Bulletin of Faculty of Science, Zagazig University (BFSZU)
e-ISSN: 1110-1555
Volume-2023, Issue-3, pp-1-10
Research Paper DOI: 10.21608/bfszu.2021.102552.1095

Isolation and identification of ochratoxigenic fungi from food and feed commodities in Sharika governorate.

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ABSTRACT: *Ochratoxin A (OTA), a mycotoxin that contaminates food and feed products, is naturally produced by Aspergillus and Penicillium species. Nephrotoxicity, hepatotoxicity, neurotoxicity, teratogenicity, mutagenicity, and immunotoxicity are only a few of the serious health risks caused by OTA. In this investigation, a total of 69 food and feed samples were collected from retail market in Sharika governorate and used as a source of the isolated fungi. Two hundreds and ninety-two fungal strains were isolated, morphologically identified and their ability to produce OTA was tested. Among these fungi, only 60 strains, belonging to 3 fungal species, were able to produce OTA. Three fungal strains viz. A. fumigatus H11, A. terreus DF6, and A. niger T2, isolated from samples of hazelnuts, dried figs, and tomatoes, respectively; were the most potent ochratoxigenic strains among these 60 ochratoxigenic fungal strains. These three fungal strains were molecularly identified, and their sequences were successfully deposited in the Genebank with accession numbers; A. fumigatus MW411792.1, A. terreus MW512515.1 and A. niger MW513392.1.*

Key words: Mycotoxins, Ochratoxin A (OTA), *A. fumigatus*, *A. terreus, A. niger*.

Date of Submission: 04-12-2022 Date of acceptance: 01-03-2023

I. INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by certain fungi that contaminate agricultural products which cause a serious health hazard throughout the world **(Abrunhosa *et al.*,2010)**. Ochratoxin A (OTA), (N-[[(3R) -5-chloro-8-hydroxy-3-methyl-1-oxo-7-isochromanyl] carbonyl] - 3-phenyl-L-alanine) (Fig. 1), is one of the most potenet mycotoxins which was first discovered and chemically characterized in 1965 in a south African corn meal inoculated with A. ochraceous by Van der Merwe **(Van der Merwe *et al.*, 1965a, b)**. The subsequent researchers found that several fungal species of Aspergillus and Penicillium were able to produce OTA **(Ismaiel and Papenbrock 2015)**.

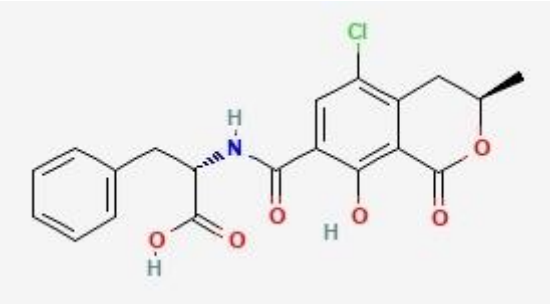


Fig. 1: Structure of OTA.

The presence of OTA has been noticed in corns, rice, soya, coffee, cocoa, bean, pea, and dried fruit **(Varga *et al.*, 2001)**, as well as corn derivatives like: flour, bread and pasta, beer **(El-Dessouki, 1992)**, grape juice, wine **(Zimmerli and Dick 1996)** and spices **(Rosa *et al.*, 2006)**. The fig, raisins and ground almond are also contaminated with OTA **(Ngundi *et al.*, 2006)**. OTA have been detected in animal meat, which feed in contaminated feed **(Jorgensen, 1998)**, milk and dairy products **(Rosa *et al.*, 2006)**. OTA contaminates different food stuff such as dried vine fruit, grape juices, pork, poultry, cereals, cereal products and chocolate. **(Hope and Hope, 2012)**. Depending on the climatic circumstances, different crops are contaminated by *Aspergillus* and

Penicillium species with varying distribution. The most significant abiotic elements that affect how these spoilage fungi grow and produce OTA in various agricultural commodities are water availability, temperature, and gas composition. Water activity could be the most critical factor influences the germination, growth and establishment of molds on these nutrients' worthy substrates **(Atumo, 2020)**. Warm and temperate zones are dominated by *Aspergillus* species, while colder locations are more frequently affected by *Penicillium* isolates. OTA production is higher at 0.98_{aw}, regardless of the temperature level, but its production tends to rise at the optimum temperature, between 25 and 30 °C in *A. ochraceus* (**Futagami *et al.*, 2011**). Not only water and temperature factors but also some of the countries are creating favorable conditions that lead to OTA production in various foods and feeds **(Atumo, 2020)**.

