(CIJST) 3007-3847 (Online) Vol. 1(1)

CAVENDISH INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF FUNGI ASSOCIATED WITH SPOILAGE OF SOME FRUITS SOLD IN MARKETS OF SOKOTO CENTRAL SENATORIAL DISTRICTS, SOKOTO STATE, NIGERIA

Open Access

Vol. 1(1):
https://journals.cavendish.ac.ug/index.php/cjst/article/view/31/25

August, 2024

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Abstract

Background: The socio-economic impact of pathogenic fungi in destructions of fruits in Sokoto Central Senatorial District were found to be very huge, hence the present study was designed to isolate and identify the species that are responsible for the spoilage of Magnifera indica, Citrullus lanatus, Citrus sinensis and Cucurbita maxima fruits in the area.

Methods: 500 fruits comprising of 80 spoiled and 20 healthy from four (4) different plant species were collected from five different markets in the area. Potato dextrose agar (PDA) was prepared as media for analysis, thin section of each rotten and healthy fruit was inoculated into a medium and incubated at 25^{o}c for five days, the isolates were identified using cultural and morphological features.

Results: The results obtained showed the presence of Aspergilus niger Rhizopus stolonifer, Rhizopus oryzae, Alternaria alternata, Mucor, and Fusarium; in this study, *A. niger* had highest frequency of 77 (32.08%), while *Fusarium* showed least frequency of 19 (07.92%), mean frequency of these fungal species was significantly different in all selected markets except Kasuwar Daji as we observed at $p \leq 0.05$. It was recommended that appropriate preventive and control measures should be considered to reduce the potential loss of the products and serious health consequences in the study area.

Keywords: *Fungi, Isolation, Identification, Frequency, Spoilage, Fruits*

INTRODUCTION

Background

Fungi are a group of eukaryotic organisms that includes microorganisms such as yeasts and molds as well as the more familiar mushrooms which were classified as separate kingdoms based on the general classification of living organisms Whittaker (Chiejina, 2008). Although fungi share some characteristics with other kingdoms of living organisms, they possess some distinct features such as the possession of cell walls that contain chitin, unlike the cell walls of plants and some protists, which contain cellulose, and unlike the cell walls of bacteria (Ademoh *et al.,* 2017). These and other differences

show that the fungi form a single group of related organisms, named the Eumycota (true fungi or Eumycetes), that share a common ancestor (is a monophyletic group). This fungal group is distinct from the structurally similar myxomycetes (slime molds) and oomycetes (water molds) (Barth *et al.,* 2009)

Fruits are the consumable part of mature ovary of flowering plants which are normally eaten raw; examples of fruits include bean pods, corn kernels, tomatoes, and wheat grains, these are important in human nutrition as they provide essential growing factors such as vitamins, proteins and minerals that are necessary for proper body metabolism in human and other animals (Tafinta *et al.,* 2013).

Fruits are widely distributed in nature and highly perishable (Droby, 2006). Due to their low pH, higher moisture content and nutrient composition are very susceptible to attack by pathogenic fungi, which in addition to causing rots may also make them unfit for consumption by producing mycotoxins (Tsao, 2001). Although many fungal species are not pathogenic, some do cause spoilage in fruits, food, meat, and other substances that are useful to humans and their properties, and spoilage is defined as the damage or injury caused by a fungal species that reduces the quality of any substance or renders it useless (Chukwuka *et al.,* 2010).

Some of the fungi identified to spoilage fruits in so many part of the world include: *Penicillium*, *Aspergillus* and *Alternaria* and some zygomycetes like *Mucor* and *Rhizopus* (Gambari and Chiejina, 2013). These fungi are almost everywhere producing spores in the fruits which is growing and handle so that they take advantage of any damage or bruising and attack the fruit at stages from harvest to consumption (El-Ghaouth *et al.,* 2002). Research had shown that fungi make way into its host tissue through natural openings such as lenticels, stomata and through the unbroken epidermis by means of germ tube (Salau *et al.,* 2012).

One of the limiting factors that influence the fruits economic value is the relatively short shelf-life period caused by pathogens attacked. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Ikhiwili, 2012).

