We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Cunninghamella bertholletiae's Toxins from Decomposing Cassava: Mitigation Strategy for Toxin Reduction Using *Nepenthes mirabilis* 'Monkey Cup' Digestive Fluids

*Elie Fereche Itoba-Tombo, Seteno Karabo Obed Ntwampe,
John Baptist Nzukizi Mudumbi, Lukhanyo Mekuto,
Enoch Akinbiyi Akinpelu and Nkosikho Dlangamandla*

Abstract

A fermentation technique was utilised to assess a fungus, i.e. *Cunninghamella bertholletiae/polymorpha*, isolated from rotting cassava, ability to produce mycotoxins and resultant oxidation by-products of the mycotoxins using liquid chromatography–mass spectrometry (LC/MS). Thus, the mycotoxins/secondary metabolites, fumonisin B¹ (FB¹) and deoxynivalenol (DON) were produced while, heptadecanone, octadecanamide, octadecenal and 3-keto-deoxynivalenol (DON) were successfully identified as biodegradation by-products in the fermentation broth treated with hydrolysing 'monkey cup' juice from *Nepenthes mirabilis*. Exposure to the mycotoxins and the biodegradation by-products through consumption of contaminated produce including contact due to the cumulative presence in arable agricultural soil can be harmful to humans and animals. Therefore, this work reports on a strategy for the mitigation and reduction of mycotoxins in agricultural soil using natural plant pitcher juices from *N. mirabilis* 'monkey cup'.

Keywords: biodegradation, carboxylesterases, *Cunninghamella bertholletiae*, LC/MS, mycotoxins, *Nepenthes mirabilis*

1. Introduction

Postharvest storage for cassava is often shortened due to product spoilage caused by bacterial and fungal infestation [1, 2]. Fungal species such as *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Cunninghamella* spp. can produce toxins and/or

secondary metabolites that affect the storage longevity and quality of agricultural product such as cassava [2, 3]. These mycotoxins, which have a negative impact on agricultural products, lead to economic losses due to the contamination of cassava tubers, which makes them inedible. Generally, toxins are biosynthetic compounds produced by numerous microorganisms in a natural or controlled environment.

These microorganisms include the fungus, *Cunninghamella bertholletiae* (also known as *Cunninghamella polymorpha* due to its morphological characteristics and mating/reproductive scheme) [4], is known to be pathogenic to humans and animals [5–7], while its toxins in the environment and on consumable commodities constitute an environmental hazard and a health risk to consumers [8–11]. Some fungi, including their metabolites, are able to contaminate several plant parts as they are endophytes, culminating in infestation of agricultural products such as tomatoes, maize, potatoes, beans, peanuts, yams and wheat, including cassava [1, 5, 12–17] and dairy products such as milk and cheese [1, 18, 19]. Humans' or animals' consumption of contaminated products may lead to foodborne toxin-related intoxication [7, 20] culminating in the degeneration of human internal organs including their functionality and the promotion of diseases such as cancer [8, 15, 21–23]. Some clinical outcomes in animals and humans include liver and oesophageal cancer [21, 23], the destruction of renal and nerve tissues, profound oxidative stress, heart and pulmonary diseases [23].

There are several varieties of mycotoxins, namely aflatoxins (AFB¹, AFB², AFG¹ and AFG²), fumonisins (FB¹, FB²), deoxynivalenol (DON), ochratoxins (A, B and C), amongst others, which are produced by numerous species, some of which are deleterious to plants/agricultural products, humans and animals [1, 5, 21, 23, 24]. Their production can occur under favourable environmental conditions, such as a high temperature and adequate moisture/humidity, including the availability of nutrients (mostly from the decaying produce) [25]. These concerns have prompted researchers to find cheap, efficient and cost-effective ways to reduce or manage mycotoxin-producing organisms, including mycotoxin contamination, when produced [11, 26] to limit sequential effects including products' contamination.

In a previous study, it was found that *C. bertholletiae/polymorpha*, a common soil organism [7, 23, 26] which was isolated from decomposing cassava, was both cyanide-resistant with the ability to biodegrade free cyanide while being antagonistic towards other soil organisms [15, 27]. Currently, there is minimal literature available on mycotoxins produced by *C. bertholletiae*. Similarly, there is minimal research on a mitigation strategy which could be classified as environmentally benign for combined toxin reduction, via oxidation or hydrolysis. The mitigation method must be implementable *in-situ* in order to minimise deleterious effects observed when other methods are used.

