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Isolation and Identification of Pathogens Associated with Postharvest White Yam (*Dioscorea rotundata* L) Tuber Rot

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Tubers of white yam varieties (*Gbangu, Dan-Anacha, Hembakwase, Pepa* and *Amula*) showing symptoms of rot were collected from five farms in five local government areas of Zone "A" Senatorial District of Benue State, Nigeria and assessed for fungal and bacteria contents. Eight isolates (five fungi and three bacteria) were isolated from the samples and identified using morphological, cultural, physiological and biochemical tests as well as standard identification guide. The fungal isolates identified were *Aspergillus niger, Botryodioplodia theobromae,*

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Zygosaccharomyces bailli, Zygosaccharomyces rouxil and *Myrothecium verrucaria,* while *Klebsiella oxytoca, Serratia marcescens* and *Pseudomonas aeruginosa* were the bacterial isolates identified. Pathogenicity tests revealed that all the organisms induced rots in the apparently healthy white yam tubers after seven days of inoculation. The result also showed a significant difference ($p < 0.05$) in the average rot measurements among the different yam varieties with *Aspergillus niger* being the most prevalent; *Botryodioplodia theobromae*, the most virulent, while *Hembakwase* was the most susceptible white yam variety in the study area.

Keywords: Pathogens; pathogenicity test; virulent; morphology; culture; postharvest.

1. INTRODUCTION

White yam (*Dioscorea rotundata* L) is a monocotyledonous plant that belongs to the genus *Dioscorea* and the family *Dioscoreaceae* [1]. It is an important food crop in West Africa and other tropical countries, including East Africa, Central Africa, the Caribbean, South America, South East Asia and India [1,2,3]. Yam tuber which is the thickened fleshy underground root serves as asexual reproductive organ or nutrient storage reserve for the dry months is one of the major sources of carbohydrates (about 78 %); minerals (phosphorus, calcium, iron, magnesium, potassium, sodium, zinc, copper and selenium); vitamins such as riboflavin, thiamine, niacin, pantothenic acid, and vitamins A, B6, C and E. It also contains large amount of water and fibre as well as small amounts of fats and proteins [4]. The yam tuber is the most economic part of the crop and is consumed roasted, boiled, pounded or fried as well as peeled, dried and made into flour for baking and steaming for swallowing with soup. It also serves as a source of raw material in the industry for the production of alcohol, starch, and other processed food products [5]. Additionally, white yam has considerable social and cultural significance among the people of South-Eastern and North-Central Nigeria.

White yam is widely cultivated in Nigeria, Ghana, Ivory Coast, Togo, Gabon, Central African Republic and Western parts of the Democratic Republic of Congo. These countries, referred to as “yam belt” produce about 93% of the World`s annual yam production, estimated at 38.5 million metric tons and Nigeria alone accounts for about 70 % (26.4 million metric tons) in the year 2000 and 36.72 million metric tons in 2006 [6,7]. Yam producing states in Nigeria include: Benue, Taraba, Nasarawa and Adamawa. Others are Cross River, Oyo, Delta, Edo, Ekiti, Kaduna, Kwara, Imo, Ogun, Ondo, Osun and Plateau states. Out of these, Benue state is said to be the largest yam producer with an average annual yam sale of 1.5 million metric tons [8].

Globally, about one-third of the food produced today is lost, wasted or discarded as a result of inefficiency in human-management food chain, which amounts to about 1.3 billion tons per year [9]. In addition, 30 to 40 % of the food crops produced in the world is not consumed as a result of damage, rotting as well as pest and diseases which affect crops after harvest [8]. It has been reported that over 25 % of the yams produced in the world is lost annually to diseases, pests, and nematodes [3,8], and in many African countries, including Nigeria, yam storage is still largely by traditional methods where postharvest losses can be as high as 50% [8,10]. Nigeria lost an annual average of 10% of her yam tubers between 1961 – 2009 [8]. The country recorded the highest yam lost in 2006 with over 3.7 million metric tons [8,11].