Although, fruits are easily spoilt and usually have active metabolism during the storage stage, the high concentration of various sugars, minerals, vitamins, amino acids, and low pH also enhances the successful growth and survival of various parasitic and saprophytic forms of fungi (Singh and Sharma, 2007). Annual reports have shown that 20% of fruits and vegetables produced are lost to spoilage, especially during post-harvest stages and this has been associated with spoilage fungi which can be pathogenic (Thiyam and Sharma, 2013).

Previous study has found and isolated toxin-producing fungus from rotten fruits (Okigbo *et al.,* 2009). Infections and allergies have both been linked to dangerous fungus. Mycotoxins and other toxic compounds produced by Aspergillus spp. can be dangerous to humans and animals worldwide (Bashar *et al.,* 2012). Microorganisms, particularly fungus, are known to degrade fruits, limiting the quantity available for consumption and the revenues generated from fruit sales. These microorganisms must be identified, particularly those that are harmful to humans, in order to limit the danger of contamination and infection associated with the handling and eating of fruits (Gaddeyya *et al.,* 2012).

Many fungal Species were known to grow readily in acidic as well as in alkaline and high-sugar environment. Thus, fruits are more likely to be spoiled by fungi rather than bacteria, airborne organisms fall into fruits and vegetables then penetrates the product through an abrasion of the skin or rind (Jasuja *et al.,* 2013). Acidic food containing carbohydrates, such as fruits juices favor fungal lives activities. Fruits are more likely to undergo mold spoilage, because the mycelium of the mold is anchored as it penetrates the skin and tissue of the fruit (Tan *et al.,* 2012).

Fungal spoilage on fruits remained major cause of severe lost of fruit production in many part of Nigeria because majority of farmers were not aware of the etiological agents, and data for the level of the spoilage are lacking for proper education campaigns on fungal spoilage in many part of Nigeria (Tafinta *et al.,* 2013).

In addition, majority of the farmers (fruits producers) in Sokoto central senatorial districts were not aware on the level damage caused by fugal species to their fruits, hence the level of damage may keep on increasing to uncontrolled level when remained unchecked; Despite this, these fungi are the most common and widespread diseases, infecting a wide range of host plants and causing severe losses of most fresh fruits and vegetables during storage and transit (Sommer, 2015). Chemical and biological agents have been utilized in various of ways to control infections (Neergaard, 2017).

It is desirable to isolate and identify pathogens in order to strategize control measures with the goal of decreasing losses due to spoiling or infections (Singleton *et al.,* 2012). Apart from mycotoxin contamination of fruits, the presence of fungi in the field eventually leads to disease development when the infected seeds in the fruits are planted, so fungi responsible for fruit spoilage must be isolated and

identified for the best control and prevention of pathogenic fungi infestation in the study area.

Study Aim:

This study was designed to isolate and identify the species that are responsible for the spoilage of Magnifera indica, Citrullus lanatus, Citrus sinensis and Cucurbita maxima fruits in Sokoto Central Senatorial District.

Materials and Methods:

Collection of Fruit materials

Forty eight (48) spoilage fruits samples were collected and studied from each selected five market of Sokoto central senatorial district, these markets includes; Kasuwar Daji of Sokoto North and Sokoto South local governments, Kware market of Kware Local Government, Binji Market of Binji Local Government, Tangaza Market of Tangaza Local Government and Wamakko Market of Warmakko Local Government, The spoilage fruits consist of twelve samples each of the four 4 species namely *Magnifera indica, Citrullus lanatus, Citrus sinensis* and *Cucurbita maxima*; another healthy fruits of each of the plant species was also collected from selected markets, the samples were inserted inside the polyether bags and transported to the Herbarium of the Department of Biological Sciences, Usmanu Danfodiyo University for identification and authentication, the voucher number was assigned to each of the identified fruits then transported to Mycology Bench of General Biology Laboratory, Department of Biological Sciences, Sokoto State University, Sokoto for fungal analysis.

Preparation of Culture Media

Potato dextrose agar (PDA) was the medium used for isolation of the fungi responsible for the spoilage of fruits in the study area, the medium was prepared by dissolving 39g of the media and antibiotic in 1000ml of distilled water, the prepared medium was transferred in to conical flask then covered with cotton wool to pug and then caped with Aluminium foil, the suspension was heated at 121^0C and dissolved completely before autoclaving for sterilization under pressure for 15 minutes, the sterilized medium was then allowed to cool down to 47^0C, the medium was brought out and allowed to cool to room temperature, few drops of streptomycin sulphate antibiotics was added to inhibit bacteria growth and then poured into sterilized Petridishes (Gambari and Chiejina, 2013).

