



**EVALUATION OF THE FUNGAL SPECIES ASSOCIATED
WITH THE SPOILAGE OF COCOYAM OBTAINED IN
BENIN CITY, NIGERIA.**

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ABSTRACT

A staple food in many cultures around the world, particularly in Nigeria, is cocoyam. However, this food product is vulnerable to pathogen invasion, especially fungus, and post-harvest deterioration. Therefore, the purpose of this study was to assess the fungal species linked to the spoiling of cocoyam purchased in Benin City. From a cocoyam barn in a nearby market, corms with signs of post-harvest rot and corms in good condition were acquired. Spoiled tissues from the corms were removed using a sterile knife. A section of the cut-out area was then infected on PDA plates, which were then incubated at 27°C for four days before being checked every day for the growth of fungus. As a control, uninoculated cocoyams without any isolation were used. For fourteen days, the cormels were cultured and monitored. *Mucor cercinelloides*, *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifer*, *Fusarium oxysporum*, and *Penicillium cyclopium* were the six fungi that were isolated from the spoiled cormels. The weight loss percentage of the cocoyam samples inoculated with various fungal infections varied from 49.57% to 64.66%, whereas the control sample showed a weight reduction of 3.77%. With the exception of *Penicillium*, whose alterations only became apparent on day 8, all isolates showed notable modifications between day 4 and day 9. If these organisms infect the cocoyam, growers and customers may suffer significant financial losses due to the drastic decrease in weight in such a short amount of time. Because cocoyams are a food plant that many people eat, they must be planted, stored, and even cooked with extreme caution. Furthermore, it's critical to take aseptic measures to totally prevent fungal growth, which can endanger consumers.

Keywords: Cocoyam, Fungi isolates, cormels, *Colocasia esculenta*, Araceae

INTRODUCTION

Cocoyam is a perennial member of the Araceae family of herbaceous roots, cocoyams are monocotyledonous. The two genera of cocoyams that are grown for food are *Colocasia* and *Xanthosoma*. They are typically made up of a big, spherical corm, or inflated underground storage stem, from which a few enormous leaves sprout. There are two edible species of cocoyam: *Xanthosoma sagittifolium* (L) Schott, popularly known as Tannia, and *C. esculenta*, widely known as Taro. (Lewu *et al.*, 2010). Both are typically grown for their larger underground corms and cormels, or tubers, which are rich in starch. The plant is cultivated mostly for its tasty roots, even though all of its components are edible. It is thought that *colocasia* evolved in the Indo-

Malaysian region and then expanded to the Eastern Mediterranean, the Pacific Islands, and ultimately Africa. Native to tropical Central and South America, as well as the Caribbean, *Xanthosoma* spp. have been grown and eaten there since pre-Columbian times, and it was from these regions that it spread to other areas of the world. *Xanthosoma sagittifolium* is known as ede-okú in Igbo, whereas *Colocasia esculenta* is known as ede-uli. The Yoruba call cocoyam "koko." It is one of the top six root and tuber crops in the world (Agu *et al.*, 2016). It is domesticated in most communities in Oceania, Africa, and Asia and is pantropical in nature. West Africa is the region with the greatest area under cultivation and, thus, the highest output volume. With an annual production of 4.55 million metric tonnes in 2012, Nigeria topped the list of countries producing

cocoyams; this amounted to 61.2 and 43.1% of total production in West Africa and Africa, respectively. With an estimated yearly production of 5.49 million metric tonnes, Nigeria produces almost 37% of the world's total, with Ghana producing 31%. However (Eze and Okorji, 2003) reported that Nigeria produced almost 40% of the world's cocoyam; this suggests that the country's cocoyam crop is diminishing (Zuhair and Hunter, 2000). Sand loamy soil that drains effectively is ideal for cocoyam growth. When grown in rich soil with a high capacity for water retention, it delivers the maximum amount of fruit (Lewu *et al.*, 2010). Planting is typically done at or just before the start of the rainy season in the wet tropics when irrigation is not available. It grows by using setts and takes 8 to 12 months to reach maturity after planting.

It is believed that cocoyam possesses more nutritional value than other major West African roots and tubers, particularly when it comes to protein digestibility and mineral composition (magnesium, phosphorus, and calcium) (Lim, 2016). It can be chopped up and boiled or fried to form crispy chips or flakes, or it can be used as a thickening in soup or flour. You can consume the leaf stalk as well. In many underdeveloped nations, cocoyams account for a sizable amount of the diet's carbohydrate content and produce edible starchy storage corms and cormels. However, it has been claimed that cocoyam contains oxalate, which when consumed in meals made from them might cause discomfort, toxicity, or both (Quash, 2000). Corms can be stored at room temperature for a respectable amount of time, but for long-term preservation, they should be kept at 7°C. Improper treatment of the cocoyam after harvest or storage is the cause of post-harvest deterioration.