Post-harvest losses of yam tubers have been attributed to high temperature, insects, nematodes, rodents, respiration of the dormant tuber, loss of water by evaporation, sprouting and microbial attack with microorganism being the major culprit [12,13]. Fungi, bacteria, and nematodes are said to be the major causes of white yam tuber rot with fungi accounting for about 80 % of storage rot in West Indies and 57 – 77 % in Nigeria [10,14]. The wounding of yam tubers by rodents, nematodes, insects, and even man during weeding, harvesting and post-harvest handling makes it easy for fungi and bacteria to penetrate the tubers and cause diseases that could be transferred to the store leading to considerable quantitative loss in weight or volume and qualitative losses like reduced nutritional value, changes to taste, colour, texture or cosmetic features with the attendant adverse effects [15]. Substantial losses occur during prolonged storage of yam. Losses up to 10 – 20 % [13] may occur during the first 3 months and 30 – 60 % after 6 months of storage [16,17].

Microbial rot of white yam tubers can be grouped into dry rot, soft rot, and wet or watery rot depending on the rot symptoms, invading pathogen, and the infected tissue. Dry rot is characterized by infected tissues becoming hard and dry with different colourations depending on invading pathogens [18,19]. *Fasarium species (Fasarium oxysporium, Fasarium monilforme*, and *Fasarium solani)*, *Penecillium spp,* and nematodes such as *Scutellonema bradys* are reported to be the causative agents of dry rot [18]. Yam tubers showing symptoms of soft rot causes the infected tissue to become soft and in sometimes ramified by the fungal mycelium that turns the tissue brown and in some cases wet with the tendency to break off due to a rapid collapse of the cell walls [20,21]. Fungi responsible for Soft rot are *Armillariella mellea, Mucurcir cinelloides, Rhizoctonia solani* and *Rhizopus* spp [22]. In wet or watery rot, the external symptoms are not visible as the decay is internal and the infected tissue disintegrates into a watery mass or whitish fluid from the tissues which can easily be released on the application of a slight pressure. This type of rot is characteristic of bacterial infection such as *Erwinia carotovora* [5,19].

Other pathogenic fungi of white yam tubers include *Mucor mucedus, Aspergiulus flavus, Rhizopus stolonifera, Aspergulus niger, Aspegillus tamari, Botrydiplodia theobromae, Penicillium oxalicum, Penecillium chrysogenom, Penecillium cyolopium, Cladosporium herbarum, Fusarium oxaclicum, Penicillium sclerotigenum, Trichoderma viride, Geotricum candidum, Gleiocladium roseum, Roselina species, Saccharomyces cerevisiae, Cylindrocapus radicola and Colletotrichum gloesporiodes* [21]. Pathogens responsible for white yam tuber rot vary with location (even on the same field) and time [11,19]. It has been observed that in white yam, rotting appeared first at the tail end of the yam and then proceeds towards the head regions [20,18].

Benue state is acclaimed “The Food Basket State of the Nation” with the largest yam market in Zaki-Biam (Zaki-Biam yam market) in Benue North-East Senatorial zone and other numerous yam markets across the state [11]. The foregoing indicates a high level of yam production and yam marketing activities in Benue and North-East zone of the state in particular. However, the finding of many researchers [11,23] on the condition of farming households in Benue, reveals that over 30 % of yam farming households in Benue state are still experiencing low income from yam production and food insecurity due to poor postharvest handing. These findings suggest that there might be a prevailing significant level of postharvest losses of yam that may have been negatively affecting the standard of living, food security, and safety of yam farming households in Benue North-East.

Studies on microbial rot of white yam tubers in the area are scarce. Hence the causative agents of white yam tuber rot and severity are relatively unknown by farmers in the study area. Therefore, this study is aimed at isolation and identification of pathogens associated with white yam tuber rot. Pathogenicity test against apparently healthy white yam tubers is also intended. Positive findings shall be communicated to all stake holders for precautionary measures against white yam tuber rot pathogens.

2. MATERIALS AND METHODS

2.1 Source of Materials

Five varieties of white yam tubers *Hembakwase, Pepa, Dan-Anacha, Amula* and *Gbangu* showing symptoms of rot were collected from five farms in Katsina-Ala, Ukum, Logo, Konshisha, and Vandeikya local government areas of the zone “A” senatorial district of Benue State for the isolation and identification of the pathogens. The samples were properly labeled at the collection points, packaged in cellophane bags and then transported to the Department of Botany, Benue State University, Makurdi for authentication by a plant Taxonomist before analysis.