Therefore, the aim of this study was to propose and assess a method for the identification of mycotoxins from the free-cyanide tolerant *C. bertholletiae/polymorpha* isolate; furthermore, to quantitatively assess a mitigation method using oxidative/hydrolysing 'monkey cup' digestive fluids from *N. mirabilis* (green chemistry approach). A *N. mirabilis* is a carnivorous plant which belongs to the genus of *Nepenthes*. This plant is characterised by a pitfall trap commonly known as a 'monkey cup' at the end of the plants' leaf, which contains an acidic and oxidative/hydrolysing fluid. The plants' pitcher juices are known to contain a variety of enzymes useful for prey digestion [28, 29]. As such, these enzymes can oxidise and/or hydrolyse mycotoxins and secondary metabolites via deamination or mechanisms biocatalytically facilitated by esterases for the decoupling of aliphatic chains in mycotoxins or secondary metabolites.

2. Mycotoxin (secondary metabolite) production in food

Several studies discussed about the presence of mycotoxins in food. Thus, during a produce life cycle from harvest, postharvest, selves' life, processing and sometimes distribution, there is a presence of mycotoxins in food worldwide [1]. These toxins occurred during poor storage, handling and processing conditions, sometimes might be the result of the rot/decay foodstuffs [2, 14, 30]. While these mycotoxins constitute a serious threat to food quality and human's health [22, 30].

2.1 Extraction and analysis of mycotoxins (secondary metabolites) and their biodegradation by-products

Literatures abound on the extraction and analysis of mycotoxins, a liquid-phase extraction method seems to be more used. Thus, [31, 32] used liquid-liquid extraction method for their studies in mycotoxins identification, while [33] used a liquid chromatography/tandem mass spectrometry for a combined analysis of aflatoxins, ochratoxin A and *Fusarium* for maize crop. Whereas [34] chose a multiplex approach of Gas chromatography–mass spectrometry (GC-MS), Liquid chromatography–mass spectrometry (LC-MS) and One-dimensional (1D) NMR spectroscopy (1D NMR) techniques for their study on a comparative metabolite profiling and fingerprinting of medicinal licorice roots, to name few.

The samples were analysed using an LC/MS-ToF 6230 (Agilent Technologies Inc., USA) and using mobile-phase parameters as listed in the table below in Supplementary Material, without optimisation as suggested by [31, 34]. The solvent extract phase was steadily evaporated using a blow-down technique to dryness at an ambient temperature for 24 h to minimise mycotoxin evaporation using nitrogen (N₂) gas (Afrox, South Africa) [31, 35].

The identification of the mycotoxins from *C. bertholletiae/polymorpha* isolate, including toxin biodegradation by-products, was done through analysis on LC/MS-ToF 6230 (Agilent Technologies Inc., USA) and analytical standard as well as profile data as per [31, 35] using a mycotoxin/biodegradation by-product database, with the assumption that samples were assumed to lose an electron with the H⁺ proton being hypothetically the lost ion. Compounds were initially mined based on their molecular features and verified by mining based on their exact formulas. The extracted ion chromatogram (EIC) of matched compounds is presented in **Supplementary Figure 2**.

3. Proposed mitigation strategy

3.1 *N. mirabilis* extracts collection, characterisation and application

The assessment of the physicochemical characteristics of the *N. mirabilis* pitcher juice used was similar to that in [36–38]. Thus, the assessment revealed the following: conductivity: 5.89 S/m, redox potential: 510 mV, specific gravity (SG): 1.02 and a pH of 2.5.

Additionally, a qualitative method for the analysis and enzymes/biochemical tests were done to determine the presence of enzymes in the pitcher juice [36–39]. Furthermore, the VITEK 2 DensiChek™ cards were used (as a supplementary method) to quantitatively determine the enzyme presence in the extracts during the physicochemical analysis of the pitcher juice according to the instrument's/device's user manual instructions [40].

3.2 Enzyme (carboxylesterase) activity: mechanism, specificity and quantification

The quantification of carboxylesterases activity was similar to the method adopted from [41–43] with minor modifications. The overall biocatalysis properties of the *N. mirabilis* pitcher constituents, with a focus on carboxylesterases, are described by [41], who suggested that hydrolysis mechanism associated with carboxylesterases facilitates the biocatalysis of reactions associated with enzymes, including arylesterase, lysophospholipase, acetylerase, acylglycerol lipase, etc. In the current study, the biodegradation of fumonisin and deoxynivalenol (DON) was achieved using a single enzyme (carboxylesterases).

Furthermore, subsequent reports on the development of a spectrophotometric method used for the determination of carboxylesterase activity for the *N. mirabilis* digestive fluid were used by [29, 42].