Mycotoxins have a wide range of possible short- and long-term health impacts, from an acute toxic response to long-term potential for teratogenic and carcinogenic effects. According to exposure evaluations of the number of nanograms (ng) of OTA taken per kilogram (kg) of body weight per day, human OTA exposure is higher in newborns, toddlers, and children than in adolescents and adults. Although OTA is nephrotoxic and teratogenic in animals, research on its effects on young children exposed during the time of reproduction and development is lacking. **(Bondy *et al*., 2018; Stevens and Tang, 1997)**.

2.Materials and Methods

2.1. Materials:

2.a. Food and feed samples for isolation of OTA-producing fungi

A total of sixty-nine samples consisting of almond (*Prunus dulcis*) (6 samples), cocoa (*Theobroma cacao*) powder (5 samples), peanuts (*Arachis hypogaea*) (3 samples), licorice (*Glycyrrhiza glabra*) (5 samples), hazelnut (*Corylus avellana*) (5 samples), walnut (*Juglans regia*) (5 samples), pistachio (*Pistacia vera*) (3 samples), raisins (*Vitis vinifera*) (4 samples), fresh grapes (*Vitis vinifera*) (1 sample), green coffee beans (*Coffea arabica*) (3 samples), roasted coffee beans (*coffea arabica*) (2 samples), nescafe (1 sample), wheat (*Triticum aestivum*) (3 samples), maize (*Zea mays*) (2 samples), dried figs (*Ficus carica*) (2 samples), fresh figs (*Ficus carica*) (1 sample), rice (*Oryza sativa*) (1 sample), barely (*Hordeum vulgare*) (1 sample), dried apricot (*Prunus armeniaca*) (2 samples), lima beans (*Phaseolus lunatus*) (2 samples), Cowpea (*Vigna unguiculata*) (1 sample), broad beans (*Vicia faba*) (2 samples), milk (1 sample), yogurt (1 sample), biscuits (4 samples), tomatoes (*Solanum lycopersicum*) (1 sample) and chicken feeds (2 samples) were used for isolation of OTA producers fungi. These samples were all collected locally and randomly from different markets (Shakira governorate, Egypt) during the period between April and June 2016.

2.b. Reagents and standard

All chemicals and solvents used in this study were of high degree of purity. The standard OTA was purchased from Sigma-Aldrich, Taufkirchen, Germany. Other solvents including *n*-hexane, dichloro methane, chloroform and methanol were used for extraction procedures and thin layer chromatographic (TLC) analysis.

2.c. Fungi used in the present study

Two hundreds and ninety-two fungal strains were isolated, morphologically identified and screened their ability to produce OTA. Three fungal strains identified as *A. fumigatus* H11, *A. terreus* DF6 and *A. niger* T2 were selected among 60 ochratoxigenic isolates according to their highest OTA-producing ability. They were isolated from hazelnuts, dried figs and tomatoes. These three fungi were identified by molecular tools.

2.d. Isolation of the experimented fungi

Isolation of OTA-producing fungi from different food and feed samples was carried out by direct plating (**Abdullah *et al*., 2002)** or dilution plating (**Nazir *et al.* 2014)** methods depending on the nature of the sample. The PDA plates of both methods were incubated at 30°C for 7 days. Each colony that appeared on the plate was considered as one colony forming unit (cfu). Representative plates were chosen for fungal purification by transferring each single colony into a sterile Petri dish containing potato dextrose agar (PDA) media **(Liu *et al*., 2009)** with a pH 5.6.

2.e. Screening of isolated fungal strains for their OTA production ability

The isolates from different food and feed samples were screened for their capability of OTA production. Fungal spores from 7-day old cultures of all strains were harvested separately by flooding of the slants with 10 mLof sterile saline solution with concentration 0.85% and gently scrapping off the spores with a sterile glass rod. The spore concentration was adjusted to 2.0×106 spores mL^{-1}using haemocytometer, and 1 mL of this spore

suspension was used as standard inoculum. Erlenmeyer conical flasks (250 mL), each containing 50 mL of fermented yeast extract sucrose (YES) broth with a pH 6 **(Frisvad and Samson, 2004)** were used in the present work. They were autoclaved. After cooling, each conical flask was inoculated with 1 ml spore suspension under aseptic conditions. Triplicate culture flasks for each fungal isolate were dark-incubated in static conditions at 30ºC for 10 days.

2.f. Analytical methods

2.f.a. Extraction of OTA from culture media

Extraction of OTA occurs by the addition of to the filtrate in order to remove fatty acids **(Valenta *et al.*, 1993 and Daradimos *et al.*, 2000)**, after which OTA was extracted from the defatted filtrate with an equal volume of dichloro methane **(Téren *et al.*, 1996)** that was shaken for approximately 30 min and allowed to stand for 30 min in a separating funnel. The dichloro methane layer was filtered over anhydrous sodium sulfate and then evaporated under vacuum till dryness.