The culture media: Nutrient broth, Potato Dextrose Agar (PDA), Nutrient Agar ((NA), MacConkey Agar, Triple Sugar Iron Agar (TSIA) and Simon citrate Agar were all purchased from, TITAN BIOTECH TM MEDIA, RAJASTHAN, INDIA through Agbe Science, Makurdi, Benue State, Nigeria.

The reagents used for the study, Grams staining reagents (Luggol`s iodine, crystal violet, safranin, and absolute ethanol); Covax reagents; catalase reagent (hydrogen peroxide); oxidase; methanol, methylated spirits and sodium hypochlorite etc were purchased from BDH Chemicals, England, M&B Laboratory, England and Agbe Science, Makurdi, Benue State, Nigeria. The chemicals and reagents used in this study were analytical grade and used without any further purification.

The equipment used for the study includes, oven, autoclave, incubator, binocular microscope (Olympus), laminar air flow chamber, weighing balance, pH metre, refrigerator mortar and pestle etc.

2.2 Sterilization of Laboratory Materials

The method of [24] was used with little modifications. The Inoculation needles, cork borers and scalpels etc were sterilized by dipping in 70 % ethanol and flaming to red hot, while glass wares were thoroughly washed with detergents, rinsed with sterile water and autoclaved at 121 ^{o}C and 15 psi for 15 minutes. The sterilization of the distilled water was done in 1 litre sterilized bottle. Petri dishes, pipettes and measuring cylinders were wrapped properly with aluminum foil before sterilization. The laminar flow was sterilized by swiping them with cotton wool soaked in 70 % ethanol. All glassware used in the experimental procedures were sterilized in 10 % sodium hypochlorite solution, rinsed thoroughly in double-distilled water and dried before used. Aseptic condition was maintained throughout the experiments.

2.3 Microbial Isolation

The yam samples were prepared by the method of [24] with little modifications. The samples were washed with sterile distilled water to remove soil debris and air dried. Each rotten yam was cut with the aid of flamed knife into small pieces of about 5mm at the interphase between healthy and the infected portions of the tuber. The small rotten yam pieces were surface sterilized in 10 % sodium hypochlorite solution for three minutes and washed with sterile distilled water twice. The pieces were blotted dry on sterile Whatman No. 1 filter paper to remove droplets of water and placed in laminar flow cabinets to dry for 5 minutes.

2.4 Preparation of Culture Media

All media were prepared according to the manufacture`s` instruction. Others, (PDA, NA, MacConkey Agar) were autoclaved in bulk and allowed to cool, then poured into sterile Petri dishes, while some (TSIA, SCA) were dispensed into bottles, autoclaved, cooled in slanting positions and incubated at 37°C for 24 hours for sterility check (observing for the absence of microbial growth).

2.5 Inoculation

The inoculation was carried out using the methods of [5,24] with slight modifications. The media for fungi and bacteria were labeled with individual yam codes respectively. Three pieces each of the sterilized yam tubers were picked with sterile forceps and platted into the already prepared Potato Dextrose Agar (PDA) and Nutrient Agar (NA) respectively. These were properly labeled. Three replicates were made for each sample. The inoculated Petri-dishes were then incubated at 37 ^{o}C and observed for 24 – 48 hr for bacteria and up to 7 days for fungi with aseptic conditions being applied throughout the procedure.

2.6 Sub-Culturing and Purification

The method of [5] as described by Gwa [24] was used with slight modifications. After the period of incubation, sub-culturing of different mycelia colonies from the inoculated plates was done to obtain pure cultures. Sterilized surgical blades were used to cut different mycelia growth (observed multi-growths) and transferred into newly prepared separate Potato Dextrose Agar (PDA) and Nutrient Agar (NA) plates for fungi and bacteria respectively. The plates were incubated at 37 ^{o}C for 2 – 5 days. Thereafter, several sub-culturing were done to purify the mixed culture plates so as to obtain pure cultures of fungal and bacterial isolates. The purified plates were transferred into Mcartney slants and stored in the refrigerator for characterization.

