

Identification and Pathogenicity of Rot-Causing Fungal Pathogens Associated with *Xanthosoma sagittifolium* Spoilage in South Eastern Nigeria

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Abstract – Molds are the chief pathogens of stored corms and as such cause huge post-harvest and economic losses to cocoyam farmers in Nigeria. This research was aimed at isolating and identifying molds associated with post-harvest loss of *Xanthosoma sagittifolium*. The spoilage molds identified were *Aspergillus tamari*, *Aspergillus niger*, *Fusarium oxysporium*, *Fusarium solani* and *Mucor circinelloides*. These isolates were subjected to pathogenicity tests using fresh healthy tubers in order to confirm their ability to elicit same spoilage symptoms in healthy corms. 30 test corms were used and were divided into groups A, B, C, D, E and F. Weights, rot types and percentage rot severity were monitored during the research. Dry rot had the highest value of 75%, soft rot had 25%; these brought to the summary of the fact that these molds are associated with the spoilage of *Xanthosoma sagittifolium* during storage.

Keywords – *Xanthosoma sagittifolium*, Fungal Pathogens, Rots, Pathogenicity.

I. 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, this may not be the case in Ghana where *Xanthosoma sagittifolium* is more popular [1], [2]. *Xanthosoma sagittifolium* is a herbaceous corm belonging to the family *Araceae* with enlarged root stock that acts as a storage organ [3]. It is about the third most important root and tuber crop after cassava and yam in Nigeria [1], [2], [4]. Again, Cocoyam production and processing in the country does not meet with other major root and tuber crops, owing to its low storability, decreasing yields, and the socio-anthropological perception of the crop as women's crop [5]. Women do not have possession of land, labour and capital in various parts of Nigeria especially South-eastern Nigeria. Furthermore, the cultivation of cocoyam in South-eastern Nigeria is jeopardised by the devastating disease, cocoyam root rot blight complex (CRRBC) [6],

[7]. Storage loss of root and tuber crops has saddled farmers because more than 40% of their harvests are lost as a result of decay [8]. Fungal rot is the main cause of root and tuber loss in storage [9].

The foremost species of microorganisms linked with cocoyam rot in Nigeria include *Aspergillus flavus*, *Penicillium digitatum*, *Botryodiplodia theobromae*, *Sclerotia rolfsii*, *Fusarium solani* and the bacterium *Erwinia carotovora*. These fungi were reported to be pathogenic to four cultivars of *Colocasia esculenta*, causing rot of cocoyam in numerous parts of southern Nigeria [10]. Other influences like vermin, prolonged linger in on-set and cessation of rainfall, lengthened dry season and moisture stress, play roles in the low cultivation of cocoyam in Nigeria [5].

Cocoyams are best kept in a cool, dry and well aerated environment. Optimal storage temperature of 7 °C is best for long term storage, while storage at room temperature will preserve the corms for a fairly reasonable period of time [11]. Cocoyams can also be stored under shade, in pits and covered with ash and plantain leaves. The ash is believed to have some fungicidal effects [12].

The dietary significance of root crops has led people to formulate several ways to evaluate the nutrient profile of food commodities. A host of authors have assayed the chemical composition of *Xanthosoma sagittifolium* and *Colocasia esculenta* corms [13], [14]. In spite of the fact that cocoyam corms are overlooked crops, their compositional significance is great with an average protein content of 6 % and 390 calories per 100g dry matter [15]. One major constraint in the utilization of cocoyam is the presence of oxalates which confer acidity or elicit irritations when foods prepared from them are ingested. Consumption of foods containing oxalates have also been discovered to have caustic effect, exert irritations to the intestinal tract and cause absorptive poisoning [16].

This research is aimed at isolating and identifying the spoilage molds found in cocoyam corms, *Xanthosoma sagittifolium*, during storage, with a view of evaluating the pathogenicity of the spoilage fungi as well as to determine the percentage severity of rots caused by them.

II. MATERIALS AND METHODS

Sample Collection

Twenty-five diseased cocoyam corms (*Xanthosoma sagittifolium*) were obtained from Eke market Nibo,

Anambra State, Nigeria and transported to the laboratory for microbiological analyses.

Examination of Tubers for Rots

This was done by hand feel and visual examination of the tubers, as to observe for discolourations of any sort as well observing the nature of the odour arising from the rotted portions. Rots were categorized into **dry rot**, when infected tissues became hard and dry with various colourations depending on the causative agent; **soft rot**: when infected tissues turned out to be soft and ramified by the fungal mycelium and **wet rot**: which was typified by the exudation of whitish fluid from the diseased yam tissue when pressed [17], [18].

