



UNIVERSITI PUTRA MALAYSIA

**CHARACTERIZATION OF MALAYSIAN NON-AFLATOXIGENIC
Aspergillus flavus ISOLATES AND THEIR POTENTIAL USE AS
BIOCONTROL AGENTS FOR AFLATOXIGENIC *A. flavus* IN SWEET
CORN**

KHAN RAHIM

FSTM 2021 10



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By

KHAN RAHIM

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

June 2020

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Associate Professor Farinazleen Mohamad Ghazali, PhD
Faculty : Food Science and Technology

This study was intended to morphologically and molecularly characterize and determine the aflatoxigenic and non-aflatoxigenic *Aspergillus flavus* isolates. Forty isolates of *A. flavus* were obtained from sweet corn kernels and soil samples collected from Kampong Raja, Rose Valley, Kea, and Klebang farms in Malaysia. They were cultured on potato dextrose agar (PDA), dichloran rose-bengal chloramphenicol (DRBC), *A. flavus*, and *parasiticus* agar (AFPA), and coconut cream agar (CCA). Macro-morphological characteristics were determined by observing the colony color, and texture while the micro-morphological characteristics were determined by examining the spore color, size, structure, conidiophore structure, and vesicle shape. The production of aflatoxin was determined by direct visualization of the UV fluorescence of *A. flavus* colonies on CCA. Aflatoxin was qualitatively detected in 18 (45%) isolates of *A. flavus* using UV fluorescence screening while the remaining 22 (55%) isolates did not exhibit any aflatoxin production. AFs production by *A. flavus* was quantitatively analyzed using High-Performance Liquid Chromatography equipped with a fluorescence detector and post-column photochemical reactor. The limits of detection for AFB₁, AFB₂, AFG₁, and AFG₂ were 0.072, 0.062, 0.028, and 0.032 ppb, while the limits of quantification were 0.220, 0.189, 0.087, and 0.098 ppb respectively. AFB₁ was detected in 24 out of 40 isolates of *A. flavus* in concentrations ranging between 0.009 and 3.848 ppb. On the other hand, AFG₁ was detected in 12 isolates in which two isolates (ARV17, ARV22) exceeded the maximum tolerable limits of 5 ppb (50.687 ppb, 40.434 ppb). AFs content was significantly different ($p < 0.05$) in *A. flavus* isolates collected from four different farms. Molecular characterization of *A. flavus* isolates and detection of AF-producing gene in AF⁺ was carried out using polymerase chain reaction (PCR). Specific primers, FLA1 and FLA2, were used to amplify the targeted sequence of *A. flavus*. The isolates were confirmed as *A. flavus*, exhibiting 100% homology with other reference strains of *A.*

flavus on the National Center for Biotechnology Information (NCBI). Multiplex PCR techniques successfully amplified *aflP*, *aflO*, and *aflR* genes. AF-producing genes were detected in 35 strains of *A. flavus* strains, while the remaining five strains did not exhibit these genes. The efficacy of A^{F-} strains in inhibiting AF⁺ growth and reducing AFB₁ production was assessed. Co-inoculation of sweet corn kernels with A^{F-} and AF⁺ strains significantly ($p < 0.05$) reduced the concentrations AFB₁ in sweet corn kernels. The concentration of AFB₁ detected in the co-inoculated treatments ranged from 0.321 to 1.093 ppb while the reduction of AFB₁ in corn kernels ranged from 23% for AKL36⁻ to 79% for AKR5⁻ strain. The AKR5⁻ and AKL34⁻ strains exhibited the maximum AFB₁ reductions of 79 and 75% while the AKL36⁻ exhibited a minimum reduction towards AFB₁ production. In conclusion, the AKR5⁻ and AKL34⁻ would be suitable candidates as potential biocontrol agents to be used in the sweet cornfield, as they significantly reduced the AFB₁ in sweet corn kernels.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENCIRIAN ISOLAT *ASPERGILLUS FLAVUS* NON-AFLATOKSIGENIK
MALAYSIA DAN POTENSI PENGGUNAANNYA SEBAGAI EJEN
BIOKONTROL UNTUK AFLATOKSIGENIK *ASPERGILLUS
FLAVUS* JAGUNG MANIS**

Oleh

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Kajian ini bertujuan untuk mencirikan secara morfologi dan molekular serta menentukan isolat *Aspergillus flavus* aflatoksigenik dan bukan aflatoksigenik. Empat puluh isolat *A. flavus* diperoleh dari biji jagung manis dan sampel tanah yang dikumpulkan dari ladang Kampong Raja, Rose Valley, Kea, dan Klebang di Malaysia. Mereka dikultur pada agar-agar dekstrosa kentang (PDA), dichloran rose-bengal chloramphenicol (DRBC), *A. flavus* dan agar *parasiticus* (AFPA), dan agar-agar krim kelapa (CCA). Ciri makro-morfologi ditentukan dengan memerhatikan warna dan tekstur koloni sementara ciri-ciri mikro-morfologi ditentukan dengan memeriksa warna spora, ukuran, struktur, struktur konidiophore dan bentuk vesikel. Penghasilan aflatoxin ditentukan oleh visualisasi langsung pendarfluor UV koloni *A. flavus* pada CCA. Aflatoxin dikesan secara kualitatif dalam 18 (45%) isolat *A. flavus* yang menggunakan pemeriksaan pendarfluor UV sementara 22 (55%) isolat selebihnya tidak mengeluarkan aflatoxin. Penghasilan AF oleh *A. flavus* dianalisis secara kuantitatif menggunakan Kromatografi Cecair Berprestasi Tinggi yang dilengkapi dengan pengesanan pendarfluor dan reaktor fotokimia pasca lajur. Had pengesanan untuk AFB₁, AFB₂, AFG₁, dan AFG₂ masing-masing adalah 0,072, 0,062, 0,028, dan 0,032 ppb, sementara had pengukuran adalah 0,220, 0,189, 0,087, dan 0,098 ppb. AFB₁ dikesan dalam 24 daripada 40 isolat *A. flavus* dalam kepekatan antara 0,009 dan 3,848 ppb. Sebaliknya, AFG₁ dikesan dalam 12 isolat di mana dua isolat (ARV17, ARV22) melampaui had maksimum toleransi 5 ppb (50.687 ppb, 40.434 ppb). Kandungan AF berbeza secara signifikan ($p < 0.05$) dalam isolat *A. flavus* yang dikumpulkan dari empat ladang yang berbeza. Pencirian molekul isolat *A. flavus* dan pengesanan gen penghasil AF⁻ dalam AF⁺ dilakukan dengan menggunakan reaksi berantai polimerase (PCR). Primer khusus, FLA1 dan FLA2 digunakan untuk memperkuat urutan *A. flavus* yang disasarkan. Isolat yang disahkan sebagai *A. flavus*, menunjukkan homologi 100% dengan jenis rujukan *A. flavus* lain di Pusat Maklumat

Nasional Bioteknologi (NCBI). Teknik PCR multiplek berjaya menguatkan gen *aflP*, *aflO*, dan *aflR*. Gen penghasil AF dikesan pada 35 strain *A. flavus* sementara 5 strain selebihnya tidak menunjukkan gen ini. Keberkesanan strain AF dalam meningkatkan pertumbuhan AF⁺ dan mengurangkan pengeluaran AFB₁ telah dinilai. Inokulasi biji jagung manis dengan strain AF⁻ dan AF⁺ secara signifikan ($p < 0.05$) mengurangkan kepekatan AFB₁ dalam biji jagung manis. Kepekatan AFB₁ yang dikesan dalam rawatan bersama-inokulasi berkisar antara 0,321 hingga 1,093 ppb sementara pengurangan AFB₁ dalam biji jagung kisar antara 23% untuk AKL36⁻ hingga 79% untuk strain AKR5⁻. Strain AKR5⁻ dan AKL34⁻ menunjukkan penurunan AFB₁ maksimum 79 dan 75% sementara AKL36⁻ menunjukkan penurunan minimum terhadap pengeluaran AFB₁. Kesimpulannya, AKR5⁻ dan AKL34⁻ berpotensi digunakan sebagai agen biokontrol di ladang jagung manis, kerana mereka mengurangkan AFB₁ secara signifikan dalam biji jagung manis.



ACKNOWLEDGEMENTS

Alhamdulillah, thank you, Allah, for the strength that He has given me, for the wisdom that He has granted me, and for the unconditional love that He has shown me until I can pursue and completed my Doctor of Philosophy. Without You, I would never have the perseverance to make it until the end.

I would like to dedicate my heartfelt gratitude to Assoc. Prof. Dr. Farinazleen Mohamad Ghazali as my supervisor, for her guidance, encouragement, and constructive suggestions given in the completion of the present work. A million thanks to Dr. Nik Iskandar Putra Samsudin and Assoc. Prof. Dr. Nor Ainy Mahyudin, who has also contributed tremendously to the completion of the present work.

Sincere thanks and appreciations also extended to all support staff of Microbiology Laboratory, Faculty of Food Science and Technology for their assistance during my laboratory work. Thousands of thanks also go to my lab mates for their help and support throughout the present work.

Words could not describe my most profound appreciation to my Mom, Mrs. Mehmanda, and all my family members for their love, encouragement, and understandings. Millions of thanks are extended to my lovely wife, Mrs. Nighat BiBi, who has always been by my side along my PhD journey. Not forgotten to my late father, Mr. Dilawar Khan, your loving and encouragement are always in my mind. Lastly, thank you very much again to all of you who had been involved in my research. I will remember you all for the rest of my life.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

A	Adenine
AF	Aflatoxin
AFB ₁	Aflatoxin B ₁
AFB ₂	Aflatoxin B ₂
AFBS	AFB ₁ + AFB ₂
AFG ₁	Aflatoxin G ₁
AFG ₂	Aflatoxin G ₂
AFGS	AFG ₁ + AFG ₂
AFM ₁	Aflatoxin M ₁
afIR	Transcriptional (regulatory) gene in Aflatoxin B ₁ biosynthesis genes cluster
afIC	A structural gene in aflatoxin B ₁ biosynthesis genes cluster
afID	A structural gene in aflatoxin B ₁ biosynthesis genes cluster
afIM	A structural gene in aflatoxin B ₁ biosynthesis genes cluster
afIP	Amplified fragment length polymorphism
AFP ₁	O-demethylation of AFB ₁
AFQ ₁	3 α -hydroxylation of AFB ₁
AF-alb	Aflatoxin albumin adducts in blood
AFPA	<i>A. flavus parasiticus</i> agar
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
AVF	Averufin
AVN	Averantin
a _w	Water Activity
BHA	2(3)-tert-Butyl-4-Hydroxyanisole

bw	Bodyweight
C	Cytosine
CEM	Corn Extract Media
CIT	Citrinin
CPA	Cyclopiazonic Acid
CYA	Czapek Yeast Agar
DH	Dehydratase domain of PKS
DHDMST	Dihydrodemethylsterigmatocystin
DHOMST	Dihydro-O-Methylsterigmatocystin
DHST	Dihydrosterigmatocystin
DMST	Demethylsterigmatocystin
DNase	Deoxyribonuclease
DON	Deoxynivalenol
EFSA	European Food Safety Authority
ER	Transacting Enoyl domain of PKS
EST	Short single-read Transcript sequences database
EC	European Commission
EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FB ₁	Fumonisin B ₁
FB ₂	Fumonisin B ₂
FBS	Fumonisin B ₁ , B ₂
G	Guanine
g	gram
G proteins	Heterotrimeric G protein

H	Hyphae
h	hour
HCC	Hepatocellular Carcinoma
HAVN	5'-Hydroxy-Averantin
HBsAG positive	HBV-positives patients
HBsAG negative	HBV-negatives patients
HBV	Hepatitis B Virus
His-rich	Section rich in Histidine in the <i>aflR</i> gene
HIV	Human Immunodeficiency Virus
HPLC	High-Performance Liquid Chromatography
HOMST	11-Hydroxy-O-Methylsterigmatocystin
HR-PKS	Highly-reducing PKS
IARC	International Agency Center for Research on Cancer
IgA	Immunoglobulin A
ITS	Internal Transcribed Spacer
KR	β -Ketoreductase domain of PKS
MA	Aerial Mycelium
MADE	Myxobacteria Aflatoxin Degradation Enzyme
mg	Milligram
MLN	Mesenteric Lymph Node
mm	Millimeter
min	Minute
NOR	Norsolorinic Acid
NLD	Nuclear Localization Domain
NR-PKS	Non-reducing PKS
OAVN	Oxoaverantin

OMST	O-Methylsterigmatocystin
OTA	Ochratoxin A
P	Phialides
PAT	Patulin
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PKS	Polyketide Synthase
PR-PKS	Partially-Reducing PKS
psia	Hydroxylated linoleic acids
ppm	Parts per million
R	Radical group of trichothecenes
RNase	Ribonucleic acid (RNA) enzyme
rpm	Revolutions per minute
RASFF	Rapid Alert System for Food and Feed
RCM	Residual Concentration in the Medium
RT-qPCR	Reverse Transcription quantitative
S	Spirales
sec	Second
spp.	Specie
SSR	Single Sequence Repeats
ST	Sterigmatocystin
T	Thymine
Tc	CVPs tethering start point
TF	Transcription factor
tsp	Transcription start point
TSP	Translational Start Point

T-2	Trichothecenes
UPM	Universiti Putra Malaysia
UV	Ultraviolet
V	Vesicle
VAL	Versiconal
VCG	Vegetative Compatibility Groups
VERA	Versicolorin A
VERB	Versicolorin B
VHA	Versiconal Hemiacetal Acetate
WHO	World Health Organization
ZEA	Zearalenone

CHAPTER 1

INTRODUCTION

Corn (*Zea mays L.*) ranks as the third main cereal crop after wheat and rice. Asia is one of the leading producers of sweet corn, where more than 62% of corn production is consumed in animals' feeds and human consumption. While sweet corn is a popular food in the U.S, China, and Brazil, it has recently gained popularity in other Asian countries such as Malaysia. According to the U.S Department of Agriculture, the total world corn production in 2017-2018 was 1,076.18 million tons. By 2025, corn is predicted to be the most cultivated cereal with market demand to be double by 2050 in developing countries (Rosegrant *et al.*, 2018). In 2017, Malaysia consumed nearly 4 million tons of grain corn. In Malaysia, the majority of grain corn consumed has been imported while sweet corn was grown locally (Wahab, 2018).

