Nig. J. Biotech. Vol. 41(1): 130-138 (June 2024)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and https://bsn.org.ng
DOI: https://dx.doi.org/10.4314/njb.v41i1.14

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Control of Aflatoxin-Producing Moulds in Melon Paste (*Ogiri*) Sold in Ogun State Using African Star Apple Oil and Cucumber Oil

***[1a]Oni E. O.[1a], [1a]Badmos A. O., [1a]Ariom T. O., [1a]Shoyinka, Z. O., [1a]Parakoyi G. O., [2]Adeleke A. J., [3]Sanusi J. O., [3]Sirajudeen A. O., [1a]Obuotor T. M., and [1b]Omemu A. M.**

[1a]Department of Microbiology, [1b]Department of Hospitality and Tourism Management, Federal University of Agriculture, Abeokuta, Nigeria. [2]Department of Microbiology, Modibbo Adama University, Yola, Nigeria. [3]Department of Biological Sciences, College of Natural and Applied Sciences, Crescent University, Abeokuta, Nigeria.

Abstract

The deleterious effects of aflatoxin-producing moulds cannot be over-emphasized in traditionally fermented food condiments like '*Ogiri*' (melon paste), irrespective of their nutritional properties. Therefore, the use of essential oils as a biocontrol agent to control aflatoxin-producing moulds in food condiments like '*Ogiri*' is a potential approach worth exploring. This study aimed to evaluate the antifungal properties of African Star Apple Oil and Cucumber Oil against aflatoxin-producing moulds. A total number of five samples of the *ogiri* (melon paste) were purchased from five different markets (Ijebu Igbo, Ijebu Awa, Ago-Iwoye, Kuto, and Osiele) in Ogun State, Nigeria. Fungal isolation and identification were carried out on the melon pastes using standard microbiological procedures. Aflatoxigenicity screening of the suspected fungal isolates was carried out using Neutral Red Dessicated Coconut Agar and viewed under an ultra-violet (UV) spectrophotometer at 365nm. The phytochemical properties and the anti-fungal potentials of the oils on the aflatoxin-producing moulds were determined using Gas Chromatography-Mass Spectrometry (GCMS) and disc well diffusion method respectively. *Aspergillus flavus, Aspergillus niger, Neurospora spp., and Saccharomyces cerevisiae* were the fungi isolated from the *ogiri* samples, with *Aspergillus flavus, Saccharomyces cerevisiae* and *Neurospora spp. h*aving the least percentage occurrence of 21.0%, 16.0%, and 5.0% respectively while *Aspergillus niger* had the highest percentage occurrence of 58.0%. The screening for aflatoxin-producing potential among the isolates indicated that only the melon samples from Osiele exhibited positive aflatoxigenicity. The result showed that fermented melon paste were highly contaminated with aflatoxigenic fungi and its consumption could pose aflatoxicosis risk to man. The antifungal efficacy of African star apple and cucumber oil against aflatoxin-producing mould displayed minimal zones of inhibition around the well, signifying limited antimicrobial activity of the oils.

Keywords: Aflatoxins, Anti-fungal, Bio-control agent, Condiments and Melon paste.

Correspondence: *Oni, Eniola Oluyemisi; onieo@funaab.edu.ng Tel. +234 8032237108

Introduction

Ogiri (fermented melon paste) is an oily paste produced from oil seeds in West Africa and they often serve as aromatic enhancements for soup. It originates from the fermentation process of melon seeds {*Citrullus vulgaris*} [Achi 2005a; Nwosu, & Ogueke, (2022)]. *Ogiri* is a culinary seasoning crafted through traditional processes, utilizing spontaneous solid-state fermentation of melon seeds, a process characterized by chance fermentation (Akinyele and Oloruntoba, 2013). Fermented condiments contribute to bolstering and fortifying our immune systems, aiding in the prevention and combating of diseases

such as tuberculosis, cancer, and cardiac disorders. Fermented condiments improve the nutritive values of foods as well as sensory properties as taste enhancers. Fermentation is proven to enhance not just the nutritional value of legume seeds but also to augment their antioxidative properties, thereby elevating their potential as functional foods and sources of nutraceuticals (Ademiluyi and Oboh, 2011).