2.7 Identification of the Pure Isolates

The fungal isolate were characterized by the methods of [5] as described by [18,24]. Few strands of the fungi isolates were mounted using a sterile mounted needle in 95 % ethanol, stained with lactophenol cotton blue and placed on a sterile slide. The specimen was spread very well with the aid of two mounted needles, a cover slip gently lowered on the slide and viewed under a binocular Olympus camera microscope (X 40) and photo micrographs were taken. The physical and structural characteristics of the mycelia, septate and non-septate, appearance and colour of colonies, nature of hyphae, growth rate, reproductive and vegetative structures, nature of spores and sporangia and branching of the hyphae, and presence of special organs such as rhizoids etc were observed and compared with fungal identification guides [25,26,27,28] for the identification of the fungal isolates.

The methods of [5,29] were used for the various tests and observation to identify the bacteria isolates. The bacteria isolates were properly coded and Gram stained. The Gram stained slides were then examined under a microscope at X 100 objective lens using oil immersion. The Grams` reactions, shapes and features such as extent and mixture of growth, colony elevation, pigmentation, colour, edge and form of colony as well as consistency were recorded. Catalase, oxidase, motility and capsule staining tests were carried out for identification. Sugar fermentation test was carried out using TSIA, while citrate utilization test was performed using Simon citrate agar.

2.8 Pathogenicity Test of the Pure Isolates

The method described by [5,18,24] were used without any modification. All the pure cultures obtained from the sub-cultured plates were used for pathogenicity test. The isolate were tested for their ability to initiate disease symptom (cause rot) on the apparently healthy white yam tubers. The fresh healthy white yam tubers (3 for each variety) were washed in sterile water to remove soil debris, surface sterilized by dipping each yam tuber in 10 % (v/v) hypochlorite solution (jik) for 2 minutes to remove surface contaminants, rinsed thrice with sterile water each time for 2 minutes, blotted with sterile filter paper in the laminar cabinet to dry for 20 minutes and labeled accordingly. Each yam tuber was measured with a sterile plastic ruler and then divided into segments for the fungi and bacteria isolates. A flamed 5 mm cork borer was used to make holes (5 mm deep) on the clean and healthy white yam tubers in an aseptic environment for each isolate, all fungi, all bacteria and all fungi and all bacteria. Petri dishes containing the pure cultures of each isolate were punctured with sterile 6 mm cork borer. Separate sterile inoculating cork borers were used to pick a colony of the disc made and introduced into the holes made in the yam tubers. The cut off flesh from each yam tubers were replaced into the hole, thus blocking the inoculated region on the yam and sealed with masking tape to prevent infestation by other microorganisms. Similar holes were made in the adjacent portion in the yam tuber and closed back with the cut flesh but without any pathogen to serve as control. The inoculated white yam tubers were placed in a safety chamber at room temperature and observed for seven days. The infected tubers were compared with the initial decayed tubers. After the incubation period, the inoculated yam tubers were cut transversely along the point of inoculation into two halves. The inner portions were exposed and the extent of rot and/or infected portions was measured with a transparent plastic ruler and recorded.

The percentage rot severity was calculated by method of [22] as follows

$$\text{Percentage rot severity} = \frac{Area of tissue infected}{Total surface area} \times 100\ \% \quad (1)$$

The percentage rot severity was ranked on a 5-point scale: 1 = No symptom, 2 = (≥ 1< 10 %) - mild rot, 3 = (≥ 10 < 25 %) - moderate rot), 4 = (≥ 25 % < 50 %)- severe rot and 5 = (≥ 50 %)- very severe rot [5,12,22].

Fig. 1. Pathogenicity test of the white yam tuber varieties

2.9 Statistical Analysis

The data obtained from the zone of inhibition (mm) was analyzed (descriptive statistics and inferential statistics to report the findings and to test hypothesis at 0.05 level of significance respectively) using statistical package for social science, SPSS Version 21. Results were reported as Mean $\pm$ SD. The statistical difference between more than 2 groups of data was evaluated using ANOVA with Tukey`s HSD Post Hoc Test. Differences between means were considered significant at $p < 0.05$.