The most important element in cocoyam spoiling is thought to be pathogen invasion through natural holes or wounds (Osuji, 2003). But because of the challenges with storage, cocoyams are typically used or eaten fresh soon after harvest because fungus cause them to decay while being stored. *Aspergillus flavus*, *Fusarium oxysporum*, *Penicillium cyclopium*, *Penicillium digitatum*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Sclerotia rolfsii*, *Mucor circinelloides*, *Rhizopus stolonifer*, *Fusarium solani*, and the bacteria *Erwinia carotovora* are among the common

species of microorganisms associated with cocoyam rot in Nigeria (Agu *et al.*, 2016). Fungi damage cocoyams by colonizing them and depolymerizing particular cell wall polymers. Given that cocoyams are a food plant that is widely consumed, handling, planting, storing, cooking, and even preservation of the plant should be done with extreme caution. It is important to take aseptic precautions, such as using clean planting tools, to completely prevent fungal growth, which can endanger consumer health. Therefore, ensuring the safety of this food product is of utmost importance and calls for everyone's participation in the hand-to-fork chain. This made it necessary for this study to look into the fungi that cause cocoyam rotting.

MATERIALS AND METHODS

Collection of Materials

We took cormels of cocoyam (*Colocasia esculenta linn*) that showed signs of post-harvest rot from a cocoyam barn in a neighborhood market in Benin City, Nigeria. From the same barn, fresh, healthy cocoyam cormels were also gathered. The cocoyam corms were moved to the lab for additional analysis.

Isolation

The spoiled tissues were removed from the cocoyam corms using a sterile knife and forceps. Using a surface-sterilized forcep, 2 mm cubes were removed from the tissue at the intersection of the healthy and infected portions of the cocoyam cormels. They were cleaned twice (one minute each wash) with sterile distilled water after being surface sterilized (to get rid of surface impurities) in 70% ethanol. The cormel piece was dried for ten minutes in a Laminar Air flow Hood chamber by being placed on sterile paper towels. According to the procedure used by (Agu *et al.*, 2016), a portion of the spoilt area of the cocoyam was cut out with a sterile knife, and the rotten parts were pushed into the center of the agar plate as an inoculum on Potato dextrose agar (PDA), which contains gentamicin, an antibacterial agent. Following the inoculation, plates were incubated for four days at 27°C, and every day they were checked to see if any fungal growth had developed. Repeated subculture techniques were used to purify the

growing cultures. Subculturing, Identifying, and Characterizing Test Fungi Pathogens.

Once growth was achieved, subcultures were made using inocula from the different species in the mixed cultures. Using flame-sterilized blades, hyphal tips were transferred from the colony edge of the mixed cultures to new PDA plates that contained gentamicin, an antibacterial agent. Until pure cultures were established, plates were subcultured at 27°C in an incubator. The Petri dishes holding the pure cultures of the test fungi were then sealed with parafin to avoid contamination. The fungal colonies' gross morphological appearance on PDA culture media was described in the Manual of Fungal Atlas, which guided the characterisation and identification of the isolates and the method of slide culture for microscopic analysis (Wagner et al., 1999).

Test for Pathogenicity

For the pathogenicity test, methodology described by (Okigbo, 2003) was used. The pathogenicity test was then conducted using the test fungal isolates that were subcultured from the spoiled cocoyam corms. After thoroughly cleaning with tap water and rinsing with distilled water, healthy cocoyam corms were used. The corms' surfaces were then cleaned of infection using a 70% ethanol solution. The corms were put in a Laminar Air flow hood and let to dry for 12 minutes on sterile paper towels. Two distinct holes were drilled into the cocoyam corms using a sterile cork borer with a 5 mm diameter. Pure fungal isolates were then added to the holes using a second sterile cork borer, and the holes were sealed with petroleum jelly. One milliliter of sterile distilled water was used to inoculate controls who were not exposed to any isolate. Following the complete inoculation of the test isolates into their corresponding healthy corms, each corm was

incubated and monitored in a humidity chamber for a duration of 14 days. Every day, the corms were inspected for signs of discoloration, shrinkage, and loss of weight. The weight reduction as a percentage aims to quantify the extent of the infection and the pathogenicity rate of the fungi that cause rot.

This was ascertained by weighing each individual yam tuber at completion and comparing it to its starting weight before inoculation. After 14 days, the weight loss of the cocoyam corms measured as a percentage.

$IW-FW \times 100 = \% \text{ weight loss in IW}$, where FW = Final weight of infected cocoyam tuber after 14 days

IW stands for initial tuber weight before to inoculation.