The percentage severity of rot was thus determined by removing the rotted portions from the whole tubers and taking the final weight of the individual yam tuber. The percentage severity of rot (Sr %) was calculated [19].

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

Where:

W = Initial weight of healthy yam tuber

w = final weight of rotted tuber portion.

Isolation of Spoilage Molds

With the aid of sterile knife and forceps, the diseased tissues were obtained from the spoilt cocoyam corms and cultured on Saboraud Dextrose Agar (SDA) incorporated with chloramphenicol to inhibit bacterial growth. The cultures were incubated for 48 hours at room temperature.

Characterisation of Spoilage Molds

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 Atlases [20]-[23].

Comparative Pathogenicity Testing

Two methods were employed in this test and a comparison was drawn between them. The first was the method of Okigbo and Nmeka [24]. Test corms were washed with distilled water and thereafter, disinfected with 70% ethanol. Cylindrical discs were removed from the sterilized corms with a sterile 4mm cork borer, thereby, exposing the inner tissues, in which the test molds were inoculated and the inoculation points sealed by replacing the cylindrical discs and vaseline applied.

The second method (method B), had a portion of the disinfected test corms pushed into solid SDA culture medium (50 ml) containing cells of the test molds in an

enclosed sterile vessel. Mold mycelia that were pathogenic were expected to be able to colonize and penetrate healthy corms by themselves. Thus, tissue traumatization of corms [24] was avoided.

In both methods, Corm weights in grams were measured prior to the pathogenicity tests, as well as on a weekly basis, throughout the entire duration of the experiment.

Corm Groups

Six groups of 5 corms each were used for the pathogenicity testing as stated below:

Corm Groups	Infection with Spoilage Molds
Group A	Control
Group B	<i>Aspergillus tamaris</i> infected corms
Group C	<i>Fusarium oxysporium</i> infected corms
Group D	<i>Aspergillus niger</i> infected corms
Group E	<i>Fusarium solani</i> infected corms
Group F	<i>Mucor circinelloides</i> infected corms

Percentage Occurrence of Molds

This was calculated thus:

% Mold occurrence =

$$\frac{\text{Total number of individual mold colonies}}{\text{Total number of stock corms}} \times 100$$

Percentage Occurrence of Specific Rot Types

This was calculated thus;

% Occurrence of rots =

$$\frac{\text{Number of corms with specific rot type}}{\text{Total number of test corms}} \times 100$$

III. RESULTS

Five species of molds were isolated from twenty-five diseased cocoyam corms sourced from Eke Nibo market, Anambra State, Nigeria. These isolates were identified as *Aspergillus tamari*, *Fusarium oxysporium*, *Aspergillus niger*, *Fusarium solani* and *Mucor circinelloides* based on their cultural and microscopic appearance.

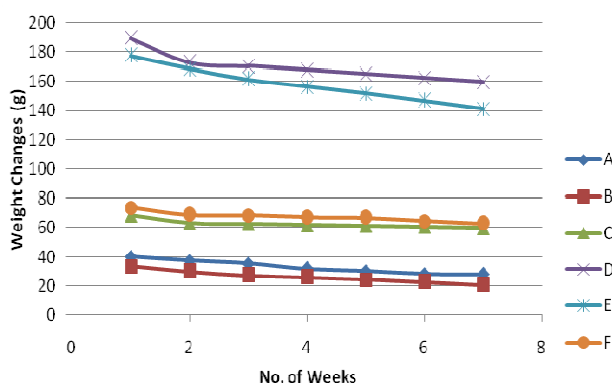
Table 1 shows the physiological changes observed on the corms during the pathogenicity tests with the method of Okigbo and Nmeka [24], whereas, Table 2 shows the physiological changes observed on the test corms using method B.

Table 1: Physiological Changes Occurring in the Corms during Pathogenicity Test using Method A.

Test groups	Test Organisms	Rot types	Symptoms observed
A	Control	None	Tuber still hard and dry maintaining its integrity
B	<i>Aspergillus tamaris</i>	Dry rot	Infected tissues appeared brown
C	<i>Fusarium oxysporium</i>	Dry rot	Infected tissues turned green. Tuber was still hard and dry.
D	<i>Aspergillus niger</i>	Dry rot	Infected tissues were discoloured brown with yellowish periphery. But tuber was still hard and dry
E	<i>Fusarium solani</i>	Dry rot	Infected tissues appeared green, with tuber being hard and dry
F	<i>Mucor circinelloides</i>	Soft rot	Tuber became wet, with brown discolouration.