2.f.b. Determination of OTA using thin layer chromatographic (TLC)

OTA was determined qualitatively using thin layer chromatographic (TLC) plates prepared according to **Stahl (1969)**. TLC plates are prepared by pouring a mixture of 10 gm of silica gel 60 GF-254 over a (20 × 20) glass plate. The plate was left to solidify at room temperature and was then dried at 110° C for 1h in an electric oven. The dry film of dichloro methane and the toxin was then dissolved in 250 µl of absolute methanol and spotted on TLC plates along with OTA standard solutions using glass capillary tubes approximately 2 cm away from the plate's margins. After that, the TLC plates were placed into a solvent tank containing a developing system consists of chloroform: methanol (93:7, v: v). the plates were left to dry at room temperature and then the spots of both OTA samples and standard were detected as a light-blue fluorescence under UV (254 nm) **(Téren *et al.*, 1996)**. The rate of flow (Rf) value of OTA was calculated by dividing the Distance traveled by compound (Ds) by the Distance traveled by the solvent front (Df) **(Snyder 2008)**. The detected spots of both OTA standard and extracted samples were scrapped off then eluted in 3 mL of methanol. OTA samples were centrifuged at 5000 rpm centrifugation for 15 minutes. The supernatant was used to quantify OTA concentration in solutions which estimated using UV spectrophotometer against 3 ml of absolute methanol as a control. Absorption of OTA was observed at 365 nm **(Nesheim, 1976)**. OTA concentration was then calculated from a standard curve.

2.g. Identification of the fungal isolates

Morphological identification of the different genera and species was made according to macroscopic and microscopic criteria in accordance with appropriate keys. **Moubasher, 1993 and Geiser *et al.*, 2007** were applied for *Aspergillus* species, **Lackner *et al.*, (2014)** for *Mucor* species, and **Leslie and Summerell (2006)** for *Fusarium* species.

Identification of the most ochratoxinogenic fungal strains; *A. terreus* (DF6), *A. fumigatus* (H11) and *A. niger* (T2), was confirmed molecularly based on the sequence of PCR-amplified ITS1-5.8S and ITS4 rRNA-gene analysis at The Animal Health Research Institute, Dokki, Giza, Egypt. Fungal DNA was extracted and isolated using QIAamp DNeasy Plant Mini kit instructions. The polymerase chain reaction (PCR) method was used to amplify the ribosomal rRNA gene (rDNA) before sequencing. The reaction mixture included the two universal fungal primers ITS1 (forward) and ITS4 (reverse). Gene amplification primers consist of the following composition: ITS1 (5' – TCC GTA GGT GAA CCT GCG G - 3'), and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC-3'). The purified PCR products (amplicons) were reconfirmed using a size nucleotide marker (100 base pairs) by electrophoreses on 1% agarose gel. Then these bands were eluted and sequenced with the incorporation of dideoxynucleotides (dd NTPs) in the reaction mixture. Each sample was sequenced in the sense and antisense directions using ITS1 and ITS4 primers **(White *et al.*, 1990)**. Sequences were further analyzed using Basic Local Alignment Search Tool (BLAST) program **(Altschul *et al.*, 1990)** from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of CLC Biosequence viewer (Version 7.7, Qiagen Aarhus, Denmark). The sequence size of the three potent fungal strains were successfully deposited in the Genebank with accession numbers; *A. fumigatus* MW411792.1, *A. terreus* MW512515.1 and *A. niger* MW513392.1.

3. Experimental results

3.a. Isolation of OTA-producing fungi

Two hundred and ninety-two fungal strains were isolated from sixty-nine food and feed samples under study.

3.b. Microbiological analysis and isolate identification

The purified fungal isolates were identified morphologically based on appropriate keys. They belonged to three genera *Aspergillus*, *Fusarium*, and *Mucor*. *A. flavus* and *A. fumigatus* were the most frequent isolates and detected in 50 food and feed samples. They showed the highest Fr% and RD%; 72.64 and 32.5 (*A. flavus*) and 71.01 and 27.39 (*A. fumigatus*), respectively (Table 1). *A. terreus* from 35 samples and *A. niger* from 32 samples recorded the relatively moderate Fr% and RD% (50.7, 18.83, 46.37, and 13.69, respectively). *Mucor* spp. and *Fusarium* spp. were isolated from 5 samples and 10 samples, respectively, were the minor components of the isolated fungal flora with Fr% (14.49 and 7.2, respectively) and RD% (5.1 and 2.39, respectively).

Table (1): The percentages of relative density (RD%) and frequency (Fr%) of fungal isolates.