RESULTS AND DISCUSSION

The purpose of this study was to assess the fungal pollutants linked to the spoiling of cocoyam that was purchased in Benin Metropolis, Edo State, Nigeria.

In Edo State's New Benin, Benin City, a local barn provided the cocoyam used for the study. It was discovered that these cocoyam corms were infected by fungi, and these fungi were isolated using potato dextrose agar, a growth medium that contains the antibiotic drug gentamicin. *Cercinelloides*, *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifer*, *Fusarium oxysporum*, and *Penicillium cyclopium* were the fungus found in this damaged cocoyam. After inoculating samples of healthy cocoyams with the identified fungal species, the samples were monitored for alterations and spoiling over a period of 14 days.

The following summarizes the findings of the analysis of the fungal pollutants linked to the spoiling of cocoyam samples

Table 1: Colonial morphology and microscopic characterizations of fungi associated with cocoyam spoilage.

No.	Colonial characteristics	Microscopic features	Rot type	Probable fungi
i.	Colonies were fluffy and cottony, and had a yellow reverse side. There was rapid growth and the plate was completely covered with growth within 3 days.	hyaline, non-septate, branching and sympodially and circinate were the characteristics of sporangiophores. No chlamydospores.	Soft	<i>M. cercinelloides</i>
ii.	Fast growth rate. Colonies that are compact and level with a yellow basal foot. Pale yellow on the back	septate hyphae with soft, simple, erect pale brown conidiophores with foot cells at the base.	Soft	<i>Aspergillus niger</i>
iii.	White-black colonies that expanded quickly. White on the back side.	Hyphae had green conidiospores and was septate. basal foot cell presence.	Soft	<i>Aspergillus fumigatus</i>
iv.	swift expansion of colonies, cottony and turn yellow, then dark grey, then back to white.	Grey, smooth-walled sporangiospores feature massive, globose sporangia and well developed subtending columellae.	Dry	<i>Rhizopus stolonifer</i>
v.	A pink, rapidly expanding cottony colony	The largest spores were packed with cells and resembled a sickle.	Soft	<i>Fusarium oxysporum</i>
vi.	The colonies are velvety, green, and growing quickly.	Conidia has a smooth, ellipsoidal shape. Mycelia arranged erratically.	Dry	<i>Penicillium cyclopium</i>

The microscopic characterizations and colonial morphology of the fungi linked to cocoyam rotting are listed in Table 1. *Mucor cercinelloides*, *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifer*, *Fusarium oxysporum*, and *Penicillium cyclopium* were the six fungal species that were isolated from the spoiled cocoyam cormels.

Table 2: Characteristic observations in the inoculated cocoyam samples observed for 14 days

Days	<i>M. mercurioides</i>	<i>A. Niger</i>	<i>A. fumigatus</i>	<i>R. stolonifer</i>	<i>F. oxysporum</i>	<i>P. cyclospium</i>
1	unchanged	Un changed	Unchanged	Unchanged	Unchanged	Unchanged
2	Un change d	Un changed	Unchanged	Unchanged	Unchanged	Unchanged
3	Un change d	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged
4	Tiny whitish mucoid material	filamentous substance and black in color observed	dust -like growth and greenish in color observed	Greyish substance with yellowish dusts	Bud -like whitish substance observed	Unchanged
5	More visible white mucoid substance	More observable filamentous blackish substance	Greenish substance became more visible	Greyi sh substance became more observable	More white buds seen	Unchanged
6	A white mucoid substance spraed on the sample	blackish filaments formed a cotton -like wool	Continued dissemination of the dust-like greenish substance	Sample became darker in colour	white bud -like substance was visible and spreading throughout	Unchanged
7	Sample began to shrink	Sample began to shrink	Sample contracted and colour darkened	Continued dispersal of the greyish substance	Weight began to decrease	Unchanged
8	Significant weight loss	Significant weight loss	Significant weight loss	Reduction in size	Noticeable color dullness and shrinkage in a single location	Whitish substance seen
9	Sample dried up and lost weight	Sample appeared dry and shrunk	Sample appeared dry	Significant weight reduction observed	Further shrinking and dryness	No spread of dot - like whitish substance
10	No further alterations	No further alterations	No further alterations	No further alterations	No further alterations	No further alterations
11	No further alterations	No further alterations	No further alterations	No further alterations	No further alterations	Fair shrink in size
12	No further alterations	No additional changes	No further alterations	No further alterations	No further alterations	Weight reduction observed
13	No further alterations	No further alterations	No further alterations	No further alterations	No further alterations	No further alterations
14	No additional changes	No additional changes	No additional changes	No further alterations	No further alterations	No additional changes

The distinctive findings in the infected cocoyam samples that were monitored for 14 days are displayed in Table 2. The six fungal species listed and reported in Table 1 were injected into the cocoyam samples.