3.RESULTS AND DISCUSSION

3.1 Isolation and Identification of Pure Isolates from Rotten White Yam Tubers

The Fungal pathogens isolated and identified from rotten white yam tubers were *Aspergillus niger, Botryodioplodia theobromae, Zygosaccharomyces bailli, Zygosaccharomyces rouxil and Myrothecium verrucaria*.

Biochemical tests confirmed the presence of *Klebsiella oxytoca*, *Serratia marcescens*, and *Pseudomonas aeruginosa* as the bacterial isolates (Table 1).

The result obtained from the area is in agreement with earlier findings [11,18,19,24]. Brown hard rot of the white yam tubers in the study area was recorded with the fungi *Apergillus niger* and the bacteria, *Pseudomonas aeruginosa* in all the white yam varieties sampled in the area. *Apergillus niger* and *Pseudomonas aerugonosa* are known to be pathogenic or opportunistic to humans and may even secrete harmful toxins which are harmful to humans [18,23,24].

3.2 Incidence of Occurrence of the Isolates

Table 2 shows the frequency of occurrence of the isolates at the sampled farms in the five local government areas per white yam varieties. The result shows that within the same local government area, an organism present in the same white yam variety from one farm may be absent at another farm. There was also similarity or variation of the occurrence across the five local government areas. Generally, the presence of micro-organisms in the different white yam tuber varieties varied from one local government to another. The staggering distributions of the isolates in the tuber varieties as well as sampling area may be attributed to weather conditions at the time of harvest. The entry of pathogens through bruised tubers during harvesting in the months of November or December to February (late) may cause bruised tuber surfaces to dry up quickly to form a barrier, which reduces microbial entry, and subsequent rot. Harvesting in humid weather condition make bruised tubers more vulnerable to pathogenic attack, with the attendant tuber rot, damage, and loss. It is observed that favourable environmental conditions may influence the presence of yam pathogens in an area. It is reported by other researchers that incidence of rotting varies with the species and its distribution as well as within each yam species , but not related to soil mineral status, which does not correlate with the type of microorganism isolated nor percentage of rot. This research finding is corroborated by many researches in which higher rot-causing microorganism were isolated and identified [5,24,30,31,32].

Table 1. Biochemical test for identification of bacteria isolates

Test	Micro-organism		
	Klebsiella oxytoca	***Serratia marcescens***	***Pseudomonas aeruginosa***
Gram staining reaction	+	+	+
Catalase	-	+	+
Oxidase	-	-	+
Citrate utilization	+	+	+
Gas production	-	-	-
H_2S production	-	-	-
Motility	-	+	+
Indole	-	-	-
Pigment	-	Red	Blue
pH (in nutrient broth medium)	7.46	7.49	7.30
Acid	-	+	+

Key; + = positive reaction; - = negative reaction

Table 2. Incidences of occurrence of the pure isolates

	Fungi					Bacteria		
	A. nigeer	*B. theobromae*	*Z. bailli*	*Z. rouxil*	*M. verrucaria*	*K. oxytoca*	*S. marcenscens*	*P.aroginosa*
Yam variety/ Sample area				***Gbangu***				
K/Ala	+	+	-	+	-	-	-	+
Ukum	+	+	+	+	-	-	-	+
Logo	+	+	-	-	-	-	-	+
Konshisha	+	+	-	-	+	+	-	-
V/Ikya	+	-	+	-	+	+	+	-
Incidence Ratio	**5/5**	**4/5**	**2//5**	**2/5**	**1/5**	**2/5**	**1/5**	**3/5**
				Dan-Anacha				
K/Ala	+	+	-	-	+	+	+	-
Ukum	+	+	-	+	-	-	-	+
Logo	+	+	+	-	-	+	-	+
Konshisha	+	-	-	+	+	-	-	-
V/Ikya	+	+	-	-	-	+	-	-
Incidence Ratio	**55**	**4/5**	**1/5**	**2/5**	**2/5**	**35**	**1/5**	**1/5**
				Hembankwase				
K/Ala	+	+	+	-	+	+	-	+
Ukum	+	+	-	-	-	+	-	+
Logo	+	+	+	+	-	-	-	-
Kinshisha	+	+	+	+	-	-	+	-
V/Ikya	+	+	-	-	+	-	-	+
Incidence Ratio	**5/5**	**5/5**	**3/5**	**2/5**	**2/5**	**2/5**	**1/5**	**3/5**
				Pepa				
K/Ala	+	+	+	-	-	+	-	-
Ukum	+	+	-	-	+	-	-	+
Logo	+	+	-	-	-	-	-	-
Konshisha	+	+	-	+	-	-	+	-
V/Ikya	+	+	-	-	-	-	-	+
Incidence Ratio	**5/5**	**5/5**	**1/5**	**1/5**	**1/5**	**1/5**	**1/5**	**2/5**