Fungal isolates	Fr%	RD%
Aspergillus flavus	72.64	32.5
A. fumigatus	71.01	27.39
A. terreus	50.7	18.83
A. niger	46.37	13.69
Fusarium spp.	14.49	5.1
Mucor spp.	7.2	2.39

3.c. Screening of the ochratoxigenic isolates

Based on TLC investigation **(Figure 2)**, Rf (0.89) and UV spectrophotometric measurements at 365 nm, sixty fungal strains were found to be ochratoxigenic. They were grown in YES broth and statically incubated at 30°C for ten days. The strains were arranged in descending order according to their OTA potentiality (**Table** 2). The most efficient ochratoxigenic strains *A. fumigatus* H11, *A. terreus* DF6, and *A. niger* T2 were isolated from hazelnut (*Corylus avellana*), dried figs (*Ficus carica)*, and tomatoes (*Solanum lycopersicum*), respectively. The OTA-producing potentiality of three strains was elucidated by HPLC, as will be shown later. They were selected for further studies and identified molecularly**.**

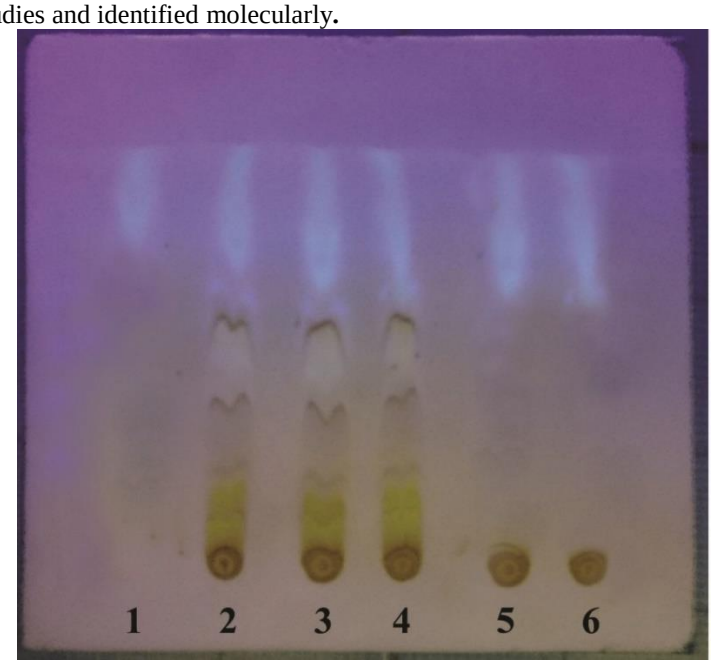


Fig. (2): TLC of the dichloro methane extracts of five fungal strains and standard OTA giving light-blue spots under UV lamp. Mobile phase, chloroform: methanol (93:7, v: v). **(1)** Standard OTA (Sigma-Aldrich, Taufkirchen, Germany), **(2)** *A. terreus* DF6 culture extract, **(3)** *A. terreus* T1culture extract, **(4)** *A. terreus* CF11culture extract, **(5)** *A. fumigatus* H11 and **(6)** *A. niger* T2.

Table (2): The ochratoxigenic fungal strains isolated from different food and feed samples understudy.