3.3 Carboxylesterase activity assay

Previous studies assessed carboxylesterase activity. Thus, the carboxylesterase activity assay was determined spectrophotometrically at an ambient temperature using *p*-nitrophenyl acetate (PNPA) as the substrate as suggested by [36, 43]. While the activity was measured by determining the rate of biocatalysis of PNPA to *p*-nitrophenol (PNP) which was spectrophotometrically monitored at 410 nm. The PNPA exhibits minimal absorbance at 410 nm, whereas the PNP absorbs strongly. The extinction coefficient used for PNP was $17,000 \text{ M}^{-1}\cdot\text{cm}^{-1}$ [36]. Activity was then expressed in U/L, where 1 unit is equivalent to $1 \mu\text{mol}/\text{min}$ (the rate of conversion for PNPA to PNP).

3.4 Spectrophotometer settings: Carboxylesterase activity assay

The JENWAY 6405 UV/Vis spectrophotometer (Agilent Pty, USA) at a kinetics setting was used 410 nm to monitor PNP formation for 2 min at 10 sec intervals, while the cell holder temperature was at 25°C . Eq. (1) Illustrates the mathematical expression used to quantify the activity of carboxylesterases [36].

$$\text{activity (U/L)} = \left[\frac{\frac{dA}{dt} * (\text{dilution factor})}{\text{extinction coefficient}} \right] * 60 * 10^6 \quad (1)$$

Where $\frac{dA}{dt}$ is the value of the reaction's initial rate.

4. Mycotoxins identification

Mycotoxins produced by the isolated *C. bertholletiae/polymorpha* were assessed via a fermentation technique in a nutrient broth medium with the liquid-liquid extraction method being done using chloroform, subsequent to a blow-down technique of the samples and reconstitution in absolute methanol. The compounds listed in **Table 1** were identified based on their molecular composition (structural features) and mass-to-charge ratio (m/z), using an LC/MS-ToF.

Toxin identification is important due to observed consequential outcomes of the infested cassava as by-products of bacterial or mycotic infestation which are hazardous to both humans and animals if such agricultural product is consumed. Thus, both fumonisin B¹ and deoxynivalenol were identified as the prevalent compounds associated with the fermentation of the cyanide resistant isolate, *C. bertholletiae*, accession no. KT275316 [15].

FB¹ detection on LC/MS-ToF was done, based on a method developed by [18, 24, 31, 44], for which the analyte produces a signal under a positive MS acquisition mode (**Table 1**).

A, mycotoxins molar mass (g/mol); B, biodegradation by-products molar mass (g/mol); A1, mycotoxins mass (*m/z*) to charge ratio-ion form [M + H]⁺; B1, biodegradation by-products mass (*m/z*) to charge ratio-ion form.

For FB¹, mean peak counts of 4 × 10³ were observed, while 1.9 × 10³ counts were for DON. Similarly, and according to [31], DON detection is easily achieved through HPLC/LC-MS and UV methods. A LC/MS-ToF method, as described above, was used without modification nor optimisation, to also identify the biodegradation by-products for each identified mycotoxins/secondary metabolite as listed in **Table 1**.

Two peaks were observed with a retention time of 23.79 and 35.12 min, with a molecular formula of C₃₄H₅₉NO₁₅ and C₁₅H₂₀O₆, analogous to FB¹ and DON, respectively. The peaks, A and B, were directly associated with ion *m/z* of 722.395 and 297.13, when the ESI was operated in a positive mode [ion form: M + H⁺]. From the analysis, a combination of the molecular weight, the structure, including *m/z* ratio, confirmed the identification of the compounds. It is paramount to indicate that FB¹ was detected in a culture in which CN⁻ (as KCN) was supplemented; hypothetically, indicating that the FB¹ production was perhaps influenced by strenuous conditions to which the culture was subjected in comparison to DON.

4.1 Biodegradation by-products' identification

To the reported residual samples of the cyanide-resistant *C. bertholletiae/polymorpha*, in which FB¹ and DON were detected, *N. mirabilis* pitcher juices were added. This was for an assessment of the fungal mycotoxins/toxins' (FB¹ and DON) biodegradation into by-products [36–38], which could be identified using the LC/MS-ToF. Thus, compounds such as heptadecanone, octadecanamide and octadecenal were successfully identified from FB¹ samples with only 3-keto-DON being identified in DON samples, respectively (**Table 1**; **Figure 1**).