According to the Malaysian Department of Agriculture (Shahniza *et al.*, 2020), approximately 10,477.30 ha of the cultivated land produced 72,560.522 tons of sweet corn in 2017. Malaysia's leading states of sweet corn are Johor, Pahang, and Kelantan. Sweet corn has been consumed as a food by humans and as a raw material in the industry. For animal consumption, Malaysia usually imports corn grains from other corn-producing countries, including Argentina, Brazil, the U.S, Pakistan, and France (FAOSTAT, 2017). Malaysia's climate, which has a constant high temperature (22–31°C) and high relative humidity (60–80%), provides ideal conditions for the growth of *Aspergillus* fungi to infect food and feedstuffs (Neme & Mohammed, 2017; Norlia *et al.*, 2018). The average annual rainfall is approximately 200-250 cm. A study of aflatoxins (AFs) in food and stored agricultural products in Malaysia revealed that corn is exceedingly vulnerable to mold and AFs contamination, except for Bt corn. Several mycotoxin-producing fungi contaminate corn crops and produce mycotoxins such as *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* (Munkvold *et al.*, 2019).

Mycotoxins are the secondary metabolites, which significantly affect food safety and cause severe complications for humans and animals (Njoroge *et al.*, 2017; Ostry *et al.*, 2017). In commodities, the most common mycotoxins are aflatoxins (AFs), fumonisins (FUM), deoxynivalenol (DON), ochratoxins-A (OTA), zearalenone (ZEA), patulin (PAT), and citrinin (CIT) (Alshannaq & Yu, 2017). Among mycotoxins, AFs have received considerable attention due to its sporadic incidence in food commodities and health effects in poultry, fish, swine, and cattle (Ali *et al.*, 2015). AFs are potent carcinogenic compounds, predominantly produced by *Aspergillus flavus* and *A. parasiticus*, which contaminate diverse cereal crops such as corn, rice, and nuts (Frisvad *et al.*, 2019; Okoth *et al.*, 2018).

While numerous AFs were presently identified, AFB₁, AFB₂, AFG₁, and AFG₂ are the four most crucial AFs. The International Agency for Research on Cancer classifies AFB₁ as the most potent, mutagenic, and Group 1 human carcinogen (McGuire, 2016). It is also considered to cause chronic and acute health diseases (aflatoxicosis) in

children and older people. Several studies have revealed that the liver is the main target organ of AFB₁ in humans and animals (Benkerroum, 2016). Furthermore, the intake of high-dose undetectable AFB₁ in food can be fatal. AFB₁ exposure in agricultural corn products has been the most significant and severe case of an epidemic of acute aflatoxicosis in Eastern and Central Kenya, resulting in 317 cases and 125 deaths (Frisvad *et al.*, 2019). In Malaysia, the first outbreak of aflatoxicosis was recorded in 1960, in pig farms of Malacca (Mohajeri *et al.*, 2018). The second outbreak erupted in 1988, in Perak, killing 13 Chinese children who consumed Chinese noodles known as *loh shi fun* (Benkerroum, 2020; Iqbal *et al.*, 2019; Norlia *et al.*, 2018). In 2009, Zulhabri and his colleagues recorded AFB₁-albumin adduct in patients with hepatocarcinoma (HCC) (Redzwan *et al.*, 2014). Thus, many countries have developed guidelines for AFs in food and other products while taking account of possible risks to human health.

The European Commission Regulation established an acceptable amount of 2 µg/kg for AFB₁ and 4 µg/kg for overall AFs (Commission Regulation (EC) No. 165/2010). In the same way, Malaysia has set an acceptable limit of 10 µg/kg for AFB₁ and 15 µg/kg for all AFs in cereal crops in the Food law of 1983 (Amendment) (No.3) Regulations of 2014. Although several methods have been implemented globally to minimize pre-harvest AFs contamination in corn and other cereals, only a few have shown some potential (Atehnkeng *et al.*, 2014; Bandyopadhyay *et al.*, 2016). Concerns about fungicides use and cost-effectiveness reduce its significance for handling AFs contamination in corn (Abdallah *et al.*, 2015). Therefore, the usage of non-aflatoxigenic *A. flavus* (AF⁻) as a biocontrol agent would be the most promising method for this purpose.

AF⁻ strains cannot produce AFs owing to genetic mutation or the lack of polyketide synthase genes on the AF gene cluster (Ehrlich *et al.*, 2015; Zanon *et al.*, 2016; Zhou *et al.*, 2015). This technique for regulating AFs has been employed on cotton (Zhang *et al.*, 2018), corn (Mauro *et al.*, 2015), and peanuts crops (Chulze *et al.*, 2015). The control of AFs contamination by this technique is attributed to the competitive exclusion of AF⁺ strains with AF⁻ strains. The removal of AF⁺ strains could be achieved through the rapid expansion of AF⁻ strains (Damann, 2015). This technique has already been employed in countries where AFs contamination in corn is a severe problem, including the U.S, China, Kenya, Nigeria, and Malaysia. Hence, the present study was conducted to characterize Malaysian non-aflatoxigenic *A. flavus* isolates and analyze their potential use as biocontrol agents for aflatoxingenic *A. flavus* in sweet corn. The following specific objectives can accomplish this:

- 1 To collect, isolate, morphologically characterize the *A. flavus*, and determine the potential aflatoxingenic and non-aflatoxingenic isolates of *A. flavus*.
- 2 To detect, analyze, and quantify aflatoxin produced by aflatoxingenic *A. flavus* by High-Performance Liquid Chromatography (HPLC).
- 3 To molecularly characterize *A. flavus* strains and detect the aflatoxin-producing genes in aflatoxingenic strains.

- 4 To assess the efficacy of non-aflatoxigenic strains of *A. flavus* against aflatoxigenic strains in sweet corn kernels, in vitro condition.



REFERENCES

- Ab Majid, A. H., Zahran, Z., Ismail, N. A., Rahman, W. A., Zubairi, K. S. M., Dieng, H., & Satho, T. (2015). Morphological and molecular characterization of fungus isolated from tropical bed bugs in Northern Peninsular Malaysia, *Cimex hemipterus* (Hemiptera: Cimicidae). *Asian Pacific Journal of Tropical Biomedicine*, 5(9), 707-713.
- Ababulgu, D., Shimelis, H., Laing, M., & Amelework, B. (2018). Phenotypic characterization of elite-quality protein maize (QPM) inbred lines adapted to tropical highlands and the association studies using SSR markers. *Australian Journal of Crop Science*, 12(1), 22-31.
- Abbas, H. K., Accinelli, C., & Shier, W. T. (2017). Biological control of aflatoxin contamination in US crops and the use of bioplastic formulations of *Aspergillus flavus* biocontrol strains to optimize application strategies. *Journal of Agricultural and Food Chemistry*, 65(33), 7081-7087.
- Abbas, H. K., Zablotowicz, R. M., Weaver, M. A., Shier, W. T., Bruns, H. A., Bellaloui, N., . . . Abel, C. A. (2013). Implications of Bt traits on mycotoxin contamination in maize: overview and recent experimental results in the Southern United States. *Journal of Agricultural and Food Chemistry*, 61(48), 11759-11770.
- Abdallah, M. F., Girgin, G., & Baydar, T. (2015). Occurrence, prevention, and limitation of mycotoxins in feeds. *Animal Nutrition and Feed Technology*, 15(3), 471-490.
- Abdel-Azeem, A. M., Abdel-Azeem, M. A., Abdul-Hadi, S. Y., & Darwish, A. G. (2019). *Aspergillus*: Biodiversity, ecological significances, and industrial applications. *Recent Advancement in White Biotechnology Through Fungi*: (1) 121-179. Springer, Cham.
- Abdel-Hadi, A. M., Awad, M. F., Abo-Dahab, N. F., & ElKady, M. F. (2014). Extracellular synthesis of silver nanoparticles by *Aspergillus terreus*: biosynthesis, characterization, and biological activity. *Biosciences Biotechnology Research Asia*, 11(3), 1179-1186.
- Abdulateef, S. M., Aljubori, M. H., & Abdulbaqi, N. J. (2014). Genetic Diversity among some *Aspergillus flavus* isolates by using inter simple sequence repeats (ISSR). *Iraqi Journal of Science*, 55(3A), 986-993.
- Accinelli, C., Abbas, H. K., Little, N. S., Kotowicz, J. K., & Shier, W. T. (2018). Biological control of aflatoxin production in corn using non-aflatoxigenic *Aspergillus flavus* administered as a bioplastic-based seed coating. *Crop Protection*, 107, 87-92.

- Adebo, O., Njobeh, P., Gbashi, S., Nwinyi, O., & Mavumengwana, V. (2017). Review of microbial degradation of aflatoxins. *Critical Studies in Food Science and Nutrition*, 57(15), 3208-3217.
- Adedara, I. A., Nanjappa, M. K., Farombi, E. O., & Akingbemi, B. T. (2014). Aflatoxin B₁ disrupts the androgen biosynthetic pathway in rat Leydig cells. *Food and Chemical Toxicology*, 65, 252-259.
- Adhikari, B. N., Bandyopadhyay, R., & Cotty, P. J. (2016). Degeneration of aflatoxin gene clusters in *Aspergillus flavus* from Africa and North America. *AMB Express*, 6(1), 1-16.
- Adhikari, M., Negi, B., Kaushik, N., Adhikari, A., Al-Khedhairi, A. A., Kaushik, N. K., & Choi, E. H. (2017). T-2 mycotoxin: toxicological effects and decontamination strategies. *Oncotarget*, 8(20), 33933–33952.
- Afsah-Hejri, L., & Jinap, S. (2013). Influence of different mobile phase compositions on detection of ochratoxin A. *Food Control*, 31(1), 244-250.
- Afsah-Hejri, L., Jinap, S., Hajeb, P., Radu, S., & Shakibazadeh, S. (2013). A review on mycotoxins in food and feed: Malaysia case study. *Comprehensive Reviews in Food Science and Food Safety*, 12(6), 629-651.
- Afzal, H., Shazad, S., & Nisa, S. Q. U. (2013). Morphological identification of *Aspergillus* species from the soil of the Larkana District (Sindh, Pakistan). *Asian Journal of Agriculture and Biology*, 1(3), 105-117.
- Agbetiameh, D., Ortega-Beltran, A., Awuah, R. T., Atehnkeng, J., Islam, M.-S., Callicott, K. A., . . . Bandyopadhyay, R. (2019). Potential of atoxigenic *Aspergillus flavus* vegetative compatibility groups associated with maize and groundnut in Ghana as biocontrol agents for aflatoxin management. *Frontiers in Microbiology*, 10, 1-15.
- Ahmad, F. N. B. (2016). The occurrence of aflatoxin m1 in urine samples, milk, and dairy products and their associated factors among residents in Terengganu, Malaysia. 1-51.
- Aimi, N. N., Anuar, H., Manshor, M., Nazri, W. W., & Sapuan, S. (2014). Optimizing the parameters in durian skin fiber reinforced polypropylene composites by response surface methodology. *Industrial Crops and Products*, 54, 291-295.
- Al-Hindi, R. R., Aly, S. E., Hathout, A. S., Alharbi, M. G., Al-Masaudi, S., Al-Jaouni, S. K., & Harakeh, S. M. (2018). Isolation and molecular characterization of mycotoxigenic fungi in agarwood. *Saudi Journal of Biological Sciences*, 25(8), 1781-1787.
- Al-Saad, L. A., Al-Badran, A. I., Al-Jumayli, S. A., Magan, N., & Rodríguez, A. (2016). Impact of bacterial biocontrol agents on aflatoxin biosynthetic genes, *aflD*, and *aflR* expression, and phenotypic aflatoxin B₁ production by

Aspergillus flavus under different environmental and nutritional regimes. *International Journal of Food Microbiology*, 217, 123-129.

- Aldars-García, L., Ramos, A. J., Sanchis, V., & Marín, S. (2016). Modeling postharvest mycotoxins in foods: recent research. *Current Opinion in Food Science*, 11, 46-50.
- Algül, I., & Kara, D. (2014). Determination and chemometric evaluation of total aflatoxin, aflatoxin B₁, ochratoxin A and heavy metals content in corn flours from Turkey. *Food Chemistry*, 157, 70-76.
- Ali, N., & Degen, G. H. (2019). Citrinin biomarkers: a review of recent data and application to human exposure assessment. *Archives of toxicology*, 1-10.
- Ali, N., Hashim, N. H., & Shuib, N. S. (2015). Natural occurrence of aflatoxins and ochratoxin A in processed spices marketed in Malaysia. *Food Additives & Contaminants: Part A*, 32(4), 518-532.
- Alkhayyat, F., & Yu, J.-H. (2014). Upstream regulation of mycotoxin biosynthesis. In *Advances in Applied Microbiology*: (86), 251-278.
- Almeida, F., Rodrigues, M. L., & Coelho, C. (2019). The still underestimated problem of fungal diseases worldwide. *Frontiers in Microbiology*, 10, 1-214.
- Alshannaq, A., & Yu, J.-H. (2017). Occurrence, toxicity, and analysis of major mycotoxins in food. *International Journal of Environmental Research and Public Health*, 14(6), 632.
- Alvarez-Dominguez, J. R., Bai, Z., Xu, D., Yuan, B., Lo, K. A., Yoon, M. J., . . . Chen, S. (2015). De novo reconstruction of adipose tissue transcriptomes reveals long non-coding RNA regulators of brown adipocyte development. *Cell Metabolism*, 21(5), 764-776.
- Amani Ghayum, S., Shams-Ghahfarokhi, M., & Razzaghi-Abyaneh, M. (2017). Expression of *aflR*, *veA*, and *laeA* as regulators of aflatoxins and cyclopiazonic acid biosynthesis pathway in *Aspergillus flavus*. *International Journal of Molecular and Clinical Microbiology*, 7(1), 787-794.
- Amare, M. G., & Keller, N. P. (2014). Molecular mechanisms of *Aspergillus flavus* secondary metabolism and development. *Fungal Genetics and Biology*, 66, 11-18.
- Arroyo-Manzanares, N., Huertas-Pérez, J. F., García-Campaña, A. M., & Gámiz-Gracia, L. (2015). Aflatoxins in animal feeds: A straightforward and cost-effective analytical method. *Food Control*, 54, 74-78.
- Astoreca, A., Vaamonde, G., Dalcero, A., Marín, S., & Ramos, A. (2014). Abiotic factors and their interactions influence on the co-production of aflatoxin B₁

and cyclopiazonic acid by *Aspergillus flavus* isolated from corn. *Food Microbiology*, 38, 276-283.