In Africa, the proliferation of fungi, especially *Aspergillus* spp., in storage poses a serious challenge exacerbated by the humid tropical climate that favours fungal proliferation (Bankole et al., 2006). These fungi can thrive in specific food and feed products, given suitable environmental conditions, leading to the production of harmful secondary metabolites known as mycotoxins, with aflatoxins being the most severe and widely recognized examples. Aflatoxins are among the major mycotoxins and are widely known as a group of cancer-causing chemicals and highly toxic secondary metabolites produced by members of the fungal Aspergillus genus (Williams et al., 2004), such as *Aspergillus parasiticus, Aspergillus flavus* and *Aspergillus nominus* (Al- Gabr et al., 2013; Rawal et al., 2010). Research conducted by Ma et al., (2014) reported that food safety issues caused by mycotoxins have caught a large attention worldwide and aflatoxins are known as the main representative toxins. It was also indicated that these aflatoxins can pose serious health risks for both humans and animals. However, the toxic properties of aflatoxins manifest themselves in diverse ways based on the dose, and duration of exposure (Dhanasekaran et al., 2011). Recent studies indicate that the aflatoxin-producing fungus, *Aspergillus flavus* is one of the main causes of invasive and non-invasive aspergillosis (Amaike and Keller 2011).

Essential oils have demonstrated high effectiveness in managing postharvest diseases through their fungicidal properties. Utilizing essential oils offers a natural alternative to synthetic fungicides (Simas et al., 2017). Fungal diseases are currently controlled with fungicides; however, reliance on this single control strategy leads to problems such as environmental damage and resistance to fungicides. Alternatives to fungicides include the use of essential oils as they inhibit the growth of several fungi and are seen as natural compounds. These oils exhibit great potential in insecticidal, anti-bacterial, anti-fungal, and anti-viral capacities, effectively combating various pests and pathogens owing to the presence of diverse functional groups, including alcohols, aldehydes, phenolics, terpenes, ketones, and other antimicrobial compounds (Sartorelli et al., 2007; Burt 2023).

The African star apple, popularly known as *Udara*, and *agbalumo* in Yoruba belongs to the *Sapotaceae* family, comprising approximately 800 species (Ehiagbonare *et al*., 2008). It stands as an evergreen tree, capable of reaching heights of up to 40 meters with a girth of about 2 meters. Characterized by a straight and elongated fluted trunk, it typically features a small buttress at its base (Adebayo et al., 2012). African star apple known as *Chrysophyllum albidum*, holds significant economic importance across tropical Africa, attributed to its wide array of medicinal and culinary uses (Adebayo et al., 2012). The plant has been found to contain a variety of phytochemicals including tannins, flavonoids, terpenoids, proteins, sugars, and resins (Edem et al., 2017 and Kamba et al., 2016). It possesses antioxidant properties with free radicals scavenging, reducing lipid peroxidation, and increasing the levels of endogenous blood antioxidant enzymes as its main mechanisms (Asare et al., 2015). Cucumbers, scientifically classified as *Cucumis sativus*, belong to the botanical category of berries, having diverse sizes, shapes, and colours. Among the prevalent varieties, the long smooth salad cucumber is widely recognized, distinguished by its smooth, dark-green skin (Lippincott et al., 2009).

This study aims to assess the efficacy of African star apple oil and cucumber oil against aflatoxigenic fungi commonly found in locally fermented condiment, *ogiri* sold in Ogun state markets. Given that *ogiri* production often involves uncontrolled fermentation practices and minimal adherence to hygiene standards, there exists a potential risk of contamination with hazardous microorganisms and mycotoxins such as aflatoxins. This underscores the necessity of evaluating the safety of this traditional food product. Consequently, addressing fungal foodborne diseases and mitigating the risk of toxic contamination in food stocks necessitates exploring novel solutions such as investigating the antifungal and phytochemical properties of African star apple and cucumber oils to inhibit fungal growth and aflatoxin production.

Materials and Methods

Five samples of *ogiri* (fermented melon paste) were procured from five markets namely Ijebu Igbo, Ijebu Awa, Ago-Iwoye, Kuto, and Osiele in Ogun State,

Nigeria. The samples were collected under aseptic conditions, stored in sterile Ziplock bags, properly labelled, and promptly transported to a laboratory for further analysis.

Isolation of Fungi from Ogiri Samples

Serial dilution and culturing techniques were employed for fungal isolation. Each sample (1 gm) was aseptically added to 9 ml of sterile distilled water to prepare a stock solution, which was then appropriately labeled. Subsequently, serial dilutions up to 10^{-2} were conducted while portions from the dilutions were plated onto sterile petri dishes, followed by the addition of freshly prepared malt extract agar. Plates were then incubated at room temperature up to 72 hours, and discrete colonies were sub-cultured onto fresh agar plates for identification and enumeration of fungal viability (Klich, 2002).

