MACROSCOPIC AND MICROSCOPIC EXAMINATIONS OF FUNGI ASSOCIATED WITH *IPOMOEA BATATAS* IN KADUNA STATE, NORTHERN NIGERIA

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Abstract
This study is aimed at establishing that fungi constitute the major causes of sweet potato tuber spoilage. A total number of 60 samples of sweet potato tubers were used for the study. 40 samples of dotted sweet potato tubers were collected from Kawo market and 20 freshest more from a farm along Kaduna Zaria express way, Kaduna, and were brought to the mycology lab for experiment. 40 samples with dotted spotwere considered as spoiled. Each of the 40 spoiled samples was cut and the liquid content inoculated on potato Dextrose Agar and incubated at 25 ºC and observed for 4-30 days after which the different colonies obtained were sub-cultured and later identified using the slide culture technique. Twenty (20)fresh and healthy potato tubers were used for pathogenicity test. A total of five (5) fungal species were identified which were Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Rhizopus stolonifer, and Penicillium species. From the results obtained, Aspergillus flavus has the highest frequency of occurrence (45%) and Aspergillus niger(30%) respectively. All fungi proved to be infectious on the sweet potato tubers, hence causing spoilage. Surface damage during handling and harvesting should be avoided as they serve as a major entry point of disease pathogens. There should be improvement of storage facilities for the harvested sweet potato tubers in other toprolong the shelf life of the crop.

Keywords: Fungi, Macroscopic, Microscopic, *Ipomoea batata*

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INTRODUCTION

Ipomoea batatas (L.) is dicotyledonous shrub plants from family Convolvulaceae. Family divide into fourty five (45) genera with nearly more than one thousand species. Among the one thousand species found in this family, *I. batatas* was found to be the only economical important species (Mohanraj and Sivasankar, 2014). Among the major world crop production, *I. batatas* is the seventh with over one million to nine production per annum. In Nigeria *I. batatas* is an important staple food especially in the northern part of the country where it is cultivated throughout the year during raining and dry season. In Nigeria it is been rated to be sixth most important root crop. 2% of the word potato is produce from Nigeria. The species have occupied a significant place in the world as source of energy food. The total requirement of the carbohydrates required by the Nigerian population *I. batatas* account for the half of it every year. The specie is a perennial, herbaceous with palmately lobed leaves. In some part of northern Nigeria, the leaves and shoots were consumed as vegetables. *I. batatas* despite being sweet is of significant importance to diabetes patient as investigation revealed it stabilised blood sugar level which lower insulin resistance. Also reported by Mohanraj and Sivasankar, (2014) the wide acceptability of the crop which lead to large cultivation of the plants have make it prone to diverse microbial infection (Ray and Ravi, 2005). The productivity and acceptability of the root crop can be significantly improved by identification of the microorganisms associated with the storage of the *I. batatas* with time. Storage of *I. batatas* are subject to many microbial infections which depend largely on the processing, storage condition and the time of harvesting. After poverty, scarcity of food is one of the world problems

persisted (Khatoon et al., 2017). Quine number of countries have issues of food scarcity. Previously it has been reported by Khatoon et al. (2017) approximately one billion people all over the world faced with hunger problem. Significant number of the estimated people 10% die annually from complications related hunger (Khatoon et al., 2017). The problem persisted as a result of lack of adequate techniques to avoid microbial spoilage of the stored agricultural product (Mohanraj and Sivasankar, 2014). Microbial infection can be introduced to the plants during planting, harvesting, storage or processing (Tortoe et al., 2010). One of the major challenge or setbacks in production or handling of *I. batatas* is susceptibility to diverse microorganisms. Losses in the post-harvest and rotten are surely due to the microbial infection that occur either before planting or post-harvest activities, through handling. Pre harvest microbial infections to the plants are usually caused by fungal pathogens (Coyne and Affokpon, 2018). Damages caused by fungi are responsible for the 80% loss of the stored staple food (Ravi and Aked, 1996). Worldwide fungi are consider to be sole responsible of yield loss, deterioration of the tuber quality and blemishing diseases in tuber (Mukhopadhyay et al., 2011). The black dot of *I. batatas* is caused by fungal infection which is becoming of great concern. In order to overcome the challenges of the fungal infection of *I. batatas* isolation and identification of the fungal strain would provide an idea on how to strategies to control this infection. In some instances, the infection was caused by a new strain of microorganism that has not been previously study. The aim of study was to isolate and identify fungal pathogens associated with the post-harvest deterioration of *I. batatas.*

MATERIALS AND METHODS

Sample Collection

Ipomea batatas (40) were collected in Kawo Kaduna metropolis in different market and later (20) *I. batatas* was also collected farm along Kaduna Zaria express way for pathogenicity test after the isolation of the fungi. The collected samples were placed in sterile polythene bag were taken to the Department of Biological Sciences, Kaduna State University for further analysis.