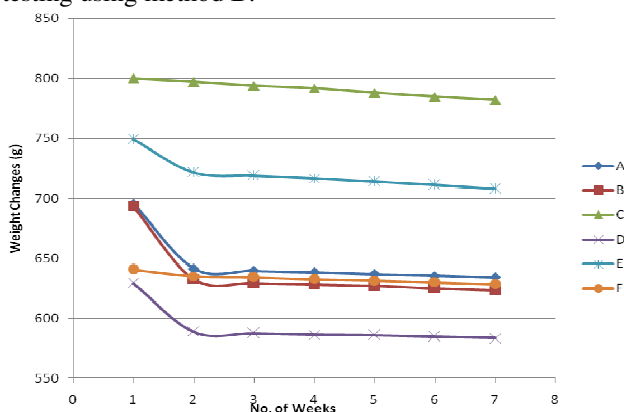
Table 2: Physiological Changes Occurring in the Corms during Pathogenicity Test with Method B

Test groups	Test Organisms	Rot types	Symptoms observed
A	Control	None	Tuber still hard and dry maintaining its integrity
B	<i>Aspergillus tamarii</i>	Dry rot	Infected tissues had pinkish dry sites
C	<i>Fusarium oxysporum</i>	Dry rot	Infected tissues turned brown with wrinkled sites that flaked off easily. Tuber was still hard and dry.
D	<i>Aspergillus niger</i>	Dry rot	Infected tissues were discoloured brown with yellowish periphery. But tuber was still hard and dry
E	<i>Fusarium solani</i>	Dry rot	Infected tissues appeared pale pink, with tuber being hard and dry
F	<i>Mucor circinelloides</i>	Soft rot	Tuber became wet, with brown discoloration.


Fig.1. Weight changes observed in corms during Pathogenicity test using method A.

- A: Uninfected with any of the mold isolates (healthy control).
- B: *Aspergillus tamari* infected corms.
- C: *Fusarium oxysporum* infected corms.
- D: *Aspergillus niger* infected corms.
- E: *Fusarium solani* infected corms.
- F: *Mucor* infected corms.

Figure 1 shows the weight changes observed in the corms undergoing pathogenicity test using the method of Okigbo and Nmeka [24]. The tubers were monitored on a weekly basis. Every group showed a decrease in weight during storage, which indicated that corms lose weight during storage, irrespective of whether they were healthy or diseased. Figure 2 also shows the monitored weekly weight differences of the corms during the pathogenicity testing using method B.


Fig.2. Weight changes observed during Pathogenicity test with method B

- A: Uninfected with any of the mold isolates (healthy control).
- B: *Aspergillus tamari* infected corms.
- C: *Fusarium oxysporum* infected corms.
- D: *Aspergillus niger* infected corms.
- E: *Fusarium solani* infected corms.
- F: *Mucor* infected corms.

Table 3 shows the percentage severity of rots obtained from the corms used for the pathogenicity testing using both methods A and B.

Table 3: Percentage Severity of Rots Obtained from Test Corms During Pathogenicity Test.

Test groups	Test organisms	% Severity of rots	
		Okigbo and Nmeka's method	Method B
A	Control	0	0
B	<i>Aspergillus tamarii</i>	40.3	88.5
C	<i>Fusarium oxysporum</i>	60.3	4.1
D	<i>Aspergillus niger</i>	81.6	80.4
E	<i>Fusarium solani</i>	44.6	80.5
F	<i>Mucor circinelloides</i>	41.9	6.5

All the molds used in the research were primarily isolated from stock diseased corms obtained from Eke Nibo market, Anambra State and their percentage occurrence at that original state is shown on table 4.

Table 4: Percentage Occurrence of Molds Isolated from the Stock Diseased Corms of *Xanthosoma sagittifolium*

Molds	Rot types	Percentage Occurrence
<i>Aspergillus tamarii</i>	Dry rot	20
<i>Fusarium oxysporum</i>	Dry rot	24
<i>Aspergillus niger</i>	Dry rot	16
<i>Fusarium solani</i>	Dry rot	16
<i>Mucor circinelloides</i>	Soft rot	24
		Total = 100

