Research Journal of Food Science and Quality Control (RJFSQC) E-ISSN 2756-5483
P-ISSN 2695-2459 Vol 10. No. 2 2024 www.iiardjournals.org (Online Version)

Isolation and Identification of Fungi Associated with Deteriorated Cocoyam

***[1]Abubakar, Z.I., [1]Idris, H.A., [1]Maliki, H.S., [1]Mustapha, F.A., [1]Abdallah, H.Y., [1]Aisha, M.B., [1]Ogbu, L.C .,[1]Yusuf,S.K.**
[1]National Biotechnology Development Agency, Abuja, Nigeria
Correspondence: ayisaterna5@gmail.com,

DOI: 10.56201/rjfsqc.v10.no2.2024.pg83.91

Abstract

One of the food crops which provides plenty yield of root and foliage is known as cocoyam. Fungal species are paramount causative agents responsible for the spoilage of Cocoyam. This study seeks to identify various Fungi associated with deterioration of cocoyam. Samples of the Cocoyam were obtained from three markets within Bida local government in Niger state. The markets include; small market, old market (Etsu Musa) and New market (Gwadabe). A total of 30 tubers were collected, ten from each market. Enumeration technique was carried out using pour plate method to isolate the fungi causing spoilage. From the results obtained a total of five fungal were isolated using Potato DextroseAgar and identified as:Aspergillusfiavus, AspergillusnigerMucorracemosus, Rhizopusstolonfer and Rhizopousoryzae. The most prevalent was Aspergillusflavus 42.5% and Mucorracemosus with less than 10%. The highest fungal count was recorded at Old market (Etsu Musa) 4.3 x 10^6 and lowest fungal load was recorded at Gwadabe(New market) 1.2x 10^4. The fungal species isolated in this study are said to be pathogenic and cause spoilage of Colocasiaesculenta. Their presence poses a threat to consumers and economic loss to farmers. This study has raised a concern over public health risk associates with consumption of deteriorated Cocoyam. Proper storage, packaging, handling with good transportation should be taken to reduce the occurrence and deterioration of Cocoyam by these fungi.

INTRODUCTION

In South-eastern Nigeria there are two type of cocoyam thatare grown, which are both herbaceous plant. Bazaar is Ede-uli in igbo (Colocasiaesculanta) is available andmost popular in South-eastern Nigeria communities it is grown in marshy areas and itscorms are used as soup thickening agent (Agu et al., 2019). *Xanthosomasagittifolium*called ede-oku in igbo is the second type which is less popular, it corms can be boiled and eaten with various soups, (Agu et al., 2019).

One of the food crop which provides plenty yield of root (or corms) and foliage is known as cocoyam. It can be grown under flooded or upland condition which makes it a tropical food

crop.(Agu et al., 2019). The presence of calcium oxalate crystals in cocoyam plant makes it toxic typically as raphides. A pinch of baking soda is added while cooking or by soaking roots in cold water or overnight to reduce toxin. Kidney stones are contributed by calcium oxalate which is highly insoluble. (Babajide and Obadina, 2020).Developing countries, in many regions, Cocoyam contribute to carbohydrate contact of the diet and provide edibles starchy storage corms and cornels. Other tropical roots such as cassava, sweet potatoes and yam is less important, they are still essential supply in some part of the sub-tropical and tropical regions (Babajide and Obadina, 2020).

