

**NATURAL INCIDENCE AND OCCURRENCE OF MAJOR MYCOTOXINS,
AFLATOXIN PRECURSORS AND *ASPERGILLUS* METABOLITES IN NIGERIAN
DATE PALM (*Phoenix dactylifera* L) FRUITS**

^{1*}Anjorin T. S., ²Adeniran L. A., ³Sulyok M., ³Krska R. and ⁴Zarafi A. Z.

¹Department of Crop Protection, Faculty of Agriculture, University of Abuja, Nigeria

²Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Abuja, Nigeria

³Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna (BOKU), Konrad Lorenzstr, 20, A-3430 Tulln, Austria

⁴ Department of Crop Protection, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria

*Correspondence Author: toba.anjorin@uniabuja.edu.ng; +2348062242866

SUMMARY

Mycotoxins profile and *Aspergillus* metabolites of 108 samples of date palm fruits collected from farmers stores (48) and markets (60) from Jigawa, Kano, Sokoto and Kaduna States, Nigeria were investigated. Analysis was carried out using liquid chromatography and mass spectrometry. There was no major mycotoxin (aflatoxin B₁ (AFB₁), ochratoxin A (OTA), and fumonisin detected in all the tested samples. Also, sterigmatocystin (STC), a key metabolite needed in the biosynthesis of aflatoxin B₁ was below the limit of detection in the samples. The incidence of Aflatoxin precursors found in the samples were versicolorin (8%), nidurufin (4%) and averufin (16%) but had comparatively low maximum concentration of 1.6, 0.03, and 0.49 µg/kg respectively. *Aspergillus* metabolites detected in both the marketed and stored samples included nigragillin, aspulvinone and aurasperon C with the least median concentration of 1.7 x10⁴ µg/kg. The absence of major mycotoxins in the investigated date palm fruits might be due to cultivation of improved cultivars and the missing key metabolites needed in the biosynthesis of aflatoxin B₁ and fumonisins. The toxicity associated with the *Aspergillus* metabolites found in the fruits deserves further investigation.

Keywords: Mycotoxins, LC-MS/MS, *Aspergillus* metabolites, aflatoxin precursors, date palm fruits, Nigeria

DATES are edible fruits of the date palm (*Phoenix dactylifera* L.) of the family Arecaceae. They are a staple food in arid and semi-arid regions of Africa, the Middle East, and South-Asian

countries (Maqsood *et al.*, 2019). Date palm fruits are highly nutritious as it contains carbohydrates (44-88%), fats (0.2 - 0.4%), fiber (6.4-11.5%), minerals (oil, calcium, sulfur, iron, potassium, phosphorous, manganese, copper and magnesium), vitamins, a higher concentration of protein (2.3-5.6%) compared with other major cultivated fruits such as oranges, bananas and grapes that contain only 0.3 %, 0.7%, 1.0 % and 1.0 % of protein respectively (Tang *et al.*, 2013). Date palm fruits have always played an important role in the economic and social lives of the people. Some health specialists have said that eating one date per day is necessary for a balanced and healthy diet (Arias *et al.*, 2016)

Some of the indices developed to assess date palm fruits quality include fruit size, shape, color, texture (chewiness), cleanliness, and freedom from mycotoxins (Chao and Krueger, 2007; Abdullah, 2010) and defects (such as sunburn, skin separation, insect damage, sugar migration to fruit surface, and fermentation) and decay-causing pathogens (Jomaa *et al.*, 2016). The drying of the fruits to 9 - 12 moisture contents helps in minimizing the activities of storage insect pests and pathogens (Degri and Zainab, 2013) thus making the commodities available at all seasons (Jacobs *et al.*, 2019) and selling at higher price (Gunathilake *et al.*, 2019).

