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# Toxigenic Mycoflora associated with *Monodora mysistica* (Gaerth). Dunal., *Xylopia aethiopica* (Dunal) A.Rich, and *Piper guineense* Schum & Thonn from Ebonyi State of Nigeria, and their Implications to Human- Health.

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**Abstract**

*Monodora myristica* (calabash nutmeg), *Piper guineense* (Ashanti pepper) and *Xylopia aethiopica* (black pepper) seeds were screened for mycotoxigenic fungi, and the results showed that they were contaminated by molds belonging to seven genera:*Aspergillus*, *Trichoderma*, *Thallospora*, *Geotricum*, *Dactyllela sp*, *Botryodema*, and *Curvularia*, *Aspergillus spp.* being the most genera, with ten species. These ten species were *A. flavus* and *A. niger* from *Monodora myristica; A. foetidus* from *Piper guineense* (Ashanti pepper) and *Xylopia aethiopica* (black pepper); *A. fumigatus*,*A. terreus*, *A. terreus*,*A. tamarii* and *A. clavatum* from *Monodora myristica* only*; A. ochraceous* and *A. japonicus* from *Xylopia aethiopica. Monodora myristica* had the highest fungal contaminations, with more of the *Aspergillu*s species affecting it, followed by *Piper guineense. Dactyllela candida* and *Curvularia lunata* were isolated from *Xylopia aethiopica* only; *Botryoderma sp* was isolated from only *Piper guineense*, while *Thallospora aspera*, *Geotricum candidum*, and *Trichoderma viride* were isolated from *Monodora myristica*

**Keywords: Human-health, *Monodora mysistica,* Mycoflora, *Piper guineense,* Toxigenic,*Xylopia aethiopica***

## 1.Introduction

In Nigeria, several spices are routinely used in homes for cooking, for example, calabash nutmeg (*Monodora myristica*, Ehuru in Igbo), red pepper, leaves and seeds of Ashanti pepper (*Piper guineense*, Uziza in Igbo), *Aframonium meliguete*(ose ensi, in Igbo)., black pepper (*Xylopia aethiopia* Uda in Igbo), and many others. The fruits of black pepper (Uda) and the seeds of Ashanti Ashanti pepper (Uziza) are boiled daily for women who were just delivered of new babies, for three months. The hot soup is used to stabilize their wombs and bowels. The fruit of the calabash nutmeg (Ehuru) is usually sold dry and used in stews, soups, cakes, and desserts. The seeds are also added to food to enhance the flavour and taste,and complement the dish.

Calabash nutmeg *Monodora myristica* (ehuru) is a perennial edible plant of the Annonaceae family. Its local (Nigeria) names include Ehuru or Ehiri (Igbo), Ariwo (Yoruba), Jamaica nutmeg, calabash nutmeg, and Airama (Ekeanyanwu *et al.*, 2010; Jonathan *et al.*, 2016). Omobuwajo *et al.*(2013) reported that it is most widely distributed in Africa, Asia, Central and South America, and Australia. According to (Uyoh *et al.*, 2014;Adeolu *et al*, 2015; Enwereuzoh, *et al.*, 2015), it is located most commonly in the evergreen forests of West African countries like Liberia, Cameroon, Angola, Uganda, West Kenya, and Nigeria. It possesses an impressive range of medicinal and nutritional properties. Different parts of this plant, such as seeds, bark, and flowers, contain essential minerals and are a good source of protein, vitamins, β-carotene, amino acids, and various phenolics, as well as bioactive substances that exhibit health-beneficial effects, including stimulation of the cardiac and circulatory systems, anti-inflammatory, antispasmodic, diuretic, antihypertensive, cholesterol-lowering, antioxidant, anti-diabetic, hepatoprotective, antibacterial and antifungal activities. Crude extracts of the plant are reported by Agiriga and Siwela (2017) to be used for the treatment of different ailments in the indigenous system of medicine,especially in South Asia. When ground to powder, the kernel is used to prepare soup as a stimulant to relieve constipation and control passive uterine hemorrhage in women immediately after childbirth, and this berry also has diuretic properties, and is used for mild fever and antiseptic (Nwozo *et al.,* 2015).

