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Effect of Fungal Infection on the Anatomical Integrity of Cells of Some Selected Tubers (*Colocasia esculenta*, *Dioscorea alata* and *Ipomoea batatas*)

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Abstract

Tubers are staple food crops in most parts of West Africa as they serve as a major source of energy to the consumers. The fungi associated with *Colocasia esculenta*, *Ipomoea batatas* and *Dioscorea alata* in storage were isolated on PDA plates using the pour plate technique. The effects of the fungal hyphae on the anatomical features of the three tubers were also studied using light microscopy. *Trichoderma harizanum*, *Rhizopus stolonifer*, *Mucor racemosus*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus brasiliensis*, *Aspergillus fumigatus*, *Botrydioplodia theobromae*, *Penicillium chrysogenum*, *Ceratocystis fimbriata*, *Trichoderma viridae*, *Aspergillus terreus*, *Fusarium oxysporium* and *Aspergillus uvarum* were identified as fungi associated with the tuber spoilage. Anatomically, the intercellular and intracellular walls of the tissues were greatly disrupted. The structural integrity of the parenchyma cells were negatively affected though the level of hyphae penetration is superficial. Indeed, the relatively harder parts- inner cortex parenchyma area and core amyloiferous layer were not yet affected by the hyphal growth most probably because the period of infection was early. This study showed that *Aspergillus* species were the most isolated fungi and also inferred that though destruction of food crops may not be visible during storage, consumption of infected tubers can lead to adverse health effect which can be avoided.

Key words: Fungal infection, tubers, pathogenic fungi, tuberous tissue, parenchyma cells.

INTRODUCTION

Postharvest diseases caused by fungi constitute a worldwide public health problem and preventing them is a major goal of societies. Microbiological foodborne diseases are typically caused by fungi or their metabolites, parasites, virus or toxins (Oloruntopa, 2015). Some fungi produce poisons in food without signs of spoilage and consumers may therefore be unaware of the contamination. Tuber crops are moist, low acidic foods that can support the growth of food poisoning fungi. Tubers are staples found in many parts of the tropic regions of the world and they belong to the class of food that basically provides energy in the human diet. There are several stem tubers (FAOSTAT 2015). The main components of tubers are water (60-90%), starch and fiber, with lesser amounts of proteins, fat, sugars, vitamins and minerals (FAO, 2006). Tubers are cheap but nutritionally rich staple foods

which are associated with high transportation cost, short shelf life, and limited market margin in developing countries (Chandrasekara and Kumar, 2016).

Ipomoea batatas L (Lam) the seventh largest food crop, grown in tropical, subtropical, and warm temperate regions in the world (Agu *et al.*, 2015) is an important food crop in Nigeria ranking third amongst important tuber crops of Sub-Saharan Africa, after yam (Anoma and Joseph, 2016) while the vines are fed to livestock. Quantitative and qualitative losses of sweet potato arising from post-harvest storage results from physical, physiological, or pathological factors or various combinations of these factors (Ogbo and Agu, 2014). *Dioscorea alata* (water yam) is ranked next to *Dioscorea rotundata* (white yam) (Pandey, 2007). It is a staple food crop as well as a cash crop in West Africa, especially in Nigeria.

Africa is the largest producer of yams in the world where it is a staple food as well as a means of foreign exchange (Pandey, 2007). In some countries where there is no white yam, *D. alata* production is the highest amongst yams. Unlike the white yam which is perennial *D. alata* is known as ten months' yam because it takes nine to ten months to mature (Onuh *et al.*, 2015). The high phytochemicals (phytate, lectins, phenolic compounds, amylase inhibitors and saponins) in *D. alata* are believed to be of great disease treatment value; these are reported to reduce blood glucose, plasma cholesterol, triglycerides level and control cancer risks (Onuh *et al.*, 2015) and often recommended for diabetic patients and those slimming due to its low glycemic index. *Colocasia esculenta* (cocoyam), otherwise known as 'koko' among the Yoruba tribe of Nigeria has the ability to withstand waterlogged and reduced conditions (Awah *et al.*, 2016), since it can transport oxygen from the leaves to the roots under such conditions. *C. esculenta* grows well on all types of soils, the ideal type being deep well drained, friable loams, particularly alluvial (Pandey, 2007). Cocoyam is used as source of food for man and livestock (Agu *et al.*, 2016). Therefore, this study aimed at isolating and identifying various fungal species associated with sweet potato, water yam and cocoyam spoilage with a view of highlighting the effect of the fungal infections on the anatomical features of the tubers.