The weight of the cocoyam before and after the fungus species inoculation is displayed in Table 3. Between 49.57% and 64.66% of the cocoyam samples that were injected with various fungal infections showed a percentage drop in weight.

Table 3: Weight of cocoyam before and after inoculation with the fungal species

Fungi	Before (g)	After (g)	Percentage weight reduction (%)
<i>M. cercinelloides</i>	71.5	30.0	58.04
<i>A. niger</i>	107.0	45.0	57.94
<i>A. fumigatus</i>	84.9	30.0	64.66
<i>R. stolonifer</i>	95.0	48.0	49.47
<i>F. oxysporum</i>	84.9	36.8	56.65
<i>P. cyclopium</i>	96.2	46.3	51.87
Control	53.0g	51.00g	3.77

With the exception of *Aspergillus fumigatus*, all six fungal species recovered from the current samples were also isolated by (Agu et al., 2016), whose study is consistent with the fungal species isolated in this investigation. (Onuegbu, 1999) examined the root and related fungal infections in cocoyam and identified *Aspergillus niger*, *Botryodiplodia spp.*, *Corticium rolfsii*, *Geotrichum candidum*, and *Fusarium spp.* as rot-causing fungi. These fungi, in particular, *Fusarium* and *Aspergillus*, were also identified way back. (Ugwuanyi, 1996) reported that the *Aspergillus* rot was so severe that the cocoyam tissues completely macerated. Four of these six fungi—*Aspergillus niger*, *Penicillium*, *Rhizopus stolonifer*, and *Fusarium spp.*—were also isolated by Ankworji, (2012) during the study of fungi that cause cocoyam rot. According to Frank and Kingsley, (2014) these organisms are true crop diseases that affect root and tuber crops. According to (Okigbo, 2003) the *Rhizopus* and *Mucor* that were isolated belonged to the fast-growing fungal species that are thought to be responsible for the cocoyam rot.

No discernible change was seen until day 3 for all of the samples infected with different fungal species, as shown in Table 2. Conversely, the *Penicillium*-inoculated sample did not exhibit any notable alterations until the eighth day of observation. Additionally, it was noted that, after day 9, no additional alterations were seen in the cocoyam samples infected with the different fungal infections (with the exception of *Penicillium cyclopium*, which showed shrinkage and weight decrease up to day 12). Perhaps the cormel's continued dramatic modifications when others had stopped could be attributed to the delayed expression of *Penicillium cyclopium* infection.

As a result of the fungal pathogens' pathogenicity, the percentage weight loss varied from 49.47% to 64.66%, while it was only 3.77% in the unvaccinated controls. One possible explanation for the 3.77% could be the typical dehydration that comes with storage. Nevertheless, the cause of a percentage loss as high as 64.66% is not simply dehydration; rather, it is

the result of the infection with these fungi. Should this food product be contaminated by such organisms, producers and customers may suffer significant financial losses because to the dramatic decrease in cocoyam weight over such a short period of time. This is consistent with report by Osuji, (2003) who concluded that fungal diseases are the main cause of the over 40% of cocoyam that may be lost to post-harvest rot. In this investigation, every organism significantly changed the cormels' size, morphological characteristics, and color. Particularly in the barns, these tubers are frequently kept for extended periods of time in storage. The identification of numerous pathogenic fungi from a single cormel indicates the potential for many infections, which when combined, might quickly lead to the rotting of crops that have roots and tubers. According to (Agu et al., 2016) because of variables including temperature and relative humidity, these fungi have been discovered to generate damaging rot blight complex (CRRBC), which poses a serious danger to cocoyam production.

The fungus infect this crop prior to harvest, but they also cause harm after harvest due to improper handling, insects, and other animal damage, as well as through direct encroachment of these pathogenic organisms into the plant's undamaged epidermis. However, all of these can be prevented by using hygienic planting supplies and equipment, as well as by properly packaging and managing them.

CONCLUSION

Cocoyams should be planted, stored, cooked, and even preserved with extreme care and caution because they are a food plant that is widely consumed. Aseptic measures are necessary to totally prevent fungal growth, which can endanger consumers. Examples of these precautions include the use of clean planting tools. The spread of knowledge about appropriate planting methods and considerations for growing cocoyam and tubers in general is necessary in order to foster a healthy country and a balanced economy.

ETHICS

The study proposal was presented and then

approved by the Research committee of the Department of Microbiology, University of Benin, Nigeria.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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