



Mycotoxins and Mytoxicogenic Fungi from Sorghum: A Comprehensive Review

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ABSTRACT: Fungi are ever-present plant and animal's microorganism that are major fermentation agents of foods and herbage. Presence of microscopic fungi or fungal spores in crops or feed made from the crop, most often compromise the nutritional quality, sensorial attributes and the safety of the food and feeds that humans and animals solely rely on. They are also known to be infamous blender of mycotoxins. Review was carried out to determine the conceivability of polymerase chain reaction (PCR) based methods to profile any fungal contaminating sorghum, to evaluate the toxicogenicity of fungal isolated, to validate the ability of, Liquid chromatography–tandem mass LC-MS/MS to profile *aflatoxin*, *ochratoxins*, *fumonisin*, *zearalenone*, *deoxynivalenol*, *HT-2 toxin*, *T-2 toxin*, *citrinin* and *ergot alkaloids* contaminating sorghum, to identify non-toxicogenic fungi as bio-control agents against mycotoxin contamination of sorghum, and to evaluate the fungicide effects of *Parkia biglobosa* (Jacq.) and *d'Eucalyptus camaldulensis* (Steud.) against fungi contaminating sorghum. Research works considered for this review were the research studies carried out from the last ten years. It was concluded that multiplex PCR (mPCR) based assay is a rapid, cost effective and user friendly that can be used for the diagnosis of major mycotoxigenic fungi in sorghum, toxicogenicity of isolated fungi depends on the environmental and climatic factors and *Aspergillus parasiticus* was reported to be the highest producer of aflatoxins while *Fusarium graminearum* is the highest producer of Zearalenone and Deoxynivalenol in Africa, (LC/MS/MS) method possesses the performance characteristics required to obtain accurate mycotoxins profiles in Sorghum, set of primers can be developed using quantitative PCR (qPCR), PCR- restriction fragment length polymorphism (PCR-RFLP) and mPCR assay which allows for the identification and quantification of non-toxicogenic fungi agents, sorghum and that *Parkia biglobosa* Stem bark extract contains large quantity of phytochemical compare to the leaf extract and the constituents are higher in acetone extract than ethanol extract also *D'Eucalyptus camaldulensis* leaves extract has the largest inhibition zones against *Aspergillus alliaceus*.

Keywords: Sorghum, fungi, Mycotoxins, *Parkia biglobosa*, *Eucalyptus camaldulensis*.

Introduction

Mycotoxins are secondary metabolites produced by filamentous fungi that can cause wide variety diseases ranging from acute skin lesions to chronic cancerous syndromes on humans and other farm animals when introduced into food chain (Priyanka *et al.*, 2013). These toxins are invincible, tasteless,

The objectives of this research work is to carry out a review to determine the conceivability of polymerase chain reaction (PCR) based methods to profile any fungal contaminating sorghum, to evaluate the toxicogenicity of fungal isolated, to determine, to validate the ability of LC-MS/MS to profile *aflatoxin*, *ochratoxins*, *fumonisin*, *zearalenone*, *deoxynivalenol*, *HT-2 toxin*, *T-2 toxin*, *citrinin* and *ergot alkaloids* contaminating sorghum, identification of non-toxigenic fungi as bio-control agents against mycotoxin contamination of sorghum, and the evaluation of fungicide effects of *Parkia biglobosa* (Jacq.) and *d'Eucalyptus camaldulensis* (Steu.) against fungi contaminating sorghum. Research works considered are from the last ten years.

Determination of the Fungal Profile Contaminating Sorghum Using Polymerase Chain Reaction (PCR) Based Methods.