	Fungi					Bacteria		
	A. nigeer	***B. theobromae***	***Z. bailli***	***Z. rouxil***	***M. verrucaria***	***K. oxytoca***	***S. marcenscens***	***P.aroginosa***
					Amula			
K/Ala	+	+	+	-	-	-	-	+
Ukum	+	+	+	+	-	+	-	+
Logo	+	+	-	-	+	-	+	-
Konshisha	+	+	-	-	-	-	+	+
V/Ikya	+	+	-	-	-	+	-	-
Incidence Ratio	**5/5**	**5/5**	**2/5**	**1/5**	**1/5**	**2/5**	**2/5**	**3/5**

Table 3. Percentage incidence occurrence of the isolates

Fungi	Percentage
Apergillus niger	**100**
Botryodiophodia theobromae	**72**
Zygosaccharomyces bailli	**36**
Zygosaccharoumyces rouxil	**32**
Myrothecium verrocaria	**28**
Bacteria	
Klesbesiella oxytoca	**40**
Serratia marcenscens	**24**
Pseudomonas aeruginosa	**52**

Table 4. Average (depth and width) pathogenicity test rot measurement results: (cm)

	Gbangu	**Dan-Anacha**	**Hembakwase**	**Pepa**	***Amula***
Fungi					
Aspergirus niger	2.52 ± 0.54^b	4.38 ± 1.66^d	7.44 ± 0.21^f	2.91 ± 0.89^b	2.70 ± 0.85^b
Botryodioplodia theoromae	2.97 ± 2.03^b	2.25 ± 1.29^b	8.90 ± 0.51^f	1.71 ± 0.81^a	2.99 ± 2.08^b
Zygosaccharomyces bailli	2.48 ± 1.33^b	2.03 ± 0.92^b	8.50 ± 0.00^f	2.13 ± 0.83^b	2.81 ± 1.80^b
Zygosaccharomyces rouxil	2.42 ± 1.75^b	3.11 ± 2.79^c	8.21 ± 0.18^f	3.11 ± 1.99^c	2.32 ± 1.30^b
Myrothecium verrucaria	4.6 ± 0.67^d	2.29 ± 1.13^b	8.43 ± 0.12^f	2.66 ± 1.53^b	2.51 ± 1.40^b
Bacteria					
Klesiella oxytoca	2.42 ± 0.48^b	2.08 ± 0.96^b	5.00 ± 2.06^e	1.86 ± 0.96^a	2.81 ± 0.50^b
Serratia marcescens	3.13 ± 1.99^c	2.94 ± 2.26^b	5.48 ± 4.27^e	2.35 ± 1.15^b	1.79 ± 0.86^a

	Gbangu	Dan-Anacha	Hembakwase	Pepa	*Amula*
Pseudomonas aeruginosa	1.30 ± 0.34[a]	1.55 ± 0.00[a]	3.29 ± 0.35[c]	1.60 ± 0.71[a]	3.35 ± 1.20[c]
All Bacteria	3.00 ± 1.27[c]	2.90 ± 1.14[b]	3.95 ± 0.35[c]	2.60 ± 1.13[b]	3.05 ± 0.78[c]
All Fungi	4.88 ± 2.43[d]	4.94 ± 2.35[d]	4.82 ± 0.68[d]	3.95 ± 1.06[c]	3.40 ± 1.13[c]
All Isolates	5.17 ± 2.31[e]	5.43 ± 1.94[e]	3.98 ± 0.74[c]	4.13 ± 0.95[d]	2.65 ± 1.20[b]

