World Journal of Advanced Engineering Technology and Sciences
eISSN: 2582-8266
Cross Ref DOI: 10.30574/wjaets
Journal homepage: https://wjaets.com/

(RESEARCH ARTICLE)

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Assessment of mycotoxin producing fungi isolated from dried tomatoes chips sold in Keffi, Nigeria

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World Journal of Advanced Engineering Technology and Sciences, 2023, 09(02), 235–241

Publication history: Received on 20 June 2023; revised on 01 August 2023; accepted on 04 August 2023

Article DOI: https://doi.org/10.30574/wjaets.2023.9.2.0222

Abstract

Mycotoxins are low-molecular-weight natural products. That is produced as secondary metabolites by filamentous fungi. Assessment of mycotoxin producing fungi isolated from dried tomatoes chips sold in Keffi, Nigeria. Fungi species were isolated using standard microbiological methods. Sixty (60) dry tomatoes were purchased from 5 different shops in Keffi market. The percentage occurrence of fungi was 73.3 %. The highest fungi were isolated from new market 13(86.6%) and the lowest was from Angwan Waje 9(60.0 %). The fungi species isolated were *A. niger*, *A. melleus*, *F. culmorum, Penicillium sp* and *Cladosporium cladosporioides*. The highest percentage of fungi isolated from Angwan Lambu was *A. niger and A. flavus* (20.0 %). From Angwan Waje the highest was A. fumigates and *A. flavus* (20.0%) and least were *F. culmorum* and *C. cladosporioides* (6.6 %). The highest recorded in new market were *Penicillium* sp and A. melleus (20.0%) and the lowest was *Penicillium Citrinum* (6.6%).Out of 6 *Fusarium culmorum* screened for mycotoxin production 4 produce fumonisins toxin. Of 3 Penicillium screened for mycotoxin production 1 produced patulin and 2 produced cyclopiazonic acid. Out of 5 Aspergillus fumigatus screened for toxin production only 1 cyclopiazonic acid. Different mycotoxin were produced by different fungi species isolated from the study area

Keywords: Mycotoxins; Fungi; Screened; Dry tomatoes; produced

1. Introduction

The more important mycotoxins producers belong to species of *Aspergillus*, *Fusarium*, *Penicillium* and *Claviceps*. These toxigenic fungi contaminate food products in different phases of production and processing especially in suitable heat and moist conditions [1]. The most important mycotoxins are Aflatoxins (AF), Ochratoxin (OT), Zearalenone (ZEA), Fumonisin (FB), Deoxynivalenol (DON), Patulin, Stergmatocystin, Trichothecence, T2-toxin (T2) and Ergot [2]. Mycotoxins can cause vomiting, abdominal pains, pulmonary edema, convulsions, coma, carcinogenic effects, immunosuppression, gene toxicity,immunological cyto-toxicity, mutagenic effects, low appetite, weight loss, faintness, depression and death [3]. Aflatoxins are structurally related to a group of toxic compounds found in most plant products such as wheat, peanut, copra, soya, maize and rice. Main Aflatoxins are B1, B2, G1 and G2. They are generally produced by special strains of *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius* [4].

The most important species producing Fumonisin are *Fusarium moniliforme, Fusarium nygamai* and *Alternaria alternaria* [4]. The Ochratoxins are produced by *Aspergillus ochraceus, Aspergillus niger, Penicillum viridicatum* and *Penicillum verrucosum*. The Ochratoxin is the most important mycotoxin which may cause disorder endocrine, chronic and acute toxicity, immune toxicity and carcinogenic in human [5, 6]. *Fusarium* genus is the most prevalent one producing toxin.

The most important toxin of this genus is Zearalenone which is produced by *Fusarium graminearum* [7]. Feeding milk cow with Zearalenone-contaminated feedstuff helps this toxin to enter the milk which is dangerous to the public health.

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In human beings, the toxin has symptoms such as enlarging breasts in young girls, early maturity, hormones imbalance leading to the breast cancer and cervices cancer. Its acute and chronic toxicity, gene toxicity, immunological cyto-toxicity, mutagenic effects, and its carcinogenicity have also been reported [8].