Isolation of Fungi

Each infected fruit was surface sterilized with cotton wool and soaked in 70% percent alcohol for 2 minutes to remove external contaminant if any, followed by rinsing with sterile distilled water, the fruits were then cut into small segments (2mm diameter) with a sterilized scalpel; the segments of the infected fruits were aseptically placed on the solidified primed PDA media; the plates were incubated at 25^0C for five (5) days; the fungal isolates were distinguished by different coloration (whitish, blackish, or creamy colors) that symbolizes the emergence of different fungal colonies, To acquire pure cultures of the fungal isolates, the colonies that developed were constantly sub-cultured. After five (5) days, other Petridishes containing fresh PDA media were used in the incubation at room for subculturing. Inoculating needle was burnt until red hot and allowed to cool for five minutes then used to transfer fungal mycelia and spore to the Petridish containing the fresh medium, the process was repeated continuously to different culture on the fresh prepared PDA, the isolates were then observed and the cultural characteristics of each colony as well as presence or absence of the aerial growth and rate of growth were observed and recorded (Adebayo *et al.,* 2012).

Isolated fungi identification

Cultural and morphological parameters such as colony development pattern, conidial morphology, and pigmentation were used to identify the fungal isolates. The cotton blue in lactophenol stain procedure used by Oyeleke and Manga (2008) was also used to identify the isolated fungi. A little section of the aerial mycelia from the representative fungal cultures was removed and placed in a drop of lactophenol after a drop of the stain was deposited on a clean slide with the aid of a mounting needle. With the needle, the mycelium was evenly distributed on the slide. To prevent air bubbles, a cover slip was softly put with minimum pressure. The slide was mounted and studied with 10 and 40 objective lenses under a light microscope. According to Adebayo-Tayo *et al.* (2012), the morphological traits and appearance of the fungal organisms were observed and recognized

Pathogenicity Tests

To conduct the pathogenicity test for each fruit, Healthy fruits were rinsed in distilled water and treated with a 0.1 percent mercury chloride solution on the base. A cylindrical core was removed from the fruits using a sterile cork borer. The open core was replaced and sealed with sterile petroleum jelly after a pure culture of the isolate was injected. For 12 days, the fruits were kept at room temperature. The development of the spoilage was recorded on two days interval (2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th} and 12^{th} days after inoculation), length and wide of the spoilage parts were measured by dividing each fruits into two equal part to determined the degree of pathogenicity of each fungal specie (Okigbo *et al.* 2009). Inoculums from diseased fruits were

obtained and cultured once disease signs were established. Pure cultures were identified using the following criteria of Main *et al*, (2004).

Statistical analysis

The data was statistically analysed using Statistical Package for the Social Sciences (SPSS). The frequency and prevalence of fungal isolates were recorded. P<0.05 was regarded significant, and analysis of variance (ANOVA) was employed to compute and arrive at statistical decisions.

Results:

Distribution of Fungal Species responsible for the spoilage of Fruits in the Study areas

The results obtained from Sokoto central senatorial District indicated that; six (6) fungal species namely, *Aspergilus niger, Rhizopus stolonifer, Rhizopus oryzae, Alternaria alternata, Mucor* and *Fusarium*were responsible for the spoilage of the four (4) selected fruits in the study area,it was clearly observed that, *A. niger* had highest frequency of 77 (32.08%), followed by *R. stolonifer* [60 (25.00%)] then *R. Oryzae* [40 (16.67)] and then Mucor [24 (10.00%)] while least frequency of 20 (8.33%) and 19 (7.92%) accounted for *A. alternata,* and *Fusarium* respectively (Table 1)

Frequency of Pathogenic Fungi among the Fruits Based on the Selected Markets

Results for the percentage frequencies of the fungi responsible for the spoilage of the fruits was study based on the selected markets of Sokoto Central Senatorial District and results were presented in Table 2. Kasuwar Daji Market results showed that; *A. niger* in *C. lanatus* and *R. stolonifer* in *C. sinnensis*had the highest percentage frequency of 5 (41.67) followed by *R. stolonifer* in *M. indica; R. orayzae* in *C. lanatus*; *Mucur* in *C. maxima* and *A. alternata* in *C. sinnensiss* with 3 (25.00%) then *A. niger,* in *C. sinnensiss*; *R. oyzae*in *C. maxima* and *M. indica*, *Mucor* in *C.lanatus* and *Fusariuum* in *C. maxima* which account for 2 (16.67%) while *R. stolonifer* in *C. maxiama,, R. oryzae* showed least frequency of 1(08.33%). Statistically, there is no significant difference (P=0.129)