N= 5, values expressed as Mean ± SD. Values in the same column with different alphabetical letters (superscript) are statistically significant at p <0.05

Table 5. Percentage rot severity of the isolates

Fungi	**Percentage**
Apergillus niger	48.17[4]
Botryodiophodia theobromae	56.44[5]
Zygosaccharomyces bailli	43.66[4]
Zygosaccharoumyce srouxil	40.75[4]
Myrothecium verrocaria	37.31[4]
Bacteria	
Klesbesiella oxytoca	45.38[4]
Serratia marcenscens	45.25[4]
Pseudomonas aeruginosa	40.98[4]
All fungi	65.03[5]
All bacteria	55.09[5]
All isolates	68.38[5]

Key: 1 = No rot, 2 = (≥ 1< 10 %) - mild rot, 3 = (≥ 10 < 25 %) - moderate rot), 4 = (≥ 25 % < 50 %)- severe rot and 5 = (≥ 50 %)- very severe rot. Note: Superscript indicates percentage rot severity [5,12,22]

The result from Table 3 shows that the highest percentage incidence (100%) was obtained from *Aspergilus niger,* followed by *Botryodiophodia theobromae* (72%) and *Pseudomonas aeruginosa* (52%). The percentage incidence occurrence for *Klesbesiella oxytoca, Zygosaccharomyces bailli,* and *Zygosaccharoumyces rouxil,* was 40%, 36%, and 32% respectively, while Serratia *marcenscens* recorded the lowest (24%).

3.3 Pathogenicity Test and Rot Severity

Pathogenicity test shows that the isolates were pathogenic to the healthy white yam tuber with varying degree of rot among the organisms and yam varieties (Table 4). The results showed that *Hembakwase* was more susceptible to pathogen causing rot, followed by *Amula, Gbangu, Dan-Anacha,* and *Pepa* respectively. There was a significant difference ($p < 0.05$) in the virulence potential of the isolates when re-inoculated to healthy white yam tubers.

Table 5 showed that *Botryodioplodia theobromae* recording the highest rot severity of 56.44%, followed by *Aspergillus niger* (48.17%), *Klebsiella oxytoca* (45.38%), *Serratia marcenscens* (45.25%), *Zygosacchromyces baill* (43.66%), *Pseudomonas aeruginosa* (40.98%), *Zygosacchromyces rouxil* (40.75%), and *Myrothecium verrucaria* (37.31%) respectively. All fungi and bacteria caused rot severity of 65.03% and 55.09% respectively while all isolates recorded rot severity of 68.38 % [33-35].

4. CONCLUSION

Post-harvest deterioration of white yam tubers is influenced by the synergetic action of fungi and bacteria. Eight isolates comprising of five fungi: *Aspergillus niger, Botryodioplodi atheobromae, Zygosaccharomyces bailli, Zygosaccharomyces* rouxil and *Myrothecium verrucaria,* and three bacteria: *Klebsiella oxytoca, Serratia marcescens* and *Pseudomonas aeruginosa* were isolated from five white yam varieties :*Gbangu, Dan-Anacha, Hembakwase, Pepa* and *Amula* showing symptoms of rot from five farms in five local government areas of Zone “A” Senatorial District of Benue State, Nigeria and identified using morphological, cultural, physiological and biochemical tests as well as standard identification guides. These pathogens have been reported to be the major cause of white yam tuber rot in Nigeria and other parts of the world [4,11,12,18,24,30].

Pathogenicity tests revealed that all the organisms induced rots in the apparently healthy white yam tubers after seven days of inoculation. The result also showed a significant difference ($p < 0.05$) in the average rot measurements among the different yam varieties with *Aspergillus niger* being the most prevalent; *Botryodioplodia theobromae*, the most virulent, while *Hembakwase* was the most susceptible white yam variety in the study area.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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