- Atehnkeng, J., Ojiambo, P., Cotty, P., & Bandyopadhyay, R. (2014). Field efficacy of a mixture of atoxigenic *Aspergillus flavus* Link: Fr vegetative compatibility groups in preventing aflatoxin contamination in maize (*Zea mays* L.). *Biological Control*, 72, 62-70.
- Ayalew, A., Kimanya, M., Matumba, L., Bandyopadhyay, R., Menkir, A., & Cotty, P. (2017). Controlling aflatoxins in maize in Africa: Strategies, challenges, and opportunities for improvement. *Achieving Sustainable Cultivation of Maize*, 2, 1-24.
- Ball, P., & Hallsworth, J. E. (2015). Water structure and chaotropicity: their uses, abuses, and biological implications. *Physical Chemistry Chemical Physics*, 17(13), 8297-8305.
- Bandyopadhyay, R., Ortega-Beltran, A., Akande, A., Mutegi, C., Atehnkeng, J., Kaptoge, L., . . . Cotty, P. (2016). Biological control of aflatoxins in Africa: current status and potential challenges in the face of climate change. *World Mycotoxin Journal*, 9(5), 771-789.
- Banu, N., & Muthumary, J. P. (2010). Screening of aflatoxigenic property of some *Aspergillus flavus* isolated from sunflower seeds and its products at sunflower oil refineries. *Research Journal of Science and Technology*, 2(5), 102-107.
- Baquião, A. C., de Oliveira, M. M. M., Reis, T. A., Zorzete, P., Atayde, D. D., & Correa, B. (2013). A polyphasic approach to the identification of *Aspergillus* section *Flavi* isolated from Brazil nuts. *Food Chemistry*, 139(1-4), 1127-1132.
- Barrett, C. B., & Bevis, L. E. (2015). The self-reinforcing feedback between low soil fertility and chronic poverty. *Nature Geoscience*, 8(12), 907-912.
- Bediako, K. A., Ofori, K., Offei, S. K., Dzidzienyo, D., Asibuo, J. Y., & Amoah, R. A. (2019). Aflatoxin contamination of groundnut (*Arachis hypogaea* L.): Predisposing factors and management interventions. *Food Control*, 98, 61-67.
- Beheshti, H. R., & Asadi, M. (2014). Aflatoxins in animal feed in Iran. *Food Additives & Contaminants: Part B*, 7(1), 40-42.
- Benkerroum, N. (2016). Mycotoxins in dairy products: A review. *International Dairy Journal*, 62, 63-75.
- Benkerroum, N. (2020). Aflatoxins: Production, structure, health issues, and incidence in Southeast Asian and Sub-Saharan African countries. 17, 423.
- Berthiller, F., Cramer, B., Iha, M., Krska, R., Lattanzio, V., MacDonald, S., . . . Stranska-Zachariasova, M. (2018). Developments in mycotoxin analysis: an update for 2016-2017. *World Mycotoxin Journal*, 11(1), 5-32.

- Bhatnagar-Mathur, P., Sunkara, S., Bhatnagar-Panwar, M., Waliyar, F., & Sharma, K. K. (2015). Biotechnological advances for combating *Aspergillus flavus* and aflatoxin contamination in crops. *Plant Science*, 234, 119-132.
- Bondy, G. S., Coady, L., Ross, N., Caldwell, D., Gannon, A. M., Kwong, K., . . . Raju, J. (2018). A reproductive and developmental screening study of the fungal toxin ochratoxin A in Fischer rats. *Mycotoxin Research*, 34(4), 241-255.
- Borisade, O., & Magan, N. (2014). Growth and sporulation of entomopathogenic *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria farinosa*, and *Isaria fumosorosea* strains concerning water activity and temperature interactions. *Biocontrol Science and Technology*, 24(9), 999-1011.
- Bouchard, M. J., Chorfi, Y., Létourneau-Montminy, M.-P., & Guay, F. (2019). Effects of deoxynivalenol and sodium meta-bisulfite on nutrient digestibility in growing pigs. *Archives of Animal Nutrition*, 73(5), 360-373.
- Bruix, J., Merle, P., Granito, A., Huang, Y.-H., Bodoky, G., Yokosuka, O., . . . Masi, G. (2016). LBA-03 Efficacy and safety of regorafenib versus placebo in patients with hepatocellular carcinoma (HCC) progressing on sorafenib: Results of the international, randomized phase 3 RESORCE trial. *Annals of Oncology*, 27(suppl_2), 56-66.
- Caceres, I., Khoury, A. A., Khoury, R. E., Lorber, S., Oswald, I. P., Khoury, A. E., . . . Bailly, J.-D. (2020). Aflatoxin biosynthesis and genetic regulation: A review. *Toxins*, 12(3), 1-26.
- Calado, T., Fernández-Cruz, M. L., Verde, S. C., Venâncio, A., & Abrunhosa, L. (2018). Gamma irradiation effects on ochratoxin A: Degradation, cytotoxicity, and application in food. *Food Chemistry*, 240, 463-471.
- Callicott, K., & Cotty, P. (2015). Method for monitoring deletions in the aflatoxin biosynthesis gene cluster of *Aspergillus flavus* with multiplex PCR. *Letters in Applied Microbiology*, 60(1), 60-65.
- Camiletti, B. X., Moral, J., Asensio, C. M., Torrico, A. K., Lucini, E. I., Giménez-Pecci, M. d. I. P. & Michailides, T. J. (2018). Characterization of Argentinian endemic *Aspergillus flavus* isolates and their potential use as biocontrol agents for mycotoxins in maize. *Phytopathology*, 108(7), 818-828.
- Carranza, C. S., Bergesio, M. V., Barberis, C. L., Chiacchiera, S. M., & Magnoli, C. E. (2014). Survey of *Aspergillus* section *Flavi* presence in agricultural soils and effect of glyphosate on nontoxigenic *A. flavus* growth on soil- based medium. *Journal of Applied Microbiology*, 116(5), 1229-1240.
- Cary, J., Han, Z., Yin, Y., Lohmar, J., Shantappa, S., Harris-Coward, P., . . . Arroyo-Manzanares, N. (2015). Transcriptome analysis of *Aspergillus flavus* reveals

the veA-dependent regulation of secondary metabolite gene clusters, including the novel aflavarin cluster. *Eukaryotic Cell*, 14(10), 983-997.

Cary, J. W., Harris-Coward, P. Y., Ehrlich, K. C., Di Mavungu, J. D., Malysheva, S. V., De Saeger, S., . . . Calvo, A. M. (2014). Functional characterization of a veA-dependent polyketide synthase gene in *Aspergillus flavus* necessary for the synthesis of asparasone, a sclerotium-specific pigment. *Fungal Genetics and Biology*, 64, 25-35.

Casquete, R., Benito, M. J., de Guía Córdoba, M., Ruiz-Moyano, S., & Martín, A. (2017). The growth and aflatoxin production of *Aspergillus flavus* strains on a cheese model system is influenced by physicochemical factors. *Journal of Dairy Science*, 100(9), 6987-6996.

Castellari, C. C., Cendoya, M. G., Marcos, F. V., Barrera, V., & Pacin, A. M. (2015). Extrinsic and intrinsic factors associated with mycotoxigenic fungi populations of maize grains (*Zea mays* L.) stored in silobags in Argentina. *Revista Argentina de Microbiología*, 47(4), 350-359.

Chan, P., Han, X., Zheng, B., DeRan, M., Yu, J., Jarugumilli, G. K., . . . Wu, X. (2016). Autopalmitoylation of TEAD proteins regulates the transcriptional output of the Hippo pathway. *Nature Chemical Biology*, 12(4), 282-289.

Chang, P.-K., Hua, S. S. T., Sarreal, S. B. L., & Li, R. W. (2015). Suppression of aflatoxin biosynthesis in *Aspergillus flavus* by 2-phenyl ethanol is associated with stimulated growth and decreased degradation of branched-chain amino acids. *Toxins*, 7(10), 3887-3902.

Chang, P.-K., Scharfenstein, L. L., Li, R. W., Arroyo-Manzanares, N., De Saeger, S., & Di Mavungu, J. D. (2017). *Aspergillus flavus* aswA, a gene homolog of *Aspergillus nidulans* oefC, regulates sclerotial development and biosynthesis of sclerotium-associated secondary metabolites. *Fungal Genetics and Biology*, 104, 29-37.

Chang, P. K. (2019). Genome- wide nucleotide variation distinguishes *Aspergillus flavus* from *Aspergillus oryzae* and helps to reveal the origins of atoxigenic *A. flavus* biocontrol strains. *Journal of Applied Microbiology*, 127(5), 1511-1520.

Chen, C., Mitchell, N. J., Gratz, J., Houpt, E. R., Gong, Y., Egner, P. A., . . . Svensen, E. (2018). Exposure to aflatoxin and fumonisin in children at risk for growth impairment in rural Tanzania. *Environment International*, 115, 29-37.

Chen, W., Lee, M.-K., Jefcoate, C., Kim, S.-C., Chen, F., & Yu, J.-H. (2014). Fungal cytochrome p450 monooxygenases: their distribution, structure, functions, family expansion, and evolutionary origin. *Genome Biology and Evolution*, 6(7), 1620-1634.

Chulze, S. N., Palazzini, J. M., Torres, A. M., Barros, G., Ponsone, M. L., Geisen, R., . . . Köhl, J. (2015). Biological control as a strategy to reduce the impact of

mycotoxins in peanuts, grapes, and cereals in Argentina. *Food Additives & Contaminants: Part A*, 32(4), 471-479.

Claudino-Silva, S., Lala, B., Mora, N., Schamber, C., Nascimento, C., Pereira, V., . . . Gasparino, E. (2018). Challenge with fumonisins B₁ and B₂ changes IGF-1 and GHR mRNA expression in the liver of *Nile tilapia* fingerlings. *World Mycotoxin Journal*, 11(2), 237-245.

Conradt, D., Schätzle, M. A., Haas, J., Townsend, C. A., & Müller, M. (2015). New insights into the conversion of versicolorin A in the biosynthesis of aflatoxin B₁. *Journal of the American Chemical Society*, 137(34), 10867-10869.

Cray, J. A., Connor, M. C., Stevenson, A., Houghton, J. D., Rangel, D. E., Cooke, L. R., & Hallsworth, J. E. (2016). Biocontrol agents promote the growth of potato pathogens, depending on environmental conditions. *Microbial Biotechnology*, 9(3), 330-354.

Dagnas, S., Onno, B., & Membré, J.-M. (2014). Modeling the growth of three bakery product spoilage molds as a function of water activity, temperature, and pH. *International Journal of Food Microbiology*, 186, 95-104.

Damann, J. (2015). Atoxigenic *Aspergillus flavus* biological control of aflatoxin contamination: what is the mechanism? *World Mycotoxin Journal*, 8(2), 235-244.

Darsanaki, R. K., Kolavani, M. H., Chakoosari, M. M. D., Shalkeh, S. E., & Tajehmiri, A. (2014). Biological control of aflatoxin B₁ by probiotic bacteria. *Trends in Life Science*, 3(1), 2319-4731.

Daryaei, A., Jones, E. E., Glare, T. R., & Falloon, R. E. (2016). pH and water activity in culture media affect the biological control activity of *Trichoderma atroviride* against *Rhizoctonia solani*. *Biological Control*, 92, 24-30.

Daryanto, S., Wang, L., & Jacinthe, P.-A. (2016). Global synthesis of drought effects on maize and wheat production. *PloS ONE*, 11(5), 1-15.

De Oliveira, H. F., Souto, C. N., Martins, P. C., Di Castro, I., & Mascarenhas, A. (2018). Mycotoxins in broiler production. *Revista de Ciências Agroveterinárias*, 17(2), 292-299.

De Vries, R. P., Riley, R., Wiebenga, A., Aguilar-Osorio, G., Amillis, S., Uchima, C. A., . . . Barry, K. (2017). Comparative genomics reveals high biological diversity and specific adaptations in the industrially and medically important fungal genus *Aspergillus*. *Genome Biology*, 18(1), 1-45.

Degola, F., Morcia, C., Bisceglie, F., Mussi, F., Tumino, G., Ghizzoni, R., . . . Restivo, F. M. (2015). *In vitro* evaluation of the activity of thiosemicarbazone derivatives against mycotoxigenic fungi affecting cereals. *International Journal of Food Microbiology*, 200, 104-111.

