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Characterization and Identification of Fungal Species Associated with the Spoilage of Cocoyam (*Colocasia esculenta* L.) in Gwandu Local Government Area, Kebbi State, Nigeria

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Abstract—fungal species are paramount important causal agent responsible for the spoilage of Cocoyam. Samples of cocoyam were obtained in Gotomo village of Gwandu local government, Kebbi State, Nigeria. Three (3) villages were selected for sample collections within the L.G.A namely; Rafin raggaye, Shiyar gobirawa and Garba gibbar. A total of Sixty (60) tubers of *Colocasia esculenta* L. were collected, from each points Twenty (20) samples of *Colocasia esculenta* tubers were collected. Pour plate method was used in the enumeration of potential spoilage fungi in cocoyam samples. From the results obtained a total number of seven (7) fungal species were isolated and identified namely; *Aspergillus niger*, *Mucor racemosus*, *Aspergillus flavus*, *Fusarium oxysporium*, *Rhizopus stolonifer*, Rhizopus *oryzae* and *Penicillium expansum.* The most prevalent fungal isolate was *Aspergillus flavus* 32.5% and *Penicillium* species was the least prevalent among the isolates with 5%. The mean fungal spore count (sfu/ml) showed that cocoyam obtained from Shiyar gobirawa had the highest fungal load 4.3 x 10^6 and the lowest was recorded in Rafin raggaye 12 x 10^4. These fungal species isolated in this research were said to be pathogenic that cause the spoilage of *Colocasia esculenta* and hence their presence on cocoyam may be additional reservoirs for the transmission of pathogenic microorganisms and this could results to infections for consumers and economic loss. This raise concern over public health risks that may be associated with the consumption of deteriorated cocoyam and proper storage, package and handling methods with good transportation should be taken to reduce the occurrence and deterioration of cocoyam by these microbes.

Keywords— Fungi, cocoyam, load and Gwandu

I. INTRODUCTION

Colocasia esculenta known as "Mankani" by Hausa speaking and Cocoyam in English is a member of the Araceae family, which is perennial monocotyledonous, herbaceous corm whose leaves grow upward with fibrous root systems [1]. They are grown primary for their edible roots although all parts of the plant are edible. The *Colocasia esculenta* is mostly grows in tropical and subtropical areas. However, the aroids as they are very often in moist or shady habitats [2]. It is used as a staple food or subsistence food by millions of people in the developing countries [3]. These crops are not that important in the developed world market but they are of significant locally by providing edible corns, cornels and leaves as well as other traditional uses and served as source of daily incomes to the farmers in the rural areas [4].

Cocoyam have less paramount important in Kebbi State than Yam, Cassava and Sweet potato, yet a staple food in most part of tropics and sub tropic, has more crude protein than other root and tubers. Its starch is highly digestible because of the small size of the starch granules [2]. Nutritionally, *C. esculenta* had higher amount of carbohydrate compared to potatoes and yield 135 kcals per 100 g [3]. It contains about 11% protein on a dry weight basis. *C. esculenta* contains 85-87% starch on dry matter basis with small granules size of 3-18 μm and other nutrients such as minerals, vitamin C, thiamin, riboflavin and niacin better than other cereals [3]. Furthermore, *C. esculenta* have interest to the various people all over the world due to their nutritional values, root tubers that are consumed as vegetable and easy in processing with good arid soil, temperature that favor their growth and market values [5].

Kebbi is an agriculture land whereby majority of its people survive on agriculture as farmers or consumers on this important crop. However, lands in Nigeria are enriched with nutrient, nitrogen and phosphorus that supports the growth of tuber crops and other plants [6]. Available data

reported by [7] states that, Nigeria produce about 37% of cocoyam and distributed it to different parts of the world. This makes Nigeria among the ranking producers of cocoyam in the world as in 2003. Similarly, pulp and tuber of cocoyam are processed as starch and flour while, the outer cover/peel are fermented with some microbes to produce poultry feeds [8]. Additionally, cocoyam are cultivated all the year round this makes it perishable tuber crops then others. According to [4], cocoyam tolerates abiotic and biotic factors that affect the growth and cultivation of many crops. Nowadays, there is an increase in demand of cocoyam in the urban markets in Nigeria and countries such Ghana, Niger, Cameroon, Ivory-cost and many others. However, fungal species has been ranked as the most important cause of decline in production of cocoyam and its deterioration in these countries [9]**.** In Kebbi state, infection by these microbes are becoming of great concern in most cocoyam cultivating communities within the State and Nigeria. The presences of these microbes on cocoyam reduce its market price by affecting its nutrient value.

II. RELATED WORK

Different research were conducted by various researchers such as [10], [11] on fungal species associated with the spoilage of cocoyam in different part of Nigeria but to the best of our knowledge no such study conducted in Kebbi State, Northern-western Nigeria during our research or before our research. Therefore, there is need to conduct further research on the types of fungal species responsible for the deterioration of this stable tuber. This work aimed to characterize and identify the fungal species responsible for the spoilage of cocoyam tubers in Gotomo village, Kebbi state Nigeria north-western.