Ashanti pepper or West African black pepper *Piper guineense* (Schumach) is a herbaceous climber commonly found in the African tropical zone, and the fruits or berries and leaves are usually sold in Nigerian markets as condiments and for food flavouring (Olanisakin *et al*, 2006). They are native to tropical regions of Central and Western Africa and are semi–cultivated in countries such as Nigeria, where the leaves (known as Uziza in Igbo) are used as a flavouring for stews and soup (Klin -Kabari *et al.*, 2011). The seeds are boiled daily as soup for women who were delivered of their babies, and are also used in making pepper soup to stabilize their wombs and bowels (Ezekiel *et al.*, 2012). *Piper guineense* berries, like other types of pepper, yield an aromatic essential oil on steam distillation (Olanisakin *et al.*, 2006).It contains 5-8% of the chemical piperine , which gives it heat and a significant proportion (10% ) of myristicin, elemicin, safrole, and dillapiol (Klin-Kabari *et al.*,2011).

Black pepper (*Xylopia aethiopica* (Dunal) A.Rich, also called Uda (in Igbo); commonly known as African guinea pepper or Ethiopian pepper, is widely spread in tropical Africa, Zambia, Mozambique, Angola, and Nigeria, and the dried fruits are also used as spices in the preparation of two special local soups named "obe ata" (Yoruba) and "isiewu" (Igbo) taken widely in the South-West and South-Eastern parts of Nigeria(Oguwike *et al.*, 2015).The chemical content of *Xylopia aethiopica* are minerals, vitamins, polysaccharides, limonoides, fibers, calcium, alkaloids, capsaicin, oxalic acid, and piperine (Oguwike *et al*, 2015).

Despite the usefulness of these local spices in various ways, they are attacked by some storage fungi, of which presence could have negative consequences to man. Some storage fungi have been reported to produce mycotoxins that inflict several health challenges on man, and some of them include *A. flavus, A. parasiticus* from African nutmeg *Monodora myristic*a (Gaertan), Black pepper *Xylopia aethiopica*

(Dunal), and Ashanti pepper *Piper g8uineense* (Schumach); and *A. fumigatus* from Black pepper *Xylopia aethiopica* (Dunal), as reported by Haruna *et al.*(2017). Greeff-Laubscher *et al.* (2019) ,reported that various filamentous fungi, of which *Fusarium, Aspergillus* and *Penicillium* were the three main genera associated with the production of the mycotoxin fumonisin contaminate grain-based feeds, *Fusarium verticillioides* being one of the most dominant toxigenic fungal species. *Aspergillus* species tend to infect plants during growth in subtropical and tropical climates, and *Aspergillus* and *Penicillium* species are frequently linked to the contamination of agricultural commodities during post-harvest storage (Agriopoulou *et al*.,2020). *Aspergillus spp* mycotoxins of most significant implication are aflatoxins, which are specific toxins, produced by *A. flavus, Aspergillus parasitics,* and *Aspergillus nominus, and* Ochratoxin A, produced by *Aspergillus ochraceus, Aspergillus carbonarius*, and *Aspergillus niger* (Matthews *et al.,* 2017). Nmom *et al.* (2020), reported that ***Aspergillus flavus***, *A. niger, Penicilluim species, Fusariurn moniliforme, Chioridium viride, Setodochiuni caesariae*, *Rhizoctonia solani* and *Mucor mucedo* with varying degrees of incidence were associated with the seeds of *X. aethiopica*, irrespective of the source of the sample and that ***Aspergillus flavus*** had a considerable high incidence of occurrence across the Deltaic zones of Rivers, Bayelsa, Delta and Imo States of Nigeria.

These storage fungi secret mycotoxins, as reported by many researchers, and they constitute a great threat to human health, animals and poultry. According to (FSAI,2009), mycotoxins are natural chemical substances produced by fungi (molds) growing as contaminants on some food crops (in the field and in storage), in particular cereals, nuts, and fruits. As reported by Bandyopadliyay *et al*., 2007 and Marx *et al.*, 2013, they are secondary metabolites produced by filamentous fungi on food crops, and are capable of causing diseases or death on humans and other animals when ingested, depending on the level and duration of exposure. Enyiukwu *et al.* (2014) , reported that contamination by mycotoxins, leads to inferior quality of products, end - products, food safety, affect marketability of agro-produce, and have been reported to be silent killers.