Characterization of Fungal Isolates

Distinct colonies were isolated and sub-cultured on malt extract agar to obtain pure cultures for morphological characterization. Macroscopic examinations, including colour, texture, and pigment, were conducted. Identification relied on morphological features such as spore types, arrangements, and mycelial characteristics. Microscopic examination using lactophenol cotton blue stain was performed with x40 and x10 objective lenses (Klich, 2002).

Fungal Counts

Rose Bengal agar was utilized for fungal enumeration. The media were prepared according to manufacturer instructions, poured into sterile petri dishes, and allowed to solidify. Plates were prepared with dilutions (10^{-1} and 10^{-2}) and then incubated at room temperature for 5 days. Colonies on Rose Bengal plates were counted, and results were expressed as colony-forming units per milliliter (cfu/ml). Subcultures were prepared for all counts to obtain pure culture isolates.

Determination of Aflatoxigenic Potential of Fungal Isolates

Desiccated Coconut Agar (DCA) following a modification of the methods outlined by Atanda et al. (2011). Initially, 100 grams of desiccated coconut was soaked in 1 litre of hot distilled water for 30 minutes (pH 4.77). The mixture was carefully blended under aseptic conditions for 5 minutes and then strained through four layers of cheesecloth. To this filtrate, 2% agar (Oxoid) was incorporated and the solution was heated until boiling, then cooled to approximately 50°C. Following this, 0.1-0.3% neutral red stain (pH 4.38) was added. The resulting media was sterilized at 121°C for 15 minutes, cooled, and poured evenly (15 ml each) into sterile petri dishes, ensuring the exclusion of air bubbles. The plates were then inoculated with suspected aflatoxigenic moulds and placed in an incubator set at 30°C for 48 hours. After the incubation period, the plates were assessed for various media characteristics including opacity, transparency, and translucency. An un-inoculated plate was utilized as a control for comparison purposes.

Observations were made on the reverse side of each plate, focusing on any large colonies present. These were examined under long-wave (365nm) ultraviolet light to detect blue/blue-green fluorescence. Furthermore, the media were visually inspected for colour changes and the minimum time required for pigmentation. Fluorescence intensity, minimal time, peak time, and duration were recorded at four-hour intervals (Atanda et al., 2011).

Antifungal Activity of African Star Apple Oil and Cucumber Oil against Aflatoxin-producing Mould using In-Vitro Assay

African star apple oil and cucumber oil were screened against isolated aflatoxin-producing mould using the disc diffusion method. The procedure involved inoculation of mould samples, preparation of Muellar-Hinton agar plates, and application of oils in wells. The plates were incubated and monitored for zones of inhibition to evaluate antifungal activity (African star apple and cucumber oil), as described by Magaldi *et al*. (2000).

Results

Viable Fungal Count of Ogiri Samples on Rose Bengal Agar

Table 1 shows the fungal count of *ogiri* samples in cfu/ml.

Among all the sample locations analyzed, the sample from Ago-Iwoye exhibited the highest dilution factor, up to 9.0x10^1 (cfu/ml).

The morphological characteristics of fungal isolates from the samples are reflected in Table 2.

A total of four (4) fungi were isolated from five *ogiri* samples obtained from five different markets (Osiele, Kuto, Ijebu Igbo, Ijebu Awa, and Ago-Iwoye) in Ogun state, Nigeria.

Frequency of Occurrence of Fungi Isolated from Ogiri Samples

Figure 1 shows *the* percentage occurrence of the fungi isolated from the ogiri samples.

Among the observed occurrences, *Aspergillus niger* showed the highest percentage occurrence of 58.0% while *Neurosposa* exhibited a lower occurrence at 5.0%.

Aflatoxigenic Potential of the Fungal Isolates from Ogiri Samples

Table 3 shows the aflatoxigenic potential of the fungal isolates.

The aflatoxigenicity screening revealed that fermented melon paste (*ogiri*) from Osiele exhibited positive aflatoxigenicity potential with *Aspergillus flavus* showing blue/bluish green fluorescence after exposure of the plate to ultraviolent light 365nm.

Antifungal Activity of Africa star apple Oils and Cucumber Oils against Aflatoxin-producing Mould

The analysis of the antifungal activity against the aflatoxin-producing mould was determined using the disc well diffusion method, which revealed a little clear zone of inhibition (ranged between 2 and 4 mm) around the well indicating limited inhibition with the oils.

