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Isolation and Identification of Fungi Associated with Post Harvest Spoilage of Sweet Potatoes (Ipomoea Batatas)

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Abstract

Sweet potato is a traditional food consumed by Bida people of Niger state but is recently observed to be in short supply. Two samples of five replicates each of decaying sweet potato were obtained randomly from small market (SM) and old market (OM), Bida and were investigated mycologically. The potatoes were homogenized and serially diluted to thin out the fungal population. The fungal load ranged from $4.2x10^{-5}$ x 10 CFU/g and the fungal isolates include Aspergillus niger, Aspergillus flavus, Mucor species, Fusarium species and Saccharomyces species. Percentage incidence of occurrence ranged from 1.3% for mucor to 79% for Saccharomyces. The result of the pathogenicity test showed that the isolates inoculated into the healthy tubers had the same features that fungi were responsible for the spoilage.

Keywords: Isolates, Potato, Fungal, Spoilage, Aspergillus.

Introduction

Sweet potatoes (ipomoea battues lam) belongs to the Convolvulaceae family and is a root crop cultivated in many countries including Nigeria, Sierra Leone, Ghana. Sweet potatoes is an important food crop in Nigeria ranking third among important tuber crops of sub -Saharan Africa, after yams second to cassava, the most important tropical root crop and are a staple crop in many parts of Africa and south east Asia (Amieyo,2007 and ogbo,2014). Sweet potato is grown in general for its storage root, which are eaten fresh, sterned or boiled. Sometimes the leaves are eaten as vegetables or may be processed into flour or starch. The vines are feed to livestock (Wu *et al.*,2004).

Sweet potatoes *(ipomoea batatas lam*) are groups in over one hundred different countries in tropical and sub-tropical regions. The production of sweet potatoes across the world is estimated to approximately 140,903000 t/a and 92% of these are reportedly produced in Asia and the pacific island (FAO, 2002). The tubers vary greatly in size, shape, color and quality of taste, depending on the variety. When the roots are stored, they are sensitive to changes in soil temperature, which is largely dependent on their storage stage of root development (William *et al.*,1980). Storage of sweet potato tubers after harvest is Imperative as this practice may prevent a surfeit of potatoes entering the food markets at any given time and prolong the period of fresh tuber availability especially when the crop is not in season or when the economic circumstances and / or required climates in a particular area of production dictate its production during the year. Food Spoilage is a metabolic process which may be brought about by microbial action and causes foods to be undesirable or unacceptable for human consumption due to deterioration in quality characteristics (Doyle, 2007). Different species of sweet potatoes have gained popularity in many countries as a result of their health benefit (leksrisonpong *et al.*,2012). Therefore, early intervention measures during crop development and harvesting

through the use of good agricultural practices will provide dramatic reduction in yield loss due to spoilage at all subsequent steps. (Barth *et al.,*2009). Food security and sustainability is one of the crops of addressing this problem of scarcity and shortage due to activities of Microorganisms after harvesting. In a FAO/WHO report of (2012), food security was defined as a situation in which all people at all times have both physical and economic access to adequate and nutritious food for an active and healthy life; the manner in which the food is produced, preserved and distributed are in a consideration of the natural processes of the earth and thus sustainable thus reducing spoilage, scarcity and malnutrition and poverty.

According to the survey carried out in Iran, 10% Pre harvest and 20% postharvest rots occurred in sweet potato (Bidarigh *et al.,*2012). These rots constitute major impediment to the drives for food security in Nigeria. It has been noted that the largest post-harvest losses in vegetable crop tubers result from microbial attacks (Ray *et al.,*2000). Reports are available on the fungi associate with spoilage of sweet potatoes during harvest storage. (Ray *et al.*2000; Doyle, 2007).

Oke *et al* (1999) and hull (1993) Postulated cropping method and increased crop cultivation as a necessity to facilitate the required long-term storage of roots, thereby meeting the world food requirements during intervening reasons. Rapid deterioration in the condition of the produce is a result of exposure to environmental conditions and is exacerbated by mechanical damage during handling and transport (Rees *et al.,*2003).

