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Isolation, Identification and Pathogenicity of Fungi Associated with Cocoyam (*Colocasia esculenta*) Spoilage

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Abstract Cocoyam *(Colocasia esculenta)* corms, purchased from a local barn in Awka, Anambra state, showing spoilage symptoms were examined for rot associated with fungal pathogens. The spoilage organisms were isolated from the corms by explanting the spoilt tissues obtained aseptically onto Sabouraud Dextrose Agar supplemented with chloramphenicol an antibacterial agent. The developing isolates were purified by repeated subculture and identified macroscopically and microscopically using the slide culture technique. The organisms recovered from spoilt cocoyam were *Aspergillus niger, Rhizopus stolonifer, Mucor circinelloides, Penicillium cyclopium* and *Fusarium oxysporum*. The rot due to *Rhizopus, Mucor* and *Fusarium* were extensive resulting in complete maceration of cocoyam tissues. The pathogenicity test also showed that the above organisms were responsible for cocoyam spoilage. These spoilage organisms may have had access into these cocoyam corms through air, wounds, working equipment (for harvesting) and pests. Proper care should be taken in the handling of these corms as some of these spoilage fungi are known to have negative impact on both humans and livestock because they produce mycotoxins.

Keywords Isolation, Pathogenicity, Fungi, Cocoyam, C*olocasia esculenta,* Spoilage

1. Introduction

Two types of cocoyams are grown in South-eastern Nigeria and are both herbaceous plants. The most popular type available in most South-eastern Nigerian bazaar is *ede-uli* in Igbo (*Colocasia esculenta*); it grows in marshy areas and its corms are used as soup thickening agents in most South-eastern Nigerian communities. The second type which is less popular is called ede-oku in Igbo (*Xanthosoma sagittifolium*), whose corms could be boiled and eaten with various soups. Cocoyam (*Colocasia esculenta*) provide substantial portion of the carbohydrate content of the diet in many regions in developing countries and provide edible starchy storage corms or cormels [1]. Cocoyam (*Colocasia esculenta*) of the Araceae family is a perennial monocotyledonous, herbaceous corm whose leaves grow upward, with fibrous root systems [2]. The exact origin of cocoyam (*Colocasia spp*) is not clear, but it may have come from India or South-East Asia. They have a long history of cultivation. *Ede-uli* is further divided into edible and ornamental cocoyam. The ornamental cocoyam has three varieties namely; *Colocasia* black-magic, *Colocasia* coffee-cup and *Colocasia* black-ruffle. On the other hand, the *ede-oku* (*Xanthosoma)* comprises of species like; *X. eggersii, X. sagittifollum, X. weeksi, and X. violaceum*[1,3]. These are grown for their starchy corms, an important staple of tropical region [1,3]. The cocoyam is known as food crop which provides high yield of roots (or corms) and foliage. It is a tropical food crop that can be grown under flooded or upland conditions [4]. The cocoyam plant is considered toxic due to the presence of calcium oxalate crystals typically as raphides [5]. The toxin is minimized by cooking especially with a pinch of baking soda or reduced by steeping roots in cold water overnight [5]. Calcium oxalate is highly insoluble and contributes to kidney stones. Cocoyam contributes significant portion of the carbohydrate content of the diet in many regions in developing countries and provides edible starchy storage corms and cormels. Although they are less important than other tropical roots such as yam, cassava and sweet potatoes, they are still a major staple in some parts of the tropics and sub tropics [3]. It could be used as thickener in soup, flour (for confectionary), or may be cut up and boiled or fried to make crispy chips or flakes. The leaf stalk can also be eaten. Post-harvest spoilage of cocoyam arises from improper handling of the cocoyam either during storage or harvest. The greatest cause of root rot and tuber loss in storage is the highest disease in cocoyam [6]. The post-harvest loss of root and tuber crops has been a very

serious problem to farmers, as more than 40% of their harvest maybe list because of decay [7]. It is estimated that in the tropics each year between 25% and 40% of stored agricultural products are lost because of inadequate farm and village-level storage [8].The principal species of microorganism associated with cocoyam rot in Nigeria are; *Aspergillus flavus, Penicillium digitatum, Botryodiplodia theobromae and Erwinia carotovora* [9]. These fungi are believed to be pathogenic to various cultivars of cocoyam, causing rot of several parts of Southern Nigeria [10]. Fungi spoils the cocoyam by colonizing it by depolymerizing certain specific cell wall polymers such as proto-protein, the cementing substance of the produce [10].