From Tangaza Market; it was also observed that, *A. niger* isolates had the highest frequency of 5(41.67%) in *C. maxima* and frequency of 4 (33.33%) in *C. sinensis* and *M. indica*; followed by 3(25.00%) of *R. stolonifer* in *C. sinnensis, C. maxima* and *M. indica*; *R. oryzae* in *C. lanatus*and *Mucor* in *C. lanatus,* then 2(16.66%) of *R. oryzae* in *C.sinensis*and *M. indica,Mucor* in *M. indica* also *Fusarium* in *C. sinensis*, and *C. lanatus* and *A. alternata* in *C. maxiam*; however, *R. stolonifer* in *C. lanatus, Mucur* in *C. sinensis, C. maxiama, Fusarium*in *C. maxima* and *A. altinata* in *M. indica* showed the least frequencies of 1(08.33%). Significant difference was observed (P=0.004)

In Kware market, it was indicated that, *R. stolonifer* in *C. lanatus* and *A. niger* in *C. sinensis*and *M. indica* had highest frequency of 5(41.67%), followed by *A. niger* in *C. sinensis* and in *C. lanatus* [4(33.33%)], then *R. stolonifer* in *C. lanatus*, *C. maxima, R. oryzae* in *C. sinensis* and *Fusarium* in *M. indica* [3(25.00%)] and then *R. stolonifer* in *M. indica*, *R. oryzae* in *C. lanatus, Mucur*in *C. maxama* and *A. altinata* in *C. sinensis*[2(16.67%)]; while 1(08.33) was the least frequency of *R. oryzae* in *C. maxima, Mucor* in *C. lanatus* and *A. alternata* in *C. maxima*. Significant difference was reported (P=0.000)

Fruits sold in Binji Market indicated that; *R. stolenifer*in *C. sinensis, C. maxima,* and *M. indica*as well as *A. niger* in *C. maxima* and *C. lanatus* showed higher frequency of 4(33.33%), followed by *A. niger* in *M. indica, R. stolonifer* in *C. lanatus* and *R. oryzae* in *M. indica* [3(25.00%)] then *A. niger* in *C. sinensis, R. oryzae* in *C. sinensis, C. maxima* and *C. lanatus; Mucor* in *C. Sinensis*and *A. alternata* in *C. cinensis*and *C. lanatus* [2(16.67%)] while least frequency of 1 (08.33%) was recorded for *Mucur* in *C. maxima, C. lanatu* and *M. indica*as well as *Fusarium* in *C. maxima* and *M. indica,* however, there is significant difference (P=0.000)

From Binji Market, it was reported that, *A. niger* in *C. lanatus* and *M. indica* had the highest frequency of 5(41.67%) followed by *R. stolonifer* in *C. sinensis* and *C. maxima* [4(33.33%)], then *A. niger* in *C. maxima*, *R. stolonifer* in *C. lanatus*, and *R. oryzae* in *C. maxima* all of which had the frequency of 3 (25.00%), and then *R. stolonifer* in *M. indica, R. oryzae* in *C. lanatus* and *M. indica, Mucur* in *M. indica, Fusarium* in *C. sinensis* and *C. maxiam*, and *A. alternata* in *C. sinensis* with frequency of 2(16.67%) while *R. oryzae* in *C. sinensis, Mucur*in *C. sinensis, Fusarium* in *C. lanatus*, and *A. alternata* in *C. lanatus* and *M. indica* had the least frequency of 1(08.33%) and there is significant difference (P=0.002)

Table 1: Distribution of Fungal Species responsible for the spoilage of the Fruits

Fungal Species (Isolates)	Frequency	Percentage (%)
A. niger	77	32.08
R. stolonifera	60	25.00
R. oryzae	40	16.67
Mucor	24	10.00
A. alternata	20	08.33
Fusarium	19	07.92
Total	**240**	**100**