Food contaminations with moulds and mycotoxins have been considered by numerous researchers: Gnonlonfin *et al.* [9] isolated *from* cassava chips of the *Rhizopusoryzae*, *Nigrospora oryzae*, *Chrysonilia sitophila*, *Cladosporium resinae*, *Cladosporium herbarum*, *A. niger* and *A. Flavus*. Romero *et al.* [10]. This study focus on assessment of mycotoxin producing fungi isolated from dried tomatoes chips sold in Keffi, Nigeria

2. Materials and methods

2.1. Methods

2.1.1. Study Area

This study was carried out in Keffi, Nasarawa State, Nigeria. Keffi is approximately 68km away from Federal Capital Territory (FCT), Abuja and 128 km away from the State Capital, Lafia and is located at located at longitude 8'5E along the Greenwich meridians and at latitude 7'5N at the equator and is 850 m above the sea level [11].

2.1.2. Sample collection

Sixty (60) dry tomatoes samples was purchased from 5 different shops in Keffi market, package using sterile polythene and transported to the Microbiology laboratory, Nasarawa State University in an ice bag for fungal isolation.

2.1.3. Isolation of fungi

The dried tomatoes were surface-sterilized in NaCl for 2 min and rinsed in two changes of sterile distilled water. Ten (10) gram of tomato sample was soaked in 90 ml of sterilized water for 15 mins and shake vigorously. 10 fold serial dilution was carried out, 1ml was picked from 90 ml stock and transfer into a test tube containing 9ml of sterilized water, another 1 ml was picked from the first 9 ml test tube and transfer into second test tube containing 9ml of sterilized water, this step was perform till 10^{th} tube. 3ml was picked from 4^{th} and 5^{th} and spread on prepared potato dextrose agar and Sabouraud dextrose agar and incubate for 5day at room temperature. The fungi growth was sub-culture into slant and store for further use.

2.1.4. Identification of the Fungi

The fungi growths were identified using a method described by Ekeleme *et al.* [12]. Identifications were based on mycology standard procedure using cultural and morphological characteristics. The cultural characteristics were determined by their appearance on culture plates while the morphological features were determined microscopically using lactophenol cotton blue staining technique where a drop of lactophenol blue was place on glass slide and mounting pin was used to pick fungal and mix with the lactophenol blue and view under x10 and x100 objective lens of microscope morphology characteristics was compared with standard fungi chart.

2.1.5. Preparation for Substrate for Mycotoxin Screening

Tomato substrate were prepared using a method described by Amadi and Adeniyi, [13]. Tomato was purchased from Keffi market and grind into powder form using clean grinding machine and sieved. Five hundred gram (500 g) powder form was added into 1 liter of distilled water and mixed to form a homogenous mixture and placed at 4 ℃ for further use.

2.1.6. Preparation of Inoculum for Screen of Mycotoxin Production

Preparation of inoculum for mycotoxin production was carried out as described by [14]. Seven milliliter (7 ml) of peptone water and glass beads were prepared and autoclaved at 5.0 lbs/in2 pressure (115 °C) for 5 min and three milliliter (3 ml) of tween 80 was added into medium. Five milliliter (5 ml) of the medium containing of 3 % tween 80, peptone water and glass beads were transferred into seven (7) days' slant culture of fungi, shaken thoroughly until spores was dislodged and the spore suspension incubated at room temperature for 6 hours and stored for further use.

2.1.7. Screening for Mycotoxin Production by Fungi Isolated

The screening was carried out using a method described by Amadi and Adeniyi, [13]. The powder form (homogenous mixture) was sterilized. Twenty-five gram (25 g) of the homogenous mixture was dispensed into sterile conical flasks. The flasks were inoculated with 5 ml of spore suspension harvested and incubated at room temperature for 12 days.

2.2. Mycotoxin Detection

2.2.1. Instrumentation for Mycotoxin Analysis

Detection of mycotoxins was performed with high-performance liquid chromatography coupled with tandem mass-spectrometry (LC/MS/MS). Chromatographic separation was carried out using Nexera X2 UHPLC (Shimadzu, Tokyo, Japan) equipped with 100 _ 2.1 mm,2.6 _m Kinetex C18 column, (Phenomenex, Torrance, CA, USA). The column was maintained at 40C and the injection volume was 2 _L. The mobile phase consisted of 2.5 mM ammonium acetate acidified with 0.1% acetic acid (A), and methanol (B). The methanol (B) concentration was raised gradually from5% to 95% within 8 min, brought back to the initial conditions at 9 min, and allowed to stabilize for3 min. The mobile phase was delivered at a flow rate of 0.4 mL/min. The LC system was coupled with API 6500 hybrid triple quadrupole/linear ion trap mass spectrometer (Sciex, Concord, ON, Canada), equipped with a turbo-ion electrospray (ESI) ion source.