III. MATERIALS AND METHODS

Study Area

Gwandu local Government area is located in the North-western corner of Nigeria, its geographical coordinates are 12° 44' 23" North, 4° 30' 54" East. The mean annual temperature varies between 20 to 35^{o}C for the coldest and hottest season, but sometimes, temperature get up to 40 to 45^{oC} on very hot days [12]. The above climatic condition supports fast run-off and erosion in the rain; while dust and wind, soil erosion is typically during the dry season. Also inclusive is the hammattan period (November to January) which is characterized by heavy fog and dust as well as extreme cold, however, March and April are usually the hottest months in the year The vegetation is Sudan/Sahel Savannah in which rainfall starts late in May or early June to late September or early October. The average monthly temperature ranges from 21 to 42^{0C} and is lowest in December to January [12]. The main occupations of the people are trading, subsistence farming, mostly dry season farming and animal rearing [12].

Collection of Sample

Colocasia esculenta L. tubers showing symptoms of rotting were randomly selected and purchased from three different areas within Gotomo village namely; Garba gibbare, Shiyar gobirawa and Raffin raggaye. Twenty (20) rotting tubers of cocoyam were collected from each collecting points making a total of sixty (60) samples all in all. The tubers were collected in a sterile polythene bags well lebelled for easy identification and taken to the Department of Plant Science and Biotechnology, Laboratory, Kebbi state University of Science and Technology, Aliero for further analysis.

Sterilization of Glass Wares

All glass wares were soaked overnight in 70% ethanol, washed with detergent, rinsed with distilled water and then air-dried. Petri dishes, glass slides and bottles used were sterilized by dried heat oven at 160^{o}C for one hour.

Preparation of Culture Media

Sabouround Dextrose Agar (SDA) was prepared according to the manufacturer's instructions. Sixty (60.0 g) of SDA was dissolved in 100ml distilled water. The water was heated to boiling point to dissolve the medium completely. The medium was then sterilized by autoclave at 12^{o}C for 15 minutes and allowed to solidified at 40^{o}C and mixed well before pouring into sterilized Petri dishes [13]. Chloramphenicol (250mg/1) was added to the media after autoclaving to prevent contamination by bacteria [14].

Fungal Isolation

Samples collected were subjected to serial dilution. One gram of each soil sample was weighed and suspended in 9ml sterile water contained in a test tube (10^{-1}) and shaken. An aliquot of 1ml was transferred from this dilution in to the second test tube containing 9.0 ml of sterilized water to arrive at 10^{-2}. Another 1.0 ml was transferred from this second test tube (10^{-2}) to the third test tube containing 9.0 ml of sterile distilled water 10^{-3}. The same procedure was repeated until 10^{-6} [14]. After the serial dilution, 0.1 ml of the dilution was transferred from 10^{-5} and 10^{-6} dilutions of each sample, which were then aseptically inoculated on the solidified plates of oil agar. The plates were incubated at (30^{o}C) for 7 days with daily observation, after the appearance of a mixed growth, each spore was sub-cultured in a fresh Potato Dextrose Agar in order to obtain a pure culture using streak method as described by [14].

Characterization and Identification of the Fungi

The fungi were characterized and identified on the basis of their colonial and microscopic characteristics. Lactophenol cotton blue staining and slide culture test were carried out to characterize the fungal species using the methods described by [14]. The fungi were identified following the morphological structures of the fungi such as hyphae (septation), reproductive structure (sporangia/conidia) in chain or single; the type of spore were observed and recorded as described by [15].

Statistical Analysis

Descriptive statistic was carried out using percentage and frequency of occurrence of the isolated fungi as follows.

$$\text{Percentage Frequency} = \frac{\text{Number of Isolated Fungi}}{\text{Total number of Isolates}} \times 100$$

IV. RESULTS AND DISCUSSION

Morphological Characteristics of Fungal Isolates from Cocoyam in Gotomo Village

The fungal associated with the cocoyam were identified based on colony appearance, morphology and cellular characteristics as shown in Table 1. The isolates are; *Aspergillus niger, Mucor racemosus, Aspergillus flavus, Fusarium oxysporium, Rhizopus stolonifer, Rhizopus oryzae* and *Penicillium expansum*.

Fungal Frequency and Distribution Associated with Cocoyam in Gotomo

The frequency of occurrence of the fungal isolates associated with cocoyam is as seen in Table 2, where *Aspergillius flavus* had the highest percentage (32.5%) and *Penicillium expansum* obtained with the lowest (5%).

Mean Fungal Spore Count (Sfu/ml) from Cocoyam Samples

Mean Fungal Spore Count (Sfu/ml) based on samples area are presented in Table 3. Shiyar gobirawa had the highest mean (4.3×10^6) and lowest was observed in Raffin raggaye area (12×10^4) respectively.