The occurrence of mycotoxins - a group of several toxic secondary metabolites of fungi like *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Claviceps* spp. and *Alternaria* spp. in date palm fruits has been documented worldwide (Shenasi *et al.*, 2002; Shi *et al.*, 2018). Available date palm fruits in the market do not always meet international quality and safety standards. Field production methods, lack of hygiene, fungal control, and loss of potency during storage have been responsible for mycotoxin contamination of medicinal and aromatic herbs (Zhang *et al.*, 2018; Qin *et al.*, 2020). Several mycotoxins of importance include aflatoxin B₁, ochratoxin A, fumonisin B₁, fumonisin B₂, fumonisin B₃, fumonisin B₄, and fumonisin B₆. Ingestion, inhalation, and absorption through the skin of mycotoxins can cause serious damage to the health of the human, animal, and damage international trade of herbs (Makun *et al.*, 2013; Adeniran *et al.*, 2013; Zhang *et al.*, 2018).

There is paucity of information on the co-occurrence of mycotoxins in date palm in Nigeria. This study is carried out to determine the occurrence of major mycotoxins, sterigmatocystin, and its biosynthetic precursors and other *Aspergillus* metabolites in date palm fruits marketed in North West, Nigeria.

MATERIALS AND METHODS

Study Area

The study area involved farmers stores and markets each in each of the three geopolitical zones of Kano State (Lat. 12.00° N; 8.31° E), Jigawa State (Lat. 12.10°N; Long. 9.56°E), Kaduna State (Lat. 10.19° N; Long. 7.45°E) and Sokoto (Lat.13.06°N; Long. 5.23°E) States, in Northern Nigeria. The vegetation of these States could be described as Northern Guinea Savanna and Sahel Savanna zones.

Sample collection

The sampling method was purposive as samples were collected from the North West zone of Nigeria where date palm fruits are most produced and sold in the country. A total of sixty samples of dried loose date palm fruits were collected from the open markets (i. e. 3 selling points from 5 markets per State), also 48 samples of semi-dried date palm fruits harvested within the past 60 days were collected from the farmers stores (i.e. 3 collection points from 4 farmers from each State). Half kilogram of date palm fruits were purchased from each location. Fruits from each point were packed in sterile prophyllene bags, well tightened and transported to the Laboratory of Pesticide and Mycotoxins in the Department of Crop Protection, Ahmadu Bello University, Zaria, Nigeria.

Analysis of mycotoxin

Reagents

Liquid chromatographic grade of methanol (CH_3COOH) and acetonitrile were purchased from Merck (Germany) and VWR (Belgium) respectively. The Mass Spectrometry grade ammonium acetate and standards for fungi metabolite were brought from Sigma-Aldrich (Austria). Decontamination of water was carried out consecutively through reverse osmotic pressure and ultra-analytic system purchased from Veolia water (UK). A total of 34 working solutions were made and kept at -20°C in the fridge but were brought to 25°C before use. Fresh final working solution was mixed accordingly for the spiking experiment.

Date palm fruit extraction

Each grain and haulm samples were milled using a cyclone pulverizer which has one millimetre square sieve (Cyclotech, Sweden) before being homogenized. Five grams each were measured into the centrifuge tube (0.05 L polypropylene). Twenty millilitres of the separation solvent (acetic acid/water/acetonitrile 1:20:79, v/v/v) were added before being vortexed using a laboratory rotary shaker (Model GFL 3017, Germany). Ratio of the dilution of the sample with the solvent was 1:1 and 5 millilitres of the dilution obtained from the extract was shot into the LC-MS/MS.

LC-MS/MS parameters

Major mycotoxins, Sterigmatocystin (STC), six aflatoxin biosynthetic precursors and 27 *Aspergillus* metabolites were tested for in all the date palm fruits samples. Analysis of the extracts was achieved with a QTrap 5500 multimycotoxin LC-MS/MS system (Applied Biosystem, California, United State of America) furnished with Turbolon spray ESI source and High Performance Liquid Chromatography (Agilent, Germany). Other liquid chromatography/Mass Spectrometry protocols applied for chromatographic separation, identifying analytes that are positive, quantification of liquid standard identification was as described (Xie *et al.*, 2011). The method precision was tested through proficiency testing organized by Bureau Interprofessionnel

des Etudes Analytique (BIPEA) (Gennevilliers, France) in accordance with ISO 13525:2015. All the 108 results of the date fruits was between $-2 < x < 2$ which was a satisfactory range.