Fungal isolate	Source	OTA Conc. (µg ml^{-1})
***A. fumigatus* H11**	Hazelnut	0.503±0.002 [a]
***A. terreus* DF6**	Dried figs	0.394±0.007 [b]
***A. niger* T2**	Tomatoes	0.348±0.002 [c]
***A. terreus* T1**	Tomatoes	0.346±0.004 [c]
***A. niger* P22**	Pistachio	0.345±0.004 [c]
***A. niger* C5**	Cocoa	0.306±0.022 [d]
***A. terreus* CF11**	Chicken feed	0.217±0.003 [e]
***A. terreus* WT5**	Wheat	0.204±0.002 [e]
***A. fumigatus* H10**	Hazelnut	0.179±0.002 [f]
***A. terreus* H23**	Hazelnut	0.178±0.002 [f]
***A. terreus* DF2**	Dried figs	0.177±0.003 [f]
***A. niger* A12**	Almond	0.164±0.002 [f-g]
***A. terreus* H9**	Hazelnut	0.153±0.003 [g]
***A. terreus* CF17**	Chicken feed	0.152±0.003 [g]
***A. niger* BI16**	Biscuits	0.133±0.002 [h]
***A. niger* WT3**	Wheat	0.120±0.002 [h-i]
***A. niger* GC2**	Green coffee beans	0.118 ±0.002 [h-i]
***A. niger* MI5**	Milk	0.117 ±0.002 [h-i]
***A. niger* RI1**	Rice	0.116 ±0.003 [i-j]
***A. niger* BI13**	Biscuits	0.114±0.003 [i-j]
***A. terreus* PN8**	Peanuts	0.112±0.003 [i-j]
***A. terreus* CF3**	Chicken feed	0.112±0.003 [i-j]
***A. terreus* WT8**	Wheat	0.111±0.002 [i-j]
***A. terreus* W22**	Walnuts	0.107 ±0.002 [i-j]
***A. niger* H19**	Hazelnuts	0.106 ±0.002 [i-k]
***A. niger* GC8**	Green coffee beans	0.100 ±0.003 [k-l]
***A. niger* A11**	Almond	0.098 ±0.006 [l-m]
***A. terreus* A21**	Almond	0.089 ±0.002 [m-n]
***A. fumigatus* W2**	Walnuts	0.086±0.003 [n-o]
***A. terreus* H8**	Hazelnut	0.085±0.004 [n-o]
***A. fumigatus* P7**	Pistachio	0.083±0.003 [n-o]
***A. terreus* A20**	Almond	0.084 ±0.003 [n-p]
***A. terreus* PN9**	Peanuts	0.084±0.002 [n-p]
***A. niger* RC6**	Roasted coffee beans	0.083±0.002 [n-p]
***A. niger* W8**	Walnuts	0.072 ±0.003 [p-r]
***A. fumigatus* Y1**	Yogurt	0.066 ±0.002 [q-s]
***A. terreus* M4**	Maize	0.062 ±0.001 [r-t]
***A. niger* A2**	Almond	0.058 ±0.002 [r-u]
***A. terreus* H22**	Hazelnut	0.057 ±0.003 [r-u]
***A. fumigatus* W10**	Walnut	0.055±0.003 [r-u]
***A. terreus* C15**	Cocoa	0.052 ±0.004 [r-v]
***A. terreus* A17**	Almond	0.051 ±0.004 [s-v]
***A. niger* G5**	Fresh grapes	0.049±0.002 [s-v]
***A. niger* A18**	Almond	0.045 ±0.003 [s-w]

***A. fumigatus* A8**	Almond	0.043 ±0.003 $^{t-w}$
***A. niger* RC3**	Roasted coffee beans	0.038 ±0.002 $^{u-x}$
***A. niger* WT2**	Wheat	0.037 ±0.003 $^{v-y}$
***A. terreus* LB9**	Lima beans	0.032±0.002 $^{v-y}$
***A. niger* CF18**	Chicken feed	0.026 ±0.002 $^{x-a}$
***A. terreus* H4**	Hazelnut	0.025 ±0.003 $^{y-a}$
***A. niger* C2**	Cocoa	0.020 ±0.003 $^{y-b}$
***A. niger* CP4**	Cowpeas	0.020±0.002 $^{y-b}$
***A. niger* GC7**	Green coffee beans	0.020±0.000 $^{y-b}$
***A. niger* GC3**	Green coffee beans	0.019 ±0.002 $^{z-b}$
***A. niger* A4**	Almond	0.018±0.002 $^{z-b}$
***A. terreus* CF15**	Chicken feed	0.017±0.003 $^{z-b}$
***A. terreus* PN11**	Peanuts	0.017±0.002 $^{z-b}$
***A. niger* F2**	Fresh figs	0.012±0.002 $^{z-b}$
***A. terreus* CF2**	Chicken feed	0.057±0.080 $^{z-b}$
***A. terreus* CF8**	Chicken feed	0.006±0.002 $^{z-b}$

Values were represented as means±SD of three replicate analyses (n=3). Means in each column with similar letters are not significantly different at the 0.05 level of significance, according to Duncan's multiple range test.

3.d. Molecular identification of the most potent ochratoxigenic strains

The most potent OTA-producer strains *A. fumigatus* H11, *A. terreus* DF6, and *A. niger* T2 were selected for the further studies and molecularly identified based on the sequence of PCR-amplified 18S rRNA–28S rRNA gene analysis. Each sequence homologous was identified in the NCBI GenBank database using BLASTP and BLASTX programs. Figure 3 showed 1% agarose gel electrophoresis for PCR using DNA of the three isolates and the expected size of PCR product is 700 bp. The rDNA sequence of the eluted PCR products of fungal strains of H11, DF6 and T2 are more identical on a nucleotide level and are closely related to the species *A. fumigatus*, *A. terreus* and *A. niger*, respectively.