Table 2: Frequency of Pathogenic Fungi among the Fruits Based on the Selected Markets

Isolates from Selected Markets	Types of fruits				Mean ± SEM
Kasuwar Daji	*C. sinensis* (n=60)	*C. maxima* (n=60)	*C. lanatus* (n=60)	*M. indica* (n=60)	
A. niger	2(16.67)	4(33.33)	5(41.67)	4(33.33)	3.75±0.63
R. stolonifer	5(41.67)	1(08.33)	0(00.00)	3(25.00)	2.25±1.11
R. oryzae	1(08.33)	2(16.66)	3(25.00)	2(16.67)	2.00±0.41
Mucor	1(08.33)	3(25.00)	2(16.67)	0(00.00)	1.50±0.65
Fusarium	0(00.00)	2(16.67)	1(08.33)	1(08.33)	1.00±0.82
A. alternata	3(25.00)	0(00.00)	1(08.33)	2(16.67)	1.50±0.65
Tangaza market					
A. Niger	4(33.33)	5(41.67)	3(25.00)	4 (33.33)	4.50±0.41^{a}
R. stolonifera	3(25.00)	3(25.00)	1(08.33)	3(25.00)	2.25±0.50^{ab}
R. oryzae	2(16.66)	0(00.00)	3(25.00)	2(16.67)	1.75±0.63^{c}
Mucor	1(08.33)	1(08.33)	3(25.00)	2(16.67)	1.75±0.48^{c}
Fusarium	2(16.67)	1(08.33)	2(16.67)	0(00.00)	1.25±0.49^{c}
A. altinata	0(00.00)	2(16.66)	0(00.00)	1(08.33)	0.75±0.48^{c}
Kware Market					
A. Niger	4(33.33)	5(41.67)	4(33.33)	5(41.67)	4.50±0.29^{a}
R. stolonifera	3(25.00)	3(25.00)	5(41.67)	2(16.67)	3.25±0.63^{a}
R. oryzae	3(25.00)	1(08.33)	2(16.67)	2(16.67)	2.00±0.41^{ba}
Mucor	0(00.00)	2(16.67)	1(08.33)	0(00.00)	0.75±0.75^{c}
Fusarium	0(00.00)	0(00.00)	0(00.00)	3(25.00)	0.75±0.48^{c}
A. alternata	2(16.67)	1(08.33)	0(00.00)	0(00.00)	0.75±0.48^{c}
Binji Market					
A. Niger	2(16.67)	4(33.33)	4(33.33)	3(25.00)	3.75±058^{a}
R. stolonifera	4(33.33)	4(33.33)	3(25.00)	4(33.33)	3.25±0.25^{ba}
R. oryzae	2(16.67)	2(16.67)	2(16.67)	3(25.00)	2.25±0.25^{b}
Mucor	2(16.67)	1(08.33)	1(08.33)	1(08.67)	1.25±0.25^{bc}
Fusarium	0(00.00)	1(08.33)	0(00.00)	1(08.67)	0.50±0.29^{c}
A. alternata	2(16.67)	0(00.00)	2(16.67)	0(00.00)	1.00±0.58^{a}
Wamakko Market					
A. Niger	2(16.67)	3(23.00)	5(41.67)	5(41.67)	3.75±0.75^{a}
R. stolonifer	4(33.33)	4(33.33)	3(23.00)	2(16.67)	3.25±0.49^{a}
R. oryzae	1(08.33)	3(25.00)	2(16.67)	2(16.67)	2.00±0.41^{a}
Mucor	1(08.33)	0(00.00)	0(00.00)	2(16.67)	0.75±0.48^{ba}
Fusarium	2(16.67)	2(16.67)	1(03.33)	0(00.00)	1.25±0.48^{b}
A. alternata	2(16.67)	0(00.00)	1(08.33)	1(08.33)	1.00±0.41^{b}

The results are shown as Mean ± SEM of four replicates. At the $P<0.05$ level, values in rows with different superscripts differ significantly (One Way ANOVA followed by Duncan Multiple Range Test)

Discussion:

Fruit production often suffers from losses in the farm, during transportation, storage, in the market or even at the consumer end (Chukwuka *et al.,* 2010; Barth *et al.,* 2013). The presence of sugars, minerals, vitamins, amino acids, and low pH in fruits all promote the growth of saprophytic and parasitic fungi (Hasan and Zanuddin, 2020; Bhale, 2011). The action of these pathogens has led to food shortages with economic consequences. Various saprophytic and parasitic fungi have been reported in Apples, Corn, Grapes, Guava, Mango, Orange, Papaya, and Pomegranate (Chukwuka *et al.,* 2010; Barth *et al.,* 2013; Alhaji *et al.,* 2020).