Statistical analysis

The percentage of the samples that tested positive (% pos) were calculated from the proportion of samples that tested positive (p pos) to each aflatoxin precursor or *Aspergillus* metabolite. Also, the median and the maximum concentration (max. conc) in $\mu\text{g/kg}$ of the mycotoxins and metabolites were determined from the data collected for each of the samples analyzed, using Microsoft Word Excel, version 2017. The scatter plots visualizing co-occurrence of aflatoxin B₁ precursors and *Aspergillus* metabolites in the stored and marketed samples were shown.

RESULTS

The major mycotoxins and STC in the date palm fruits from the farmers stores and in the markets were below level of detection (Table 1). The incidence of Versicolorin C, nidurufin and averufin detected were 8%, 4% and 16% respectively but their concentration level were generally low with the highest concentration of 1.64 $\mu\text{g/kg}$.

Table 1: Major mycotoxins and aflatoxin biosynthetic precursors detected in date palm fruits from Northern Nigeria

Major Mycotoxins					Aflatoxin biosynthetic precursors				
Mycotoxin (Target analyte)	p pos	% pos	Median ($\mu\text{g/kg}$)	Max. Conc ($\mu\text{g/kg}$)	Precursors	p pos	% pos	Median ($\mu\text{g/kg}$)	Max. Conc ($\mu\text{g/kg}$)
Aflatoxin B ₁	-	-	-	-	Sterigmatocystin	-	-	-	-
Ochratoxin A	-	-	-	-	Versicolorin A	-	-	-	-
Fumonisin B ₁	-	-	-	-	Versicolorin C	7/108	8	0.8	1.64
Fumonisin B ₂	-	-	-	-	Nidurufin	4/108	4	0.3	0.3
Fumonisin B ₃	-	-	-	-	Averantin	-	-	-	-
Fumonisin B ₄	-	-	-	-	Averufin	17/108	16	0.3	0.5
Fumonisin B ₆	-	-	-	-	Seco- Sterigmatocystin	-	-	-	-

The percentage of date palm fruits samples that tested positive to versicolorin C, nidurufin and averufin AF precursors were comparatively higher in marketed samples than those from farmers stores (Table 2). Averufin had the highest incidence of 25% from the marketed samples. However, their concentration levels were generally low.

The incidence and concentration levels of *Aspergillus* metabolites were also generally higher in the marketed samples than from the farmers stores (Table 2). Nigragillin had the highest incidence of 80% and 27.1% in the marketed and farm store samples respectively. *Aspergillus* metabolites detected in both the marketed and stored samples were nigragillin, aspulvinone and aurasperon C and they had comparatively high median concentration levels. The metabolite with the highest concentration level was aurasperon G ($5.7 \times 10^5 \mu\text{g/kg}$) in the marketed samples but it was below detectable levels in the samples from the farmers stores.

Table 2: Aflatoxin B₁ precursors and *Aspergillus* metabolites detected in date palm fruits samples from markets and farmers store in Northern Nigeria