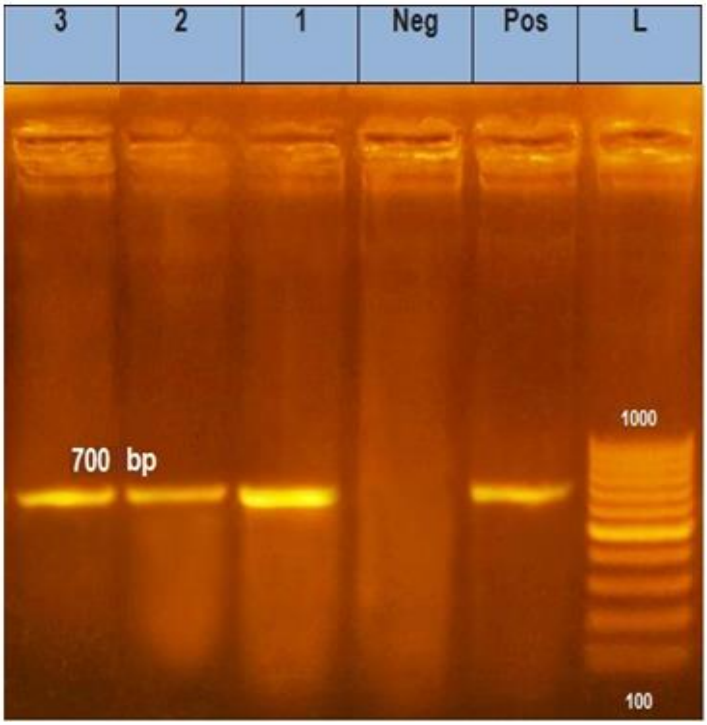
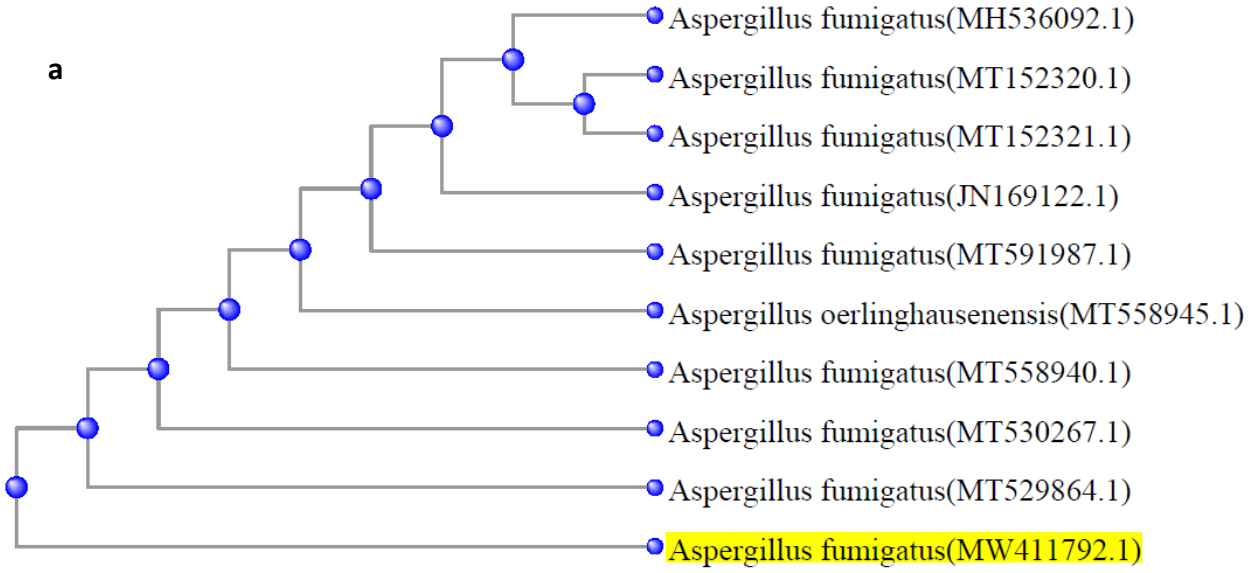
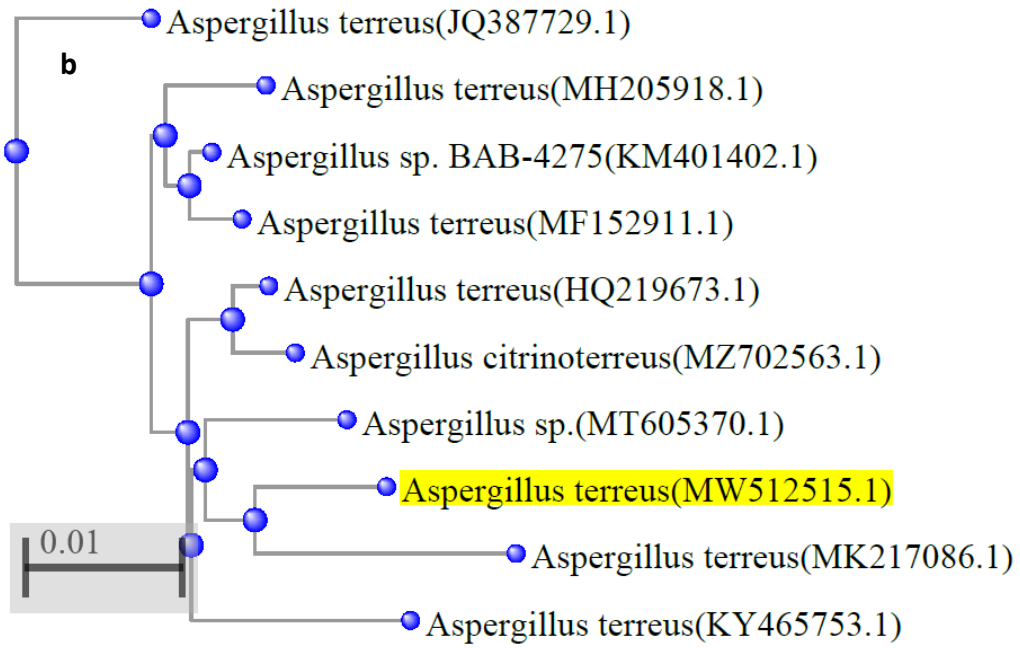