In soup recipe it can be used as thickener, flour (for confessionaries) or it can be sliced up and boil or fry to make flakes or chips. The leaf stalk is also edible. It is a perennial monocotyledonous herb, the plant consist of central corns which lies below soil surface height of 1-2metres, whose roots grows down word, leaves up word, with cormels, runners (stolons) and daughter corms grows laterally, the root systems lies mainly in the top one meter of soil, it is a food crop which provides high yield of roots (or cormels) and foliage. (Chayty et al., 2018).Cocoyam is less important than cassava, sweet potatoes and yam, it contains an essential protein than other root and tubers, its starch is easily digestible due to small size of the starch granules, nutritionally *Colocasiaesculanta* has greater amount of carbohydrate thanpotatoes and it yield l35kcals per 100g (Melese and Negussie, 2021), On a dry weight *C.esculenta* contains about 11% protein, 85-87% starch on dry matter basis, 3-1 8um on small granules and some other nutrients such as Vitamin C, riboflavin, niacin and minerals (Melese, and Negussie, 2021). Morever, *C. esculenta* is important to many people all over the world because of its nutritional values, easy in processing with arid soil, and root tubers which are consumed as vegetable, temperature that improves their growth and market value (Rao et al., 2020). *Colocasiaesculenta* known as ‘kwaza’ by the hausa, Ede by the igbos, ko’ko’ by the Yorubas and known as cocoyam in English, it is one of the member of *Araceae* family, which is Perennial monocotyledonous, herbaceous corn whose leaves grows in an up word direction with fibrous root system.(Deni, 2019). They are mainly planted for their edible roots; also all parts of the plant are edible. The *Colocasiaesculenta* is mostly planted n sub-tropical and tropical areas, so also the aroids are usually found in moist or shady environment, it serves as food for many people in some countries.(Melesa, 2021). Cocoyam is not well known in some developed markets, but they are of great importance by providing edible corns, Cormels and leaves with other traditional benefits, it also serves as source of income to farmer. (Ounniyi 2022).Cocoyam is nutritiously rich in carbohydrates (13-30%), mineral and vitamins, also containers protein (1.4- 3.0%), the leaves are rich in vitamin B6, vitamin C, thiamin (B1), niacin, and riboflavin (B2).

Furthermore they are also rich in minerals such as zinc, iron, copper, phosphorus, potassium and manganese, the corner and corners are good source of dietary fiber and also oxalic which can cause skin irritation when raw corns come in contact with the skin. (Zuhair and Hunteic 2020).Loss of root and rubber crop has been a serious issue to Farmer as more than 40% of their harvest maybe loss due to decay. The most common spoilage of root and tuber crops aredue to fungal rot. The species of microorganisms associated with deterioration of cocoyamincludes *Penicilliumdigitatum*, *Fusariumsolani*, *Aspergillusflavis*,

Scleroliarolfsii,Botryodiplodiatheobromae, and*Erwiniacaralovora*,these fungi were reported to be virulent to four cultivars of *Colocasiaesculenta,* causing deterioration of cocoyam in several parts of Nigeria. (Brunt et al., 2021).Fungi cause a post-harvest disease which constitute a public health problem worldwide and one of the major goal of the society is to prevent them. Fungi or there metabolite, virus, parasites or toxins cause a typical microbiological food born disease, (Oloruntoba, 2021).

Some Food do not show any sign of spoilage but the food is contaminated by fungi poison and consumers are not aware of it, tuber crops can support the growth of food poisoning fungi due to their moist and low acid foods (FAOSTAT 2019). Water (60-90%) is the main component of tubers, starch and fibre; with smaller amount of protein, sugar, fat, minerals and vitamins (FAOSTAT, 2019). Tubers are nutritionally rich staple foods and they are cheap, which are associated with high transportation cost, they have short shelf life, and there market Margarita is limited in development countries (Chandraseicara and Kumat 2022). The aim of this study is to identify and isolated various fungi associated with deterioration of cocoyam and to characterize fungi associated with post-harvest spoilage of cocoyam.

MATERIALS AND METHOD

The sample location and sample site Bida Town is located on North central part of Nigeria (Niger State) on latitude 9.05° and longitude 5.96° with large and small traders like farmers, fishers and entrepreneurs.

Sample Collection

Samples of *Colocasiaesculenta* showing some symptoms of spoilage were selected and purchase from three major markets within Bida local government in Niger state. Themarkets include; Small market, old market (Etsumusa) and New Market (Gwadabe). It Atotal of 30 tubers were collected 10 from each market. Also 5 tubers of *Colocasiaesculenta* showing no symptoms of deterioration were also selected from each market which will serve as control. Each sample were collected in a sterile polythene bags and well label for proper identification and transported to microbiology laboratory for microbial analysis. Samples collected were surface Sterilized with 1% sodium hypo chloride (NaOCl) solution for one minute to remove surface Charshpetriaion after washing off soil and roots debris from the corns and cormels and then rinse three times in a distilled water.