AF biosynthetic precursor and <i>Aspergillus</i> metabolites	Marketed date palm fruits samples				Farmers stores date palm fruits samples			
	p pos*	% pos	Median ($\mu\text{g/kg}$)	Max. Conc ($\mu\text{g/kg}$)	p pos	% pos	Median ($\mu\text{g/kg}$)	Max. conc. ($\mu\text{g/kg}$)
Versicolorin C	8/60	13.3	0.6	1.6	2/48	4.2	0.9	0.9
Nidurufin	3/60	5	0.3	0.3	2/48	4.2	0.3	0.3
Averufin	15/60	25.0	0.3	0.5	4/48	8.3	0.3	0.4
Pyranonigrin	10/60	16.7	2810	3.5×10^4	2/48	4.2	614	814
Nigragillin	48/60	80	1.7×10^4	1.95×10^7	13/48	27.1	5.1×10^4	2.0×10^5
Aspulvinone	9/60	15.0	1.8×10^5	2.6×10^7	2/48	4.2	7.9×10^4	7.9×10^4
Malformin A	52/60	86.7	65.23	334	11/48	22.9	0.3	4.4
Malformin A ₂	10/60	16.7	4.49	6.9	-	-	-	-
Malformin C	18/60	30	0.21	10.5	4/48	8.3	0.2	0.2
Aurasperon B	3/60	5	9.1×10^4	9.1×10^4	-	-	-	-
Aurasperon C	10/60	16.7	4.2×10^4	5.97×10^6	2/48	4.2	1.8×10^5	1.8×10^5
Aurasperon G	7/60	11.7	5.7×10^5	4.9×10^6	-	-	-	-

*Proportion of samples that tested positive

The co-occurrence of *Aspergillus* metabolites at various concentration levels in the contaminated market samples and contaminated farmers' stores samples of date palm fruits from Northern Nigeria is as shown in Figure 1. The six main metabolites in the marketed samples were aurasperon G □ aspulvinone □ nigragillin □ aurasperon B □ aurasperon C □ pyranonigrin in descending order of concentration level. Also the three main metabolites in the farmer's stores samples were aurasperon C □ aspulvinone □ nigragillin in descending order of concentration level.