From the results obtained in this research, it was observed that of the six fungal species isolated (*A. niger, R. stolonifer, R. oryzae, A. alternata, Mucor,* and *Fusarium*), *A. niger* is the most frequent with over 30% of the total. This finding is consistent with those of Baiyewu *et al.* (2007), and Chukwuka *et al.*, 2010; Mailafiya *et al.* (2017), *A. niger* fungi specie is majorly responsible for the spoilage of the fruits under study. The filthy condition of most of our markets and warehouses encourages the growth of these microorganisms (Mailafiya *et al.,* 2017). They thrive most in high temperatures and high relative humidity areas (Toma *et al.,* 2021) like Sokoto. *A. niger* though, very useful in the food and drug industry (Toma *et al.,* 2021) is said to be one of the commonest in the genus *Aspergillus* that are normally responsible for the appearance of black molds on fruits. In fact most spoilt fruits in Sokoto Market have black molds on them. These microorganisms could be dangerous to human health as *A. niger* could cause diseases such as otomycosis (Toma*et al.,*2021). Al-Najada and Al-Suabey (2014) have shown that *A niger* is one of the commonest fungi species affecting mango fruits.

The second most frequent Fungi, *Rhizopus stolonifer* (black bread mold) is classified under Zygomycota. It is mostly found in the soil or air and can be found in all parts of the world and more commonly in the tropics. It is a common contaminant of storage foods especially the moldy ones. Because it is known to grow rapidly especially in closed environments it is most likely to infest the fruit during storage and transportation while *Rhizopus oryzae,* is the third most frequent fungi specie with a percentage frequency of about 16%. It is said to be the most common agent of spoilage especially on ripe fruits. Most of *Rhizopus* species are saprobes and help in decomposing dead matter, some of them are parasitic or pathogenic, *Rhizopus* is troublesome in that it spreads and infects neighboring fruit thereby accelerating spoilage of fruits. (Marasas*et al.*, 1984).

Fusarium fungi specie was the least frequent among the species isolated with a frequency of 7%. The fungi are however a very destructive type as it has been reported to pose real threat to food safety and human health. It is known to produce a trichothecenes, zearalenone, and fumonisins (Yao, 1998). These are secondary toxic metabolites that are very destructive and have been estimated to cause global destruction of plants worth billions of dollars yearly (Marasas*et al.*, 1984; Aoki *et al.,* 2014). They are also known to cause infections of cornea and nails (Donnell *et al.,* 2015; Marasas *et al.*, 1984).

In terms of fungi distribution it could be seen that all the six fungi species are present in all the market locations considered. The mean frequencies of these fungal species were significantly different in all

selected markets of Sokoto Central Senatorial districts as observed at p≤ 0.05 level of significance with the exception of Kasuwar Daji only. The reason for the presence of the fungi in all the markets could be as a result of the fact that all the fruits are mainly sourced from the main central market in Sokoto. The differences in the frequencies are probably as a result of transportation to the local markets, handling at the different markets, and duration of storage.

Conclusion:

This study has isolated those fungi that cause fruit spoilage in some selected markets in Sokoto State of Nigeria. The fungi isolated were *A. niger, R. stolonifer, R. oryzae, A. alternata, Mucor,* and *Fusarium*. The harm and destruction caused by these fungi to the economy, agriculture, and health sectors is huge. There is a need to adopt measures to appropriately control and reduce these losses. Farmers, marketers, transporters, consumers and even those in the health sector must be educated on the consequences of not taking measures to curtail the menace of these fungi. There is need to adopt measures to appropriately control and reduce these losses. Farmers, marketers, transporters and consumers must be educated on the consequences of not taking measures.

Acknowledgment

The lead author wishes to extend his heartfelt gratitude to all co-authors for their contribution to the success of the manuscript.

Competing Interests

The author declares that there are no conflicts of interest related to this study.

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