Table 1: Average fungi count of *ogiri* samples in CFU/mL

Market samples	Average CFU/mL
Ijebu Igbo	2.95×10^2
Ijebu Awa	7.0×10^1
Ago-Iwoye	$2.95.0 \times 10^2$
Osiele	1.2×10^2
Kuto	7.55×10^1

Table 2: Morphological characteristics of fungi isolated from melon paste (*ogiri*) on malt extract agar

Isolates Code	Color Of Colony	Hyphae	Type Of Spor	Organisms
OIj1	Black	Septate	Conidia	*Aspergillus niger*
	White	Aseptate	Ascoconidia	*Saccharomyces cerevisiae*
OIj2	Orange	Septate	Conidia	*Neurospora spp.*

OIj3	Black	Septate	Conidia	*Aspergillus niger*
	Yellowish green	Septate	Conidia	*Aspergillus flavus*
OO	Black	Septate	Conidia	*Aspergillus niger*
	White	Aseptate	Ascoconidia	*Saccharomyces cerevisiae*
	Yellowish green	Septate	Conidia	*Aspergillus flavus*
OK	Black	Septate	Conidia	*Aspergillus niger*
	White	Aseptate	Ascoconidia	*Saccharomyces cerevisiae*

KEY: OIj= *Ogiri* Ijebu, OO= *Ogiri* Osiele, OK= *Ogiri* Kuto.

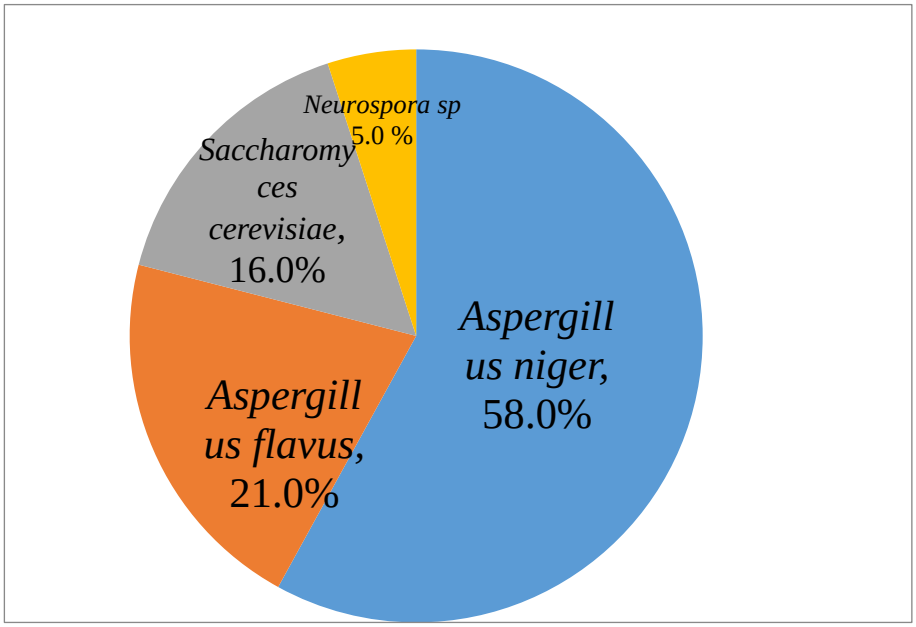

Fig 1: Percentage Occurrence of Fungal Isolates from *Ogiri* Samples

Table 3: Aflatoxigenic Potential of Fungal Isolates from *Ogiri* Sample

Fungi isolates	**Aflatoxigenicity**

Aspergillus flavus	Aflatoxigenic
Neurospora spp.	Non-aflatoxigenic
Aspergillus niger	Non-aflatoxigenic
Saccharomyces cerevisiae	Non-aflatoxigenic