IV. DISCUSSION

The isolation and identification of spoilage molds from spoilt cocoyam corms was carried as to be able to know the rot-causing molds and the type of spoilage they caused. This study was carried out based on the fact that microbial spoilage poses a problem to post-harvest storage of healthy cocoyam corms. This research was also patterned to evaluate the pathogenicity of the various spoilage molds. Two different methodologies were employed in infecting the healthy susceptible corms with the different spoilage molds that were isolated in pure cultures. These methodologies were the methods of Okigbo and Nmeke [24], and a novel method designated as B. Okigbo and Nmeke's method compromised the tissues of the healthy tubers with the aid of a cork borer, to infect the test corms with the isolated spoilage molds while method 'B', made use of innate ability of the spoilage molds to colonise, penetrate and infect the susceptible healthy corms. Furthermore, the results of the method of Okigbo and Nmeke [24], recorded some inconsistencies as only *Aspergillus niger* and *Mucor circinelloides* elicited the same symptoms they produced in the diseased corms in the susceptible healthy test corms while those of *Aspergillus tamari*, *Fusarium oxysporium* and *Fusarium solani* did not show similar symptoms with those observed in the diseased corms probably due to secondary infections from more competitive molds that colonized the susceptible tubers, since they were stored in barn-like shelves during the entire experimental period. This was not so for the results obtained for method B. For method B, all the test molds elicited the same symptoms they displayed in the diseased corms, this may be as a result of the fact that the test corms were stored in sterile air-tight containers during the entire period of the experiment.

Thirty healthy corms were used for the research and were divided into 6 groups of 5 corms each as described in the materials and methods. All the molds used for infection during the pathogenicity were isolated from the original sets of diseased corms purchased from the market, to see if they would elicit the same symptoms they caused on the diseased corms on the healthy corms. Weight comparison of the test corms, using both research methods, showed a decrease in corm weights throughout the research period as shown on figures 1 and 2, thus suggesting that corms lose weight during storage.

The percentage severity of rots observed from the corms using both methodologies is shown on Table 3. In summary of the percentage rot occurrence rate in the diseased corm population, soft rot had 25% and was elicited by *Mucor circinelloides*, while dry rot had 75% and was elicited by *Aspergillus tamarii*, *Aspergillus niger*, *Fusarium oxysporium* and *Fusarium solani*.

These molds were able to elicit same symptoms as they did to the initial corms from which they were isolated. This implied that these molds are integral factors that pose a problem to post-harvest storage of cocoyam corms and showed the need to create measures to control them, in order to avert impending economic loss. This work

corresponds with the work that reported that isolated rot causing fungi from *Colocasia esculenta* contribute to post-harvest loss of cocoyam during storage [25]. Ugwuanyi and Obeta [26] also isolated fungi associated with rots of *Colocasia esculenta* and identified them as *Aspergillus niger*, *Fusarium oxysporium*, *Fusarium solani* and *Geotrichum candidum*; thus, corresponding with the isolates in this work save for *Geotrichum candidum*. Ogaraku and Usman [27] isolated rot causing fungi from yam tubers (*Dioscorea* sp.) and found some of the fungi isolated from *Xanthosoma sagittifolium* in this work; thus, suggesting the menace created by these molds in the spoilage of stored roots, tubers and corms. Fungi isolated in their work included *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporium*, *Rhizopus stolonifer* and *Sclerotium rolfsii*. Although, most documented researches on corms have been done on *Colocasia esculenta*, with very little documented on *Xanthosoma sagittifolium*; the most striking factor about the pathogenicity methodology of the researchers is the use of tissue abrasion with sterile cork borer, and this makes the novel method 'B' employed in this work more reliable because no artificial portal of entry was originally created for the microbes. Aderolu and Sogbesan [4] posited in their research that these microbial attacks affect the nutritional composition of these corms and as such proper preservation processes should be employed in order to curb post-harvest losses.

V. CONCLUSION AND RECOMMENDATIONS

Mold infestation contributes to the problems encountered during post-harvest storage of cocoyam corms. The methodology of Okigbo and Nmeke [24] shows that these molds can attack the corms when their barks have lost their integrity, hence, the tissue traumatization method adopted during the pathogenicity testing. This suggests that corms to be stored should be wholesome (wound-free) and firm, without any kind of tissue trauma such as abrasions on the outer bark, so as not to create a portal of entry for these spoilage molds; corm barks serve as mechanical barriers. Method 'B' which brought into focus the integrity of the corms and subsequent colonization and penetration of the tubers by the spoilage molds, suggests that healthy corms should be stored in a dry place, with the low amount of moisture. Thus, Research efforts aimed at the improvement of storage techniques will help reduce post-harvest losses during storage and thus improve yam production.

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