Sterilization of Glasswares

All the glassware to be used were soaked overnight in 70% ethanol, washed with detergent, rinse with distilled water and are dried. The glasswares were then sterilize by dry heat using hot air oven at 160°c for one hour.3.4 Microbiological methods

Preparation of Potato Dextrose Agar

Sabouraund Dextrose Agar (SDA) were prepared according to manufactures standard instructions which is suspend 39g of SDA in l000ml distilled water. Sterilized by autoclaving at 121°c for l5minutes, it was allowed to cool to 40°c and then mix properly before pouring into sterilize petri dish.

Enumeration and Serial Dilution

The deteriorated samples were weighed and grounded using a sterile pestle and motor. 1g of each sample was added into a 9m1 of distilled water in a sterile test tube. The content was shaked to homogenate (10^{-1}). Then 1ml was transferred to the second test tube containing 9ml of distilled water to arrive at (10^{-2}). Another 1ml was transferred from the second test tube (10^{-2}) to the third test tube containing 9m1 of distilled water (10^{-3}). The procedure were repeated up to 10^{-6}. After the serial dilution, 0.1ml of the dilution were transferred from ($l0^{-5}$ and 10^{-6}) dilution of each sample were aseptically inoculated on the solidified PDA plate and incubated at 37°c for 7 days with daily observation.

Sub-culturing

After 7days incubation and daily observation, appearance of a mixed culture growth were observed, each spore was sub-cultured into a fresh PDA plate to obtain pure culture this was done by transferring hypal tips from the colony edges of the mixed cultures to fresh plates of SPDA using flame-sterilized blades. After sub-culturing, the plates were incubated at 27°c until pure culture were obtained.

Identification and Characterization of Fungi

The morphological appearance of the fungal colonies on the pure culture plates were observed such as size, shape and spore formation. It was obtained by placing mycelium containing the spore was placed on the slide with the aid of an inoculating needle sterilized with ethanol and subsequently stained with lactophenol cotton blue and was observed under light microscope. The fungi were identified following the morphological structure of the fungi such as hype (septation), reproductive structure (sporangia/conidia) in chain or single.

Statistical Analysis

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

$$\text{Percentage Frequency} = \frac{Number\ of\ isolated\ fungi}{total\ number\ of\ isolate} \text{ x100}$$

RESULTS AND DISCUSSION

RESULTS

Morphological characteristics of fungal isolated from cocoyam in Bida markets.

The fungal associates with deterioration were identified based on colony appearance, characteristics and morphological and this is illustrated in the table 1 below. The isolates include; *AspergillusJiavus, RhizopusstoloniferMucorracemosus, AspergillusnigerRhizopusoyzae.*

Fungal frequency and distribution associated with Cocoaan in Bida markets.

The frequency of occurrence of the fungal isolates associated with Cocoyam deterioration is shown in table two (2) where *Apergillusflavus* had the highest percentage (42.5%) and *Rhizopusoryzae* had the lowest (10%).

Mean fungal spore count (sfu/ml) from the cocoyam samples.

Mean fungal spore count (sfu/ml) based on sample area are displayed in table 3. Old market (Etsu Musa) had the highest mean ($8.0x10^6$) and lowest was observed in new market area ($l.2x10^4$) respectively.

Data Presentation

Table 1: morphological and microscopic examination of fungal isolates from cocoyam

Fungal	Colony apparatus	Microscopic Examination
Aspergillusflavus	Dense colonies, dark green in color	Aeriel hyphae bearing conidiosphore conidial head are columnar
Rhizopusoryzae	Fast growing colonies with the tendency to collapse. White cottony at first becoming brownish grey and leter turning to blackish grey due to increased sporulation	Sporangiosphore are smooth walled, non septate, simple or branched
Aspergilluniger	Black coloured colonies with white edges	Large conidial head dark brown in color radiation and split to columns
Rhizopusstolonnifa	Whitish colonies turning brownish – black	Non septate mycelia with branching sporangiosphore

Mucorracemosus	Sporangia with zygospores, dark in color, hyphae with ong branched septate	Colonies are grey in colour

The results obtained as shown in table 1, indicates the morphology and microscopic characteristics of fungi associated with deteriorated cocoyam. A total of five fungi isolates were obtained from rotten cocoyam's, the isolates includes *Aspergilusfiavus, Rhizopusstolonijê,çMucorracemosus, AspergillusnigerRhizopusoryzae. Aspergillus*species are most prevalence fungal species and are usually isolated during isolation.