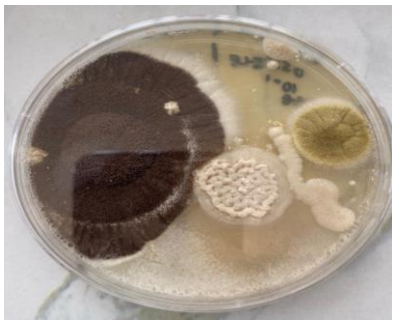

PLATE 1: Fungi isolated from *ogiri* on malt extract agar

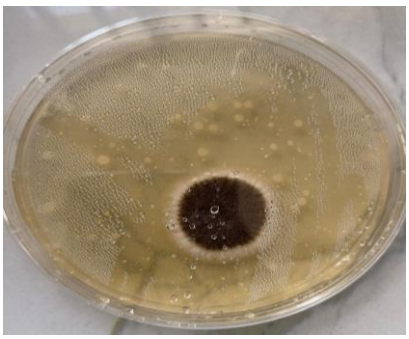

PLATE 2: Fungi isolated from *ogiri* on malt extract agar

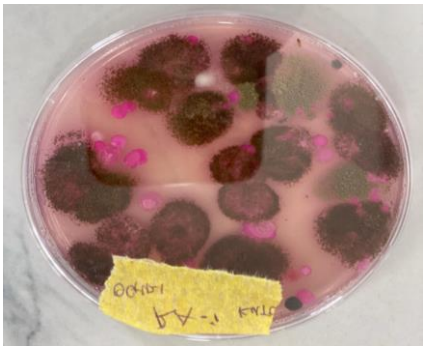

PLATE 3: Fungal Colonies on Dichloran Rose Bengal Chloramphenicol Agar

PLATE 4: Control the aflatoxin-producing mould using cucumber oil

Discussion

The result from this analysis reveal that the fungal species isolated from the fermented melon paste '*Ogiri*' samples include *Aspergillus niger, Aspergillus flavus, Neurospora spp., and Saccharomyces cerevisiae*. This finding aligns with Nwagu and Amadi (2023) who discovered that various microorganisms, including *Aspergillus niger, Aspergillus flavus, Neurospora spp., and Saccharomyces cerevisiae*, are commonly present in fermented melon paste ('*Ogiri*'). The study highlighted the microbial diversity involved in the fermentation process and the potential for aflatoxin-producing moulds to develop in such environments. The introduction of this organism can occur through various means such as air, water, utensils, and throughout the processing and production stages Nwagu et al. (2010). Melon paste (*ogiri*), as a fermented product with a permissible limit of 2 ppb for aflatoxins in Nigeria, provides a favourable environment to the growth of aflatoxin-producing moulds, potentially leading to the synthesis of aflatoxins, which pose numerous health risks when present in contaminated products. This mycological observation aligns with earlier studies, by Azi et al., (2017).

The presence of aflatoxigenic fungi (*Aspergillus flavus*) in the melon sample from Osiele necessitates for good agricultural practices, processing, handling and storage practices. The presence of high fungal counts above acceptable standard calls for concern and the detection of aflatoxigenic fungi and aflatoxin > 20 ppb is a cause for alarm.

The *Saccharomyces cerevisiae* strains isolated in this study exhibited a non-aflatoxigenic nature. Enhancements in traditional fermentation techniques, including post-processing methods such as heating, freezing, and thawing, play a crucial role in preserving the authentic flavors of foods while simultaneously bolstering food safety measures. Research indicates that adhering to traditional preparation techniques, such as prolonged soaking and repeated washing, may effectively mitigate the formation of harmful substances in food (Bhagavathi et al., 2018).

The significant mould contamination observed in *ogiri* from Osiele may stem from suboptimal environmental conditions and production practices, underscoring the necessity for improved agricultural processes, production standards, and a safer working environment (Azi et al., 2017).

The utilization of essential oils, such as African star apple and cucumber oil, has demonstrated potential as antimicrobial and anti-mycotic biocontrol agents. In this study, both African star apple and cucumber oils exhibited minimal zones of inhibition ranging from 2 mm to 4 mm, showing comparable antimicrobial activity. This research aligns with previous research by Okigbo and Ogbonnaya (2006), which opined that various plant essential oils often show almost similar ranges of inhibitory effects against microbial growth. The similar efficacy observed in this study suggests that both oils contain active compounds capable of inhibiting microbial activity to a modest extent. These results underscore the potential of using plant-derived essential oils as natural alternatives in microbial control strategies, though further research is needed to fully establish their mechanism of action and optimize their applications.

Effective management of aflatoxin-producing moulds is imperative. As outlined by Edris (2007),

bio-control agents, particularly essential oils, are categorized as "Generally Recognized as Safe" (GRAS) by the Food and Drug Administration (FDA). They represent eco-friendly and alternative solutions compared to synthetic controls, mitigating potential environmental and human health risks.