- Devadatha, B., Sarma, V., Jeewon, R., Wanasinghe, D. N., Hyde, K. D., & Jones, E. G. (2018). Thyridariella, a novel marine fungal genus from India: morphological characterization and phylogeny inferred from multigene DNA sequence analyses. *Mycological Progress*, 17(7), 791-804.
- Dias, C., Aires, A., & Saavedra, M. J. (2014). Antimicrobial activity of isothiocyanates from cruciferous plants against methicillin-resistant *Staphylococcus aureus* (MRSA). *International Journal of Molecular Sciences*, 15(11), 19552-19561.
- Diba, K., Mirhendi, H., Kordbacheh, P., & Rezaie, S. (2014). Development of the RFLP-PCR method for the identification of medically necessary *Aspergillus* species using single restriction enzyme MwoI. *Brazilian Journal of Microbiology*, 45(2), 503-507.
- Dini, A., Khazaeli, P., Roohbakhsh, A., Madadlou, A., Pourenamdari, M., Setoodeh, L., . . . Moradi, H. (2013). Aflatoxin contamination level in Iran's pistachio nut during the years 2009–2011. *Food Control*, 30(2), 540-544.
- Divakara, S. T., Aiyaz, M., Hariprasad, P., Nayaka, S. C., & Niranjana, S. R. (2014). *Aspergillus flavus* infection and aflatoxin contamination in sorghum seeds and their biological management. *Archives of Phytopathology and Plant Protection*, 47(17), 2141-2156.
- Dohnal, V., Wu, Q., & Kuča, K. (2014). Metabolism of aflatoxins: key enzymes and interindividual as well as interspecies differences. *Archives of Toxicology*, 88(9), 1635-1644.
- Doster, M. A., Cotty, P. J., & Michailides, T. J. (2014). Evaluation of the atoxigenic *Aspergillus flavus* strain AF36 in pistachio orchards. *Plant Disease*, 98(7), 948-956.
- Drott, M. T., Fessler, L. M., & Milgroom, M. G. (2019). Population subdivision and the frequency of aflatoxigenic isolates in *Aspergillus flavus* in the United States. *Phytopathology*, 109(5), 878-886.
- Ehrlich, K., & Mack, B. (2014). Comparison of expression of secondary metabolite biosynthesis cluster genes in *Aspergillus flavus*, *A. parasiticus*, and *A. oryzae*. *Toxins*, 6(6), 1916-1928.
- Ehrlich, K., Moore, G., Mellon, J., & Bhatnagar, D. (2015). Challenges facing the biological control strategy for eliminating aflatoxin contamination. *World Mycotoxin Journal*, 8(2), 225-233.
- Ehrlich, K. C. (2014). Non-aflatoxigenic *Aspergillus flavus* to prevent aflatoxin contamination in crops: advantages and limitations. *Frontiers in Microbiology*, 5, 50.

- Ehrlich, K. C., & Mack, B. M. (2014). Comparison of expression of secondary metabolite biosynthesis cluster genes in *Aspergillus flavus*, *A. parasiticus*, and *A. oryzae*. *Toxins*, 6(6), 1916-1928.
- El-Serag, H. B., Sweet, S., Winchester, C. C., & Dent, J. (2014). Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut*, 63(6), 871-880.
- El-Serag, H. B. (2020). Epidemiology of hepatocellular carcinoma. *The Liver: Biology and Pathobiology*, 758-772.
- Etemadi, F., Hashemi, M., Zandvakili, O., Dolatabadian, A., & Sadeghpour, A. (2018). Nitrogen contribution from winter-killed faba bean covers crop to spring-sown sweet corn in conventional and no-till systems. *Agronomy Journal*, 110(2), 455-462.
- Ezekiel, C., Adetunji, M., Atanda, O., Frisvad, J., Houbraken, J., & Samson, R. (2014). Phenotypic differentiation of species from *Aspergillus* section *Flavi* on neutral red desiccated coconut agar. *World Mycotoxin Journal*, 7(3), 335-344.
- Ezekiel, C. N., Oyeyemi, O. T., Oyedele, O. A., Ayeni, K. I., Oyeyemi, I. T., Nabofa, W., . . . Dada, A. (2018). Urinary aflatoxin exposure monitoring in rural and semi-urban populations in Ogun state, Nigeria. *Food Additives & Contaminants: Part A*, 35(8), 1565-1572.
- Fanelli, F., Geisen, R., Schmidt-Heydt, M., Logrieco, A., & Mulè, G. (2016). Light regulation of mycotoxin biosynthesis: New perspectives for food safety. *World Mycotoxin Journal*, 9(1), 129-146.
- Fani, S. R., Moradi, M., Probst, C., Zamanizadeh, H. R., Mirabolfathy, M., Haidukowski, M., & Logrieco, A. F. (2014). A critical evaluation of cultural methods for the identification of atoxigenic *Aspergillus flavus* isolates for aflatoxin mitigation in pistachio orchards of Iran. *European Journal of Plant Pathology*, 140(4), 631-642.
- FAOSTAT, R. (2017). FAOSTAT database. *Food and Agriculture Organization United Nations*. <http://www.fao.org/faostat/en/#data/OA>.
- Fischer, G. J., & Keller, N. P. (2016). Production of cross-kingdom oxylipins by pathogenic fungi: an update on their role in development and pathogenicity. *Journal of Microbiology*, 54(3), 254-264.
- Frisvad, J. C., Hubka, V., Ezekiel, C., Hong, S.-B., Nováková, A., Chen, A., . . . Mahakarnchanakul, W. (2019). Taxonomy of *Aspergillus* section *Flavi* and their production of aflatoxins, ochratoxins, and other mycotoxins. *Studies in Mycology*, 93, 1-63.

- Gacem, M. A., & El Hadj-Khelil, A. O. (2016). Toxicology, biosynthesis, bio-control of aflatoxin, and new methods of detection. *Asian Pacific Journal of Tropical Biomedicine*, 6(9), 808-814.
- Gakuubi, M. M., Maina, A. W., & Wagacha, J. M. (2017). Antifungal activity of essential oil of *Eucalyptus camaldulensis* Dehn against selected *Fusarium* spp. *International Journal of Microbiology*, 2017, 1-8.
- Gallo, A., Ferrara, M., & Perrone, G. (2017). Recent advances in the molecular aspects of ochratoxin A biosynthesis. *Current Opinion in Food Science*, 17, 49-56.
- Gallo, A., Knox, B. P., Bruno, K. S., Solfrizzo, M., Baker, S. E., & Perrone, G. (2014). Identification and characterization of the polyketide synthase involved in ochratoxin A biosynthesis in *Aspergillus carbonarius*. *International Journal of Food Microbiology*, 179, 10-17.
- Gallo, A., Solfrizzo, M., Epifani, F., Panzarini, G., & Perrone, G. (2016). Effect of temperature and water activity on gene expression and aflatoxin biosynthesis in *Aspergillus flavus* on almond medium. *International Journal of Food Microbiology*, 217, 162-169.
- Garnier, L., Valence, F., Pawtowski, A., Auhustsinava-Galerie, L., Frotté, N., Baroncelli, R., . . . Mounier, J. (2017). Diversity of spoilage fungi associated with various French dairy products. *International Journal of Food Microbiology*, 241, 191-197.
- González-Jartín, J. M., de Castro Alves, L., Alfonso, A., Piñeiro, Y., Vilar, S. Y., Gomez, M. G., . . . Rivas, J. (2019). Detoxification agents based on magnetic nanostructured particles as a novel strategy for mycotoxin mitigation in food. *Food Chemistry*, 294, 60-66.
- Gouvêa, V., Batistel, F., Souza, J., Chagas, L., Sitta, C., Campanili, P., . . . Santos, F. (2016). Flint corn grain processing and citrus pulp level in finishing diets for feedlot cattle. *Journal of Animal Science*, 94(2), 665-677.
- Grenier, B., Loureiro-Bracarense, A.-P., Leslie, J. F., & Oswald, I. P. (2014). Physical and chemical methods for mycotoxin decontamination in maize. *Mycotoxin Reduction in Grain Chains*, 116-129.
- Grenier, B., Schwartz-Zimmermann, H., Gruber-Dorninger, C., Dohnal, I., Aleschko, M., Schatzmayr, G., . . . Applegate, T. (2017). Enzymatic hydrolysis of fumonisins in the gastrointestinal tract of broiler chickens. *Poultry Science*, 96(12), 4342-4351.
- Grubisha, L. C., & Cotty, P. J. (2015). Genetic analysis of the *Aspergillus flavus* vegetative compatibility group to which a biological control agent that limits aflatoxin contamination in US crops belongs. *Applied and Environmental Microbiology*, 81(17), 5889-5899.

- Habib, M., Abdu, P., Kwanashie, C., Kabir, J., & Negedu, A. (2015). Isolation and identification of *Aspergillus* species from poultry feed in Kaduna State, Nigeria. *Microbiology Research International*, 3(2), 27-32.
- Hammami, W., Fiori, S., Al Thani, R., Kali, N. A., Balmas, V., Migheli, Q., & Jaoua, S. (2014). Fungal and aflatoxin contamination of marketed spices. *Food Control*, 37, 177-181.
- Han, J.-E., Seo, M.-J., Shin, K.-C., & Oh, D.-K. (2016). Production of 10R-hydroxy unsaturated fatty acids from hemp seed oil hydrolyzate by recombinant *Escherichia coli* cells expressing *PpoC* from *Aspergillus nidulans*. *Applied Microbiology and Biotechnology*, 100(18), 7933-7944.
- Hassan, N. A. (2018). Consumer knowledge, attitude, and concern in consuming contaminated peanut-based products.
- Hassan, Z. U., Al-Thani, R. F., Migheli, Q., & Jaoua, S. (2018). Detection of toxigenic mycobiota and mycotoxins in the cereal feed market. *Food Control*, 84, 389-394.
- Herbst, D. A., Townsend, C. A., & Maier, T. (2018). The architectures of iterative type I PKS and FAS. *Natural Product Reports*, 35(10), 1046-1069.
- Heussner, A. H., & Bingle, L. E. (2015). Comparative ochratoxin toxicity: A review of the available data. *Toxins*, 7(10), 4253-4282.
- Hontanaya, C., Meca, G., Luciano, F., Mañes, J., & Font, G. (2015). Inhibition of aflatoxin B₁, B₂, G₁, and G₂ production by *Aspergillus parasiticus* in nuts using yellow and oriental mustard flours. *Food Control*, 47, 154-160.
- Hosseini, H. M., Pour, S. H., Amani, J., Jabbarzadeh, S., Hosseinabadi, M., & Mirhosseini, S. A. (2020). The effect of Propolis on inhibition of *Aspergillus parasiticus* growth, aflatoxin production, and expression of aflatoxin biosynthesis pathway genes. *Journal of Environmental Health Science and Engineering*, 1-6.
- Houshyarfard, M., Rouhani, H., Falahati-Rastegar, M., Malekzadeh-Shafaroudi, S., Mehdikhani-Moghaddam, E., & Chang, P. (2014). Gene deletion patterns in non-aflatoxigenic isolates of *Aspergillus flavus*. *Mycologia Iranica*, 1(2), 87-97.
- Hu, Y., Zhang, J., Kong, W., Zhao, G., & Yang, M. (2017). Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (*Curcuma longa* L.) on *Aspergillus flavus*. *Food Chemistry*, 220, 1-8.
- Hua, S. S. T., Palumbo, J. D., Parfitt, D. E., Sarreal, S. B. L., & O'Keeffe, T. L. (2018). Development of a droplet digital PCR assay for population analysis of aflatoxigenic and atoxigenic *Aspergillus flavus* mixtures in soil. *Mycotoxin Research*, 34(3), 187-194.

- Hua, S. S. T., Parfitt, D. E., Sarreal, S. B. L., & Sidhu, G. (2019). Dual culture of atoxigenic and toxigenic strains of *Aspergillus flavus* to gain insight into repression of aflatoxin biosynthesis and fungal interaction. *Mycotoxin Research*, 35(4), 381-389.
- Huitt-Roehl, C. R., Hill, E. A., Adams, M. M., Vagstad, A. L., Li, J. W., & Townsend, C. A. (2015). Starter unit flexibility for engineered product synthesis by the nonreducing polyketide synthase PksA. *ACS Chemical Biology*, 10(6), 1443-1449.
- Hussain, A., Afzal, A., Irfan, M., & Malik, K. A. (2015). Molecular detection of aflatoxin producing strains of *Aspergillus flavus* from peanut (*Arachis hypogaea*). *Turkish Journal of Agriculture Food Science and Technology*, 3(5), 335-341.
- Hymery, N., Vasseur, V., Coton, M., Mounier, J., Jany, J. L., Barbier, G., & Coton, E. (2014). Filamentous fungi and mycotoxins in cheese: A review. *Comprehensive Reviews in Food Science and Food Safety*, 13(4), 437-456.
- Iqbal, M., Abbas, M., Adil, M., Nazir, A., & Ahmad, I. (2019). Aflatoxins biosynthesis, toxicity, and intervention strategies: A review. *M. Iqbal, M. Abbas, M. Adil, A. Nazir, and I. Ahmad. Aflatoxins biosynthesis, toxicity, and intervention strategies: A review. Chemistry International*, 5(3), 168-191.
- Iqbal, S. Z., Asi, M. R., Hanif, U., Zuber, M., & Jinap, S. (2016). The presence of aflatoxins and ochratoxin A in rice and rice products; and evaluation of dietary intake. *Food Chemistry*, 210, 135-140.
- Ishadi, A., & Mazlan, N. (2015). Insecticide use impacts on pest resistance: An evidence from diamondback moth. *International Journal of Science: Basic Applied Research*, 22, 131-150.
- Islam, M.-S., Callicott, K. A., Mutegi, C., Bandyopadhyay, R., & Cotty, P. J. (2018). *Aspergillus flavus* resident in Kenya: High genetic diversity in an ancient population primarily shaped by clonal reproduction and mutation-driven evolution. *Fungal Ecology*, 35, 20-33.
- Jafari, A. A. (2018). Mycotoxins, a major challenge in global food security. *Journal of Nutrition and Food Security*, 3(1), 1-3.
- Jahanshiri, Z., Shams-Ghahfarokhi, M., Allameh, A., & Razzaghi-Abyaneh, M. (2015). Inhibitory effect of eugenol on aflatoxin B₁ production in *Aspergillus parasiticus* by downregulating the expression of significant genes in the toxin biosynthetic pathway. *World Journal of Microbiology and Biotechnology*, 31(7), 1071-1078.
- Jantapan, K., Poapolathep, A., Imsilp, K., Poapolathep, S., Tanhan, P., Kumagai, S., & Jermnak, U. (2017). Inhibitory effects of Thai essential oils on potentially

aflatoxigenic *Aspergillus parasiticus* and *Aspergillus flavus*. *Biocontrol Science*, 22(1), 31-40.