These spices, *Monodora myristica*, *Xylopia aethiopica* and *Piper guineense are* consumed by almost every family in Ebonyi State in one way or the other because of their inherent health benefits, but no one considers the negative implications of their regular consumption, and the possible mycoflora associated with theses spices, considering their mode of storage and preservation by the marketers. Hence, this work is aimed at the isolation and identification of the storage mycoflora associated with these spices, and the possible health harzads they constitute to human health by virtue of the mycotoxigenic fungi that could be associated with them, the type of mycotoxins they produce and the extent of health hazards consumers are exposed to in Ebonyi State. The results obtained will go a long way towards advising on the mode and rate of consumption.

## 2.Materials and methods

### 2.1 Collection of spices

Sample of spices were collected from thirteen major markets of major towns in the thirteen local government areas of Ebonyi State Eke market, Afikpo in Afikpo North local government Area, Uburu main market in Ohaozara local government area, Nkwegu Isu market, Onicha local government area, Owutu market, in Afikpo South local government area, Abakpa main market in Abakaliki local government area, Onueke market in Ezza South local government area, Nkalagu market, Ishielu local government are, Ezanmgbo market in Ohaukwu local government area, Nwofemarket Ebonyi local government area, Igboji market in Izzi local government area,Nwakpu market in Ikwo local government area, Eke Imoha market Ezza North local government area. And Ishiagu market, Ivo local government area. The samples of each spice from each local government were mixed to obtain a composite sample, while the representative samples of each sample for the work were obtained from the composite samples.

### 2.2 Isolation and Identification of Fungi from seeds

The standard blotter method as described by Ibiam and Egwu (2011) was used for the isolation of the seed-borne fungi associated with *Monodora myristica* (Ehuru), *Piper guineense* (Uziza), and *Xylopia aethiopica* (Uda) seed samples. The seed samples were first sterilized in 4 % sodium hypochlorite (Chlorox) for five minutes and rinsed in several changes of sterile distilled water. The sterilized seed samples were inoculated on three moistened 9.0 cm filter papers in 9.0 cm Oswald Petri-dishes. For *Monodora myristica* ,five seeds were arranged at the periphery of the plate, followed by four and one at the centre.

The control for each sample per preparation, per variety was not treated with sodium hypochlorite, but was only washed with distilled water. Both the control and the treatment were incubated in the dark at a temperature of 25±2 °C in the incubator. Identification of the fungi was made based on their habit character and spore characterization following the Manuals of (Raifai (1969;Barnett and Hunter,2000 and Klich,2002). Where the identification of any fungus was in doubt, spore suspensions of it were made on the slide and viewed under the microscope at x40. magnification

## 3.Result

A total of sixteen fungal species were isolated in this study. About 95% of the sample seeds of *Monodora myristica* (calabash nutmeg), *Piper guineense* (Ashanti pepper) and *Xylopia aethiopica* (black pepper); were contaminated by moulds belonging to seven genera *:Aspergillus, Trichoderma, Thallospora, Geotricum, Dactyllela sp, Botryodema* and *Curvularia,* with *Aspergillus* spp. being the most predominant genera, with ten species. These ten species were *A. flavus,A. niger,A. fumigatus, A. terreus, A. tamarii,A. clavatus,A. carbonarus,A. foetidus, A. ochraceous* and *A japonicus. Monodora myristica,* had the highest fungal contamination, with more of the *Aspergillu*s species affecting it: *A. flavus, A. niger,A. fumigatus, A. terreus, A. tamarii,A. clavatus,* and other fungal species-*Thallospora aspera, Geotricum candidum* and *Trichoderma viride;*followed by *Piper guineense. With four Aspergillud spp-Aspergillus flavus, A. niger,A. carbonarus* and *A. foetidus* and one

different fungal species *Botryoderma* sp, which *X. aethiopica* brought the rear with three *Aspergllus* spp-*A.foetidus,. ochraceous* and *A. japonicus* and two other different fungal species *Dactyllela candida* and *Curvularia lunata,*as shown in table 1.