Fungai disease which can afflict sweet potatoes surface rot and root rot caused by various species of the Genus *Fusarium*; soft rot caused by the fungus *Rhizopus stolonifer*; jawa black rot caused by the fungus *Diplodia gossypina;* scurf disease caused by the fungus *monilochaetes infuscans;* black rot of the tuber which is caused by the species *Ceratocystis fimbriata* among others. The activities of the spoilage causing organisms are usually supported by microbial enzymes, which are secreted into the sweet potato tubers. These enzymes constitute the main agent of the deterioration, and it is also worth noting that enzymes of microbial origin have been exploited in food and medical research, paper and textile industries as well as in waste utilization and biotechnologies (lima *et al.,* 1997).

The shelf life of sweet potato roots varies from a few days to a few weeks according to the cultivar, as well as the condition prevailing at the time of harvest and during storage (Balagopalan *et al.,* 1991). Many methods have been used in the postharvest preservation of sweet potato with the singular aim of preventing deterioration and there by prolong shelf life. These methods include but are not restricted to curing under controlled temperature and relative humidity, the use of disinfectants and cold storage. These methods are often use in Isolation or in limited combination in other to impede the progress of spoilage agents. However, it is evidence that these approaches have not achieved the anticipated or desired results in terms of preventing post-harvest spoilage of sweet potatoes or prolonging their shelf life.

Sweet potatoes have been described as having thin, delegate skin that is easily damaged by cuts and abrasion during harvesting, transportation or distribution. Striking roots with harvesting equipment's or dropping them into containers injures their skin. The sweet potato may be cut or bruised if they are placed in containers having sharp edges or roughly hauled or handled, and these may give rise to microbial infestation (Rupsa *et al.,* 2017).

Therefore, this study examines the isolation of fungi associated with postharvest spoilage of sweet potato (*Ipomoea batata*)

Sample Collection

Replicate sample of decaying sweet potatoes were obtained at different locations, small market (SM) and Old market (OM) in Bida metropolis. The samples were collected and labelled according to source of collection in a sterile polythene bags and brought into the laboratory the Department of Biological sciences, The Federal Polytechnic, Bida for analysis.

Materials Used

The glass that were used in this research includes; petri plates, conical flask (1000ml), measuring cylinder (1000ml), pestle and mortal, glass slides and cover slide. Chemicals used include; potato dextrose agaqr (PDA), ethanol and streptomycin. Other materials used are: microscope autoclaves, hot plates, aluminum foil, distilled water, making tape, Bunsen burner, facemask, spatula, weighing balance, inoculating needles, hand gloves and cotton sterilization of glass wares.

All the glass wares were first washed with tap water and detergent solution. They were then rinsed distilled water and air dried. The glass petri-dishes were wrapped with aluminum foil and autoclaved at 160°c for pone hour (1hr). they were allowed to drop for 30 minutes before usage to avoid cracking.

Preparation and sterilization of media

Thirty-nine grams (39g) of potato dextrose agar was weighed on weighing balance and was mixed with 1000ml of distilled water in a conical flask. The conical flask was placed on a heating mantle for homogenization. After homogenization, 4ml of streptomycin (0.6g per liter) was added aseptically (after the medium has cooled) to the molten PDA to inhibit the growth of bacteria.

Preparation of samples

Cotton wool moistened with 70% ethanol was used to sterilize the workbench, hands and knife. The sterile knife was then used to cut the sample open and the decaying inner part was cut into pieces. A sterile pestle and mortar were used for homogenization. A weighing balance was then used to weigh one gram of the homogenized solid.

Isolation of organisms from sample

Using the method of Harrigan and Mc cane (2005), one gram of sample was poured into 9ml of sterile distilled water and the tube was shaken gently to ensure thorough mixing. After, mixing, a serial dilution was then carried out by transferring 1ml from the first test tube into the second test tube that contains 9ml of distilled water. The second tube was mixed gently and 1ml was taken from the second tube into the third – test tube and so on till the fifth test – tube and 1ml was discarded.