(Garba *et al.*, 2021) carried out analysis to profiled fungi isolates from sorghum in six Agro- ecological zones of Nigeria namely; Derived savannah, Northern guinea savannah, Southern guinea savannah, Sudan savannah, Sahel savannah and Mid- altitude using a Gene Amp PCR System 9700 and an automated sequencer—ABI PRISM 3700 Genetic analyser. It was observed that 82.5% of the fungal isolates co-occurred in the samples from all the Agro-ecological zones and there is high prevalence of two *Aspergillus* species of *A. flavus* and *A. parasiticus* in all the samples from the six Agro-ecological zones. It was reported that *A. flavus*, *A. niger* and *A. ochraceus* are predominant in the Northern guinea savannah while all isolated of *Penicillium* species with exemption of *P. aurentiogresum* and *P. paxillii* are found to be predominant in the derived savannah and southern guinea savannah respectively. *Fusarium* species constitute the highest fungal contaminants in the Southern Guinea savannah. It was concluded that the prevalence of fungi in each agro-ecological zone is species dependent and largely depend on the prevailing environmental conditions therein. (Masitha *et al.*, 2020) collected samples of yellow maize, white maize, millet, cowpeas, sorghum and china peas in the central district of Botswana in order to identify the fungi contaminating the cereal grains Samples. Polymerase Chain reaction was used for the identification of the fungi. It was shown from the results of the study that yellow maize was contaminated by *Fusarium*, *A. niger* and *A. flavus*; white maize was contaminated by *F. proliferatum*, *F. fujikuroi* and *Gibberella moniliformis*; red sorghum was contaminated by *A. flavus*, *A.oryzae*, *Penicillium*, *Alternaria* and *Chaetomium muelleri*; millet was contaminated by *Epicoccum sorghinum* and *curvularia branchyspora* and cowpeas were contaminated by *Aspergillus* and *Alternaria* species. It was reported that only 4% of red sorghum was contaminated. Alsohaili and Bani-hasan(2018) carried out the amplification of the extracted fungal DNA filamentous fungi gotten from different environmental sources in the northern eastern Jordanian desert using PCR through specific internal transcribed spacer primer (ITS1/ITS4).The PCR products were sequenced and eight fungal species were identified as: *Aspergillus niger*, *Aspergillus tubingensis*, *Alternaria tenuissima*, *Alternaria alternata*, *Alternaria gaisen*, *Rhizopus stolonifer*, *Penicillium citrinum*, and *Fusarium oxysporum*. It was observed from the results that the *Aspergillus niger* was the most abundant fungus obtained from all the locations and resources, while the *Alternaria tenuissima* was the less prevalent one. (Priyanka *et al.*, 2013) was able to reach a Conclusion that a developed mPCR based assay was rapid, cost effective and user friendly and can be used for diagnosis of major mycotoxigenic fungi. An evaluation was carried out to develop and evaluate a multiplex PCR assay for simultaneous detection of Aflatoxin (AF), Ochratoxin (OTA), Trichothecene (TRI), Zearalenone (ZEN) and Fumonisin (FUM) producing fungal species from contaminated foods. It was detected in the study that the standardized mPCR assay can actually detect all the five major mycotoxin metabolic genes along with artificially

inoculated maize seeds with mycotoxigenic *Fusarium*, *Penicillium* and *Aspergillus* spores. When the developed mPCR assay was applied on to 177 contaminated maize, paddy and sorghum, the detection limit of this mPCR assay was 1×10^3 spores per gram of artificially inoculated samples upon 48 h of incubation at room temperature, many of the samples (100 out of 177) were contaminated with either one or more mycotoxins. (Wei *et al.*, 2011) developed a novel nested polymerase chain reaction (n-PCR) assay to identify *Sorghum nitidum* (*S. nitidum*). Primers of typical PCR Snit5/Snit2 were used to amplify the genomic DNA extracted from a single seed of *S. nitidum*. It was concluded that the assay was able to specifically identify *S. nitidum* fast and effectively, which could be applied widely in field inspection, agriculture production and plant protection.

Evaluation of the Toxicogenicity of Isolated Fungi

(Garba *et al.*, 2021) carried out a toxigenicity studies on strains representing species of *Aspergillus*, *Penicillium*, *Fusarium* to determine their ability to produce aflatoxin B1 (AFB1); aflatoxin B2 (AFB2); aflatoxin G1 (AFG1); aflatoxin G2 (AFG2); OTA, ZEN, DON and FB1 in the six Agro- ecological zones of Nigeria. The toxigenicity of the Isolated *Aspergillus* fungal species from sorghum in Nigeria as shown in table 1, it shows that *A. parasiticus* has the highest percentage of Aflatoxins followed by *A. flavus*, *A. ochraceus* and *A. niger* respectively. It can be seen from table 2 that *Fusarium proliferatum* has the highest percentage of aflatoxin B1 from the tested samples which is 80% while *Fusarium graminearum* has the highest percentage of Zearalenone and Deoxynivalenol which are 77.78% of the tested samples. (Osman *et al.*, 2017) carried out the analysis to determine the ability of an isolated fungi collected from different Egyptian Governorates (i.e. Cairo, Kaluobia. Al-Gharbia, Alexandria, Assute and Sohag) to produce mycotoxins. It was reported in table 3 that *Aspergillus parasiticus* isolated from sorghum grain samples collected from Cairo has the ability to produce aflatoxin in a concentration ranged from 0.03 to 0. 80 µg/kg sorghum grains.

