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Full Length Research Paper

Isolation and identification of microorganims associated with yam rot of yam sold in Bauchi markets, Nigeria

Suleiman M. A.[1*], Agbo E. B[2] and Wasa, A. A.[1]

[1]Department of Biological Sciences, Faculty of Science, Gombe State University, Gombe, Nigeria.
[2]Biological Sciences Programme, School of Science, Abubakar Tafawa Balewa University Bauchi, Bauchi State, Nigeria.

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The present investigation was carried out to isolate and identify microorganisms associated with yam rot. Yams with symptoms of rot were collected from three markets Yelwa market, Wunti market and Muda Lawal market within Bauchi, the species of yam identified was *Dioscorea rotundata* (white yam). The markets were visited four (4) times and three yam sellers from each market were interviewed. The percentage of yams lost to microbial attack ranged from 33.7-36.0%. The fungi isolated included *Aspergillus flavus, Aspergillus niger, Botrydioplodia* sp. and *Rhizopus oryzae* with frequency of occurrence of 29.33, 30.67, 22.67 and 17.33%, respectively and the bacteria isolated included *Micrococcus* species, *Bacillus cereus, Proteus vulgari, Clostridium welchi* and *Pseudomonas aeruginosa.* The microorganisms isolated were major soil microorganisms penetrating through the wound in the tuber which might have been caused by insect, nematode or poor handling before, during or after harvesting. Microbial attack caused huge lost to farmers and yam traders but the level of infections could be brought to minimal by planting healthy seeds, avoid contamination during and after harvesting, and yam tubers should be stored under optimum storage conditions.

Key words: Yam rot, market, isolated microorganisms.

INTRODUCTION

Yams (*Dioscorea* spp.) belong to the family *Dioscoreceae* and to the genus *Dioscorea* (Coursey, 1967; Kay, 1987). The most cultivated yam species in Nigeria are the *Dioscorea rotundata* (white yam), *Dioscorea cayenesis*, (yellow or guinea yam) and *Dioscorea alata* (water yam). There are also species of wild yam growing in Nigeria whose tubers are collected for eating in times of food shortage (Amusa, 1999). Yams are perennial herbaceous vines which are cultivated for the consumption of their starchy tubers and valuable source of carbohydrate to the people of the tropical and subtropical Africa, Central and South America, parts of Asia, the Caribbean and Pacific Islands (Coursey, 1967; Adelusi and Lawanson, 1987).

They are a primary agricultural commodity in West African and New Guinea. They were first cultivated in Africa and Asia about 8000 BC. Due to their abundant and consequently, their importance to survival, the yam was highly regarded in Nigerian ceremonial culture and used as a vegetable offered during blessings.

The word "*yam"* is related to Portuguese *inhame* or Spanish *ñame*, which both ultimately derive from the Wolof word "*nyam"*, meaning "to sample" or "taste". In Nigeria, each different language has different name for Yam, "*Isu*" is the Yoruba translation or "*Iyan*" when it is prepared to be consumed as a main course for dinner, In Hausa language, it is called *"doya*" Yams are the staple crop of the Igbo people of Nigeria, in their language it is

*Corresponding author. E-mail: suleimanmuhammedabubakar@gmail.com.

known as *ji*, and they commemorate it by having yam festivals known as *Iri-ji* or *Iwa-Ji* depending on the dialect. *Dioscorea* spp constitutes a staple food in the tropics (Han et al., 1987). Yams after peeling the tuber can be cooked in various ways by boiling and mashing, barbecuing, but roasting and frying are also widely used, they are pounded into paste to make traditional dish called fufu. Yam in Nigeria is also processed into various staple, intermediate and end product forms (Okaka et al., 1991; Okaka and Anajekwu, 1990), which are used for direct consumption by animals, used as the basic ingredient for snacks or made into flour used for making instant puree (Coursey, 1983; Okaka and Okechukwu, 1987). Out of the World production of over 30 million tonnes per annum, Nigeria alone produces 22 million tonnes (FAO, 1998). Despite this, the demand for yam tubers in Nigeria has always exceeded its supply. However, it has been estimated that an average of over 25% of the yield is lost annually to diseases and pests (Arene, 1987; Ezeh, 1998; FAO, 1998). Onayemi (1983) also reported that over 50% of the yam tubers produced and harvested in Nigeria is lost in storage.