Five milliliters of the broth sample was mixed with 20 mL of 25:75 (v/v) water/methanol and was shaker for 30 min. After centrifugation at 8500_ g for 15 min, 5 mL of the supernatant was transferred to a 15-mL glass tube and evaporated under a stream of water bath at50°C. The dry residue was reconstituted with 0.25 mL of a 95:5 (v/v) water/methanol mixture and centrifuged for 10 min at 17,000_ g, at 4°C; the supernatant was used directly for the analysis. Samples, in which the concentration exceeded the highest level of calibration, was diluted and re-injected

3. Results

3.1. The Occurrence of Fungi Isolated from Stored Dried Tomatoes

The occurrence of fungi isolated is as shown in Table 1. Out of 60 samples collected total of 44(73.3 %). The highest fungi were isolated from new market with occurrence of 13(86.66 %) followed by old market 12(80.0 %), Angwan Lambu 10(66.6 %) and the lowest was from Angwan Waje 9(60.0 %) respectively.

3.2. Cultural Characteristics of Fungi Species Isolated from Stored Dried Tomatoes

Table 2 shows the culture characteristic of different fungi species isolated from stored dried tomatoes sold in Keffi. *Aspergillus* niger grow white, later becoming black with pale yellow on the reverse side. *Aspergillus melleus the* colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age. *Fusarium culmorum* growing slowly, surface usually orange to deep apricot due to confluent conidial slime. *Penicillium* sp Slow grower white edge/margin, velvety furrowed center with bluish green color, microscopic brush-like conidiophores with septate hyphae. *Cladosporium cladosporioides* Colonies are olive-grey to dull green, velvety and tufted. The edges of the colony can be olive-grey to white, and feathery. The colonies are diffuse and the mycelia form mats and rarely grow upwards from the surface of the colony

Table 1 Percentage Occurrence of Fungi Isolated From Stored Dried Tomatoes

Location	No. sample	No. (%) fungi isolated
Angwan Lambu	15	10(66.6 %)
Angwan Waje	15	9(60.0 %)
New market	15	13(86.6 %)
Old market	15	12(80.0 %)
Total	60	44(73.3 %)

Table 2 Cultural and Microscopic characteristics of fungi species isolated from stored dried tomatoes

Cultural characteristics	Microscopic characteristics	Inference
Initial growth is white, later becoming black with pale yellow on the reverseside	Ball like conidiophores and hyphae is septate	*Aspergillus niger*
Colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age. Conidial heads are typically radiate, later splitting to form loose columns biseriate but having some heads with phialides borne directly on the vesiclecleistothecia	Conidiophore stipes are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to sub-globose	*Aspergillus* sp
Colonies growing slowly; surface usually orange to deep apricot due to confluent conidial slime; aerial mycelium sometimes floccose and whitish	Conidiophores loosely branched, with short, often swollen phialides. Macroconidia strongly curved and pointed at the apex, mostly one septate, Microconidia absent	*Fusarium culmorum*
Slow grower white edge/margin, velvety furrowed centre with bluish green color	Brush-like conidiophores with septate hyphae	*Penicillium* sp
Colonies are olive-grey to dull green, velvety and tufted. The edges of the colony can be olive-grey to white, and feathery. The colonies are diffuse and the mycelia form mats and rarely grow upwards from the surface of the colony	The conidia are small, single lemon-shaped They form long, fragile chains up to 10 conidia in length with distinctive connective tissue between each spore	*Cladosporium cladosporioides*