**Table 1.** List of fungal species isolated from raw local spice samples.

| Fungal species | *Monodora myristica* | *Piper guineense* | *Xxylopia aethiopica* |
|---|---|---|---|
| *Aspergillus flavus* | + | + | - |
| *A. fumigatus* | + | - | - |
| *A. niger* | + | + | - |
| *A. terreus* | + | - | - |
| *A. tamari* | + | - | - |
| *A. clavatus* | + | - | - |
| *A. carbonarus* | - | + | - |
| *A. foetidus* | - | + | + |
| *A. ochraceous* | - | - | + |
| *A. japonicus* | - | - | + |
| *Botryoderma sp* | - | + | - |
| *Dactyllela candida* | - | - | + |
| *Curvularia lunata* | - | - | + |
| *Thallospora aspera* | + | - | - |
| *Geotricum candidum* | + | - | - |
| *Trichoderma viride* | + | - | - |

+ Present, - Absent

## 1.Discussion

In this study, it was observed that *Monodora myristica* (calabash nutmeg), *Piper guineense* (Ashanti pepper) and *Xylopia aethiopica* (black pepper); were contaminated by moulds belonging to seven genera *:Aspergillus*, *Trichoderma*, *Thallospora, Geotricum*, *Dactyllela sp, Botryoderma* and *Curvularia,* with *Aspergillus* spp. being the most predominant genera. *Monodora myristica,* had the highest fungal contamination, with more of the *Aspergillu*s species affecting it: *A. flavus, A. niger,A. fumigatus, A. terreus, A. tamarii,A. clavatus,* and other fungal species-*Thallospora aspera, Geotricum candidum* and *Trichoderma viride* as shown in Table 1. Oranusi *et al.*(2013), isolated only *Rhizopu sp* from the seeds of *M myristica,*Fovo *et al.*(2017), reported the isolation of *Mucor* spp from their source , while Maduike and Anuna (2018), observed *Cercospora purpurea ,Fusarium oxysporum* and *Aspergillus flavus* as the most frequently isolated fungal species from the seeds of this spice. Apart from *Aspergillus flavus*, all the fungi reported in this work are quite different from those reported by researchers above. Hence, report of this work seems to be the latest existing literature on the fungi associated with the seeds of the spice, and eight new fungi has been added to what exists in literature on fungi associated with the seeds of the spice.

*P. guineense* seeds were associated with four *Aspergillus spp -Aspergillus flavus, A. niger,A. carbonarus* and *A. foetidus* and one different fungal species, *Botryoderma* sp

as shown in table 1.Omafuvbe and Kolawole (2004), isolated *Aspergillus, Fusarium, Botrydiplodia, Penicillium, Mucor,Candida,* from the seeds of *P. guineense,*while Ugwulumba (2005), isolated *Rhizopus stolonifer* and *Trichoderma* sp from the seeds of spiceS from South Eastern Nigerian forest.The fungi reported by the above researchers, are different from those reported in this work.Apart from *Botryoderma sp, Aspergillus* spp isolated from *P. guineense* the result of this work agrees with those reportedly isolated by Oranusi *et al*.(2013),though there were organisms like *Penicillium* pp. *Fusarium* spp. and others which were not encountered in this study, which could be due to some environmental factors from where the test samples were procured. As a result of result of this work, six new fungi have been added to those existing in scholarly literature.

Three *Aspergllus* spp-*A. foetidus, A. ochraceous* and *A. japonicus* and two other different fungal species *Dactyllela candida* and *Curvularia lunata were isolated* from the seeds of *X. aethiopica*as shown in Table 1.Oranusi *et al.*(2013) also isolated *Aspergillus foetidus, A. japnicum* and *A. ochraceus* but *Rizopus spp* and *Penicilliu spp* isolated from the seeds of the spice, but they were not isolated in this study.Nmom *et al.*(2020), reported that *A. flavus*, *A. niger, Penicillium spp, Fusarium moniliforme, Chioridium viride, Setodochiuni caesariae, Rhizoctonia solani* and *Mucor mucedo* with varying degrees of incidence were associated with the seeds of *X. aethiopica*, irrespective of source of sample, and that *A. flavus* had a considerable high incidence of occurrence across the Deltaic zones, Rivers, Bayelsa, Delta and Imo States of Nigeria.The organisms isolated from the seeds of the spice are different from those reported by the above researcher, and two new fungi, *Dactyllela candida* and *Curvularia lunata* have been added to those available in scholarly literature on fungi associated with the seeds of the spice.This disparity in the number and species of fungi isolated from the seeds of these spices in this study, and those from the previous study might have been a result of the temperature and humidity of the environment from which the text samples were obtained, and the sanitary quality of the sellers, and storage.