Isolation of organisms was done using the pour plate method (Harrigan and Mc cane, 2005). One milliliter (1ml) each from the third test-tube was pipetted using new sterile pipette into two empty sterile petri- dishes. Also, 1ml Each was pipetted from the fifth test – tube and poured into two empty sterile petri-dishes.

After the sample have been poured into petri-dishes, the media were poured into their respective plates (about 15ml was poured into the plates). After the plates had set, the PDA plates were incubated at 25°c for 3- 5 days.

Representative colony types were purified by sub – culturing on fresh PDA plates. Pure cultures were transferred to slants of PDA. Pure cultures of the isolate were grown singly on PDA. For identification. The isolated fungi were identified based on the isolates colonial characteristics on culture plates and microscopic features in slide cultures. Using a sterile inoculating needle portion of

each mycelial colony was aseptically taken and placed on a clean microscopic slide and teased in a drop of lacto-phenol cotton blue stain and placed on a clean grease- free slide. A small portion of the filamentous part of the fungal isolates was emulsified in the stain. Then, the slide was covered with a coverslip avoiding bubbles. The slide was then viewed under the microscope. This technique shows various microscopic characteristics of the fungal isolates (onuorah etal 2015).

Pathogenicity test

Fresh and healthy tubers were washed with tap water and surface sterilized with 0.1% mercuric chloride solution for 2-3 minutes. Cylindrical cores were removed from the tubers with the help of 5mm cork borer. Four millimeters (4mm) agar disc containing 7 days old cultures of the isolated were introduced into the holes and sealed with the sterile Vaseline. Uninoculated tubers (control) were also set up (Okigbo and Emeka,2010). Except that the inocula consist of uninoculated potato dextrose agar blocks. All the treated tubers were put singly into sterile polythene bags and incubated at 28+- 2^{O}C for 7 days. At one-week intervals, the sample were sectioned through the site of inoculation and examined for lesion development. Infected or decayed portions were aseptically transferred onto PDA to confirm that the infection was caused by the inoculants.

Determination of percentage occurrence of isolates

This was done to determine the percentage occurrence of different fungal isolates. Kisolations were made from four different rotted banana fruits and were cultured differently. The number of occurrence for each of the isolates in the four different samples were recorded and calculated as a ratio of occurrence and was then expressed as a percentage. The formula of Mohammed et al, (2004) was followed Percentage colonization = Number of colonies for a pathogens/total number of colonies x 100

Results

The fungal load mean value of two different samples of sweet potatoes obtained within Bida metropolis is presented in Table 1. The total fungi count was measured in colony forming unit per gram (CFU/g). The morphological characteristics of the fungal isolates were shown in Table 4.

The percentage frequency of occurrence of the isolates from the two samples of five replicates are shown in Table 2 and 3. Among the fungi isolated from sweet potatoes from the small market, *Aspergillus flavus* appeared most frequently followed by Fusarium. On the other hand, the isolates from old market showed that Fusarium species occurred most frequently followed by Aspergillus species. On the whole yeast were isolated and they appeared to be the most frequently occuring organisms in the both samples investigated (Table 2 and 3). The diameter of rots got from pathogenicity test is presented in Table 5.

Table 3. The average fungi count of the spoilt potato tuber samples in CFU/g.

Sample	Fungal Load Counts	Mean Value of Count	Fungi Isolate
Small Market	4.2×10^{5} - 5.4×10^{5} cfu/g^{5}	4.8×10^{5} cfu/g	*Aspergillus niger* Aspergillus flavus, Mucor species, Fusarium species, Saccharomyces.

Old Market (OM)	4.2×10^5 - 4.6×10^5 cfu/g	4.4×10^5 cfu/g	*Aspergillus species, Mucor species Fusarium species, Yeast.*

Table 4: Percentage frequency of occurrence of the isolates from small market (SM).

Sample	Isolates	Incidence of fungi isolates	% frequency of occurrence
Small market	Aspergillus niger	4	5.3
	Aspergillus flavus	6	7.9
	Mucor species	1	1.3
	Fusarium species	5	6.6
	Saccharomyces species	60	79
		76	

% frequency = $\frac{Nmber\ of\ Isolates}{Total\ number\ of\ Isolates}\ x\ \frac{100}{1}$

Table 5: Percentage frequency of occurrence of the isolates from old market (OM).