3.3. Percentage Occurrence of Fungi Species Isolated

The percentage of occurrence of different fungi isolated from stored dried tomatoes is as given in Table 3. The highest percentage of fungi isolated from Angwan Lambu was *A. niger and A. flavus* (20.0 %) followed by *Aspergillus melleus and F. culmorum* (13.3 %). From Angwan Waje the highest was *A. fumigates* and *A. flavus* (20.0 %) followed by *A. niger* (20.0 %) and the least were *F. culmorum* and *C. cladosporioides* (6.6 %). The highest recorded in new market were *Penicillium* sp and *A. melleus* (20.0%) followed by *A. fumigatus, A. flavus* and *F. culmorum* (13.3 %) and the lowest was *P. Citrinum* (6.6 %). The highest observed from old market was *A. flavus* (26.6 %) followed by *A. niger* (20.0 %), *A. melleus* (13.3 %) and the lowest were *P. citrinum, F. culmorum* and *C. cladosporioides* (6.6 %) respectively and also the highest fungi species isolated was *A. flavus* (80.0 %) and the lowest was *C. Cladosporioides* (13.3 %).

3.4. Screening for Production of Mycotoxins by Different Fungi Species Isolated

Different mycotoxins produced by fungi species isolated from stored dried tomatoes sold in Keffi is as shown in Table 4. out of 6 *Fusarium culmorum* screened for mycotoxin production it was observed that 4 were able to produce fumonisins toxin. Of 3 *Penicillium* screened all the 3 produced different toxin 1 produced patulin and 2 produced cyclopiazonic acid. Five isolated *Aspergillus fumigatus* isolated were screened for toxin production it was observed that only one (1) was able to produce toxin which is cyclopiazonic acid. Seven (7) *Aspergillus melleus* isolated from stored dried tomatoes were screened for mycotoxins production one (1) produced Ochratoxin, three (3) produced aflatoxin *and one (1) produced* cyclopiazonic acid toxin. Out of 12 Aspergillus aflatoxiformans (*flavui*) isolated and screened for toxin production 8 recorded toxin productions, 2 produced Ochratoxin. 5 produced aflatoxin and 1 produced cyclopiazonic acid. Only 1 Penicillium Citrinum was isolated, screened for toxin production and it was observed to have ability to produced cyclopiazonic acid toxin. Out of 8 *Aspergillus niger* isolated, screen for toxin production it was observed that 2 was able to produce mycotoxins 1 produced Ochratoxin and the other 1 produced aflatoxin. Of 2 *C. cladosporioides* isolated and screened for mycotoxins none was able to produce toxin as shown in Table 4.4 respectively.

Table 3 Percentage Occurrence of Different Fungi Species Isolated

Fungi	**No. sample**			**Location**		
		Angwan Lambu	**Angwan Waje**	**New market**	**Old market**	**Total**
Aspergillus niger	15	3(20.0 %)	2(13.3 %)	0(0.00 %)	3(20.0 %)	8(53.3 %)
Penicillium expansum	15	0(0.00 %)	0(0.00 %)	3(20.0 %)	0(0.00 %)	3(20.0 %)
Aspergillus fumigatus	15	0(0.00 %)	3(20.0 %)	2(13.3 %)	0(0.00 %)	5(33.3 %)
Aspergillus melleus	15	2(13.3 %)	0(0.00 %)	3(20.0 %)	2(13.3 %)	7(46.6 %)
Aspergillus flavus	15	3(20.0 %)	3(20.0 %)	2(13.3 %)	4(26.6 %)	12(80.0 %)
Penicillium citrinum	15	0(0.00 %)	0(0.00 %)	1(6.6 %)	1(6.6 %)	2(13.3 %)
Fusarium culmorum	15	2(13.3 %)	1(6.6 %)	2(13.3 %)	1(6.6 %)	6(40.0 %)
C. cladosporioides	15	0(0.00 %)	1(6.6 %)	0(0.00 %)	1(6.6 %)	2(13.3 %)

Table 4 Different Mycotoxins Produced By Fungi Species Isolated From Dried Tomatoes

Fungi isolated	**No. Screened**	**No. positive**	**Mycotoxins produced**				
			Patulin	**Ochratoxin**	**Aflatoxin**	**Fumonisins**	**cyclopiazonic acid**
Aspergillus niger	8	2	-	+	+	-	-
Penicillium expansum	3	3	+	-	-	-	++
Aspergillus fumigatus	5	1	-	-	-	-	+
Aspergillus melleus	7	5	-	+	+++	-	+
Aspergillus flavus (aflatoxiformans)	12	8	-	++	+++++	-	++
Penicillium Citrinum	1	1	-	-	-	-	+
Fusarium culmorum	6	4	-	-	-	++++	-
C. cladosporioides,	2	0	-	-	-	-	-