- Kachapulula, P. W., Akello, J., Bandyopadhyay, R., & Cotty, P. J. (2017). *Aspergillus* section *Flavi* community structure in Zambia influences aflatoxin contamination of maize and groundnut. *International Journal of Food Microbiology*, 261, 49-56.
- Kamala, A., Kimanya, M., Haesaert, G., Tiisekwa, B., Madege, R., Degraeve, S., . . . De Meulenaer, B. (2016). Local postharvest practices associated with aflatoxin and fumonisin contamination of maize in three agro-ecological zones of Tanzania. *Food Additives & Contaminants: Part A*, 33(3), 551-559.
- Keller, N. P. (2015). Translating biosynthetic gene clusters into fungal armor and weaponry. *Nature Chemical Biology*, 11(9), 671-677.
- Khatoon, A., Khan, M. Z., Abidin, Z. u., & Bhatti, S. A. (2018). Effects of feeding bentonite clay upon ochratoxin A-induced immunosuppression in broiler chicks. *Food Additives & Contaminants: Part A*, 35(3), 538-545.
- Khayoon, W. S., Saad, B., Salleh, B., Manaf, N. H. A., & Latiff, A. A. (2014). Micro-solid phase extraction with liquid chromatography-tandem mass spectrometry for the determination of aflatoxins in coffee and malt beverages. *Food Chemistry*, 147, 287-294.
- Kistler, H. C., & Broz, K. (2015). Cellular compartmentalization of secondary metabolism. *Frontiers in Microbiology*, 6, 68, 1-11.
- Koch, M. S., Ward, J. M., Levine, S. L., Baum, J. A., Vicini, J. L., & Hammond, B. G. (2015). The food and environmental safety of Bt crops. *Frontiers in Plant Science*, 6, 283, 1-22.
- Köhl, J., Scheer, C., Holb, I., Masny, S., & Molhoek, W. (2016). *Biological control of apple scab (Venturia inaequalis) epidemics by Cladosporium cladosporioides H39*. Paper presented at the Proceedings of the XIII Meeting "Biocontrol of Plant Diseases: From the field to the laboratory and back again" at Uppsala (Sweden), June 15-18, 2014, 213-215.
- Komsky-Elbaz, A., Saksier, M., & Roth, Z. (2018). Aflatoxin B₁ impairs sperm quality and fertilization competence. *Toxicology*, 393, 42-50.
- Kong, Q., Chi, C., Yu, J., Shan, S., Li, Q., Li, Q., . . . Bennett, J. W. (2014). The inhibitory effect of *Bacillus megaterium* on aflatoxin and cyclopiazonic acid biosynthetic pathway gene expression in *Aspergillus flavus*. *Applied Microbiology and Biotechnology*, 98(11), 5161-5172.
- Kong, W.-J., Li, J.-Y., Qiu, F., Wei, J.-H., Xiao, X.-H., Zhang, Y., & Yang, M.-H. (2013). Development of a sensitive and reliable high-performance liquid chromatography method with fluorescence detection for high-throughput

analysis of multi-class mycotoxins in Coix seed. *Analytica Chimica Acta*, 799, 68-76.

- Kosegarten, C. E., Ramírez-Corona, N., Mani-López, E., Palou, E., & López-Malo, A. (2017). Description of *Aspergillus flavus* growth under the influence of different factors (water activity, incubation temperature, protein and fat concentration, pH, and cinnamon essential oil concentration) by kinetic, probability of growth, and time-to-detection models. *International Journal of Food Microbiology*, 240, 115-123.
- Kourousekos, G., & Theodosiadou, E. (2015). Effects of aflatoxins on the male reproductive system: A review. *Journal of the Hellenic Veterinary Medical Society*, 66(4), 201-210.
- Kovalsky, P., Kos, G., Nährer, K., Schwab, C., Jenkins, T., Schatzmayr, G., . . . Krska, R. (2016). Co-occurrence of regulated, masked, and emerging mycotoxins and secondary metabolites in finished feed and maize-An extensive survey. *Toxins*, 8(12), 363.
- Kumar, M., Rana, S., Kumar, H., Kumar, P., Dikhit, M. R., Mansuri, R., . . . Sahoo, G. C. (2017). Computational, structural, and functional aspects of the hypothetical protein of *Aspergillus flavus* Pheromone Receptor Pre-A (PRP-A). *Journal of Applied Pharmaceutical Science*, 7(07), 089-097.
- Kuzukiran, O., Filazi, A., Yurdakok-Dikmen, B., Ozansoy-Cengiz, G., Gurcan, I. S., Karabulut, E., & Sireli, U. T. (2018). The effects of aflatoxin residues on nutritional contents in ground red chili peppers (*Capsicum annuum*). *Toxin Reviews*, 1-10.
- Laflotte, A., Aubry, L., Mahanna, B., & Owens, F. (2016). Starch digestibility by lactating cows fed flint or dent corn silage stored two or six months before feeding. *Journal of Animal Science*, 94, 704-704.
- Lahouar, A., Crespo-Sempere, A., Marín, S., Saïd, S., & Sanchis, V. (2015). Toxigenic molds in Tunisian and Egyptian sorghum for human consumption. *Journal of Stored Products Research*, 63, 57-62.
- Lahouar, A., Marin, S., Crespo-Sempere, A., Saïd, S., & Sanchis, V. (2016). Effects of temperature, water activity, and incubation time on fungal growth and aflatoxin B₁ production by toxigenic *Aspergillus flavus* isolates on sorghum seeds. *Revista Argentina de Microbiologia*, 48(1), 78-85.
- Lan, H., Wu, L., Sun, R., Keller, N. P., Yang, K., Ye, L., . . . Wang, S. (2019). The *HosA* histone deacetylase regulates aflatoxin biosynthesis through direct regulation of aflatoxin cluster genes. *Molecular Plant-Microbe Interactions*, 32(9), 1210-1228.

- Len, A. C., Starling, S., Shivkumar, M., & Jolly, C. (2017). HIV-1 activates T cell signaling independently of antigen to drive viral spread. *Cell Reports*, 18(4), 1062-1074.
- Li, R., Oliver, R. A., & Townsend, C. A. (2017). Identification and characterization of the sulfazecin monobactam biosynthetic gene cluster. *Cell Chemical Biology*, 24(1), 24-34.
- Li, S., Muhammad, I., Yu, H., Sun, X., & Zhang, X. (2019). Detection of Aflatoxin adducts as potential markers and the role of curcumin in alleviating AFB₁-induced liver damage in chickens. *Ecotoxicology and Environmental Safety*, 176, 137-145.
- Liang, D., Xing, F., Selvaraj, J. N., Liu, X., Wang, L., Hua, H., . . . Liu, Y. (2015). Inhibitory effect of cinnamaldehyde, citral, and eugenol on aflatoxin biosynthetic gene expression and aflatoxin B₁ biosynthesis in *Aspergillus flavus*. *Journal of Food Science*, 80(12), M2917-2924.
- Liew, W.-P.-P., & Mohd-Redzwan, S. (2018). Mycotoxin: its impact on gut health and microbiota. *Frontiers in Cellular and Infection Microbiology*, 8, 60, 1-17.
- Liu, P., Li, B., Yin, R., Weng, Q., & Chen, Q. (2014). Development and evaluation of *ITS*-and *aflP*-based LAMP assays for the rapid detection of *Aspergillus flavus* in food samples. *Canadian Journal of Microbiology*, 60(9), 579-584.
- Liu, X., Guan, X., Xing, F., Lv, C., Dai, X., & Liu, Y. (2017). Effect of water activity and temperature on the growth of *Aspergillus flavus*, the expression of aflatoxin biosynthetic genes, and aflatoxin production in shelled peanuts. *Food Control*, 82, 325-332.
- Loomis, D., Guha, N., Hall, A. L., & Straif, K. (2018). Identifying occupational carcinogens: an update from the IARC Monographs. *Occupational and Environmental Medicine*, 75(8), 593-603.
- Lozano-Ojalvo, D., Rodríguez, A., Bernáldez, V., Córdoba, J. J., & Rodríguez, M. (2013). Influence of temperature and substrate conditions on the *omt-1* gene expression of *Aspergillus parasiticus* in relation to its aflatoxin production. *International Journal of Food Microbiology*, 166(2), 263-269.
- Luzardo, O. P., del Mar Bernal-Suarez, M., Camacho, M., Henríquez-Hernández, L. A., Boada, L. D., Rial-Berriel, C., . . . Díaz-Díaz, R. (2016). Estimated exposure to EU regulated mycotoxins and risk characterization of aflatoxin-induced hepatic toxicity through the consumption of the toasted cereal flour called “gofio”, a traditional food of the Canary Islands (Spain). *Food and Chemical Toxicology*, 93, 73-81.
- Maeda, K., Nakajima, Y., Motoyama, T., Kondoh, Y., Kawamura, T., Kanamaru, K., . . . Osada, H. (2017). Identification of a trichothecene production inhibitor

by the chemical array and library screening using trichodiene synthase as a target protein. *Pesticide Biochemistry and Physiology*, 138, 1-7.

- Mahmoud, M. A. (2015). Detection of *Aspergillus flavus* in stored peanuts using real-time PCR and the expression of aflatoxin genes in toxigenic and atoxigenic *A. flavus* isolates. *Foodborne Pathogens and Disease*, 12(4), 289-296.
- Malachová, A., Sulyok, M., Beltrán, E., Berthiller, F., & Krska, R. (2014). Optimization and validation of a quantitative liquid chromatography-tandem mass spectrometric method covering 295 bacterial and fungal metabolites, including all regulated mycotoxins in four model food matrices. *Journal of Chromatography A*, 1362, 145-156.
- Male, D. (2017). *Challenges Facing Foodborne Mycotoxin Regulation and Public Health Consequences of Exposure in Children Under 5 Years of Age*: Michigan State University. 1-166.
- Matabaro, E., Ishimwe, N., Uwimbabazi, E., & Lee, B. H. (2017). Current immunoassay methods for the rapid detection of aflatoxin in milk and dairy products. *Comprehensive Reviews in Food Science and Food Safety*, 16(5), 808-820.
- Mauro, A., Battilani, P., & Cotty, P. J. (2015). Atoxigenic *Aspergillus flavus* endemic to Italy for biocontrol of aflatoxins in maize. *BioControl*, 60(1), 125-134.
- Mayer, E., Novak, B., Springler, A., Schwartz-Zimmermann, H. E., Nagl, V., Reisinger, N., . . . Schatzmayr, G. (2017). Effects of deoxynivalenol (DON) and its microbial biotransformation product de epoxy-deoxynivalenol (DOM-1) on a trout, pig, mouse, and human cell line. *Mycotoxin Research*, 33(4), 297-308.
- McGuire, S. (2016). World cancer report, 2014. Geneva, Switzerland: World Health Organization, the international agency for research on cancer, WHO Press, 2015, *Advances in Nutrition*, 7, (2), 418–419.
- Medina, A., Mohale, S., Samsudin, N. I. P., Rodriguez-Sixtos, A., Rodriguez, A., & Magan, N. (2017). Biocontrol of mycotoxins: dynamics and mechanisms of action. *Current Opinion in Food Science*, 17, 41-48.
- Medina, A., Schmidt-Heydt, M., Rodriguez, A., Parra, R., Geisen, R., & Magan, N. (2015). Impacts of environmental stress on growth, secondary metabolite biosynthetic gene clusters, and metabolite production of xerotolerant/xerophilic fungi. *Current Genetics*, 61(3), 325-334.
- Mellon, J. E. (2015). Extracellular xylanolytic and pectinolytic hydrolase production by *Aspergillus flavus* isolates contributes to crop invasion. *Toxins*, 7(8), 3257-3266.