Sample	Isolates	Incidence of fungi isolates	% frequency of occurrence
Small market	Aspergillus niger	5	9.4
	Mucor species	2	3.8
	Fusarium species	6	11.3
	Yeast species	40	75.5
		53	

% frequency = $\frac{Nmber\ of\ Isolates}{Total\ number\ of\ Isolates}\ x\ \frac{100}{1}$

Table 6: Morphological identification of fungi isolates.

Isolates	Microscopic features
Aspergillus niger	The hyphae are septate consisting of a compact white basal felt with a dense layer of dark brown to dark conidiophores conidia head radiate tending to split into loose columns with age.
Aspergillus flavus	Consist of septate hyphae, conidiophores are dense felt or yellow green. Conidial heads radiate, later splitting into several loose columns, yellow green becoming dark green yellow green.
Mucor species	Colonies are whitish to olivaceous-bluff, odour aromatic, in the dark differentiated into tall and short sporangiosphores. Sporangia blackish with ellipodial sub glucose chlamydosphores.
Fusarium species	Multicellular distinctive sickle shaped conidia which were septate.
Saccharomyces species	Appear in green flat circular colony. Spherical spores often in group of fours. Appear spherical to avoid in shape. Have simple psuedohyphae.

Table 7: Decay diameter of the isolates in healthy sweet potato tubers in millimeters.

S/N	Fungi isolates	Diameter of rots (mm)
1	Aspergillus niger	30
2	Aspergillus flavus	25
3	Mucor species	10
4	Fusarium species	15
5	Saccharomyces species	35

Discussion

The research work has shown that the fungi associated with rots of sweet potato tubers in Bida in Bida local government area of Niger State were Aspergillus niger, Aspergillus flavus, Mucor species Fusarium species and the yeasts. Some of these results are similar to the findings of Gambari and Gambari and Okinedo, 2020 who identified Aspergillus niger, Mucor species, Fusarium species and Penicillium species were responsible for postharvest rots of sweet potato tubers. Anaienyo and Ataga, 2007 reported that Aspergillus flavus is the most frequently isolated fungus from spoilt sweet potato tubers in South West Nigeria. The results of this study are in agreement with the findings of other researchers (Agu *et al.*, 2014) that fungi constituent menace in storage rots of many agricultural commodities. Salami and Popoola, 2007 reported that Fusarium as the most virulent among the fungi associated with storage rots of sweet potato tubers in South Western Nigeria. This is probably close to the findings in this work where Fusarium species had percentage frequency of occurrence 11.3%. The results of pathogenicity of the identified isolates showed that isolates inoculated into the healthy potato tubers had the same features as the one re-isolated from them indicating that the fungi isolates in this work were responsible for the spoilage. The overall effect of fungi spoilage of the potato tubers leaks to hydrolysis of the starch in the tubers leading to elaboration of a lot of simple sugars for carbon and energy sources and subsequent colonization by yeasts typically Saccharomyces cerevisiae. Postharvest rots of sweet potato tubers maybe due to low pH, moisture content and nutritional composition which make it susceptible to infection by fungi. The high Incidence of storage rots of sweet potato tubers maybe related to prevailing climatic factors, storage conditions, and poor handling procedure during harvest, transit and marketing. Postharvest loss will continue to be a serious problem to Farmers and food insecurity at large.

Conclusion

Fungi associated with poor harvest rots have been identified as species of *Aspergillus niger*, *Aspergillus flavus*, *Saccharomyces cerevisiae*, Mucor and Fusarium. Evidence from this work has shown that they cause reduction in the availability of sweet potato in circulation.

In order to mitigate the rate at which these fungi cause deterioration of sweet potato, several control measures have to put in place for example proper washing of the harvested tubers, cleaning of transit containers, proper handling of the tubers in order to avoid injuries, good hygienic practices by the handlers, provision of good and healthy facilities and many more.

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