Fig. (3): Agarose gel electrophoresis (1%) of PCR-amplified 18S rRNA–28S rRNA gene analysis of the isolates. **(1)** Lane of *A. terreus* MW512515.1, **(2)** Lane of *A. fumigatus* MW411792.1, **(4)** Lane of *A. niger* MW513392.1, **(L)** Lane L, Gene Ruler™ 100 bp Plus DNA ladder (1000, 900, 800, 700, 600, 500, 400, 300, 200, 100 bp). **(Pos)** lane of positive standard and **(Neg)** lane of negative standard.

The retrieved sequences 611bp (*A. fumigatus* H11), 762 bp (*A. terreus* DF6), and 480 bp (*A. niger* T2) were deposited in the GenBank under accession numbers MW411792.1, MW512515.1 and MW513392.1, respectively. The phylogenetic analyses showed maximum homology 100% with *A. fumigatus* JN169122.1 (Figure 4a), 98.13% with *A. terreus* HQ219673.1 (Figure 4b), and 97.96% with *A. niger* MT735324.1 (Figure 4c).





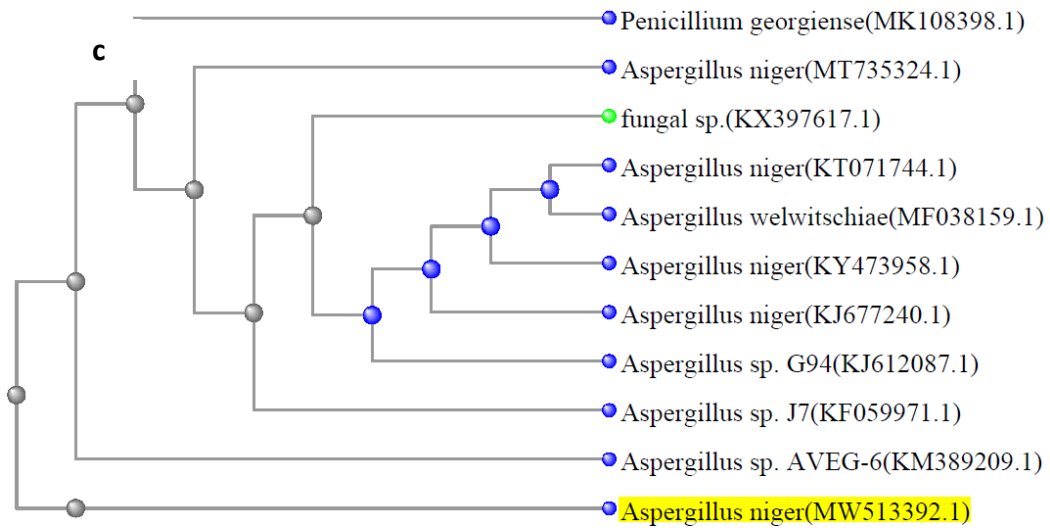


Fig. (4): The phylogenetic relationships between the fungal strains *Aspergillus fumigatus* Mw411792.1 (**a**), *A. terreus* MW512515.1 (**b**), and *A. niger* MW513392.1 (**c**) and the ITS sequences of closely related fungal strains retrieved from NCBI GenBank. The analysis was conducted by constructing a rooted tree using neighbor-joining method in MEGA6.