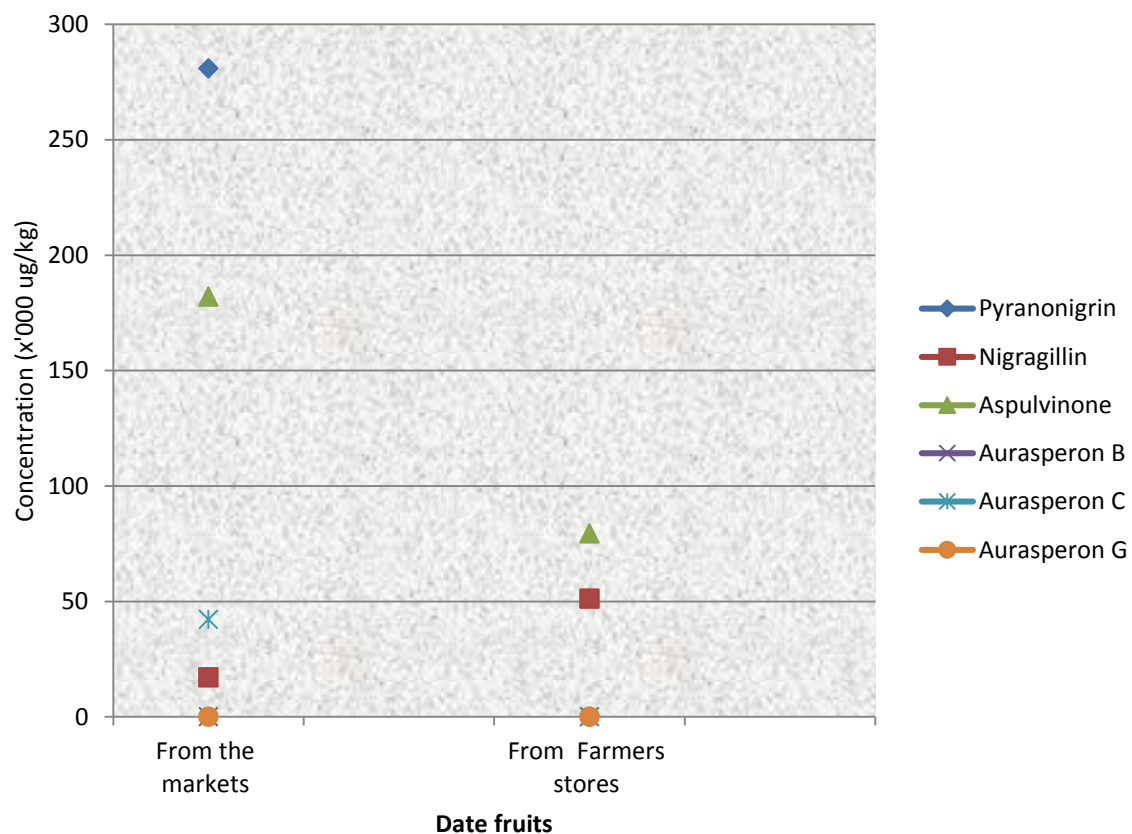


Figure 1: Co-occurrence of *Aspergillus* metabolites in the six highest contaminated market samples and three highest contaminated farmers' stores samples of date palm fruits from Northern Nigeria

DISCUSSION

Date palm fruits are staple food that contains essential nutrients and played an important role in the economic and social life of the people of Northern Nigeria (Tang *et al.*, 2013). However, recent studies have recorded microbial contamination of date palm fruits due to unfavorable pre-harvest condition and post-harvest handling which are of a major public and safety concern (Abo-el-Saad and El-Shafie, 2014). The result obtained from this study showed that AFB₁, OTA, and fumonisin B₁, B₂, B₃, B₄, and B₆ were not detected in the samples tested. The absence of major mycotoxins contamination in both fruit samples from the markets and the farmers' stores in the region indicated it is wholesome for consumption.

Also, aflatoxin key biosynthetic precursor, STC was not detected in the date samples, this may be adduced to natural resistance of the native date palms to mycotoxigenic fungi, improved breeding practices or due to good storage conditions resulting into the absence of some major mycotoxin biosynthetic precursors. This report is in line with report from the analysis of sixteen varieties of date palm fruits that all samples showed an absence of aflatoxins and their precursor, STC after adverse storage for 14 days, although aflatoxin producing *A.flavus* were identified in 10 varieties (Shenasi *et al.*, 2002). The absence of key aflatoxin precursors like sterigmatocystin in the fruits for instance might be responsible for their inability to produce aflatoxin at a detectable limit (Streit *et al.*, 2013).

A contrary report was obtained from the study conducted on 28 samples of dried date palm fruits in Egypt where fumonisin B₂ was identified in 2/28 (7%), OTA in 11%, and aflatoxins in one sample (3.6%) of the tested samples (Abdallah, 2018). Studies of date palm fruits samples obtained from Tunisia, Algeria, Spain, and Israel also showed the detection of OTA in 38% and aflatoxins B in 46% in the samples tested (Azaiez *et al.*, 2015). Contamination of food and feed samples by mycotoxin has been described as a severe health hazard through losses incurred from livestock disease and mortality; and affects international trade by inadmissibility or rejection of

products by international market. Other *Aspergillus* metabolites detected in this study include nigragillin 52% and malformin A 54%. Nigragillin toxicity has not been well defined but its insecticidal activities have been described (Nielsen *et al.*, 2009). Malformin A, A₂, and C were found at different concentrations. Malformin is derived from the malformation in the plant due to the presence of malformin it has antibacterial activity its toxicity has been determined (Shenasi *et al.*, 2002). Aurasperon was also in defective date palm fruits at the concentration range of (9.1x 10⁴ - 5.97x10⁵) µg/kg. Aurasperons are naphthol- γ -pyrones produced by *A. niger*. Aurasperon B, C, and G have shown antitumor, antibacterial, and antifungal activities details of their toxicity are not available (Ezekiel *et al.*, 2020).

CONCLUSION

This study indicated that there was no major mycotoxins contamination in the sampled date palm fruits from farmers' stores and the markets. This may be adduced to palm fruits from improved cultivars; appropriate drying of fruits and the missing key metabolite needed in the biosynthesis of aflatoxin B₁ and the fumonisins. Some *Aspergillus* metabolites were confirmed in both marketed and stored date palm fruits, however the incidence and concentration levels was higher in the marketed samples. The toxicity associated with such metabolites found in the fruits deserves further investigation.

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