Media Preparation

The media used were prepared according to the manufacturer instructions. Sabouraud Dextrose Agar (SDA) (28g) was dissolved in 1000mls of distilled water. The mixture was stirred together carefully, it was then sealed with an aluminum foil paper and then heated on a hot plate to aid in dissolution of the mixture. The solution was then sterilized by autoclaving at 15 pounds pressure at 121 º C for 15 minutes. 0.39g of chloramphenicol was added to the solution before pouring into sterile Petri dishes under aseptic conditions and is left to solidify.

Isolation of Fungi

The infected *I.batatas* were surface sterilize with 70% alcohol. *I.batatas* was subsequently cut into small pieces of about 2-3cm using sterile razor. The cut pieces of *I.batatas* were placed on solidified Sabouraud Dextrose Ager plates. The inoculated plates were then incubated at room temperature (28±30 °C) for 4 to 7 days.

Sub-culture

Individual colonies from the original cultured plates were picked using a sterile wire loop and were streaked on the freshly prepared plates. This is necessary to obtain pure culture. The plates were labeled accordingly, incubated at room temperature for five days.

Fungal Identification

The obtained pure culture of fungal isolates based on the morphological characteristics with respect to the pattern of growth colony, pigmentation and conidial morphology. Microscopic examination was through methylene blue was dropped on a glass slide and a small portion of fungal colony from the subculture plates was picked using sterile wire loop and placed on the glass slide, the sample were emulsified and covered with a cover slip. The slide was placed on the stage and observed under low and high objectives (x10 and x40 respectively) for identification. The macroscopic features and the morphological features of the isolated fungi were

authenticated and confirmed by mycological atlas of Robert and Ellen (1988).

Pathogenicity Test

Twenty (20) fresh and healthy potato tubers were washed with tap water, rinsed with distilled water and surface sterilized with 70% ethanol. Cylindrical discs were then removed from the tuber with a sterile knife. A disc of a five days old culture of the isolated fungi were transferred into holes created in the tubers, vaseline was used to seal each side and pieces of cotton was placed on the vaseline. The inoculated tubers were then placed in separate airtight containers and were labeled accordingly and incubated for 14 days at room temperature. After incubation period of 14 days at room temperature, the tubers were examined for infection and disease development. Pathogenicity test was carried out to confirm the fungal isolated were responsible for the spoilage of *I. batatas*.

Ethnobotanical Collection

Ethnobotanical survey was carried out by using a semi-structured open questionnaire in Kaduna state metropolis. Prior to the visits No appointment has been made. Ethnobotanical and other relevant information pertaining the farming, storage and spoilage of *I.batatas*were investigated. Total of hundred (100) informants were interviewed (Abdulrahman et al., 2018). Ethnobotanical data gathered in the course of the interview were subjected to the following analysis:

✓ A simple descriptive analysis was used to determine the percentage and frequencies of sociodemographic data of the respondent.

✓ Information consensus factor was also determine in other to know the agreement of the respondents on the methods of storage of the *I. batatas* (Abdulrahman et al., 2018). The ICF was calculated using the following equation: ICF = (Nur-nt) / nur-1. Where Nur is the number of each methods been cited by the respondents, nt is the number of plant species reported

Analysis of Data

Completed Randomized Design (CRD) was employed as the experimental design with three replications. The data was analyzed using Statistical Analysis System (SAS) software (University version 9.4).One way repeated ANOVA procedure was carried out and means were subjected to post hoc Duncan's Multiple Range Test (DMRT) to find out significant differences in the means at $p \leq 0.05$ level.

RESULTS

From the result of the study four fungal species were found to be associated with the rotten of *I. batatas* namely *Aspergillus niger*, *Aspergillus flavus*, *Penicillium spp*, *Fusarium oxysporum* and *Rhizopus stolonifer* (Table 1). From the findings of the study *Aspergillus flavus* was found to be more abundance with occurrence frequency of 45%, *Aspergillus niger*30%, *Penicillium* spp23.3 %, Fusarium oxysporum20.0 % and *Rhizopus stolonifer* have the least frequency of occurrence at 10% (Table 1). From the pathogenicity carried out *Aspergillus flavus* was found to have highest pathogenic inhibition zone of 65 mm, followed by *Fusarium oxysporum* (56 mm) and the least pathogenic zone was recorded from *Rhizopus stolonifer* with 12 mm (Table 1).