4. Discussion

Ochratoxins (OTs) are a broad family of mycotoxins that include more than 20 distinct metabolites, in which OTA being the most prevalent and toxic one **(Cano *et al.*, 2018)**. *Penicillium* and *Aspergillus* species (*P. verrucosum, P. nordicum, A. niger, A. ochraceus,* and *A. carbonarius*) produce two commonly recognized OTA analogs, OTB and OTC, under different environmental conditions. OTA is at least ten times more toxic than OTB or OTC **(Gupta *et al.*, 2018)**. It occurs naturally with a great frequency in a variety of cereal grains (barley, wheat, oats, corn and beans), peanuts, dried fruits, grapes/raisins, cheese, and other food products. It accumulates in the food chain because of its long half-life **(Gupta *et al.*, 2018)**. OTA and its analogs can produce a variety of toxic effects, referred to as "ochratoxicosis," including mutagenesis, carcinogenesis, embryotoxicity, reproductive and developmental toxicity, and immune suppression, by damaging mitochondria, DNA, protein, and RNA by lipid peroxidation and oxidative injury **(Adeyeye, 2016)**. Exposure to OTA has been linked with Balkan endemic nephropathy, a chronic kidney disease associated with tumors of the renal system, which can be fatal **(Trucksess and Diaz-Amigo, 2019)**. For humans, the estimated daily intakes were 0.65–5.72 ng OTA/kg body weight/day at the upper bound accounting 4.67%–40.8% of provisional tolerable weekly intake (PTWI) **(Zhang *et al.*, 2022)**. Therefore, it is recommended that OTA levels in food and feed should be reduced as much as technologically possible.

The present study was aimed to isolate fungal species that contaminate different food and feed samples. The potentiality of the isolated fungi for production of OTA was also screened. The most potent ochratoxigenic strains were molecularly identified and selected for the further studies.

In the present study two hundred and ninety-two fungal strains were isolated from sixty-nine food and feed samples under study. They belonged to three genera *Aspergillus*, *Fusarium*, and *Mucor*. The highest Fr% and RD% were recorded in the case of *Aspergillus* spp. (*A. flavus* and *A. fumigatus*). The most predominant occurrence of these species may be attributed to their ability to produce highly resistant spores. *Aspergillus* spp. existed in samples as a result of high temperatures and moisture, unseasonal rains during harvest and flash floods **(Chaleshtori and Salehi, 2018)**. *Fusarium* spp. and *Mucor* spp. showed the least Fr% and RD%.

In screening experiments the extraction of OTA from culture filtrates of the fungal isolates was carried out in two steps. At first the filtrate was defatted with *n*-hexane, and then OTA was extracted with an equal volume of methylene chloride. This is in agreement with several studies **(Daradimos *et al.*, 2000, and Dhanshetty and Banerjee, 2019)**. Based on GF-245 TLC investigation, Rf (0.89), a light-blue fluorescence under UV (254 nm) and UV spectrophotometric measurements (365 nm), sixty fungal strains out of two hundred and ninety-two ones, which represent 20.55 of total isolates, were confirmed to be ochratoxigenic. *A. fumigatus* MW512515 showed the highest producing ability of OTA (0.503 µg mL^{-1}) followed by *A. terreus* MW411792 (0.394 µg mL^{-1}) and finally *A. niger* MW513392 (0.348 mg L^{-1}). ochratoxigenic isolates. In literature, the OTA

concentrations produced by fungi are variable and differ among fungal strains. Téren *et al*. **(1996)** reported that *Aspergillus* spp. were able to produce OTA with concentrations ranging from 20 to 150 ng mL^{-1}. Abarca *et al*. **(2001)** reported that OTA was produced by *A. fumigatus* and *A. niger* with concentrations of 6.27 and 1.1 ppm, respectively. It was also reported that *Aspergillus* spp. Were able to produce OTA with concentrations ranging from 0.04±0.05 to 4.2±1.9 µg mL^{-1} in PD broth and from 1.6±2.2 to 11±10 µg mL^{-1} in YES broth according to Bayman *et al*. **(2002)**. The economically most important OTA producers belong to *Aspergillus* sections Circumdati and Nigri (**da Silva *et al.*, 2021)**. There were few studies on the production of OTA by *A. fumigatus.* **(Riba *et al.*, 2008, and Ali *et al.*, 2013)**, This met an agreement with **Varga *et al.* (1996)**, who tested the ability of sixty-one *A. fumigatus* strains, and forty-five other isolates representing fifteen species of section *Fumigati*, according to their results, none of the studied *A. fumigatus* or other representing fifteen species of section *Fumigati* were able to produce OTA.

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