Key: + = no of fungi that produced myctoxin; - = did not produced mycotoxin

4. Discussion of the Findings

Mycotoxins are low-molecular-weight natural products. That is produced as secondary metabolites by filamentous fungi. These metabolites constitute toxigenically and chemically heterogeneous assemblages that are grouped together only because the members can cause disease and death in human beings and animals [15]. This study is focused on assessment of mycotoxin producing fungi isolated from dried tomatoes chips sold in keffi.

In this study the overall occurrence of fungi isolated from dried tomatoes sold in Keffi was 73.3% higher than 52.3%, early reported by Shinkafi *et al*., [16] in Gusau, Zamfara State, Nigeria. Also highest occurrence of fungi species was observed from samples from new market, the high occurrence may not be related to location but due to nutrient content or steps used in process the dried tomatoes that make it a good medium for fungi growth.

The different species of fungi observed in this study were *Fusarium culmorum, Penicillium* sp, *Aspergillus melleus, Aspergillus niger, Cladosporium cladosporioides Aspergillus flavus and Aspergillus fumigatus* and the highest occurring

species was *Aspergillus species namely Aspergillus flavus as recorded in this study which similar to study recently reported by* Shinkafi, *et al.* [16] and Hegazy [17] the high occurrence of *Aspergillus species but in disagreement on the species that had the highest occurrence which they reported to Aspergillus niger. It was also reported by [18, 19]* that these species of fungi are responsible or cause postharvest spoilage of tomatoes as it was observed from Gwagwalada market in Abuja, Nigeria, from Otukpo and Makurdi Local Government Areas in Benue State , Nigeria and in Abakaliki, Nigeria respectively. These fungi species are also known to be abundant in the soil which may serve as source of contamination to the tomatoes during harvesting of the fruits before drying it, the contamination of these fungi may be also from the processes used during drying or the storage because most of the fungi spore are also found on the air which may land on the tomatoes during drying process.

In this study, different species of fungi isolated from dried tomatoes sold in Keffi were screened for mycotoxin production and it was observed that almost all the specie were able to produced one mycotoxin or the other ranging from aflatoxin, ochratoxin, cyclopiazonic acid toxin, fumonisins and patulin respectively. It was observed that *Aspergillus specie isolated were able to produced* aflatoxin, ochratoxin and cyclopiazonic acid which in agreement with work reported by Hegazy [17] and Motta and Soares [20] that *Aspergillus niger and Aspergillus flavus were found to produce these mycotoxins in tomatoes. Also the species of Penicillium* such as *P. expansum* were found to produce patulin and aflatoxin and it is also in line with Sanyaolu [21] who reported the presence of these mycotoxin in dried tomatoes and Pepper contaminated with *Penicillium* sp. The isolation of these mycotoxins producing fungi from dried tomatoes sold in Keffi is call for worry because these toxins are known to cause harm or foodborne infection that may cause damage on human and animal organs which lead to death such as ochratoxin [22]**.** There is need to develop a better ways to reduce the contamination of these mycotoxin producing from right from harvesting to preserving the tomatoes [23]

5. Conclusion

From this study different fungi species were isolated from stored dried tomatoes sold in different location in Keffi and molecularly identify to be *Fusarium culmorum, Penicillium citrinum, Aspergillus flavus, Aspergillus melleus, Aspergillus fumigatus, Aspergillus niger* and *Penicillium expansum* respectively. After screening for mycotoxins production it was observed that these different fungi species were able to produce different mycotoxins such as fumonisins, cyclopiazonic acid, ochratoxin, aflatoxin and patulin.

Compliance with ethical standards

Acknowledgments

With immensely thank all the staff in Department of Microbiology Laboratory Nasarawa State University Keffi for the help during the bench work and analysis.

Disclosure of conflict of interest

No conflict of interest among of the authors

Statement of informed consent

Informed consent was obtained from all individual participants included in this study

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