MATERIALS AND METHODS

Source of Microorganisms

Samples of fresh and infected tubers of *Colocasia esculenta*, *Ipomoea batatas* and *Dioscorea alata* were purchased between the months of May and July, 2017 from three localities (Egbeda market, Ayobo market and Bariga market) in Lagos, Nigeria. These were singly placed in sterile labelled polythene bags and transported to the lab. The infected tubers showed some rot symptoms of black spots, browning, pale colours and unpleasant odour. While the fresh tuber samples were still in their natural fresh colours without blemish which showed that they were still healthy. After the collection of these samples from study sites, they were then carried to the Botany Research Lab, University of Lagos where fungal pathogens were isolated from the infected tubers.

Isolation of Fungi from Samples

The isolation technique of Samuel and Adekunle (2015) was adopted in this study. A small section of infected *C. esculenta*, *I. batatas* and *D. alata* tissues containing

the advancing margin of rot and adjoining healthy tissue were cut using sterilized scalpel and cork borer while the surfaces were sterilized by dipping completely in a concentration of 40% hypochlorite solution for 60 seconds; the sterilized sections to be inoculated were then removed and rinsed with three changes of sterile distilled water. The tuber pieces were made to dry by blotting with sterile filter paper in a laminar airflow cabinet. With the aid of a sterile forceps four pieces of each cut samples were separately inoculated (90° apart) on solidified potato dextrose agar (PDA) plates which were prepared according to manufacturer's specification (Oxoid, Basingstoke, England). Two replicates for each sample were made. The plates were incubated a temperature of 28-30 °C in an incubator. After 72 hours, emerging fungal colonies were sub cultured individually on new PDA plates until pure cultures were obtained. Fungi associated with the tubers spoilage were observed.

Identification of fungi

For identification, morphological studies of the pure culture plates, i.e., the shape, size and spore formation after 72 hours were observed. The mycelium containing the spores were teased out on a slide with the aid of an inoculating needle cleaned with ethanol and subsequently stained with lactophenol blue and was observed under light microscope. Microscopic features of the organisms were taken using Motic Camera 2000. The fungi identified were confirmed by comparing their morphology with fungi descriptions of Talbot (1971), Deacon (1980) and Bryce (1992).

Anatomical analysis

Thin sections of the tissues were obtained from five different layers of both diseased and healthy tubers namely: secondary tissue, cambial layer, sub-apical meristem, primary tissue and delayed multiplying layer (Fig. 1) (Queva, 1894; Degras and Mathurin, 1978) for the study. Sectioning was done with the aid of sharp dissecting blade because of softness of the tissue. Several thin slices of the tissues of *C. esculenta*, *I. batatas* and *D. alata* used for the study were obtained from longitudinal, transverse, radial and tangential sections. Cell contents were eliminated using Sodium hypochlorite (Commercial bleach) while the cells were hardened with some drops of 50%-100% ethyl alcohol that were added in series. Each preparation was stained with Safranin O, the mountant used was glycerin and then, they were covered with cover slips and the edges of the slips were coated with nail polish. The features of each sample were captured using a microscope camera

'Toup view 3.2" attached to an Olympus microscope.

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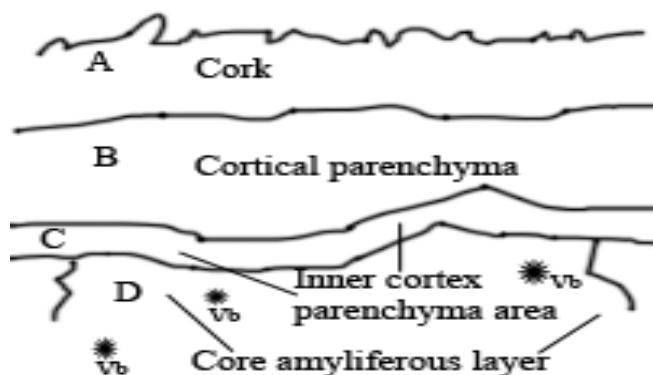


Fig. 1 A schematic diagram of layers of cells of tuberous tissue. Fahh (1967).