Table 1: Morphological and Microscopic Examination of Fungal Isolates from Cocoyam

Fungi Isolates	Colony Appearances	Microscopic Examination
Aspergillus flavus	Colonies consisting dense felt dark green in color	Conidiphore intermediate with aerial hyphae bearing Conidisphore, conidial head typically columnar
Mucor racemosus	Sporangia are dark with zygospores. Hyphae long branched with septate	Colonies were grey in color
Fusarium oxysporium	Colonies are usually fast-growing pale or bright in colour	Chlamatosphore in hyphae or in candida hypae
Penicillium expansum	Dark green spore. The culture was green diamond in color and sparkling as it matured	The conidia are globe-like and resemble glass beads under the microscope. The hyphae appeared branching and septate conidiophores were erect, septate and faint
Aspergillus niger	Black color with white edges	Large conidial heads, dark brown becoming radiate and split to columns
Rhizopus stolonifer	Whitish becoming brown-black	Non-septate mycelia with branching sporangiosphores
Rhizopus oryzae	Colonies are very fast growing, with some tendency to collapse, white cottony at first becoming brownish grey to blackish grey depending on the amount of sporulation.	Sporangiophores smooth walled, non septate, simple or branched

Table 2: Frequency of Occurrence of fungal Isolated from Cocoyam in Gotomo

Isolates	Frequency of Distribution	Percentage of Occurrence (%)
Aspergillus flavus	13	32.5
Mucor racemosus	7	17.5
Fusarium oxysporium	6	15
Penicillium expansum	2	5
Aspergillus niger	5	12.5
Rhizopus stolonifer	4	10
Rhizopus oryzae	3	7.5
Total	**40**	**100**

Table 3: Mean Fungal Spore Count (Sfu/ml) Based on Sample Areas

Areas	Sample Type	Sfu/ml
Garba gibbare	Rotten Tuber	7.0×10^6
Shiyar gobirawa	Rotten Tuber	4.3×10^6
Raffin raggaye	Rotten Tuber	12×10^4

Discussion

The sample used in this study were collected in three (3) localities within Gotomo villages namely, Garba gibbare, Shiyar gobirawa and Raffin raggaye to determine the fungi species associated with the spoilage of cocoyam. Seven (7) fungal species were isolated and identified from the samples collected namely; *Aspergillus niger, Aspergillus flavus, Penicillium expansum, Fusarium oxysporum, Rhizopus oryzae, Rhizopus stolonifer* and *Mucor recamosus*. These findings were in line with the study conducted by Khatoon *et al*. [10] who reported that, *Aspergillus niger, Rhizopus oryzae, Aspergillus flavus* and *Geotrichum candidum* were found *Colocasia esculenta* tubers **at** Bhubaneswar city, India. However, one fungal specie was not detected in this present study, these might be due to differences in the study area with certain environmental factors that favor the growth of this specific specie. Similar, species were reported by Agu *et al*. [11]. The higher number of these fungi in this present studied could probably be due to environmental conditions such as temperature and relative humidity that favor the growth and activity of these fungi species in the study area.

Aspergillus species were the most prevalence fungal species obtained in this presence research. [16], Joon *et al.* [17], reported that *Aspergillus* species were most frequently encountered during isolations in many parts of

the tropical and humid regions of the world, these statement supported our findings. According to [18], fungi create local discoloration and disruption of surrounding tissues of infected tubers resulting in changes appearance, deterioration of texture and possibly flavor or taste. [19], were of the view that these rot fungi cause postharvest losses reduction in the market value and misfortune to farmers. On the other hand [20], [11] indicated that factors such as ambient temperature, light and air moisture as well as mechanical damage of tubers also accelerate the degradation of the tubers. The presented of *Aspergillus niger* was characterized by softening of internal tissue, development of black spore mass over the infected area. Also, fungi *Aspergillus niger* isolated are known to be human pathogenic or opportunistic human pathogenic organisms.

V. CONCLUSION

The existence of these fungi species isolated and identified from cocoyam showed that, these species are responsible for the deterioration of this tubers which included; *Aspergillus niger*, *Aspergillus flavus*, *Penicillium expansum*, *Fusarium oxysporum*, *Rhizopus oryzae*, *Rhizopus stolonifer* and *Mucor recamosus*. Thus, the presences of these species may result to potential problems to farmers by reducing the yield and economic loss to the farmers. Therefore, poor personal hygiene, improper handling and poor knowledge of cocoyam farmers towards food borne disease were associated risk factors to contamination of cocoyam in the study area. Mass education of farmers on proper package and handling, clean planting equipment, healthy planted seedlings, modern storage techniques uses and transportation of this tubers is hereby recommended to avoid post-harvest fungal contamination is hereby recommended.

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