Validation of the Ability of LC-MS/MS Method to Determine Aflatoxin, Ochratoxins, Fumonisin, Zearalenone, Deoxynivalenol, HT-2 Toxin, T-2 Toxin, Citrinin and Ergot Alkaloids) Profiles in Sorghum.

(Mohammed *et al.*, 2022) carried out a study to assess fungal species and multi-mycotoxins associated with sorghum grain in post-harvest samples collected from eastern Ethiopia using a liquid chromatography-tandem mass spectrometric (LC-MS/MS) for the quantification of multiple mycotoxins/fungal metabolites. Fungal genera of *Aspergillus*, *Alternaria*, *Bipolaris*, *Fusarium*, *Mucor*, *Penicillium*, and *Rhizoctonia* were recovered in the infected grain. All metabolites were detected either in one or more samples. Among major mycotoxins and derivatives, deoxynivalenol (137 µg/kg), zearalenone (121 µg/kg), ochratoxin A (115 µg/kg), and fumonisin B1 (112 µg/kg) were detected with maximum concentrations, while aflatoxin B1 had relatively lower concentrations (23.6 µg/kg). Different emerging mycotoxins were also detected, with tenuazonic acid (1515 µg/kg) occurring at the maximum concentration among *Alternaria* metabolites. Fusaric acid (2786 µg/kg) from *Fusarium* metabolites and kojic acid (4584 µg/kg) were detected with the maximum concentration among *Fusarium* and *Aspergillus* metabolites, respectively. Unspecific metabolites were recognized with neoechinulin A (1996 µg/kg) at the maximum concentration, followed by cyclo (L-Pro-L-Tyr) (574 µg/kg) and cyclo (L-Pro-L-Val) (410 µg/kg).

(Ok *et al.*, 2016) carried out an inter-laboratory study in eight laboratories to validate a liquid chromatography–tandem mass spectrometry (LC/MS/MS) method for the simultaneous determination of aflatoxins and sterigmatocystin (STC) in white rice and sorghum (*Sorghum bicolor*). The results of the inter-laboratory study of aflatoxins and sterigmatocystin (STC) in sorghum was presented in table 6. The mean ARs for aflatoxins and STC were in the ranges 79%–99% and 85%–97%, respectively. One outlier was observed in the sorghum sample spiked with 1.0 µg/kg of aflatoxins. The RSDr and RSDR for aflatoxins were in the ranges 8.1%–24.4% and 13.6%–42.5%, respectively. The HorRat values of aflatoxins ranged from 0.3 to 1.0. For STC, the AR, RSDr, and RSDR were in the ranges 85%–97%, 14.2%–29.0%, and 32.0%–54.7%, respectively. It was concluded LC/MS/MS method possessed the performance characteristics required to obtain accurate results when the results is compare with EU guidelines.

Table 1 Results of the Inter-Laboratory Study Of Aflatoxins and Sterigmatocystin in Sorghum(Ok *et al.*, 2016)