Mycotoxins/secondary metabolites	Biodegradation by-products identified	Molar mass (g/mol)		(m/z) ion form [M + H] ⁺	
		A	B	A1	B1
Fumonisin B ¹ (C ₃₄ H ₅₉ NO ₁₅)	Heptadecanone C ₁₇ H ₃₄ O	721	254.45	722.395	256.270
	Octadecanamide C ₁₈ H ₃₇ NO		283.29		284.282
	Octadecenal C ₁₈ H ₃₄ O		266.46		267.268
Deoxynivalenol (DON) (C ₁₅ H ₂₀ O ₆)	3-keto-DON C ₁₅ H ₁₈ O ₆	296	294.91	297.13	295.115

Table 1.
C. bertholletiae's mycotoxins/toxins and mycotoxins biodegradation by-products identified using LC/MS-ToF.

The findings of this study are similar to those from previous studies which revealed that a biodegradation of FB¹ yielded by-products such as heptadecanone, octadecanamide and octadecenal (**Supplementary Figure 2a–c**) [26, 45]. While a degradation of DON led to an intermediate by-product such as 3-keto-DON [46, 47] (**Supplementary Figure 2d**). By using a similar identification strategy to that used to identify FB¹ and DON, it was clear that *N. mirabilis* had a deleterious effect on both DON and FB¹. The findings of this study are in agreement with those by [38, 48]. From the spectra, the by-product counts indicated octadecenal (1.1×10^2) > octadecanamide (1×10^2) > heptadecanone (0.9×10^2) with molecular ion peaks at m/z [M + H⁺], 267.268, 284.282 and 256.270, respectively.

Furthermore, for DON residual samples, the by-products observed when subjected to the *N. mirabilis* pitcher juice were indicative of 3-keto-DON; that is, with the ESI spectra showing a molecular ion peak at m/z [M + H⁺], 295.115 in a positive ion mode which was consistent with the molecular formula (C₁₅H₁₈O₆) (see **Supplementary Figure 2d**). Due to the nature of the proposed *in-situ* mitigation strategy, it is prudent to indicate that the applied *N. mirabilis* pitcher juice comprises biocatalytic agents or enzymes [39, 49] known to facilitate the

Figure 1. Summary of a biodegradation process and associated oxidation/hydrolysing enzymes.

biodegradation of mycotoxins, using both qualitative and quantitative techniques. Thus, a degrading ability of the pitcher juice is due to the presence of enzymes such as carboxylesterase, β -glucuronidase, phosphatidyl inositol phospholipase C, xylanases, etc., which are able to biodegrade several organic matters, i.e. agro-waste, hemicellulose, etc., as well as mycotoxins/toxins [36–39, 49–51]. The enzymes found in the *N. mirabilis* pitcher juice originate from decayed multitude of trapped preys/species (insects) and microbial community (fungal and bacterial, etc.) within the plant’s fluid [28, 37, 39, 41, 49, 51, 52].

4.2 Enzyme/biochemical activity assays for *N. mirabilis* pitcher juice

The samples’ carboxylesterase activity (quantitative) and other biochemical assays (using the VITEK system, qualitative) were also done at room temperatures, whereas the *N. mirabilis* pitcher juice for carboxylesterase, *P*-nitrophenyl acetate (PNPA) were used as a substrate at 75% dilution and 410 nm absorbance which was similar to [36, 37]. For biochemical assays, numerous enzymes (as highlighted in Table 2) were positively identified, while the calculation of carboxylesterase activity was found to be 7.8 U/L.

5. Mycotoxin identification from cyanide-resistant *Cunninghamella* spp.

Due to the multitude of methods developed and assessed, a method modified by [44], for toxin extraction from a fermentation of broth, was adopted. It was thus used to produce mycotoxins (FB1 and DON) from the cyanide-resistant *C. bertholletiae/polymorpha*, with the extracts being used for LC/MS-ToF analysis due to the method’s usability, reproducibility and rapidity, while incurring minimal input/sample-processing costs.

5.1 Biodegradation by-products: outcomes of the mitigation strategy

A digestive fluid of *N. mirabilis* was used as a feasible alternative for the biodegradation of fungal mycotoxins/toxins (Fumonisin and DON) with assays ($n = 2$) confirming the prevalence of carboxylesterases. However, previous studies mentioned the existence of several enzymes [28, 39, 41, 49, 50] within a *N. mirabilis* digestive fluid/pitcher juice, which counts as a larger enzymatic profile than individual microbial species, as highlighted in Table 2.