RESULTS

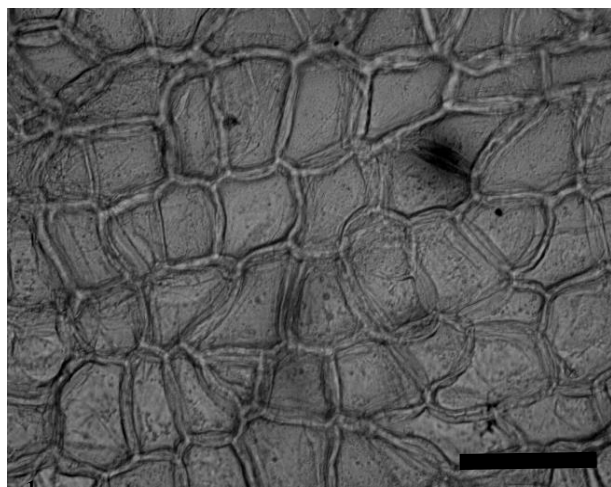
Identification of Fungi Isolates

A total of fifteen different kinds of fungi were isolated on the PDA medium from infected water yam, cocoyam and sweet potato. These fungi isolates are; *Trichoderma harizanum*, *Rhizopus stolonifer*, *Mucor racemosa*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus brasiliensis*, *Aspergillus fumigatus*, *Botrydiopodia theobromae*, *Penicillium chrysogenum*, *Ceratocystis fimbriata*, *Trichoderma viridae*, *Aspergillus terrus*,

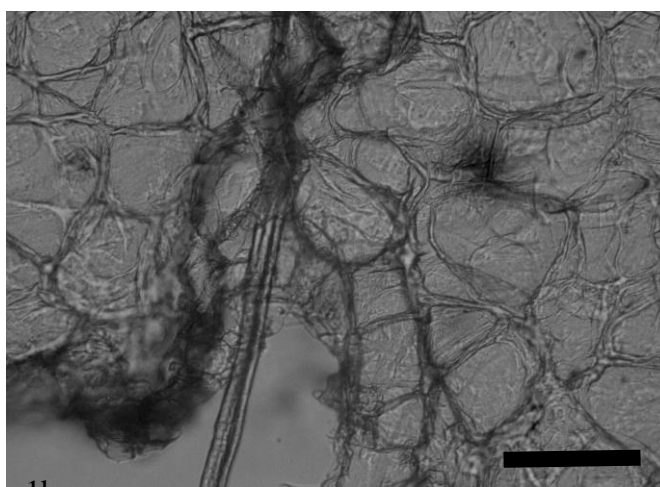
Fusarium oxysporium and *Aspergillus uvarum*.

Anatomical review on epidermal layers of selected tubers

The five different layers of both diseased and healthy tubers namely: secondary tissue, cambial layer, sub-apical meristem, primary tissue and delayed multiplying layer revealed different features of the samples. An illustration of these layers is presented in Figure 1. The sign of infection was noticeable in the secondary tissue, a layer that is next to the cortical layer. Infection is still partially superficial in the tissues examined, hence it has not penetrated deep to the amyloiferous zone where there is higher food reserve. Infections were visible in all sections; but more features of the tissues were seen through the longitudinal section. The affected cells are parenchymatous (Fig. 1). In the sweet potato tubers (*Ipomoea batatas*), the healthy tissue, parenchyma cells have normal configuration (Plate 1a) whereas, symptoms of spoilage were indicated by ruptured tissues and there was an evidence of growing hyphae in the tissue (Plate 1b). In the spoilt water yam, parenchyma cells appeared broken and roughened and the cell walls were damaged (Plate 3a, b). For the cocoyam, the cell walls and contents were distorted and there was growth of fungal hyphae penetrating the cells (Plate 2b).