Also cocoyams may be attacked by pests like mealybug, aphids, catapillers etc. these pests and diseases can be controlled by the use of chemicals, fertilizers rich in potassium. Insecticides from nurseries (for pests) use of well cleaned planting materials, initiation of early planting (April/May) and planting in well-drained soil with no water logging [10].

This study is aimed at isolating and characterizing various fungal species associated with cocoyam spoilage with a view of highlighting and describing the types of fungal rots associated with post-harvest loss of cocoyam.

2. Materials and Methods

2.1. Collection of Materials

Cocoyam corms were obtained from a local barn in Awka, Anambra, Nigeria. The cocoyam corms were transported to the laboratory of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka.

2.2. Isolation

Sterile knife and forceps were used to obtain spoilt tissues from the cocoyam corms. A portion of the spoilt part of the cocoyam was cut out using the sterile knife and was inoculated into the agar plate by pushing rotted parts of the cocoyam into the centre of the plate. After the inoculation, plates were incubated at room temperature and the growth was monitored and recorded daily. The developing cultures were purified by repeated subculture technique.

2.3. Characterization and Identification of Isolates

This was done based on the description of the gross morphological appearance of fungal colonies on the SDA culture medium and the slide culture technique for microscopic evaluation with reference to the Manual of Fungal Atlas [11].

2.4. Pathogenicity Test

The method of Ogbo and Agu, 2015 [12] was used for the pathogenicity test. Nine healthy cocoyam corms were properly washed with tap water and then rinsed with distilled water. Then the surfaces of the corms were dis-infected with 75% ethanol. The severity of rot seeks to measure the magnitude of the infection as well as the rate of the pathogenicity of the rot-causing fungi. This was determined by obtaining the rotted portions off the whole tubers and taking the final weight of the individual yam tuber. Un-inoculated controls were placed in clean polyethylene bags. The percentage severity of rot (Sr %) was calculated thus:

$$Sr\ (\%) = \frac{FW - w \times 100}{w}$$

where, FW = Final weight of infected yam tuber,
w = weight of rotted tuber portion.

3. Results

The results of colonial morphology and microscopic characteristics of fungi associated with post-harvest loss of cocoyam and their pathogenicity on the cocoyams are presented in table 1. A total of five fungal isolates were obtained from rotting cocoyams. The isolates include *Mucor circinelloides, Fusarium oxysporum and Rhizopus stolonifer responsible for soft rots.* The only dry rot producing molds *was Penicillum cyclopium.* In the pathogenicity species of *Rhizopus, Mucor, and Penicillum* were very pathogenic while, *Aspergillus niger* was mildly pathogenic. Table 2 records the frequency of occurrence of the different disease conditions. Dry rot had 37.5% percentage occurrence while soft rot had 62.5% percentage occurrence.

Table 1. Frequency of Occurrence of the Different Disease Conditions

Disease condition	Frequency of occurrence	% Occurrence
Dry rot	3	37.5
Soft rot	5	62.5
Total	8	100

Table 2. Colonial Morphology and Microscopic Characterizations of Fungi Associated with Cocoyam Spoilage.