Frequency and percentage occurrence

Table 2: frequency occurrence of fungal isolated from cocoyam in Bida Market

Isolate	Frequency distribution	Percentage of occurrence (%)
Aspergillusflavus	17	42.5
Rhizopusoryzae	5	12.5
Aspergillusniger	8	20
Rhizopusstolonifer	6	15
Mucorracemosus	4	10
Total	40	100

The result obtained from table 2, records the frequency of occurrence of the different disease conditions were *Aspergillusflavus* had (42.5%) , *Rhizopusoryzae* has (12.5%), *Aspergillusniger* has (20%), *Rhizopusstolonfer* has (15%) and *Mucorracemosus* has (10%). *Aspergillusflavus* has the highest percentage (42.5%) and *Rhizopusoryzae* had the lowest (10%).

Total mean fungal count

Table 3: Mean Fungal spore count (SFULML) based on sample

Areas	Sample type	SFULML
Small market	Rotten tuber	$4.3x10^6$
Old market (Etsu market)	Rotten tuber	$8.0x10^6$
New market (gwadabe)	Rotten tuber	$1.2x10^4$

The result obtained from table 3 shows the fungal spore count (sfu/ml). The samples of cocoyams obtained from three markets which old market (Etsumusa) has a fungal count of($8.0x\ 10^6$) which is the highest while the lowest count was obtained from the new market (Gwadabe) sample with the fungal count of($l.2x10^4$).

DISCUSSION

The sample of cocoyam uses in the study were acquired from three (3) different markets within Bida namely small market, old market (Etsumusa) and New market (Gwadabe) to determine the fungi species associates with cocoyam deterioration. Five fungal species were isolated and identified from the samples collected namely *Aspergillusniger,Aspergillusflavus, Mucorracemosus, Rhizopusstolinifer, Rhizopûsoryzae*.

These findings were in accordance with (khatoon et al., 2022) who reported that *Aspergilus Niger, Rhizopusoryzae*and*Aspergillusflavus*were found at Bhubaneswar city in India. However one species that was reported by Khatoon that is Geotrichumcandidumwas not detected in this study and it could be as a result of differences in location or continent and also certain environmental factors that could favor the growth of this particular species.

Similarly, some species were also by (Agu et al., 2019). The higher number of these fungi in this present study could be probably due to environmental condition such as temperature and humidity that favors the activities and growth of these fungal speciesin the study area.*Aspergillus* species were most prevalent fungal species obtained from this present study.(Ibrahim, 2015 &Joon et al., 2021) reported that *Aspergillus* species were most frequently encountered during isolation in many parts of the tropical and humid regions of the world, these statements supported our findings. According to (Snowdon2019), fungi create local disruption and discoloration of surrounding tissues of infected tubers resulting in appearance changes, deterioration of the texture and possibly flavour or taste. (Clark and Moyer 2020) were of the view that these rot fungi cause post harvest losses, reduction in market value, and misfortune to farmers. On the other hand, Okigbo, (2023) and (Agu et al., 2019) indicated that factors such as ambient temperature, light and air moisture as well as mechanical damage of tubers also accelerate the degradation of tuner.

The presence of *Aspergillusniger* was characterized by softening of internal tissue, development of black spore mass over the infected area. Also the *Aspergillusniger*isolated are known to be human pathogenic or opportunistic human pathogenic organisms.

CONCLUSION AND RECOMMENDATION

CONCLUSION

The existence of these fungi species isolated and identified from cocoyam showed that these species are responsible for the deterioration of these cocoyam tubers which includes*AspergillusnigerAspergillusflavus, Rhizopusoryzae, Rhizopus sto1inifer* and*Mucorracemosus.* Thus the presence of these species may result to potential problems to

farmers by reducing the yield and economic loss to the farmers as well. Poor personal hygiene, improper handling due to poor knowledge of cocoyam farmers towards food borne diseases were associated risk factors to the contamination of cocoyam in this presentstudy.