MATERIALS AND METHODS

A total number of three yam tubers with symptoms of rot were obtained from three major different markets such as Yelwa market, Wunti market and Muda Lawal market within Bauchi metropolis. One yam tuber was obtained from each of these markets. The diseased yam tubers obtained were labeled using the initial names of the markets from which they were obtained. The yam tubers were packed in a sterile polythene bag and transported to the school laboratory for further analysis. The collected yam samples were taken to School of Agricultural Sciences for identification.

Isolation of fungal pathogens

The methods of Jha (1995) and Ogaraku and Usman (2008) were adopted. The yams' fleshes were removed using sterile knife Small sections of yam tissues containing the advancing margin of rot and adjoining healthy tissue were cut open with a sterilized knife surface sterilized by immersion in 0.1% mercuric chloride solution for 1-2 min and rinsed three times in sterile distilled water. It was then blended using sterile blender. One (1) g from the blended sample was then placed in 10 ml of distilled water and mixed thoroughly to give a good homogenous solution, it was then used as stock. The stock solution was inoculated on a solidified Potato Dextrose Agar (PDA) agar, and incubated at 27ºC and observations made daily for possible fungal growth for 7 days. Sub-culturing was done to obtain pure cultures of the isolates.

Identification of isolated fungi pathogens

The method of James and Natalie (2001) was adopted. The identification of the fungi isolates was done by examining the isolates:

Macroscopically: The colony characteristics, spores, mycelium either septate or not, sporangia and conidia were taken note of. These structural features were matched with standardized one (Figure1).

Microscopically: A cotton blue in lactophenol was used. A drop of the stain was placed on a clean slide with the aid of a mounting needle; small portion of the mycelium from the fungi culture was removed and placed in the drop of the lactophenol. The mycelium was spread very well on the slide with the aid of the needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and observed with x10 and x40 objectives respectively under the microscope.

Isolation of bacteria pathogens

The method described by Ibrahim (2008) and WHO (1998) were adopted. Several dilutions of the stock solution as described for fungi above were achieved up to five (5) folds (10^{-5}), for each prepared sample. One (1) ml from the stock homogenate was serially diluted with 9 ml sterile distilled water.

The bacterial pathogens were isolated using the pour plating method. One (1) ml was taken from 1-5 folds dilution and dispensed in Petri dishes containing the media (ManConkey agar and nutrient agar) and introduced into clean sterilized Petri dishes, this was then followed by pouring out the media which had been allowed to cool down to temperature of about 45ºC and swirled. Thus they were allowed to set firmly for five (5) min afterward inoculated plates were incubated at 37ºC for 24 h.

Identification of isolated bacteria pathogens

Characterization and identification of the colony isolates were achieved by initial morphological examination of the colonies in the plate (macroscopically) for colony appearance, size, elevation, form, edge, colour, odour, opacity, and pigmentation. Colonies were selected at random and subcultured to obtain pure isolates on fresh plates and then incubated at 37°C for 24 h. The stock cultures were obtained, labeled carefully. Biochemical tests were also conducted on the isolated organisms for proper identification.