Sample	Colonial characteristics	Microscopic characteristics	Suspected Organisms (species)	Rot type	Pathogenicity
A	On SDA, colonies were floccose (cottony in texture), pale greyish-brown. Growth rate was rapid, thus, colonies filled the entire petri-dish in 3 days. Colour on the reverse side was yellow. Colonies were incubated at 30 ^{o}C for 5 days.	Sporangiophores were hyaline, erect, non-septate and branched sympodially and circinate. Sporangia were terminal, dark-brown, finely echinulate to smooth and spherical (20-80 µm in diameter). Sporangiospores were hyaline or pale-brown. Collumellae were ellipsoidal and 4.5-7 x3.5-5 µm in size. Chlamydospores were absent.	*Mucor circinelloides*	Soft	+++
B	Fast growing pink and colony cottony	Largest spores are sickle shaped and may contain several cells	*Fusarium oxysporum*	Soft	+++
C	On SDA, Colonies are very fast growing, cottony to fluffy, white to yellow, becoming dark-grey.	Sporangiospores are hyaline, grey or brownish, globose to ellipsoidal, and smooth-walled, and erect, simple or branched, forming large, terminal, globose to spherical, multispored sporangia, without apophyses and with well-developed subtending columellae.	*Rhizopus stolonifer*	Dry	+++
D	On SDA, colonies had rapid growth rate. However, colonies were flat and compact with yellow basal felt covered by a dense layer of black conidial heads with powdery texture. The colour on the reverse side was pale yellow. Colonies were incubated at 30 ^{o}C for 5 days.	Septate hyphae with Conidiophores were hyaline or pale-brown to black, erect, simple, with foot cells basally, inflated at the apex forming globose vesicles, bearing conidial heads split into over 4 loose conidial columns with over 4 fragments apically composed of catenulate conidia.	*Aspergillus niger*	Soft	++
E	Colonies are velvety and fast growing, with shades of green sometimes white.	Conidia are smooth and ellipsoidal. Conidiophores are smooth and short. Mycelia are arranged irregularly with branches of various length	*Penicillium cyclopium*	Dry	+++

Key:++: mildly pathogenic (>10<80mm in diameter);+++: very pathogenic (>/50mm in diameter).

4. Discussion

The cocoyam used for the research was obtained from a local barn in Awka, Anambra state. These cocoyam corms were observed to be infected by fungi and these fungi were isolated using sabouraud dextrose agar (containing chloramphecol which acts as an antibacterial agent), as growth medium. The fungi in the spoilt cocoyam were identified as *Fusarium oxysporum, Penicillium cyclopium, Aspergillu niger, Mucor circinelloides* and *Rhizopus stolonifer*. These fungi especially *Aspergillus* and *Fusaruim* were also identified by Ugwuanyi (1996) [13] who carried out an examination on root and associated fungal pathogens in cocoyam and discovered *Aspergillus niger, Botryodiplodia spp.*, *Corticium rolfsii Geotrichum candidum*, *Fusarium spp.* to be causes of rot. This rot due to *Aspergillus*, according to Ugwuanyi (1996) [13] was extensive resulting in complete maceration of cocoyam tissues. More recent reports by Frank and Kingsley (2014a) [14] and Frank and Kingsley (2014b) [15] showed that the above named organisms are actual pathogens of root and tuber crops.

Okigbo (2003) [16], isolated *Rhizopus* and *Mucor* species which belonged to the group of fast growing fungi that cause rot in cocoyam. Onuegbu (1999) [10], also isolated *Aspergillus* and *Fusarium* species from spoilt cocoyams.

In this study, most organisms tend to cause severe rot in cocoyam, as observed during the pathogenicity test. The severe rot occurrence may be due to improper storage and harvesting of cocoyam and also due to injuries caused after harvest. The above fungi have been found to cause devastating rot blight complex (CRRBC) which is a major threat to cocoyam production. These fungi inhabit the cocoyam through factors like; temperature and relative humidity.

The fungi infect this crop before harvest, though, injuries caused after harvest by careless handling or by insects or other animal damage, and also by direct penetration of the intact skin of the plant by these pathogenic organisms. But all these can be avoided by proper packaging and handling and use of clean planting equipment and healthy planting materials.

5. Conclusions

Since cocoyam is a food plant consumed by a large population, great care and precaution should be taken in it's handling, that is during planting, storage and cooking, even in preservation. Aseptic measures like use of clean planting equipment should be adopted to totally inhibit fungal growth, which can impose ill-health on consumers. Awareness should be created by the government, on good planting techniques and factor to be considered when growing cocoyam and tubers in general, so as to ensure a healthy

nation and in turn have a balanced economy.

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