Conclusion

The finding of this study revealed that *ogiri* sold in the selected markets are contaminated with aflatoxin producing mould signifying a concern for food safety. Developing safety strategies (such as basic hygiene) to inhibit fungal growth and aflatoxin production is essential during production and processing. It is imperative that these safety strategies should be enforced and monitored. In addition, the utilization of essential oils, such as African star apple and cucumber oils, has demonstrated efficacy as antimicrobial and anti-mycotic biocontrol agents.

Recommendations

Aflatoxin control is needed in pre-harvest, post-harvest handling and storage. To mitigate aflatoxin contamination in fermented melon paste (*ogiri*), it is essential to implement good agricultural and processing practices, along with regular monitoring and testing for aflatoxins. Additionally, adopting bio-control agents such as essential oils can effectively manage aflatoxin-producing moulds in an eco-friendly manner.

References

Achi, O.K. (2005a). The Upgrading of traditional fermented foods through biotechnology. Afr. J. Biotechnol. **4**: 375-380.

Ademiluyi, A. O. and Oboh, G. (2011). Antioxidant Properties of Condiment Produced from Fermented Bambara Groundnut (Vigna subterranean L.Verdc). J. Food Biochem. **35** (4):1145-1160.

Akinyele, B. J. and Oloruntoba, O. S. (2013). Comparative Studies on *Citrullus vulgaris*, Citrullus colocynthis and Cucumeropsis mannii for *Ogiri* Production. Br. Microbiol. Res. J. **3** (1):1-18.

Al-Gabr, H.M., Ye, C., Zhang, Y., Khan, S., Lin, H. and Zheng, T. (201s3). Effects of carbon, nitrogen and pH on the growth of *Aspergillus parasiticus* and aflatoxins production in water. J. Environ. Biol. **34**: 353.

Amaike, S. and Keller, N.P. (2011). Aspergillus flavus. Annu. Rev. Phytopathol. **49**: 107-133.

Asare, I.K. and Abenaa, A.O. (2015). Nutritional and phytochemical constituents of the African Star Apple. Ann. Food Sci. Technol. **16**:138-46.

Bankole, S. A., Schollenberger, M. and Drochner, W. (2006). Mycotoxins in food systems in sub Saharan. Afr. Rev. Mycotoxin Res. **22**:163–169.

Burt, S. (2023). Essential oils: Their antibacterial properties and potential applications in foods—a review. Int. J. Food Microbiol. 362, 109509.

Edem, D.O., Eka, O.U. and Ifon, E.T. (2017). Chemical evaluation of the value of the fruit of African Star Apple (Chrysophyllum albidum). Food Chem. **14**:303-311.

Kamba, A.S. and Hassan, L.G. (2016). Phytochemical Screening and antimicrobial activities of African Star Apple (Chysophyllum albidum) leaves, stem, against some pathogenic microorganisms. Int. J. Pharm. Front. Res. **1**(2):119-129.

Klich, M.A. (2002). Identification of common Aspergillus species. In Peterson, S.W. (Ed.) Mycologist, p. 1-116. Utrecht, The Netherlands: Centraalbureau voor schimmelcultures.

Ma, X., Wang, W., Chen, X., Xia, Y., Wu, S., Duan, N. and Wang, Z. (2014). Selection, identification, and application of aflatoxin B1 aptamer. Eur. Food Res. Technol. **238**: 919-925.

Magaldi, S., Mata, S., and Hartung, C. (2000) 'In vitro' susceptibility of 137 Candida sp. isolates from HIV positive patients to several antifungal drugs. Mycopathol. **149**: 63-68

Rawal, S., Kim, J.E. and Coulombe Jr.R. 2010. Aflatoxin B1 in poultry: toxicology, metabolism and prevention. Res. Vet. Sci. **89**: 325-331.

Nwagu, T. N., and Amadi, L. O. (2023). Microbial diversity and biochemical properties of traditionally fermented melon seed (Citrullus vulgaris) 'Ogiri'. Food Microbiol. 104, 103949.

Nwosu, J. N., and Ogueke, C. C. (2022). Fermentation and sensory characteristics of '*Ogiri*' produced from melon seeds (Citrullus vulgaris). J. Food Sci. Technol. **59** (3): 915-924.

Okigbo, R. N., and Ogbonnaya, U. O. (2006). Antifungal effects of two tropical plant extracts (Ocimum gratissimum and Aframomum melegueta) on post-harvest yam (Dioscorea spp.) rot. Afr. J. Biotechnol. 5(9): 727-731.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M. and Aggarwal, D. (2004). Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am. J. Clin. Nutr. **80**: 1106-1122