Laboratory	AFB ₁ ^a			AFB ₂ ^b			AFG ₁ ^c			AFG ₂ ^d			STC ^e		
	1.0	2.5	5.0	1.0	2.5	5.0	1.0	2.5	5.0	2.0	5.0	10.0	0.30	0.75	1.50
1	1.00	2.44	4.64	0.89	2.04	4.79	0.95	2.24	4.77	1.81	4.26	9.50	0.21	0.51	1.06
2	1.03	1.69	2.87	0.74	1.55	2.94	0.79	1.71	2.98	2.22	3.50	6.03	0.14	0.35	0.76
3	1.10	2.39	4.56	0.93	2.22	4.23	1.01	2.25	4.31	0.82	3.38	7.67	0.27	0.65	1.45
4	0.62	1.84	3.49	0.71	1.86	4.46	0.58	2.46	4.24	1.28	3.96	9.18	0.25	0.66	1.35
5	0.90	2.35	4.88	0.93	2.19	4.88	0.89	2.23	4.68	1.87	4.31	9.36	0.30	0.72	1.46
6	1.04	2.62	5.40	1.07	2.57	5.42	1.01	2.55	5.68	2.01	4.75	10.34	0.32	0.76	1.56
7	0.97	1.94	3.96	0.93	1.83	4.04	0.50	1.43	3.63	1.54	3.04	7.10	0.47	0.72	1.23
8	0.92	2.37	3.95	0.92	2.42	3.46	0.91	2.32	3.48	1.65	4.31	6.38	0.33	0.77	1.39
Mean (µg/kg)	0.99	2.21	4.22	0.89	2.08	4.28	0.83	2.15	4.22	1.65	3.94	8.19	0.29	0.64	1.28
AR (%) ^f	99.0	88.4	84.4	89.0	83.2	85.6	83.0	86.0	84.4	82.5	78.8	81.9	96.7	85.3	85.3
Outlier ^g	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RSD _r (%) ^h	11.9	14.6	8.1	18.3	17.4	9.6	21.6	14.6	17.4	19.3	24.4	12.8	29.0	24.2	14.2
RSDR (%) ⁱ	13.6	24.8	29.4	23.0	26.9	28.8	38.5	28.6	32.7	42.5	28.1	30.8	54.7	38.0	32.0
HorRat	0.3	0.6	0.8	0.5	0.7	0.8	0.9	0.7	0.9	1.0	0.8	1.0	1.0	0.8	0.8

^a AFB₁: aflatoxin B₁; ^b AFB₂: aflatoxin B₂; ^c AFG₁: aflatoxin G₁; ^d AFG₂: aflatoxin G₂; ^e STC: sterigmatocystin; ^f AR: apparent recovery; ^g Outlier: cochrane and single Grubbs parameters;

^h RSD_r: relative standard deviation of repeatability; ⁱ RSDR: relative standard deviation of reproducibility.

(Kim *et al.*, 2017) carried out a study to simultaneous determine 13 mycotoxins (deoxynivalenol, nivalenol, 3-acetylivalenol, aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, fumonisin B1, fumonisin B2, T-2, HT-2, zearalenone, and ochratoxin A) in 5 types of commercial grains (brown rice, maize, millet, sorghum, and mixed cereal) from South Korea using liquid chromatography–tandem mass spectrometry (LC/MS/MS) method, after a single immunoaffinity column clean-up. It was observed that the method showed a good linearity, sensitivity, specificity, and accuracy in mycotoxin determination when the results were compare with Korean Food and Drug Administration (KFDA) guidelines. Mean and range of levels and incidence of 13 mycotoxins in a total of 507 brown rice, millet, sorghum, maize, and mixed cereal collected are presented in table 7.

Conclusion

Based on the review of the research studies carried out from the last 10 years about the objectives of this review work, the following conclusions can thus be deduced. MPCR-based assay is a rapid, cost effective and user friendly that can be used for the diagnosis of major mycotoxigenic fungi in sorghum and that *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus ochraceus* are the predominant fungi species in the Northern guinea savannah while all *Penicillium* species with

exemption of *Penicillium aurentiogresum* and *Penicillium paxillii* are found to be predominant in the derived savannah and southern guinea savannah respectively and *Fusarium* species was found to constitute the highest fungal contaminants in the Southern Guinea savannah of Nigeria. Other PCR methods like Noble nested polymerase chain reaction (n-PCR) assay and quantitative (q) real-time (PCR) are possible methods that can also be used in the identification of Fungal Profile Contaminating Sorghum.

Toxicogenicity of Isolated Fungi depends on the environmental and climatic factors. *Aspergillus parasiticus* was reported to be the highest producer of aflatoxins. *Fusarium proliferatum* is the highest for aflatoxin B1 while *Fusarium graminearum* is the highest producer of Zearalenone and Deoxynivalenol in Africa. In Riyadh region of Saudi Arabia, *Aspergillus niger* isolates were reported to be the highest producers of aflatoxins, *Fusarium verticillioides* was the highest producer of the *Fusarium* toxins fumonisin (19.1 ppb) and Zearalenone (21.4 ppb). *Fusarium nygamai* was the highest producer of vomitoxin (31.3 ppb). *Penicillium funiculosum* was reported as the highest producer toxin Patulin (41 ppb), while *Penicillium oxalicum* was the highest producer of citreoviridin (10 ppb). It was also reported that that *aspergillus* and *fusarium* spp. and their mycotoxins do not pose a threat to sorghum production.

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Data Availability: The author holds all the data employed in this study and is open to sharing it upon reasonable request.

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