- Midorikawa, G. E., Maria de Lourdes, M., Silva, O. F., Jurema do Socorro, A. D., Kanzaki, L. I., Hanada, R. E., . . . Bittencourt, D. M. (2014). Characterization of *Aspergillus* species on Brazil nut from the Brazilian Amazonian region and development of a PCR assay for identification at the genus level. *BMC Microbiology*, *14*(1), 1-9.
- Milani, J., & Maleki, G. (2014). Effects of processing on mycotoxin stability in cereals. *Journal of the Science of Food and Agriculture*, *94*(12), 2372-2375.
- Milicevic, D., Lakicevic, B., Petronijevic, R., Petrovic, Z., Jovanovic, J., Stefanovic, S., & Jankovic, S. (2019). Climate change: impact on mycotoxins incidence and food safety. *Theory and Practice of Meat Processing*, *4*(1), 9-16.
- Mitema, A., Okoth, S., & Rafudeen, M. S. (2018). Vegetative compatibility and phenotypic characterization as a means of determining the genetic diversity of *Aspergillus flavus* isolates. *Fungal Biology*, *122*(4), 203-213.
- Mohajeri, M., Behnam, B., Cicero, A. F., & Sahebkar, A. (2018). Protective effects of curcumin against aflatoxicosis: A comprehensive review. *Journal of Cellular Physiology*, *233*(4), 3552-3577.
- Mohale, S., Medina, A., & Magan, N. (2013). Effect of environmental factors on *in vitro* and *in situ* interactions between atoxigenic and toxigenic *A. flavus* strains and control of aflatoxin contamination of maize. *Biocontrol Science and Technology*, *23*(7), 776-793.
- Mohammed, A., Chala, A., Ojiewo, C., Dejene, M., Fininsa, C., Ayalew, A., . . . Arias, R. S. (2018). Integrated management of *Aspergillus* species and aflatoxin production in groundnut (*Arachis hypogaea* L.) through the application of farmyard manure and seed treatments with fungicides and *Trichoderma* species. *African Journal of Plant Science*, *12*(9), 196-207.
- Mohapatra, D., Kumar, S., Kotwaliwale, N., & Singh, K. K. (2017). Critical factors are responsible for fungi growth in stored food grains and non-chemical approaches for their control. *Industrial Crops and Products*, *108*, 162-182.
- Molo, M. S., Heiniger, R. W., Boerema, L., & Carbone, I. (2019). Trial summary on the comparison of various non- aflatoxigenic strains of *Aspergillus flavus* on mycotoxin levels and yield in maize. *Agronomy Journal*, *111*(2), 942-946.
- Montemarani, A., Nesci, A., & Etcheverry, M. (2014). Production of *Kluyveromyces* spp. and environmental tolerance induction against *Aspergillus flavus*. *Annals of Microbiology*, *64*(3), 935-944.
- Moon, Y.-S., Kim, H.-M., Chun, H. S., & Lee, S.-E. (2018). Organic acids suppress aflatoxin production via lowering expression of aflatoxin biosynthesis-related genes in *Aspergillus flavus*. *Food Control*, *88*, 207-216.

- Moore, G. G., Mack, B. M., & Beltz, S. B. (2013). Testing the efficacy of eGFP-transformed *Aspergillus flavus* as biocontrol strains. *Food and Nutrition Sciences*, 4(04), 469-479.
- Moretti, A., Susca, A., Mulé, G., Logrieco, A., & Proctor, R. (2013). Molecular biodiversity of mycotoxigenic fungi that threaten food safety. *International Journal of Food Microbiology*, 167(1), 57-66.
- Mousa, W., Ghazali, F. M., Jinap, S., Ghazali, H. M., Radu, S., & Salama, A. E.-R. (2016). Temperature, water activity, and gas composition effects on the growth and aflatoxin production by *Aspergillus flavus* on paddy. *Journal of Stored Products Research*, 67, 49-55.
- Mtimet, N., Walke, M., Baker, D., Lindahl, J., Hartmann, M., & Grace, D. (2015). *Kenyan awareness of aflatoxin: an analysis of processed milk consumers* (No. 1008-2016-79993), 1-23.
- Munkvold, G. P., Arias, S., Taschl, I., & Gruber-Dorninger, C. (2019). Mycotoxins in corn: occurrence, impacts, and management. *Corn*, (3), 235-287.
- Mutegi, C., Cotty, P., & Bandyopadhyay, R. (2018). Prevalence and mitigation of aflatoxins in Kenya (1960-to date). *World Mycotoxin Journal*, 11(3), 341-357.
- Mwakinyali, S. E., Ding, X., Ming, Z., Tong, W., Zhang, Q., & Li, P. (2019). The recent development of aflatoxin contamination biocontrol in agricultural products. *Biological Control*, 128, 31-39.
- Nadira, A. F., Rosita, J., Norhaizan, M., & Redzwan, S. M. (2017). Screening of aflatoxin M₁ occurrence in selected milk and dairy products in Terengganu, Malaysia. *Food control*, 73, 209-214.
- Nair, S. C., Bhagobaty, R. K., Nampoothiri, K., Kalaigandhi, V., & Menon, K. (2014). Detection of aflatoxin production by fungi in spice samples using HPLC and direct visual cultural methods. *Innovative Romanian Food Biotechnology*, 14, 1-12.
- Naseer, R., Sultana, B., Khan, M., Naseer, D., & Nigam, P. (2014). Utilization of waste fruit-peels to inhibit aflatoxins synthesis by *Aspergillus flavus*: a biotreatment of rice for safer storage. *Bioresource technology*, 172, 423-428.
- Navya, H., Hariprasad, P., Naveen, J., Chandranayaka, S., & Niranjana, S. (2013). Natural occurrence of aflatoxin, aflatoxigenic, and non-aflatoxigenic *Aspergillus flavus* in groundnut seeds across India. *African Journal of Biotechnology*, 12(19), 2587-2597.
- Nayak, S., Dhua, U., & Samanta, S. (2019). Morphological and toxigenic study of *Aspergillus flavus* from indoor air. *Journal of Environmental Biology*, 40(4), 648-654.

- Nazir, K., Hassan, J., Durairaj, P., & Yun, H. (2014). Isolation and identification of *Aspergillus flavus* from poultry feed samples using combined traditional-molecular approach and expression of CYP64A1 AT mRNA level. *Pakistan Journal of Agricultural Sciences*, 51(2), 287-291.
- Ndemera, M., De Boevre, M., & De Saeger, S. (2020). Mycotoxin management in a developing country context: A critical review of strategies aimed at decreasing dietary exposure to mycotoxins in Zimbabwe. *Critical Reviews in Food Science and Nutrition*, 60(4), 529-540.
- Neme, K., & Mohammed, A. (2017). Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategy. A review. *Food Control*, 78, 412-425.
- Neppelenbroek, K., Seó, R., Urban, V., Silva, S., Dovigo, L., Jorge, J., & Campanha, N. (2014). Identification of *Candida* species in the clinical laboratory: a review of conventional, commercial, and molecular techniques. *Oral Diseases*, 20(4), 329-344.
- Nguyen, H. D., McMullin, D. R., Ponomareva, E., Riley, R., Pomraning, K. R., Baker, S. E., & Seifert, K. A. (2016). Ochratoxin A production by *Penicillium thymicola*. *Fungal Biology*, 120(8), 1041-1049.
- Nierman, W. C., Yu, J., Fedorova-Abrams, N. D., Losada, L., Cleveland, T. E., Bhatnagar, D., . . . Payne, G. A. (2015). Genome sequence of *Aspergillus flavus* NRRL 3357, a strain that causes aflatoxin contamination of food and feed. *Genome Announcements*, 3(2), 1-2.
- Njoroge, S. M., Matumba, L., Kanenga, K., Siambi, M., Waliyar, F., Maruwo, J., . . . Monyo, E. S. (2017). Aflatoxin B₁ levels in groundnut products from local markets in Zambia. *Mycotoxin Research*, 33(2), 113-119.
- Noman, E., Al-Gheethi, A., Rahman, N., Talip, B., Mohamed, R., & Kadir, O. (2018). Single Spore Isolation as a Simple and Efficient Technique to obtain pure fungal culture. *Environmental Science and Pollution Research*, 23 (19), 19806-19824.
- Nooh, A., Amra, H., Youssef, M., & El-Banna, A. A. (2014). Mycotoxin and toxigenic fungi occurrence in Egyptian maize. *International Journal of Advanced Research*, 2(2), 521-532.
- Nor-Khaizura, M., Jinap, S., Norlia, M., Radu, S., & Samsudin, N. I. P. (2019). *Aspergillus* section *Flavi* and aflatoxins: Occurrence, detection, and identification in raw peanuts and peanut-based products along the supply chain. *Frontiers in Microbiology*, 10, 2602.
- Norlia, M., Jinap, S., Nor-Khaizura, M., Son, R., & Chin, C. (2018). A polyphasic approach to the identification and characterization of aflatoxigenic strains of

Aspergillus section *Flavi* isolated from peanuts and peanut-based products marketed in Malaysia. *International Journal of Food Microbiology*, 282, 9-15.

- Nurtjahja, K., Zuhra, C. F., Sembiring, H., Bungsu, A., Simanullang, J., Silalahi, J. E., . . . Sartini, S. (2019). Fungal contamination spices from Indonesia with an emphasis on *Aspergillus flavus*. *Czech Journal of Food Sciences*, 37(5), 338-344.
- Ohkura, M., Cotty, P. J., & Orbach, M. J. (2018). Comparative genomics of *Aspergillus flavus* S and L morphotypes yields insights into niche adaptation. *G3: Genes, Genomes, Genetics*, 8(12), 3915-3930.
- Okoth, S., De Boevre, M., Vidal, A., Diana Di Mavungu, J., Landschoot, S., Kyallo, M., . . . De Saeger, S. (2018). Genetic and toxigenic variability within the *Aspergillus flavus* population is isolated from maize in two diverse environments in Kenya. *Frontiers in Microbiology*, 9, 57, 1-14.
- Okoth, S., Nyongesa, B., Joutsjoki, V., Korhonen, H., Ayugi, V., & Kang'ethe, E. (2016). Sclerotia formation and toxin production in large sclerotial *Aspergillus flavus* isolates from Kenya. *Advances in Microbiology*, 2016, 6, 47-56.
- Olarte, R. A., Worthington, C. J., Horn, B. W., Moore, G. G., Singh, R., Monacell, J. T., . . . Carbone, I. (2015). Enhanced diversity and aflatoxigenicity in interspecific hybrids of *Aspergillus flavus* and *Aspergillus parasiticus*. *Molecular Ecology*, 24(8), 1889-1909.
- Ortega-Beltran, A., & Bandyopadhyay, R. (2019). Comments on "Trial Summary on the comparison of various non-aflatoxigenic strains of *Aspergillus flavus* on mycotoxin levels and yield in maize" by MS Molo, *et al.* *Agronomy Journal* 111: 942–946 (2019). *Agronomy Journal*, 111(5), 2625-2631.
- Ortega-Beltran, A., & Cotty, P. (2018). Frequent shifts in *Aspergillus flavus* populations associated with maize production in Sonora, Mexico. *Phytopathology*, 108(3), 412-420.
- Ostry, V., Malir, F., Toman, J., & Grosse, Y. (2017). Mycotoxins as human carcinogens-the IARC Monographs classification. *Mycotoxin Research*, 33(1), 65-73.
- Ozer, H., Basegmez, H., Whitaker, T., Slate, A., & Giesbrecht, F. (2017). Sampling dried figs for aflatoxin—part 1: variability associated with sampling, sample preparation, and analysis. *World Mycotoxin Journal*, 10(1), 31-40.
- Pandya, U., & Saraf, M. (2014). In vitro evaluation of PGPR strains for their biocontrol potential against fungal pathogens. In *Microbial Diversity and Biotechnology in Food Security*: 293-305.

- Pankaj, S., Shi, H., & Keener, K. M. (2018). A review of novel physical and chemical decontamination technologies for aflatoxin in food. *Trends in Food Science & Technology*, 71, 73-83.
- Park, J. H., Shin, J. H., Choi, M. J., Choi, J. U., Park, Y.-J., Jang, S. J., . . . Shin, M. G. (2017). Evaluation of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for identification of 345 clinical isolates of *Aspergillus* species from 11 Korean hospitals: comparison with molecular identification. *Diagnostic Microbiology and Infectious Disease*, 87(1), 28-31.
- Paulussen, C., Hallsworth, J. E., Álvarez- Pérez, S., Nierman, W. C., Hamill, P. G., Blain, D., . . . Lievens, B. (2017). Ecology of aspergillosis: insights into the pathogenic potency of *Aspergillus fumigatus* and some other *Aspergillus* species. *Microbial Biotechnology*, 10(2), 296-322.
- Peng, Z., Chen, L., Zhu, Y., Huang, Y., Hu, X., Wu, Q., . . . Yang, W. (2018). Current primary degradation methods for aflatoxins: A review. *Trends in Food Science & Technology*, 80, 155-166.
- Peromingo, B., Rodríguez, A., Bernáldez, V., Delgado, J., & Rodríguez, M. (2016). Effect of temperature and water activity on growth and aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus* on cured meat model systems. *Meat Science*, 122, 76-83.
- Perrone, G., Haidukowski, M., Stea, G., Epifani, F., Bandyopadhyay, R., Leslie, J. F., & Logrieco, A. (2014). Population structure and aflatoxin production by *Aspergillus* section *Flavi* from maize in Nigeria and Ghana. *Food Microbiology*, 41, 52-59.
- Pfannenstiel, B. T., Zhao, X., Wortman, J., Wiemann, P., Throckmorton, K., Spraker, J. E., . . . Lim, F. Y. (2017). Revitalization of a forward genetic screen identifies three new regulators of fungal secondary metabolism in the genus *Aspergillus*. *mBio*, 8(5) 1-15.
- Picot, A., Doster, M., Islam, M.-S., Callicott, K., Ortega-Beltran, A., Cotty, P., & Michailides, T. (2018). Distribution and incidence of atoxigenic *Aspergillus flavus* VCG in tree crop orchards in California: A strategy for identifying potential antagonists, the example of almonds. *International Journal of Food Microbiology*, 265, 55-64.
- Pirouz, A., Selamat, J., Iqbal, S., Mirhosseini, H., Karjiban, R. A., & Bakar, F. A. (2017). The use of innovative and efficient nanocomposite (magnetic graphene oxide) for the reduction of *Fusarium* mycotoxins in palm kernel cake. *Scientific Reports*, 7(1), 1-9.
- Pitt, J. (2019). The pros and cons of using biocontrol by competitive exclusion as a means for reducing aflatoxin in maize in Africa. *World Mycotoxin Journal*, 12(2), 103-112.