Fungi that occur as a result of storage are mostly encountered on plants under moist conditions, and these fungi are mostly *Aspergillu*s and *Penicillium* species found in stored products (Amadi and Adeniyi, 2009). Fungi are known to produce spores and they are also common environmental contaminants that survive unfavourable environmental conditions; hence,this could explain why they are present in local spices and seeds.The species of fungi encountered in these raw local spices varied from one sample to another, and the occurrence of *Aspergillus* species in the raw seeds predominated over the other fungi encountered. These were *A. flavus, A. niger, A.fumigatus* and *A. ochraceus.*Other *Aspergillus* sp with some other fungi like *Thallospora aspera, Geotrichum candidum, Curvulria lunata* and *Dactyllelas candida* were also encountered in this study as shown in Table 1.

Pathological fungi like *A. flavus* a fungus with the ability to produce aflatoxins was isolated from the seeds of two of the spices.Aflatoxins are acutely toxic, mutagenic, immune-suppressive, teratogenic and carcinogenic compounds and cause

aflatoxicoses in episodic poisoning outbreaks (Lizarraga-Paulin *et al.*, 2010; Ahene *et al.*, 2011;Tiffany, 2013;Enyiukwu *et al.*, 2014;IARC, 2017). It is reported that *A. flavus* causes a broad spectrum of diseases in humans, ranging from hypersensitivity reactions to invasive infections associated with angio-invasion (Hedayati *et al*., 2007). Other toxigenic fungi isolated like *A. niger* are reported to possess the ability to produce certain mycotoxins which are hepatocarcinogenic, nephrogenic immunological in nature, and are causative agents of many rot diseases in plants. Mycotoxins produced by *A. niger* are not only linked to discoloration, quality deterioration, reduction in commercial values, but could also cause several ailments of the liver, kidney, nervous system, muscles, skin, respiratory organs, digestive tract, genital organs etc.**.** *A. niger* may also cause infections of the inner and outer ear (otomycosis) as well as pulmonary aspergillosis (Agnieszka, 2010). It is reported to synthesize Ochratoxin A which is classified as a possible human carcinogen by the (IARC , 2017),and target mainly the kidney, and the toxin is nephrotoxic, teratogenic, carcinogenic and immuno-suppressive (Agnieszka,2010).It also causes Otomycosis, and pulmonary aspergillosis (Egbuta *et al.*, 2017). *A fumigatus* is reported to be a life-threatening pulmonary or sinus diseases in severely immuno-compromised patients (Hedayati *et al*., 2007). *A fumigatus* may cause acute and chronic inhalatory respiratory tract infections (aspergillosis, aspergilloma) as well as infections of the hematopoietic system, digestive system, genitourinary tract, skeletal muscles, and nervous system (Agnieszka, 2010).

*Aspergillus terreus* is reported to have spores that may cause an allergic reaction or invasive aspergillosis of the respiratory system, as well as infections of the skin, eye or liver (Agnieszka 2010). *A tamarrii* has been implicated in a case of eyelid infection (Hedayati *et al*., 2007). *A carbonerius* and *A foetidus* have the ability to produce ochratoxin A with high frequency (Scheuster *et al*., 2002). *Geotrichum candidum* causes pulmonary cancer in immuno-suppressed patients (Bilman and Yetik, 2017). Domschi *et al.*, (2013), had reported that it affected the bronchi, lungs, mouth, or intestinal tracts .It affected mainly patients with systematic diseases like diabetes mellitus and those with neoplasms, leucosis, renal transplant, and HIV (Andreas *et al*, 2007 and Manisha and Panwar, 2012). *Trichoderma* spp are reported to cause invasive fungal diseases in patients with lung /haematological malignancies and peritoneum in CAPD patients, and according to Sal *et al*.(2022), in spite of anti-fungal remedial treatments and surgery, infections caused by Trichoderma, are life-threatening complications in immune-compromised patients , especially, after HSCT or SOT. No literature exists on health challenges caused by *Curvularia lunata* and *Dactyllella candida* and *Thallospora aspera.*

It is worthy of note that these fungi associated with the seeds of these three spices cause some health challenges, hence regular consumption of them, as done locally, could lead to these challenges, hence, consumer discretion is advised for optimum health.

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