Furthermore, a few sceptics could express concern about the use of a plant’s pitcher juice on mycotoxin-contaminated matrices because of its low pH (2.5), as well as availability, which can be addressed by using appropriate buffers and suitable plant

Enzymes	Activity/outcome	References
Carboxylesterase	7.8 (U/L)	In this study
β -glucosidase	++	[38, 39]
β -glucuronidase	++	[48]
Phosphatidyl inositol phospholipase C	++	[49]
++, positively identified in previous studies.		

Table 2.
Carboxylesterase activity and qualitatively identified enzymes.

extracts with similar enzymatic characteristics. Overall, the application of a low pH extract in a matrix such as agricultural soil should not be a major concern because a soil's pH can be amended by an application of lime. A study by [53] revealed that the application of lime on agricultural soil with a low pH increases the soil's pH, improving its respiration capacity, while retaining the soil's microbial community profile at an acceptable level.

6. Conclusions

The identification through LC/MS-ToF of toxins ((fumonisin B¹ and deoxynivalenol (DON)) from a free-cyanide-resistant *Cunninghamella bertholletiae/polymorpha* as well as a mitigation strategy for toxins reduction through a biodegradation/fermentation process using 'monkey cup' juice from *N. mirabilis* (which yielded by-products such as heptadecanone, octadecanamide, octadecenal and 3-keto-DON) is an important step towards ensuring food safety and mitigating humans' health hazards through toxins exposure. As, an exposure or intoxication from these mycotoxins, through consumption of contaminated food or agricultural product, can be hazardous to humans and animals. Therefore, control measures for food and animal feed contamination are needed in order to decrease the levels of these compounds. Additionally, preventative protocols and/or mitigation strategies that would ensure the eradication of these hazardous compounds, using an environmentally benign approach such as *N. mirabilis* digestive fluid/pitcher juices, are paramount. Thus, the application of the digestive fluid to a liquid matrix which culminated in the biodegradation of mycotoxins (fumonisin B1 and DON), with the subsequent formation of the biodegradation by-products such as heptadecanone, octadecanamide, octadecenal for fumonisin B1 and 3-keto-DON for DON, which are easier to biodegrade by other microbial communities, should be encouraged.

However, it is worth noting that at this stage, there is a need to find alternative indigenous plant extracts with similar characteristics to that of the *N. mirabilis*.

Acknowledgements

The authors would like to express their gratitude to: Ogheneochuko Oputu, all BioERG members as well as staff from the Environmental Management Programme and Biotechnology department for their support.

Funding

The research is funded by the Cape Peninsula University of Technology, through the University Research Fund (URF)—Cost code R980.

Conflicts of interest

The authors declare no conflicts of interest with respect to the research, authorship and/or publication of this manuscript.

Appendix

Supplementary Figure 2.
Molecular features and the extracted ion chromatograms (EICs)/mass spectrum of mycotoxins/toxins' biodegradation by-products: (a) heptadecanone, (b) octadecanamide, (c) octadecenal and (d) 3-keto-DON.

Gradient (min)	A (H ₂ O)*	B (MeOH)Y	Flow (mL/min)
0	85	15	0.4
30	0	100	0.4
33	0	100	0.4
45	85	15	0.4
50	85	15	0.4

* , water contained, 0.1% formic acid, pH 3.
Y, analytical grade methanol.

Supplementary Table S1.
LC/MS-ToF elution and mobile phase parameters.

IntechOpen

Author details

Elie Fereche Itoba-Tombo^{1*}, Seteno Karabo Obed Ntwampe²,
John Baptist Nzukizi Mudumbi³, Lukhanyo Mekuto⁴, Enoch Akinbiyi Akinpelu³
and Nkosikho Dlangamandla⁵

1 Department of Environmental and Occupational Studies, Cape Peninsula University of Technology, Cape Town, South Africa

2 School of Chemical and Mineral Engineering, North-West University, Potchefstroom, South Africa

3 Bioresource Engineering Research Group (BioERG), Department of Biotechnology, Cape Peninsula University of Technology, Cape Town, South Africa

