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Isolation and Identification of Fungi Associated with the Spoilage of Cocoyam Corms

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Abstract:
Cocoyam (Colocasia esculenta) is an important tropical root crop grown purposely for its starchy corms. This study was carried out to isolate and identify fungi associated with the spoilage of cocoyam corms. These organisms were isolated from spoilt corms by direct inoculation of the rotten tissues on Sabouraud Dextrose Agar. The fungal isolates were identified using cultural and morphological features such as colony appearance and also through microscopic characteristics. The isolated organisms include Rhizopus stolonifera, Rhizopus arrhizus, Fusarium oxysporum, and Aspergillus niger. Out of the 70 samples that were analyzed, 45 (64%) had Rhizopus stolonifera, 36 (51%) had Fusarium oxysporum, 52 (74%) had Rhizopus arrhizus while 61 (87%) had Aspergillus niger. A pathogenicity test was carried out on healthy corms using isolated microorganisms. The result showed that the organisms were responsible for the deterioration of the corms. These organisms may have infected these corms through contaminated working tools during harvest or improper storage by farmers. Corms are always infected by molds if not properly handled or stored. Hence, proper care should be taken while harvesting, handling, or storing these corms to avoid being infected by these pathogenic organisms since they can be detrimental to human health. It can also cause huge economic losses to farmers.

Keywords: *Cocoyam, spoilage, pathogenicity, Rhizopus stolonifera, Rhizopus arrhizus, Fusarium oxysporum, Aspergillus niger*

1. Introduction

Cocoyam (*Colocasia esculenta)* is an important tropical root crop grown purposely for its starchy corms or underground stem (Odebunmi *et al.,* 2017). It is widely cultivated and used for food in Nigeria, Ghana, and other countries, including China (Mbanaso *et al.,* 2018). Although its production has increased from 643000 to 1.7 million tons during the past decade, its contribution to root-crop production increased from only 39-6.8% (Shutt *et al.,* 2022).

In Nigeria, *Colocasia* species are the most important food and the third most important root/tuber crop after yam and cassava (Ugwuanyi & Obeta, 2014). It is also considered a source of starch for use in biodegradable film materials and for other industrial and pharmaceuticals purposes because of the interesting properties of the amylopectin fraction of starch, which is the principal form of cocoyam starch (Coronell-Tovar, 2019). Nutritionally, cocoyam has more carbohydrates than potatoes, yielding 135 kcals per 100 g (Mubarak *et al.,* 2021). It contains about 11% protein and 85-87% starch and other nutrients such as minerals, vitamin C, thiamin, riboflavin, and niacin (Melese & Negussie, 2015). Cocoyam is of great benefit because of its nutritional value. (Rao *et al.,* 2016).

Cocoyams have a very short post-harvest storage life of a few days to weeks (Mairami *et al.,* 2018; Ugwunwanyi & Obeta, 2014). Differing and often conflicting reports have been made on the post-harvest losses of cocoyam. Post-harvest storage life is often short, biodegradative losses are extensive, and up to 100% losses are common due to the fungi, which associate with the spoilage of cocoyam during storage (Khatoon *et al.,* 2016). However, substantial post-harvest losses caused by the fungi associated with the spoilage of cocoyam tubers during storage are attributed to several physical and physiological damages arising from harvesting, storage, or transportation. This is often implicated as some of the predisposing factors that lead to spoilage or deterioration (Agu *et al.,* 2016; Mubarak *et al.,* 2021). Invasion by pathogens, either through natural openings or cuts, is considered the most critical factor in cocoyam spoilage (Anukworji *et al.,* 2019).

A wide range of microorganisms (particularly moulds) have been associated with cocoyam decay, but relatively few are implicated as primary pathogens (Agu *et al.,* 2016). The degree of pathogenicity varies and is highly dependent upon storage conditions. It has been stated that fungi take the lead in the spoilage of cocoyam during storage (Anukworji *et al.,* 2019). Under these storage conditions, the fungi which are liable to attack cocoyam include; *Fusarium solani*, *Botryodiplodia theobromae*, *Rhizopus stolonifer*, *Aspergillus niger*, *Sclerotium roltsii*, *Trichoderma hamatum* and *Pythium* species (Mubarak *et al.,* 2021).

2. Materials and Methods

2.1. Collection of Samples

Seventy cocoyam corms were purchased from the new market in Enugu, Enugu State, Nigeria. The samples were transported to the Laboratory of Applied Microbiology and Brewing, Enugu State University of Science and Technology, for fungal analysis.

2.2. Sample Preparation and Isolation

Sterile knives and forceps were used to cut out spoilt tissues from the spoilt portion of the cocoyam corms. The spoilt tissues were inoculated into already prepared Sabouraud dextrose agar (SDA) plates by placing them directly in the middle of the plates. The plates were incubated at 37^{o}C for 5 to 7 days and the growths were monitored and recorded accordingly.

2.3. Purification of the Isolates

The developing colonies were purified by subculturing them repeatedly on freshly prepared Sabouraud Dextrose Agar (SDA) prior to identification (Cheese).

2.4. Identification of Isolates

2.4.1. Morphological Identification

It was done based on the colony appearance on the plates: colour, pigmentation, mycelia growth, texture, structure, size, and shape (Cheseebrough, 2006).

2.4.2. Microscopic Identification

The lactophenol cotton blue staining wet mount preparation was used for staining the isolates. They were observed under the microscope using 40x objective with reference to the Manual of Fungal Atlas.