RECOMMENDATION

Mass education of farmers on proper packaging, handling, clean planting equipment, healthy planted seedlings, modern storage techniques uses and transportation of these tubersare recommended to avoid post-harvest fungal contamination.

REFERENCES

Agu, K.C., Awah, N.S., Nnadozie, A.C., Okeke, B.C., Orji, M.U., Iloanusi, C.G., Anaukwu, H.C., Eneite, M.C., Ifediegwu, N.D., Umeoduagu, E.E., and Udoh, S. R. (2019) Isolation, Identification and Pathogenicity of Fungi Associated with Cocoyam (*Colocasiaesculenta*) Spoilage. *Journal of Food Science and Technology*. 10(3):106-111.

Babajide, O.A. and Obadina, O.B. (2020).An assessment of the microbiological safety of dry yam processed in south West Nigeria.*Africa Journal of Biotechnology*. 6:157-161.

Brunt, J., Hunter ,D., and Delp, C. (2021). A Bibliography of Taro Leaf Blight. Secretariat of the Pacific Community; genetic resources: conservation and utilization 22:123-143.

Chandrasekara, A. and Kumar, T.J. (2022). Roots and tuber crops as functional foods: A review on phytochemical constituents and their potential health Nigeria. *Journal of Pure and Applied.Science*. 31:3180-3185.

Chayty, A., Asano, K., and Sone, T. (2018).“A Molecular Phylogeny-Based Taxonomy of the Genus Rhizopus”.*Bioscience, Journal of Biotechnology, and Biochemistry*. 74 (7): 1325 - 1331.

Clark, C.A. and Moyer, T. (2020). Compenduim of sweet potato disease phytopathology.Journal ofphytological research. 2:70-74.

Deni, B. (2019). Aroids plants of the arum family. Timber press, Portland.11: 201-215.

FAOSTAT, (2019).Food crop and storage.Corporate Document Repository and Database.

Ibrahim, S.D. (2022). Fungal pathogens associated with store onions in Maiduguri and Bama Towns of Borno state. Unpublished Microbial Sciences University of Miaduguri 1:35-40.

John, T.L., Don, W.B., Seun, H.P. and Change, S.K. (2021). Occurrence and Biological control of Post harvest decay in onions caused by Fungi Plant journal of Pathology, 17(3) 141-148.

Khatoon, A., Mohapatra, K.B., and Satapathy, A. (2022). Fungi associated with storage rots of *Colocasiaesculenta L*. Tubers in Bhubaneswar city, Odisha. *British Microbiology Research Journal*. 3, 1-5.

Melesa, B. (2021). "Patulin accumulation in apples by Penicilliumexpansum during postharvest stages".*Journal of Applied Microbioliogy*.44(1): 30 - 5.

Melese, T. and Negussie, R. (2021). Nutritional potential, health and food security benefits of taro *Colocasiaesculenta L*: A review. Food Science and Quality Management, 1:23-30.

Okigbo R.N. (2023). Occurrence and control of fungal rot pathogens of yam (*Dioscorearotundatapoir*) with leaf extracts of *Chromolenaodorata, Carica papaya and Aspilia Africana*. Nigeria. *Journal of Mycology*. 2(1): 154-165.

Oloruntoba, E.O. (2021). Microbial Flora Associated with Postharvest Spoilage of White Yam (*Dioscorearotundata*) and Implication for Health of Consumers in a Canteen in Ibadan. 12, 234-245.

Ounniyi, B. A. (2022). Composition of four cocoyam cultivars and their tolerance to corm rot. *Journal of Tropical Science*. 39: 136-139.

Rao, V.R., Hunter, D. Eyzaguirre, P.B. and Matthews P.J. (2020).Ethno-botany and global diversity of taro. In: The GlObal Diversity of Taro Ethno-botany and Conservation. Biodiversily International, Rome, lialy. 1:5-21.

Snowdon, A. (2019). A colour Atlas of Post-harvest Disease and Disorder of fruits and vegetables.*Col.1 Wolfe Scientific Ltd. London*. 302.

Zuhair, M. and Hlunter, D. (2020). Taro cultivation and use in the maldwes. IPGR Session 12th Symp.of IATRC, TSUKUBA. Japan.