4 Department of Chemical Engineering, University of Johannesburg, Johannesburg, South Africa

5 Department of Chemical Engineering, Durban University of Technology, Durban, South Africa

*Address all correspondence to: elie.tombo@gmail.com

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Pitt JI, Hocking AD. The ecology of fungal food spoilage. In: Pitt JI, Hocking AD, editors. *Fungi and Food Spoilage*. Boston, MA: Springer; 2009. pp. 3-9
- [2] Zidenga T, Leyva-Guerrero E, Moon H, Siritunga D, Sayre R. Extending cassava root shelf life via reduction of reactive oxygen species production. *Plant Physiology*. 2012;**159**:1396-1407
- [3] Matumba L, Sulyok M, Monjerezi M, Biswick T, Krska R. Fungal metabolites diversity in maize and associated human dietary exposures relate to micro-climatic patterns in Malawi. *World Mycotoxin Journal*. 2014;**8**(3):269-282
- [4] Weitzman I, Crist MY. Studies with clinical isolates of *Cunninghamella* I. Mating behavior. *Mycologia*. 1979;**71**:1024-1033
- [5] Wagacha J, Muthomi J. Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology*. 2008;**124**(1):1-12
- [6] Nguyen TT, Choi YJ, Lee HB. Isolation and characterization of three unrecorded zygomycete fungi in Korea: *Cunninghamella bertholletiae*, *Cunninghamella echinulata*, and *Cunninghamella elegans*. *Mycobiology*. 2017;**45**(4):318-326
- [7] Zhang R, Zhang JW, Szerlip HM. Endocarditis and hemorrhagic stroke caused by *Cunninghamella bertholletiae* infection after kidney transplantation. *American Journal of Kidney Diseases*. 2002;**40**(4):842-846
- [8] Ueno Y, Iijima K, Wang SD, Sugiura Y, Sekijima M, Tanaka T, et al. Fumonisin as a possible contributory risk factor for primary liver cancer: A 3-year study of corn harvested in Haimen, China, by HPLC and ELISA. *Food and Chemical Toxicology*. 1997;**35**(12):1143-1150
- [9] Brakhage AA. Regulation of fungal secondary metabolism. *Nature Reviews Microbiology*. 2013;**11**(1):21-32
- [10] Selvaraj JN, Lu Z, Yan W, Zhao YJ, Xing FG, Dai XF, et al. Mycotoxin detection—Recent trends at global level. *Journal of Integrative Agriculture*. 2015;**14**(11):2265-2281
- [11] Amadi JE, Adeniyi DO. Mycotoxin production by fungi isolated from stored grains. *African Journal of Biotechnology*. 2009;**8**(7):1219-1221
- [12] Adetunji M, Atanda O, Ezekiel CN, Sulyok M, Warth B, Beltrán E, et al. Fungal and bacterial metabolites of stored maize (*Zea mays*) from five agro-ecological zones of Nigeria. *Mycotoxin Research*. 2014;**30**(2):89-102
- [13] Delgado RM, Sulyok M, Jirsa O, Spitzer T, Krska R, Polišenská I. Relationship between lutein and mycotoxin content in durum wheat. *Food Additives & Contaminants: Part A*. 2014;**31**(7):1274-1283
- [14] Njumbe Ediage E, Hell K, De Saeger S. A comprehensive study to explore differences in mycotoxin patterns from agro-ecological regions through maize, peanut, and cassava products: A case study, Cameroon. *Journal of Agricultural and Food Chemistry*. 2014;**62**(20):4789-4797
- [15] Itoba-Tombo EF, Waxa A, Ntwampe SKO. Isolation of an endophytic cyanide resistant fungus

Cunninghamella bertholletiae from (*Manihot esculenta*) and cassava cultivated soil for environmental engineering applications. In: 7th International Conference on Latest Trends in Engineering and Technology (ICLTET'2015); 26-27 November 2015; Pretoria, South Africa. 2015. pp. 150-153

[16] Itoba-Tombo EF, Ntwampe SKO, Mudumbi JBN, Mekuto L, Akinpelu EA, Oputu OU. Rapid identification of *Cunninghamella bertholletiae*'s toxins/secondary metabolites via a fermentation technique. In: 10th Int'l Conference on Advances in Chemical, Agricultural, Biological and Environmental Sciences (ACABES-18); 19-20 November 2018; Cape Town, South Africa. 2018 [Retrieved: 17 September 2018]

[17] Streit E, Schwab C, Sulyok M, Naehrer K, Krska R, Schatzmayr G. Multi-mycotoxin screening reveals the occurrence of 139 different secondary metabolites in feed and feed ingredients. *Toxins*. 2013;5(3):504-523

[18] Warth B, Petchkongkaew A, Sulyok M, Krska R. Utilising an LC-MS/MS-based multi-biomarker approach to assess mycotoxin exposure in the Bangkok metropolitan area and surrounding provinces. *Food Additives & Contaminants: Part A*. 2014;31(12):2040-2046