2.5. Pathogenicity Test

To access the potency of the fungal organisms causing rots, 10 fresh corms of cocoyam were washed with tap water and then rinsed with distilled water. The surfaces were then sterilized with 70% ethanol and allowed to air dry. Cylindrical discs were removed from the sterilized corms using a sterile 4mm cork borer. The exposed tissues of 8 corms were inoculated with the already isolated molds in replicate while the other 2 corms were used as control. They were kept at room temperature and were observed daily for rot formation or deterioration. The results were recorded accordingly.

3. Results

The result of the colonial morphology and microscopic characteristics of the fungal isolates from spoilt cocoyam corms are presented in table 1. Out of the 70 samples that were analyzed, 45 (64%) had *Rhizopus stolonifera*, 36 (51%) had *Fusarium oxysporum*, 52 (74%) had *Rhizopus arrhizus* while 61 (87%) had *Aspergillus niger* (table 2). The result of the pathogenicity test is shown in table 3. The fungal isolates obtained from spoilt corms were tested for their ability to cause the same spoilage/rot in healthy corms.

Morphology	Microscopy	Probable Organism
Growth was initially white but changed to black after a few days producing conidial spores. The edges of the colonies appeared pale yellow, producing radial fissures on SDA	Smooth-coloured conidiophores and conidia were present. The conidiophores were protrusions from septate hyaline hyphae. The conidial heads appeared radial, and they splitted into columns.	*Aspergillus niger*
Dense growth of cotton-candy-like rhizoids with white to grey or yellowish brown colonies containing black spores	Non-septate mycelia with unbranched sporangiophores appearing brown in colour	*Rhizopus arrhizus*
Colonies were characterized by abundant white-cottony mycelium and a dark purple undersurface. Microconidia were oval to ellipsoidal or kidney-shaped microconidia were oval tapering and septated in 3 cells	Produced both macro and microconidia from slender phialides Macroconidia are hyaline, two to several-celled, sickle-shaped, mostly with an elongated apical cell and pedicellate basal cell	*Fusarium oxysporum*
Branched mycelia, rounded black sporangia at the tips of the sporangiophores with non-motile multinucleated spores	Broad hyphae, scarcely septate, and rhizoids and stolons were present. Brownish ovoid sporangiophores with tuft on the stolons diverging from the point at which the rhizoids were formed.	*Rhizopus stolonifer*

Table 1: Morphological and Microscopic Characteristics of the Fungal Isolates

Organism	Number of Samples	Number Positive (%)	Number Negative (%)
Rhizopus stolonifer	70	45 (64%)	25 (36%)
Rhizopus arrhizus	70	52 (74%)	18 (26%)
Fusarium oxysporum	70	36 (51%)	34 (49%)
Aspergillus niger	70	61 (87%)	9 (13%)

Table 2: Prevalence of the Isolated Organisms

Test Organisms	Rot Types	Symptoms Observed
Control (uninfected)	None	Corm was still hard and dry, maintaining its integrity
Aspergillus niger	Dry rot	The tissues were infected and had brown with yellowish periphery; infected corms were hard and dry
Rhizopus arrhizus	Dry rot	Infected tissues appeared green, with infected corms being hard and dry
Fusarium oxysporum	Dry rot	The tissues were green, and the corms were hard and dry
Rhizopus stolonifer	Soft rot	Mushy brown with white mycelia and black fruiting bodies on the corms

Table 3: Result of Pathogenicity of the Isolated Organism on the Healthy Corms

4. Discussion

Fungal spoilage of cocoyam corms has always been a post-harvest problem for farmers. Hence, this study was carried out to determine the fungal pathogens that are responsible for corm rot and the type of spoilage they cause. Also, to determine the pathogenicity of the isolated organisms using healthy corms.

The results obtained showed that out of the 70 samples that were analyzed, 45 (64%) had *Rhizopus stolonifera*, 36 (51%) had *Fusarium oxysporum*, 52 (74%) had *Rhizopus arrhizus* while 61 (87%) had *Aspergillus niger*. This agrees with the work of Ugwuanyi and Obetta (2014), who also isolated similar organisms from spoilt corms and identified that the organisms were responsible for the complete maceration of cocoyam tissues. More recent reports by Anukworji *et al.* (2019), Mubarak *et al.* (2021), and Eze and Ameh (2011) also showed that the above organisms were responsible for the spoilage of corms and root tubers.

Also, Khatoon *et al.* (2016), Agu *et al.* (2016), and Gwa and Ekefan (2018) also isolated similar organisms from *Colocasia esculenta* tubers. The presence of these organisms could be due to environmental conditions such as storage temperature and relative humidity that favored the growth and activity of these fungal pathogens. It may also be due to improper storage and the method of harvesting of these corms using contaminated implements as well as mechanical damage of corms which leads to direct penetration of these pathogenic organisms and accelerates the rate of deterioration of the corms.

The result of the pathogenicity test showed that there was severe spoilage or rot on the corms, which was caused by these organisms. This conforms with the work of Agu *et al.* (2014), who also discovered that the isolated fungi were actually responsible for the spoilage of these corms. This shows that the spoilage was actually caused by these fungal pathogens. These organisms caused discoloration and disruption of surrounding tissues, which led to the complete maceration of infected corms. This can be prevented by harvesting the corms using clean/sterile equipment, and mechanical damage to the corms should be avoided. Also, proper practices and modern technologies should be employed for better storage and preservation of these corms since these organisms can be dangerous to human health when consumed.

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