- Pitt, J., Taniwaki, M. H., & Cole, M. (2013). Mycotoxin production in significant crops as influenced by growing, harvesting, storage, and processing, with emphasis on the achievement of Food Safety Objectives. *Food Control*, 32(1), 205-215.
- Prakash, B., Kedia, A., Mishra, P. K., & Dubey, N. (2015). Plant essential oils as food preservatives to control molds, mycotoxin contamination, and oxidative deterioration of agri-food commodities—Potentials and challenges. *Food Control*, 47, 381-391.
- Prendergast, A. J., & Humphrey, J. H. (2014). Stunting syndrome in developing countries. *Paediatrics and International Child Health*, 34(4), 250-265.
- Probst, C., Bandyopadhyay, R., & Cotty, P. (2014). Diversity of aflatoxin-producing fungi and their impact on food safety in sub-Saharan Africa. *International Journal of Food Microbiology*, 174, 113-122.
- Proctor, R. H., McCormick, S. P., Kim, H.-S., Cardoza, R. E., Stanley, A. M., Lindo, L., . . . Vaughan, M. M. (2018). Evolution of structural diversity of trichothecenes, a family of toxins produced by plant pathogenic and entomopathogenic fungi. *PLoS Pathogens*, 14(4), 1-38.
- Puntscher, H., Cobankovic, I., Marko, D., & Warth, B. (2019). Quantitation of free and modified *Alternaria* mycotoxins in European food products by LC-MS/MS. *Food Control*, 102, 157-165.
- Qazi, M. A., Kanwal, T., Jadoon, M., Ahmed, S., & Fatima, N. (2014). Isolation and characterization of a biosurfactant- producing *Fusarium* spp. BS- 8 from oil-contaminated soil. *Biotechnology Progress*, 30(5), 1065-1075.
- Ramalingam, S., Bahuguna, A., & Kim, M. (2019). The effects of mycotoxin patulin on cells and cellular components. *Trends in Food Science & Technology*, 83, 99-113.
- Ramdial, H., Latchoo, R., Hosein, F., & Rampersad, S. (2017). Phylogeny and haplotype analysis of fungi within the *Fusarium incarnatum*-exquisite species complex. *Phytopathology*, 107(1), 109-120.
- Rangel, D. E., Braga, G. U., Fernandes, É. K., Keyser, C. A., Hallsworth, J. E., & Roberts, D. W. (2015). Stress tolerance and virulence of insect-pathogenic fungi are determined by environmental conditions during conidial formation. *Current Genetics*, 61(3), 383-404.
- Rasul, M., Islam, M. S., Yunus, R. B. M., Mokhtar, M. B., Alam, L., & Yahaya, F. (2017). Spatial and temporal variation of water quality in the Bertam Catchment, Cameron Highlands, Malaysia: Rasul *et al.* *Water Environment Research*, 89(12), 2088-2102.

- Redzwan, S. M., Rosita, J., Sokhini, A. M., Nurul'Aqilah, A., Wang, J.-S., Kang, M.-S., & Zuraini, A. (2014). Detection of serum AFB₁-lysine adduct in Malaysia and its association with liver and kidney functions. *International Journal of Hygiene and Environmental Health*, 217(4-5), 443-451.
- Rharmitt, S., Hafidi, M., Hajjaj, H., Scordino, F., Giosa, D., Giuffrè, L., . . . Romeo, O. (2016). Molecular characterization of patulin producing and non-producing *Penicillium* species in apples from Morocco. *International Journal of Food Microbiology*, 217, 137-140.
- Rogowska, A., Pomastowski, P., Sagandykova, G., & Buszewski, B. (2019). Zearalenone and its metabolites: Effect on human health, metabolism, and neutralization methods. *Toxicon*, 162, 46-56.
- Rosegrant, M. W., Magalhaes, E., Valmonte-Santos, R. A., & Mason-D'Croz, D. (2018). Returns to investment in reducing postharvest food losses and increasing agricultural productivity growth. *Prioritizing development: A cost-benefit analysis of the United Nations' sustainable development goals*, 322-338.
- Roze, L. V., Laivenieks, M., Hong, S.-Y., Wee, J., Wong, S.-S., Vanos, B., . . . Linz, J. E. (2015). Aflatoxin biosynthesis is a novel source of reactive oxygen species—a potential redox signal to initiate resistance to oxidative stress? *Toxins*, 7(5), 1411-1430.
- Rubin, J. N., Gilman, C. A. L., Gasmí, B., Stern, W. R., Hernandez, J. M., & Koh, C. (2020). Hepatocellular Carcinoma where you least expect it. *American Journal of Gastroenterology*, 115(7), 1134-1136.
- Runa, F., Carbone, I., Bhatnagar, D., & Payne, G. A. (2015). Nuclear heterogeneity in conidial populations of *Aspergillus flavus*. *Fungal Genetics and Biology*, 84, 62-72.
- Rushing, B. R., & Selim, M. I. (2019). Aflatoxin B₁: A review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. *Food and Chemical Toxicology*, 124, 81-100.
- Sabino, R., Veríssimo, C., Parada, H., Brandão, J., Viegas, C., Carolino, E., . . . Stevens, D. A. (2014). Molecular screening of 246 Portuguese *Aspergillus* isolates among different clinical and environmental sources. *Medical Mycology*, 52(5), 519-529.
- Sabran, M. R., Jamaluddin, R., & Ahmad, Z. (2013). A mini-review on aflatoxin exposure in Malaysia: past, present, and future. *Frontiers in Microbiology*, 4, 334.
- Sahebi, M., Hanafi, M. M., van Wijnen, A. J., Akmar, A. S. N., Azizi, P., Idris, A. S., . . . Foroughi, M. (2017). Profiling secondary metabolites of plant defense

mechanisms and oil palm in response to the *Ganoderma boninense* attack. *International Biodeterioration & Biodegradation*, 122, 151-164.

Samson, R. A., Visagie, C. M., Houbraken, J., Hong, S.-B., Hubka, V., Klaassen, C. H., . . . Tanney, J. B. (2014). Phylogeny, identification, and nomenclature of the genus *Aspergillus*. *Studies in Mycology*, 78, 141-173.

Samsudin, N. I. P., & Abdullah, N. (2013). A preliminary survey on the occurrence of mycotoxigenic fungi and mycotoxins contaminating red rice at the consumer level in Selangor, Malaysia. *Mycotoxin Research*, 29(2), 89-96.

Samsudin, N. I. P., Medina, A., & Magan, N. (2016). Relationship between environmental conditions, carbon utilization patterns, and Niche Overlap Indices of the mycotoxigenic species *Fusarium verticillioides* and the biocontrol agent *Clonostachys rosea*. *Fungal Ecology*, 24, 44-52.

Sarma, U. P., Bhetaria, P. J., Devi, P., & Varma, A. (2017). Aflatoxins: health implications. *Indian Journal of Clinical Biochemistry*, 32(2), 124-133.

Sarrocchio, S., & Vannacci, G. (2018). Preharvest application of beneficial fungi as a strategy to prevent postharvest mycotoxin contamination: A review. *Crop Protection*, 110, 160-170.

Scala, V., Giorni, P., Cirilini, M., Ludovici, M., Visentin, I., Cardinale, F., . . . Battilani, P. (2014). LDS1-produced oxylipins are negative regulators of growth, conidiation, and fumonisin synthesis in the fungal maize pathogen *Fusarium verticillioides*. *Frontiers in Microbiology*, 5, 669, 1-14.

Schelstraete, W., Devreese, M., & Croubels, S. (2020). Comparative toxicokinetics of *Fusarium* mycotoxins in pigs and humans. *Food and Chemical Toxicology*, 137, 1-14.

Schwartz-Zimmermann, H. E., Hametner, C., Nagl, V., Fiby, I., Macheiner, L., Winkler, J., . . . Berthiller, F. (2017). Glucuronidation of deoxynivalenol (DON) by different animal species: identification of iso-DON glucuronides and iso-de epoxy-DON glucuronides as novel DON metabolites in pigs, rats, mice, and cows. *Archives of Toxicology*, 91(12), 3857-3872.

Seth, R. K., & Alam, S. (2016). Management of seed-borne fungi of wheat through *Trichoderma* species. *Journal of Eco-friendly Agriculture*, 11(2), 149-153.

Shahbazi, Y., Nikousefat, Z., & Karami, N. (2017). Occurrence, seasonal variation, and risk assessment of exposure to aflatoxin M₁ in traditional Iranian cheeses. *Food Control*, 79, 356-362.

Shahniza, S. S., Firdaus, I. M., & Roslan, I. (2020). Effect of time of application and concentrations of plant growth regulators on growth and yield of sweet corn (*Zea mays* L.). *Research on Crops*, 21(1), 46-53.

- Sharma, H., Jadhav, V. J., & Garg, S. R. (2020). Aflatoxin M₁ in milk in Hisar city, Haryana, India, and risk assessment. *Food Additives & Contaminants: Part B*, *13*(1), 59-63.
- Shekhany, K. A., & Rostam, S. R. (2016). Detection of aflatoxigenic *Aspergillus flavus* in maize grains and soils in Sulaimani Province using molecular approaches. *Journal of Zankoy Sulaimani*, *18*, 25-36.
- Shi, T., McAllister, D. A., O'Brien, K. L., Simoes, E. A., Madhi, S. A., Gessner, B. D., . . . Aguayo, C. (2017). Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modeling study. *The Lancet*, *390*(10098), 946-958.
- Shirima, C. P., Kimanya, M. E., Routledge, M. N., Srey, C., Kinabo, J. L., Humpf, H.-U., . . . Gong, Y. Y. (2015). A prospective study of growth and biomarkers of exposure to aflatoxin and fumonisin during early childhood in Tanzania. *Environmental Health Perspectives*, *123*(2), 173-178.
- Shoukouhi, P., Hicks, C., Menzies, J. G., Popovic, Z., Chen, W., Seifert, K. A., . . . Liu, M. (2019). Phylogeny of Canadian ergot fungi and a detection assay by a real-time polymerase chain reaction. *Mycologia*, *111*(3), 493-505.
- Shuib, N. S., Makahleh, A., Salhimi, S. M., & Saad, B. (2017). Natural occurrence of aflatoxin M₁ in fresh cow milk and human milk in Penang, Malaysia. *Food Control*, *73*, 966-970.
- Singh, N. D., Sharma, A. K., Dwivedi, P., Leishangthem, G. D., Rahman, S., Reddy, J., & Kumar, M. (2016). Effect of feeding graded doses of citrinin on apoptosis and oxidative stress in male Wistar rats through the F₁ generation. *Toxicology and Industrial Health*, *32*(3), 385-397.
- Smith, J. S., Williams, W. P., & Windham, G. L. (2019). Aflatoxin in maize: a review of the early literature from "moldy-corn toxicosis" to the genetics of aflatoxin accumulation resistance. *Mycotoxin Research*, *35*(2), 111-128.
- Soto-Muñoz, L., Teixidó, N., Usall, J., Viñas, I., Abadías, M., & Torres, R. (2015). Molecular tools applied to identify and quantify the biocontrol agent *Pantoea agglomerans* CPA-2 in postharvest treatments on oranges. *Postharvest Biology and Technology*, *100*, 151-159.
- Spraker, J. E., Jewell, K., Roze, L. V., Scherf, J., Ndagano, D., Beaudry, R., . . . Keller, N. P. (2014). A volatile relationship: profiling an inter-kingdom dialogue between two plant pathogens, *Ralstonia solanacearum* and *Aspergillus flavus*. *Journal of Chemical Ecology*, *40*(5), 502-513.
- Sserumaga, J. P., Ortega-Beltran, A., Wagacha, J. M., Mutegi, C. K., & Bandyopadhyay, R. (2020). Aflatoxin-producing fungi associated with pre-

harvest maize contamination in Uganda. *International Journal of Food Microbiology*, 313, 108376.

- Stasiewicz, M. J., Falade, T. D., Mutuma, M., Mutiga, S. K., Harvey, J. J., Fox, G., . . . Nelson, R. J. (2017). Multi-spectral kernel sorting to reduce aflatoxins and fumonisins in Kenyan maize. *Food Control*, 78, 203-214.
- Sulaiman, G. M., T Hussien, H., & Saleem, M. M. (2015). Biosynthesis of silver nanoparticles synthesized by *Aspergillus flavus* and their antioxidant, antimicrobial, and cytotoxicity properties. *Bulletin of Materials Science*, 38(3), 639-644.
- Sun, L.-H., Lei, M.-y., Zhang, N.-Y., Gao, X., Li, C., Krumm, C. S., & Qi, D.-S. (2015). Individual and combined cytotoxic effects of aflatoxin B₁, zearalenone, deoxynivalenol, and fumonisin B₁ on BRL 3A rat liver cells. *Toxicol*, 95, 6-12.
- Sun, Z., Wang, X., Tang, Z., Chen, Q., & Liu, X. (2019). Development of a biotin-streptavidin-amplified nanobody-based ELISA for ochratoxin A in cereal. *Ecotoxicology and Environmental Safety*, 171, 382-388.
- Taniwaki, M. H., Pitt, J. I., & Magan, N. (2018). *Aspergillus* species and mycotoxins: occurrence and importance in significant food commodities. *Current Opinion in Food Science*, 23, 38-43.
- Täubel, M., & Hyvärinen, A. (2016). The occurrence of mycotoxins in indoor environments. In *Environmental Mycology in Public Health*, 299-323. Academic Press.
- Thathana, M. G., Murage, H., Abia, A. L. K. & Pillay, M. (2017). Morphological characterization and determination of aflatoxin-production potentials of *Aspergillus flavus* isolated from maize and soil in Kenya. *Agriculture*, 7(10), 1-14.
- Torres, A., Palacios, S., Yerkovich, N., Palazzini, J., Battilani, P., Leslie, J., . . . Chulze, S. (2019). *Fusarium* head blight and mycotoxins in wheat: Prevention and control strategies across the food chain. *World Mycotoxin Journal*, 12(4), 333-355.
- Torres, A. M., Barros, G. G., Palacios, S. A., Chulze, S. N., & Battilani, P. (2014). Review on pre-and postharvest management of peanuts to minimize aflatoxin contamination. *Food Research International*, 62, 11-19.
- Torres, O., Matute, J., Gelineau-van Waes, J., Maddox, J., Gregory, S., Ashley-Koch, A., . . . Riley, R. (2015). Human health implications from co-exposure to aflatoxins and fumonisins in maize-based foods in Latin America: Guatemala as a case study. *World Mycotoxin Journal*, 8(2), 143-159.
- Tran-Dinh, N., Pitt, J., & Markwell, P. (2018). Use of microsatellite markers to assess the competitive ability of nontoxicogenic *Aspergillus flavus* strains in studies on

biocontrol of aflatoxins in maize in Thailand. *Biocontrol Science and Technology*, 28(3), 215-225.