1a



1b

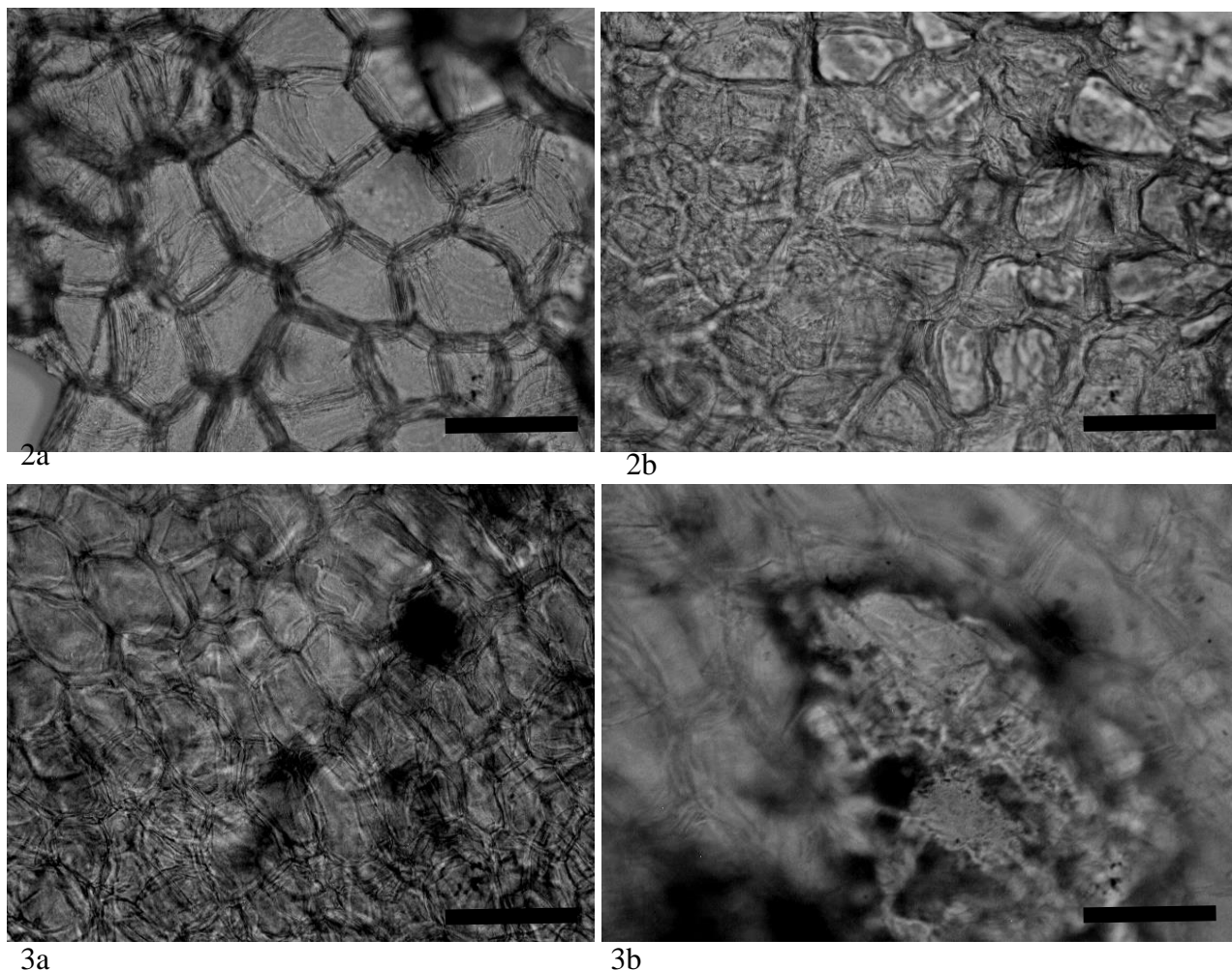


Plate.....Photomicrographs of the parenchyma cells of the tubers of *Ipomoea batatas*- Plate 1a: Fresh (Mag x400), Plate 1b: Infected (Mag x400); *Colocasia esculenta*- Plate 2a: Fresh cocoyam (Mag x400). Plate 2b: Infected cocoyam (Mag x400) and *Dioscorea alata*- Plate 3a: Fresh (Mag x400), Plate 3b: Infected (Mag x400). Scale bar 25µm.