Table 1: Frequency of occurrence and Pathogenic Decay Rate of collected *I.batatas*

S/N	Identified Fungi	Frequency of occurrence	Pathogenicity Decay Rate (mm)
1	*Aspergillus niger*	30.0^{b}	25
2	*Aspergillus flavus*	45.0^{a}	65
3	*Penicillium spp*	23.3^{c}	12
4	*Fusarium oxysporum*	20.0^{c}	56
5	*Rhizopus stolonifer*	10^{d}	12

Note: S/N= Serial Number. Values with same alphabet have no significant difference at $p \leq 0.05$.

The data obtained from the study revealed the following morphological appearance of the fungi from the examined collected *I. batatas* from selected farms and Kaduna market respectively. *Aspergillus niger* was found to be dark velvet brown which later change to black, *Aspergillus flavus* was seen to be yellow to greenish bordered with whitish, *Fusarium oxysporum* were observed to be purple with violet, whitish and later change to peach color, *Penicillium* were document in the present study to be fluffy whitish color and *Rhizopus stolonifer* were initially recorded to be whitish and later change to brownish (Table 2).

Total of hundred respondents were interviewed. Seventy nine are males and twenty one are females (figure 2). Fifty of the respondents interviewed were farmers, twenty five are farmers and vendors and twenty five are vendors of *I. batatas* (figure 3). Only respondents with minimum of five years were interviewed. All the respondents interviewed have attend basic level of education excepts nineteen (figure 4). They either spend minimum of five years farming or selling of *I. batatas*. All the respondents reported only identification of rotting *I. batatas* when it is physically damage as shown in (figure 1).

Table 2: Macroscopic and Microscopic Appearance of examined *I.batatas*

S/N	Pathogenic Fungi	Macroscopic Appearance	Microscopic Features
1	*Aspergillus niger*	Velvet dark brown to black colour	Conidial heads are large, globose and dark brown.
2	*Aspergillus flavus*	Surface is greenish-yellow colour and may have a white border.	Septate hypha with long coni diospores which have rough texture and spiny bellow the vesicle.
3	*Fusarium oxysporum*	Violet purple pigment, whitish or peach tinge.	Hypha were hyaline and Septate. Micro conidia are septate, conidiophores are branched.
4	*Penicillium*	White fluffy luxuriant growth	Ellipsoidal conidia which are cylindrical. Septate hyphae, with simple conidiophores
5	*Rhizopus stolonifer*	White colon and later brownish grey to Blackish	Smooth sporangiospores, walled, hyphae non septate and powdery in appearance are observed