RESULTS

The percentage of yam lost to microbial attack ranged

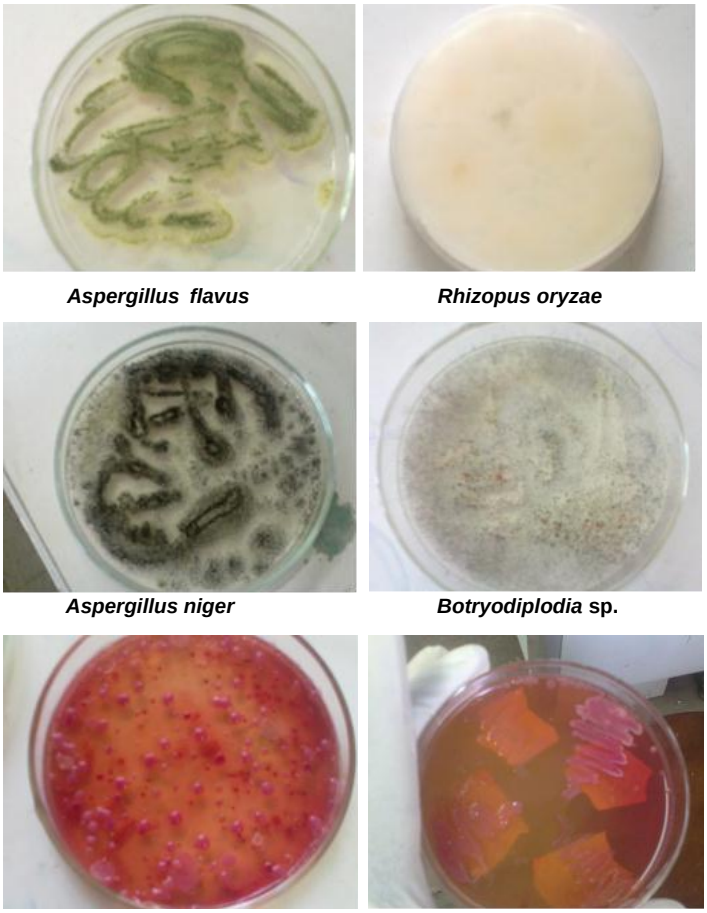

Figure 1. Colony morphology of microrganisms isolated.

Table 1. Percentage of spoilage caused by Microorganisms per 100 tubers of yam.

Market	No. of storage Visited	No. with Microbial infection	Percentage (%)
Yelwa	3	23	30.7
Wunti	3	25	33.3
Muda Lawal	3	27	36.0

from 30.7-36.0 (Table 1). The species of fungi isolated and identified from the yam samples collected were *Aspergillus flavus, Aspergillus niger, Botryodiplodia* sp. and *Rhizopus oryzae* (Table 2). The frequency of occurrence of the fungi isolated ranged from 17.33-30.67% (Table 2). Five species of bacteria were also isolated and identified and these include *Bacillus cereus, Pseudomonas aeruginosa, Clostridium welchi, Proteus vulgaris* and *Micrococcus* species (Tables 3, 4 and 5).

DISCUSSION

The species of yam identified and used for this study was *Dioscorea rotundata* (white yam). The survey had shown that 30.7 - 36.0 % of yam tubers were lost in storage due to microbial attack (Table 1), the result obtained agreed with the work of Arene (1987) and Ezeh (1998) which showed that an average of over 25% lost of yield is lost to diseases.

It was obvious from the study that wide range of microorganisms is responsible for the storage rot of yam and these include both the fungi and bacteria. The fungi isolated and identified from the yam samples were *Aspergillus flavus* 29.33%, *Aspergillus niger* 30.67%, *Botrydioplodia* sp. 22.67% and *Rhizopus oryzae* with frequency of occurrence of 29.33, 30.67, 22.67 and 17.33%, respectively (Table 2). The results showed slight different from those isolated and identified by Ogaraku and Usman (2006) (*Aspergillus flavus* 19.3%, *Aspergillus niger* 38.6%, *Rhizopus stolonifer* 18.1%, *Sclerotiumrolfisii*

Table 2. Frequency of occurrence of fungi isolates.