Tsygankova, V., Andrusevich, Y., Shtompel, O., Hurenko, A., Solomyannyj, R., Mrug, G., . . . Brovarets, V. (2016). Stimulating effect of five and six-membered heterocyclic compounds on seed germination and vegetative growth of maize (*Zea mays* L.). *International Journal of Biology Research*, 1(4), 1-14.

Umereweneza, D., Kamizikunze, T., & Muhizi, T. (2018). Assessment of mycotoxins types in some foodstuff consumed in Rwanda. *Food Control*, 85, 432-436.

Unija, E. (2019). Commission Implementing Regulation (EU) 2019/947 of 24 May 2019 on the rules and procedures for the operation of unmanned aircraft. *Official Journal of the European Union*. http://data.europa.eu/eli/reg_impl/2019/947/oj

Union, E.-E. (2016). RASFF-The Rapid Alert System for Food and Feed: 2015 annual report. In: European Commission. Health and Food Safety Brexellas.

Verheecke- Vaessen, C., Monte, J., Garcia- Cela, E., Magan, N., & Medina, A. (2020). Proof of concept: could snake venoms be a potential source of bioactive compounds for control of mold growth and mycotoxin production. *Letters in Applied Microbiology*, ISSN 0266-8254.

Vicente, S., Pok, P. S., García Londoño, V. A., & Pacin, A. (2019). Aflatoxins distribution in fractions derived from tofu production. *Food Additives & Contaminants: Part A*, 36(10), 1559-1566.

Vila-Donat, P., Marín, S., Sanchis, V., & Ramos, A. (2018). A review of the mycotoxin adsorbing agents, with an emphasis on their multi-binding capacity, for animal feed decontamination. *Food and Chemical Toxicology*, 114, 246-259.

Wahab, A. (2018). GAIN REPORT: Biofuels Annual, Global Agricultural Information Network. 2-11.

Waliyar, F., Osiru, M., Ntare, B., Kumar, K., Sudini, H., Traore, A., & Diarra, B. (2015). Postharvest management of aflatoxin contamination in groundnut. *World Mycotoxin Journal*, 8(2), 245-252.

Wang, B., Han, X., Bai, Y., Lin, Z., Qiu, M., Nie, X., . . . Yuan, J. (2017). Effects of nitrogen metabolism on growth and aflatoxin biosynthesis in *Aspergillus flavus*. *Journal of hazardous materials*, 324, 691-700.

Wang, J., Tang, L., Glenn, T. C., & Wang, J.-S. (2016). Aflatoxin B₁ induced compositional changes in gut microbial communities of male F344 rats. *Toxicological Sciences*, 150(1), 54-63.

- Wang, J. H., Zhang, J. B., Li, H. P., Gong, A. D., Xue, S., Agboola, R. S., & Liao, Y. C. (2014). Molecular identification, mycotoxin production, and comparative pathogenicity of *Fusarium temperatum* isolated from maize in China. *Journal of Phytopathology*, 162(3), 147-157.
- Warburton, M. L., Tang, J. D., Windham, G. L., Hawkins, L. K., Murray, S. C., Xu, W., . . . Williams, W. P. (2015). Genome-wide association mapping of *Aspergillus flavus* and aflatoxin accumulation resistance in maize. *Crop Science*, 55(5), 1857-1867.
- Watson, S., Gong, Y. Y. & Routledge, M. (2017). Interventions targeting child undernutrition in developing countries may be undermined by dietary exposure to aflatoxin. *Critical Reviews in Food Science and Nutrition*, 57(9), 1963-1975.
- Weaver, M. A., Abbas, H. K., Falconer, L. L., Allen, T. W., Pringle III, H. L., & Sciombato, G. L. (2015). Biological control of aflatoxin is effective and economical in Mississippi field trials. *Crop Protection*, 69, 52-55.
- Weaver, M. A., Abbas, H. K., Jin, X., & Elliott, B. (2016). Efficacy of water-dispersible formulations of biological control strains of *Aspergillus flavus* for aflatoxin management in corn. *Food Additives & Contaminants: Part A*, 33(2), 346-351.
- Wei, C., Yu, L., Qiao, N., Zhao, J., Zhang, H., Zhai, Q., . . . Chen, W. (2020). Progress in the distribution, toxicity, control, and detoxification of patulin: A review. *Toxicon*, 184, 83-93.
- Wei, D., Zhou, L., Selvaraj, J. N., Zhang, C., Xing, F., Zhao, Y., . . . Liu, Y. (2014). Molecular characterization of atoxigenic *Aspergillus flavus* isolates collected in China. *Journal of Microbiology*, 52(7), 559-565.
- Wu, F. (2015). Global impacts of aflatoxin in maize: trade and human health. *World Mycotoxin Journal*, 8(2), 137-142.
- Wu, Q., Wang, X., Nepovimova, E., Miron, A., Liu, Q., Wang, Y., . . . Kuca, K. (2017). Trichothecenes: immunomodulatory effects, mechanisms, and anti-cancer potential. *Archives of Toxicology*, 91(12), 3737-3785.
- Wu, Y.-Z., Lu, F.-P., Jiang, H.-L., Tan, C.-P., Yao, D.-S., Xie, C.-F., & Liu, D.-L. (2015). The furofuran-ring selectivity, hydrogen peroxide-production, and low Km value are the three elements for highly effective detoxification of aflatoxin oxidase. *Food and Chemical Toxicology*, 76, 125-131.
- Wu, Z., Ma, H., Wang, L., Song, X., Zhang, J., Liu, W., . . . Wang, Z. (2019). Tumor suppressor ZHX2 inhibits NAFLD-HCC progression via blocking LPL-mediated lipid uptake. *Cell Death & Differentiation*, 1-16.
- Wyatt, T. T., Van Leeuwen, M. R., Golovina, E. A., Hoekstra, F. A., Kuenstner, E. J., Palumbo, E. A., . . . Hallsworth, J. E. (2015). Functionality and prevalence of

trehalose- based oligosaccharides as novel compatible solutes in ascospores of *Neosartorya fischeri* (*Aspergillus fischeri*) and other fungi. *Environmental Microbiology*, 17(2), 395-411.

- Xie, L., Chen, M., & Ying, Y. (2016). Development of methods for the determination of aflatoxins. *Critical Reviews in Food Science and Nutrition*, 56(16), 2642-2664.
- Xu, D., Wang, H., Zhang, Y., Yang, Z., & Sun, X. (2013). Inhibition of non-toxicogenic *Aspergillus niger* FS10 isolated from Chinese fermented soybean on growth and aflatoxin B₁ production by *Aspergillus flavus*. *Food Control*, 32(2), 359-365.
- Xu, H., Hao, S., Gan, F., Wang, H., Xu, J., Liu, D., & Huang, K. (2017). *In vitro* immune toxicity of ochratoxin A in porcine alveolar macrophages: A role for the ROS-relative TLR4/MyD88 signaling pathway. *Chemical-Biological Interactions*, 272, 107-116.
- Yabe, K., Hatabayashi, H., Ikehata, A., Zheng, Y., & Kushiro, M. (2015). Development of the dichlorvos-ammonia (DV-AM) method for the visual detection of aflatoxigenic fungi. *Applied Microbiology and Biotechnology*, 99(24), 10681-10694.
- Yang, K., Liang, L., Ran, F., Liu, Y., Li, Z., Lan, H., . . . Nie, X. (2016). The DmtA methyltransferase contributes to *Aspergillus flavus* conidiation, sclerotial production, aflatoxin biosynthesis, and virulence. *Scientific Reports*, 6(1), 1-13.
- Yang, M., Lu, L., Pang, J., Hu, Y., Guo, Q., Li, Z., . . . Wang, C. (2019). Biocontrol activity of volatile organic compounds from *Streptomyces alboflavus* TD-1 against *Aspergillus flavus* growth and aflatoxin production. *Journal of Microbiology*, 57(5), 396-404.
- Yao, Y., An, C., Evans, D., Liu, W., Wang, W., Wei, G., . . . Gao, S.-S. (2019). Catalase involved in the oxidative cyclization of the tetracyclic ergoline of Fungal ergot alkaloids. *Journal of the American Chemical Society*, 141(44), 17517-17521.
- Yin, G., Hua, S. S. T., Pennerman, K. K., Yu, J., Bu, L., Sayre, R. T., & Bennett, J. W. (2018). Genome sequence and comparative analyses of atoxigenic *Aspergillus flavus* WRRL 1519. *Mycologia*, 110(3), 482-493.
- Yin, Z., Wang, Y., Wu, F., Gu, X., Bian, Y., Wang, Y., & Deng, D. (2014). Quantitative trait locus mapping of resistance to *Aspergillus flavus* infection using a recombinant inbred line population in maize. *Molecular Breeding*, 33(1), 39-49.
- Yoshida, H., Mamada, Y., Taniai, N., & Uchida, E. (2016). Spontaneous ruptured hepatocellular carcinoma. *Hepatology Research*, 46(1), 13-21.

- Yu, J. (2012). Current understanding of aflatoxin biosynthesis and future perspective in reducing aflatoxin contamination. *Toxins*, 4(11), 1024-1057.
- Yu, J., Chang, P.-K., Ehrlich, K. C., Cary, J. W., Bhatnagar, D., Cleveland, T. E., . . . Bennett, J. W. (2004). Clustered pathway genes in aflatoxin biosynthesis. *Applied Environmental Microbiology*, 70(3), 1253-1262.
- Zakaria, L. (2017). Mycotoxigenic *Fusarium* species from crops in Malaysia. *JSM Mycotoxins*, 67(2), 67-75.
- Zampieri, M., Ceglar, A., Dentener, F., Dosio, A., Naumann, G., Van Den Berg, M., & Toreti, A. (2019). When will current climate extremes affecting maize production become the norm? *Earth's Future*, 7(2), 113-122.
- Zanon, M. S. A., Barros, G. G., & Chulze, S. N. (2016). Non-aflatoxigenic *Aspergillus flavus* as potential biocontrol agents to reduce aflatoxin contamination in peanuts harvested in Northern Argentina. *International Journal of Food Microbiology*, 231, 63-68.
- Zhang, F., Li, X.-L., Zhu, S.-J., Ojaghian, M. R., & Zhang, J.-Z. (2018). Biocontrol potential of *Paenibacillus polymyxa* against *Verticillium dahliae* infecting cotton plants. *Biological Control*, 127, 70-77.
- Zhang, X., Song, M., Yu, X., Wang, Z., Ke, Y., Jiang, H., . . . Wen, K. (2017). Development of a new broad-specific monoclonal antibody with uniform affinity for aflatoxins and magnetic beads-based enzymatic immunoassay. *Food Control*, 79, 309-316.
- Zhang, Z., Zheng, X., Yang, J., Messing, J., & Wu, Y. (2016). Maize endosperm-specific transcription factors O₂ and PBF network the regulation of protein and starch synthesis. *Proceedings of the National Academy of Sciences*, 113(39), 10842-10847.
- Zhong, Y., Xia, X., Deng, S., Zhan, J., Fang, R., Xia, Y., . . . Tu, J. (2018). Popcorn inspired porous macrocellular carbon: rapid puffing fabrication from rice and its applications in lithium-sulfur batteries. *Advanced Energy Materials*, 8(1), 1701110.
- Zhou, L., Wei, D.-D., Selvaraj, J. N., Shang, B., Zhang, C.-S., Xing, F.-G., . . . Liu, Y. (2015). A strain of *Aspergillus flavus* from China shows potential as a biocontrol agent for aflatoxin contamination. *Biocontrol Science and Technology*, 25(5), 583-592.
- Zhou, P., Hirsch, C. N., Briggs, S. P., & Springer, N. M. (2019). Dynamic patterns of gene expression additivity and regulatory variation throughout maize development. *Molecular Plant*, 12(3), 410-425.
- Zhu, Z., Yang, M., Bai, Y., Ge, F., & Wang, S. (2020). Antioxidant-related catalase CTA1 regulates development, aflatoxin biosynthesis, and virulence in

pathogenic fungus *Aspergillus flavus*. *Environmental Microbiology*, 22(7) 2793-2810.

Zhuang, Z., Lohmar, J. M., Satterlee, T., Cary, J. W., & Calvo, A. M. (2016). The master transcription factor *mtfA* governs aflatoxin production, morphological development, and pathogenicity in the fungus *Aspergillus flavus*. *Toxins*, 8(1), 1-16.



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PUBLICATIONS

Khan, R., Mohamad Ghazali, F., Mahyudin, N. A., & Samsudin, N. I. P. (2020). Morphological Characterization and Determination of Aflatoxigenic and Non-Aflatoxigenic *Aspergillus flavus* Isolated from Sweet Corn Kernels and Soil in Malaysia. *Agriculture*, 10(10), 450.

Khan, R., Mohamad Ghazali, F., Mahyudin, N. A., & Samsudin, N. I. P. (2021). Co-inoculation of Aflatoxigenic and Non-Aflatoxigenic Strains of *Aspergillus flavus* to Assess the Efficacy of Non-Aflatoxigenic Strains in Growth Inhibition and Aflatoxin B1 Reduction. *Agriculture*, 10(10)





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