[19] Mantle P, Copetti MV, Buddie A, Frisvad J. Comments on "Mycobiota and Mycotoxins in Traditional Medicinal Seeds from China. *Toxins* 2015, 7, 3858-3875"—In attributing ochratoxin A biosynthesis within the genus *Penicillium* occurring on natural agricultural produce. *Toxins*. 2016;8(6):1-4

[20] Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D. Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential

health consequences, and interventions. *The American Journal of Clinical Nutrition*. 2004;80(5):1106-1122

[21] Pestka JJ, Smolinski AT. Deoxynivalenol: toxicology and potential effects on humans. *Journal of Toxicology and Environmental Health, Part B*. 2005;8(1):39-69

[22] Afsah-Hejri L, Jinap S, Hajeb P, Radu S, Shakibazadeh S. A review on mycotoxins in food and feed: Malaysia case study. *Comprehensive Reviews in Food Science and Food Safety*. 2013;12(6):629-651

[23] Rickerts V, Böhme A, Viertel A, Behrendt G, Jacobi V, Tintelnot K, et al. Cluster of pulmonary infections caused by *Cunninghamella bertholletiae* in immunocompromised patients. *Clinical Infectious Diseases*. 2000;31(4):910-913

[24] Srey C, Kimanya ME, Routledge MN, Shirima CP, Gong YY. Deoxynivalenol exposure assessment in young children in Tanzania. *Molecular Nutrition & Food Research*. 2014;58(7):1574-1580

[25] Van Der Fels-Klerx HJ, Olesen JE, Madsen MS, Goedhart PW. Climate change increases deoxynivalenol contamination of wheat in north-western Europe. *Food Additives & Contaminants: Part A*. 2012;29(10):1593-1604

[26] Vanhoutte I, Audenaert K, De Gelder L. Biodegradation of mycotoxins: Tales from known and unexplored worlds. *Frontiers in Microbiology*. 2016;7:1-20

[27] Itoba-Tombo EF, Ntwampe SKO, Waxa A, Paulse A, Akinpelu EA. Screening of fungal (*Cunninghamella bertholletiae*) pathogenic activity on microbial community in cassava (*Manihot esculenta crantz*) cultivated soil. In: Int'l Conf. on Advances in Science,

Engineering, Technology and Natural Resources (ICASETNR-16); 24-25 November 2016; Parys, South Africa. 2016. pp. 112-117

[28] Adlassnig W, Peroutka M, Lendl T. Traps of carnivorous pitcher plants as a habitat: Composition of the fluid, biodiversity and mutualistic activities. *Annals of Botany*. 2010;**107**(2):181-194

[29] Lee L, Zhang Y, Ozar B, Sensen CW, Schriemer DC. Carnivorous nutrition in pitcher plants (*Nepenthes* spp.) via an unusual complement of endogenous enzymes. *Journal of Proteome Research*. 2016;**15**(9):3108-3117

[30] Peraica M, Domijan AM. Contamination of food with mycotoxins and human health. *Arhiv za Higijenu Rada i Toksikologiju*. 2001;**52**(1):23-35

[31] Sulyok M, Beed F, Boni S, Abass A, Mukunzi A, Krska R. Quantitation of multiple mycotoxins and cyanogenic glucosides in cassava samples from Tanzania and Rwanda by an LC-MS/MS-based multi-toxin method. *Food Additives & Contaminants: Part A*. 2015;**32**(4):488-502

[32] Rubert J, Soler C, Marín R, James KJ, Mañes J. Mass spectrometry strategies for mycotoxins analysis in European beers. *Food Control*. 2013;**30**(1):122-128

[33] Lattanzio VMT, Solfrizzo M, Powers S, Visconti A. Simultaneous determination of aflatoxins, ochratoxin A and *Fusarium* toxins in maize by liquid chromatography/tandem mass spectrometry after multitoxin immunoaffinity cleanup. *Rapid Communications in Mass Spectrometry*. 2007;**21**(20):3253-3261

[34] Farag MA, Porzel A, Wessjohann LA. Comparative metabolite profiling and fingerprinting of medicinal licorice roots

using a multiplex approach of GC–MS, LC–MS and 1D NMR techniques. *Phytochemistry*. 2012;**76**:60-72

[35] Zide D, Fatoki O, Oputu O, Opeolu B, Nelana S, Olatunji O. Zeolite 'adsorption' capacities in aqueous acidic media; the role of acid choice and quantification method on ciprofloxacin removal. *Microporous and Mesoporous Materials*. 2018;**255**:226-241