Isolated organism	No. of occurrence			Total	Percentage (%)
	YM	MM	WM		
Aspergillusflavus	9	5	8	22	29.33
Aspergillusniger	7	10	6	23	30.67
Rhizopusoryzae	6	3	4	13	17.33
Botryodiplodia sp.	4	6	7	17	22.67

Table 3. Colony morphology and biochemical characteristics of bacteria isolated from Yelwa market.

Colony	Cultural morphology	Gr	Microscopy	Mo	Ca	Co	Cit	Ind	Ur	Gl	Su	La	H2S	Probable genus
A	Moderate, raised colony	+	Cocci in pairs and tetrads	-	+	-	+	-	-	-	-	-	-	*Micrococcus sp.*
B	Raised, circular, colourless colony	+	Long rod in cluster	+	+	-	-	+	-	-	-	-	-	*B. cereus*
C	Flat, spreading, rough, translucent colony	+	Rod shaped cells	-	-	-	+	-	-	+	+	+	+	*C. welchi*
D	Large, smooth, mucoid colony	-	Rod shaped cells	+	+	-	-	-	-	+	+	+	+	*P. aeruginosa*

Table 4. Colony morphology and biochemical characteristics of bacteria isolated from Wunti market.

Colony	Cultural morphology	Gr	Microscopy	Mo	Ca	Co	Cit	Ind	Ur	Gl	Su	La	H2S	Probable genus
A	Moderate, raised colony	+	Cocci in pairs and tetrads	-	+	-	+	-	-	-	-	-	-	*Micrococcus sp.*
B	Raised, circular, colourless colony	+	Long rod in cluster	+	+	-	-	+	-	-	-	-	-	*B. cereus*
C	Confluent swarming *colony*	-	Rods in pair and single	+	+	-	+	+	+	+	+	-	+	*P. vulgaris*
D	Flat, spreading, rough, translucent colony	+	Rod shaped cells	-	-	-	+	-	-	+	+	+	+	*C. welchi*

Table 5. Colony morphology and biochemical characteristics of bacteria isolated from Muda Lawal market.

Colony	Cultural morphology	Gr	Microscopy	Mo	Ca	Co	Cit	Ind	Ur	Gl	Su	La	H2S	Probable genus
A	Moderate, raised colony	+	Cocci in pairs and tetrads	-	+	-	+	-	-	-	-	-	-	*Micrococcus sp.*

B	Raised, circular, colourless colony	+	Long rod in cluster	+	+	-	-	+	-	-	-	-	-	*Bacillus cereus*
C	Confluent swarming *colony*	-	Rods in pair and single	+	+	-	+	+	+	+	+	-	+	*Proteus vulgaris*
D	Flat, spreading, rough, translucent colony	+	Rod shaped cells	-	-	-	+	-	-	+	+	+	+	*Clostridium welchi*
E	Large, smooth, mucoid colony	-	Rod shaped cells	+	+	-	-	-	-	+	+	+	+	*Pseudomona aeruginosa*

Key: WM = Wunti market, **YM =** Yelwa market and **MM =** Muda Lawal market
Key: Gr = Gram reaction, **Mo =** Motility test, **Ca =** Catalase test, **Co =** Coagulase test,
Cit = Citrate utilization, **Ind =** Indole test, **Ur=** Urease production, **Gl =** Glucose utilization, **Su =** Sucrose utilization
La = Lactose utilization.

20.0%, *Fusarium oxysporium* 7.2% and *Rhizoctonia sp*. 4.8%).

The bacteria isolated and identified based on their macroscopic, microscopic and biochemical characteristics were *Micriococcus* species, *Bacillus cereus, Proteus vulgari, Clostridium welchi* and *Pseudomonas aeruginosa* (Tables 3, 4, and 5), no earlier work was carried out on bacteria associated with rot of yam. Therefore, result was not compared with any previous work. The organisms isolated and identified are major soil microorganisms and they played important role in losses of yam in storage. The microorganisms penetrate through the wounds in the tubers and infect the inner tissues. Such wound could have been caused by insects, nematodes, poor handling before, during and after harvesting.

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