Note: S/N= Serial Number

Figure 1: Arrow indicating the physical damage of *I.batatas*

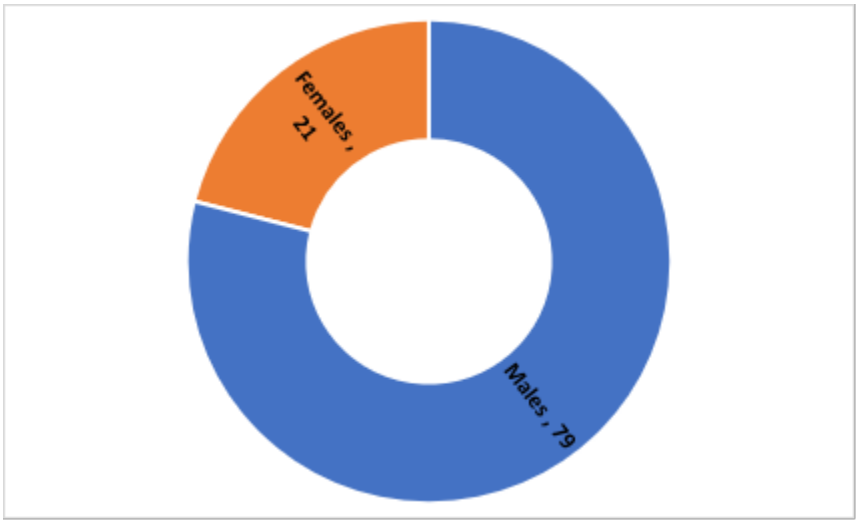

Figure 2: Respondents gender profile

Figure 3: Respondents occupational profile

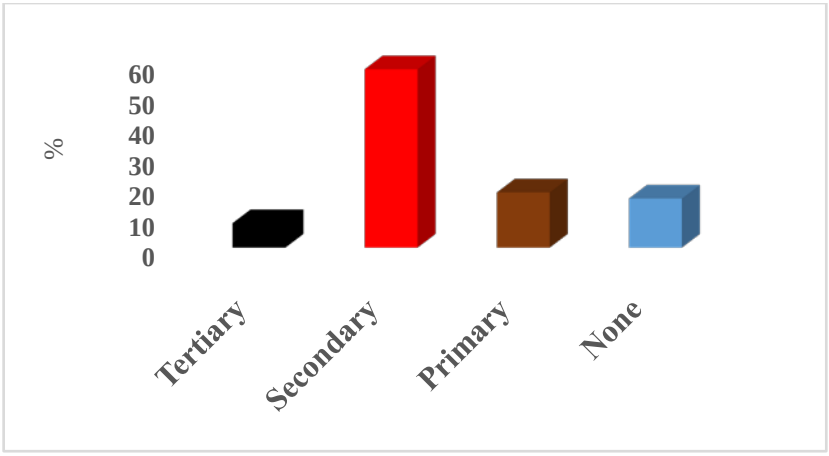

Figure 4: Respondents educational profile

The study also tries to establish if there are any methods employed by the farmers or vendors in storage of the *I. batatas* to avoid its early spoilage. The respondent reported keeping of *I. batatas* in room temperature 60ºC at least for a period of two to four weeks. Information consensuses factors was determined to know the agreement of the respondents on the usage

of particular for preservation of *I. batatas* with the value 0.8901.

DISCUSSION

Ipomoea batatas is a vegetable that is delicious and versatile that high possesses nutritional contents (Mohanraj and Sivasankar, 2014). It is a medicinal plant with antidiabetic, anti-cancer and anti-inflammatory potentials (Mohanraj and Sivasankar, 2014). *Ipomoea batatas* (Sweet potato) from the family Convolvulaceae, *Ipomoea batatas* is one of the major root crops cultivated and consumed in the world (Mukhopadhyay et al., 2011). Cassava and yam are the major leading important tuber crop in Nigeria followed *I. batatas* which were also known to be African staple food (Khatoon et al., 2017). This particular tuber crop is consumed freshly in some parts of Nigeria or processed before eating by boiling or steaming. *Ipomoea batatas* is also processed to starch or flour. Food spoilage is described as changes, damages, loss of its desired physical or nonphysical qualities that renders it unfit for human consumptions (Nweke, 2015). Deteriorations of *Ipomoea batatas* caused by invasion of microbial at post-harvest is one of the major reasons responsible huge lost for the unachievable its long term storage (Nweke, 2015). Fungi has been the major causal agents for the spoilage of economically vegetables and fruits all over the world (Ilondu, 2013; Nweke, 2015; Udoh et al., 2015). The present study has found and documented five strains of fungi responsible for the deterioration of *I. batatas*. The study is in agreement Khatoon et al. (2017) where they report also five strain of fungi (*Aspergillus niger, Aspergillus flavus, Geotrichum candidum, Fusarium oxysporum, and Rhizopus oryzae*) on the studies of fungi associated with the storage of Sweet potato in India. *Aspergillus flavus* was reported with high frequency of occurrence in the present study. The study disagreed with Khatoon et al. (2017) whrere the reported *Rhizopus oryza* with highest percentage of occirance. One of the reason for the deteriontion of the *I. batatas* might be a result of the damage incured during harbvest and transportation which has a detrimental effect on the storage (Tortoe et al., 2010). Damaged on the *I. batatas* leads to the lost of moisture and an avenue for the entrance of microbial organisms (Coyne and Affokpon, 2018). Application of fungicide, improper pre harvest, poor handling or washing method inadequate culling of vegetables results in the infestation of spoilage bacteria or fungal which lead to the damage of the fruits or vegatables (Udoh et al., 2015). The pathological deacy or spoilage of the *I. batatas* was due to the post harvest latent infection that later become active during storage was due to the presence of the pathological microorganisms coupled with the conditions of the enviroments makes it deteriot in a short period of time. Tortoe et al. (2010) suggested a proper handling method and preservation has has to be proposed. *Ipomea batatas* should be handle properly at the time of harvest, storage and distribution in order to minimse bruising, injuring and cull all kind of physical or non physical diseases on the harvested products.

Survey was caried out to determine from the respondents on how they handle I.batas from the time of harvesting to processing. Previously Abdulrahman et al. (2018) carried out survey in other to established of utilisation of medicinal plants around their immediate community. The ICF value showed that the respondents have a uniform agreement on the methods used for preservation of *I. batatas*, with value 0.8901. The ICF values were high due to the facts, belief of the respondents on the efficiency and best methods of preservation after harvesting. In similar way Abdulrahman et al. (2018) used ICF to determine respondents agreement on the type of medicinal plants used for treatments of ailments.

CONCLUSSIONS

Deterioration of *I. batatas* was sole responsible of fungal infection at the time of harvest. Study recommends careful handling process of *I. batatas* should be employed. Herbicidal should found from plant origin, nontoxic for the spray of the *I. batatas* to avoid mycotoxins contamination that led deterioration of the *I.*

batatas. Further studies should also be carried out to confirm the taxonomic identification of fungal species isolated to the molecular level.

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