[36] Dlangamandla N, Ntwampe SKO, Angadam JO, Chidi BS, Mewa-Ngongang M. Kinetic parameters of *Saccharomyces cerevisiae* alcohols production using *Nepenthes mirabilis* pod digestive fluids-mixed agro-waste hydrolysates. *Fermentation*. 2019;**1**(5):1-14

[37] Dlangamandla N, Ntwampe SKO, Angadam JO, Itoba-Tombo EF, Chidi BS, Mekuto L. Integrated hydrolysis of mixed agro-waste for a second generation biorefinery using *Nepenthes mirabilis* pod digestive fluids. *PRO*. 2019;**7**(2):64

[38] Kanokratana P, Mhuanthong W, Laothanachareon T, Tangphatsornruang S, Eurwilaichitr L, Krueetreepradit T, et al. Comparative study of bacterial communities in *Nepenthes pitchers* and their correlation to species and fluid acidity. *Microbial Ecology*. 2016;**72**(2):381-393

[39] Takeuchi Y, Salcher MM, Ushio M, Shimizu-Inatsugi R, Kobayashi MJ, Diway B, et al. In-situ enzyme activity in the dissolved and particulate fraction of the fluid from four pitcher plant species of the genus *Nepenthes*. *PLoS One*. 2011;**6**(9):1-9

[40] Pincus DH. Microbial Identification Using the Bio Merieux VITEK® 2 System. *Encyclopedia of Rapid Microbiological Methods*. Bethesda, MD: Parenteral Drug Association; 2006

- [41] Schomburg D, Brenda. The comprehensive enzyme information system (online). 2015. Available from: <http://www.brenda-enzymes.org/enzyme.php> [Retrieved: 10 October 2018]
- [42] Wheelock CE, Severson TF, Hammock BD. Synthesis of new carboxylesterase inhibitors and evaluation of potency and water solubility. *Chemical Research in Toxicology*. 2001;**14**(12):1563-1572
- [43] Ljungquist A, Augustinsson KB. Purification and properties of two carboxylesterases from rat-liver microsomes. *European Journal of Biochemistry*. 1971;**23**(2):303-313
- [44] Bily AC, Burt AJ, Ramputh AI, Livesey J, Regnault-Roger C, Philogène BR, et al. HPLC-PAD-APCI/MS assay of phenylpropanoids in cereals. *Phytochemical Analysis*. 2004;**15**(1):9-15
- [45] Benedetti R, Nazzi F, Locci R, Firrao G. Degradation of fumonisin B1 by a bacteria strain isolated from soil. *Biodegradation*. 2006;**17**(1):31-38
- [46] He JW, Bondy GS, Zhou T, Caldwell D, Boland GJ, Scott PM. Toxicology of 3-epi-deoxynivalenol, a deoxynivalenol-transformation product by *Devosia mutans* 17-2-E-8. *Food and Chemical Toxicology*. 2015;**84**:250-259
- [47] He JW, Hassan YI, Perilla N, Li XZ, Boland GJ, Zhou T. Bacterial epimerization as a route for deoxynivalenol detoxification: The influence of growth and environmental conditions. *Frontiers in Microbiology*. 2016a;**7**:572
- [48] Duvick J, Maddox J, Gilliam J. Compositions and methods for fumonisin detoxification. U.S. Patent No. 6,538,177. Pioneer Hi-Bred International Inc.; 2003
- [49] Terra WR, Ferreira C. Insect digestive enzymes: Properties, compartmentalization and function. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*. 1994;**109**(1):1-62
- [50] Borgen BH. Functional analysis of plant idioblasts (myrosin cells) and their role in defense, development and growth. In: Thesis Submitted for the degree of Doctor Scientiarum at the Norwegian University of Science and Technology (NTNU). UNIGEN Center of Molecular Biology and Department of Biology, Faculty of Natural Sciences and Technology; 2002
- [51] Hassan YI, He JW, Perilla N, Tang K, Karlovsky P, Zhou T. The enzymatic epimerization of deoxynivalenol by *Devosia mutans* proceeds through the formation of 3-keto-DON intermediate. *Scientific Reports*. 2017;**7**:6929
- [52] Carere J, Hassan YI, Lepp D, Zhou T. The enzymatic detoxification of the mycotoxin deoxynivalenol: Identification of DepA from the DON epimerization pathway. *Microbial Biotechnology*. 2018;**11**(6):1106-1111
- [53] Bezdicek DF, Beaver T, Granatstein D. Subsoil ridge tillage and lime effects on soil microbial activity, soil pH, erosion, and wheat and pea yield in the Pacific Northwest, USA. *Soil and Tillage Research*. 2003;**74**(1):55-63