.65 ± 0.04 ^a	72.00 ± 2.46 ^a	76.03 ± 2.17 ^a	22.13 ± 2.17 ^a	54.51 ± 2.85 ^b	3.84 ± 0.83 ^a	4.49 ± 0.14 ^a
Aberkan	0.47 ± 0.08 ^a	0.67 ± 0.03 ^a	71.00 ± 2.97 ^a	78.88 ± 2.50 ^a	21.10 ± 1.99 ^a	79.35 ± 5.65 ^a	6.18 ± 2.25 ^a	4.52 ± 0.18 ^a

Note: ^{a,b}Mean ± SD values in the same column heading followed by different alphabet are significantly different (Student t-test).

pH: Potential of hydrogen.

Conclusion

Microscopic fungi are ubiquitous microorganisms of which some species can contaminate many agricultural commodities intended for human or animal consumption. The fungal flora isolated from dried figs 'Thaamriouth' and 'Abarkan' varieties was dominated with 70% of 'Black *aspergilli*' species following by some species belonging to *Penicillium* genus and some *Mucorales* strains as a bad conditions of storage and conservation. The physicochemical analysis reveals that the

dried figs is favorable micro ecosystem for fungal growth due to the high carbohydrates concentration. The determination of levels contamination by Aflatoxins and Ochratoxin A using HPLC/FLD method revealed none detectable levels. Despite the absence of detectable aflatoxins and ochratoxin A in the fig samples, the potential risk to human health cannot be ruled out, particularly due to the presence of toxigenic fungal species. Therefore, proper agricultural and storage practices remain essential, using essential oils with high antifungal potential and mycotoxin destruction ability for future studies.

Acknowledgment

We thank the dried figs organisation of Beni maouche director and workers for the kind gift of dried figs samples. We are grateful to the Algerian ministry of high education and scientific research (DGRSDT) for the financial support, My supervisor Dr. BOUKHALFA Farid and all the staff of Biophysic, Biochemistry, Biomathematic and Scientometric laboratory. Dr. Javad FEIZY, Dr. Behrouz Ghorani and Alireza Rahimian coordinators of international relations and all the staff of Research Institute of Food Science and Technology in Iran.

Author contributions

Younes ARROUL: Data collection, Data analysis, Data analysis and interpretation, Presentation and discussion of the results, Writing the draft of the manuscript; **Farid Boukhalfa:**

Data analysis and interpretation, Revising and editing the article; **Mohand Teffane:** Data analysis and interpretation; **Amar Riba:** Supervising the study, Presenting the research idea and study design; **Amira Oufighou:** Approval of the final version, Revising and editing the manuscript; **Abdeslem Taibi:** Data analysis and interpretation; **Ahcene Kadi:** Analysis and interpretation of the data; **Javad Feizy:** Supervising the study, Presenting the research idea and study design, Revising and editing the; **Fahimeh Mahmoodi:** Supervising the study, Data collection; **Sareh Hadjiabadi:** Supervising the study, Data analysis and interpretation.

Conflicts of interest

The authors declare no conflicts of interest, financial or otherwise.

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