DISCUSSION

Tuber crops which are economical to man due to the way they are handled from the field during cultivation, harvesting, packaging and transportation before finally marketing them are mostly exposed to pathogenic fungi. Most of these pathogenic fungi may not reflect their symptoms at the early stages and could cause harmful infections to liable consumers. Tubers that develop symptoms early enough are separated from the healthy ones which result in economic loss to farmers and marketers. Most of the fungi we isolated from this study have been reported to cause deterioration and losses to agricultural products from so many parts of the world (Oyeyipo, 2012; Ogbo and Agu, 2014; Onuh *et al.*, 2015). Observation during this study showed that fungi

grew easily with available nutrients (PDA) which affirms that once there is sufficient nutrient supply fungi flourish thereby affecting the healthy condition of the host in this case the agricultural produce. Members of the genus *Aspergillus* were the most frequent observed in our study supporting other findings that this group of fungi causes deterioration in farm produces like tubers during storage. The results from this study correlates with the reports of Essono *et al.* (2007), Onuh *et al.*, (2015) and Ogunleye *et al.* (2014). These authors reported *Aspergillus niger* as an acute agent of tuber decay.

The isolation and identification of *Rhizopus* and *Mucor* species from this present work also aligned with the report of Agu *et al.* (2016) that these two fungi belong to

the fast growing group of fungi which cause rot in cocoyam.

The study also surmised the presence of *Botrydioploda theobromae* in the diseased water yam. It is remarkable to note that *B. theobromae* sporulates on several varieties of *Dioscorea* media because the fungus has curiously been found associated with the storage of yams (Nwawuisi *et al.*, 2012 and Adeogun *et al.*, 2014). Furthermore, *B. theobromae* identified in this study has been reported to cross infect other crops like cocoa, mango, banana and yam with significant tissue damage and economic losses.

The intercellular and intracellular connection of the penetrating fungal hyphae greatly disrupted the structural integrity of the parenchyma cells of the studied tubers. Anatomy of the sweet potato, cocoyam and water yam observed. This was very obvious in the sweet potato samples where hyphae were spotted causing lesions in the parenchyma cells and the water yam where the infection was in the advanced stage which led to destruction of the cell properties. The cortical parenchyma of the three studied tubers was affected by the fungi. Fungal hyphae were noticeable in the affected tissues of *Ipomoea batatas* and *Colocasia esculenta* whereas, total rupturing of the tissue was observed in the damaged tissue of *Dioscorea alata*. It is evident that the level of infection is still superficial in all the three species. Hyphal growth has not penetrated to the inner cortex parenchyma area and core amyloiferous layer. Ayensu (1972) reported that striking uniformity of the general anatomy can be observed in the tubers of Dioscoreaceae. The inner cortex and the amyloiferous parenchymatous layers can be lignified and possess some strengthening substances (Mantell *et al.* 1977). This may hinder the growth of fungal hyphae in the tissue as the tissues were macerated. Similarly, Davies *et al.*, (1981) noted an extensive maceration of colonized tissues of carrot root in storage by *Mycocentrospora acerina*. They reported that the obstruction imposed by the network of invading hyphae impaired the proper functioning of the tuber tissues which may result into rot. According to Impullitti and Malvick (2014), penetration of fungal hyphal can cause a disruption in vessel functions which also leads to disruption in water movement. They also stated that colonization of pathogenic fungi hyphae may leads to abnormal function of the tuber tissues thereby resulting into rot and anatomical changes as observed in the tubers.

Our report has also shown that though some tubers may appear physically healthy to consumers, they could pose a threat to consumers' health. Fungi pathogens though

are microscopic in nature, cause damages and deterioration before the symptoms become visible. The difference can be seen on the anatomy between the healthy and infected tubers where invasion and colonization of host tissues is noticed in some tubers until the whole cell is degraded.

CONCLUSION

Postharvest spoilage by pathogenic fungi has been found to reduce economic shelf life in tuber crops. For there to be an increase in economic value and safety of lives, postharvest handling and facilities should be improved to avoid losses and meet the economic demand of tuber crops in Nigeria. Proper practices and modern technologies should also be employed for better storage and preservations.

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CONFLICT OF INTEREST

Authors declare that no conflict of interest.

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