Studies on Microbial Profiles and Control of Postharvest Rot of *Dioscorea alata* and *Dioscorea rotundata* using Plants Extracts

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Background

The main causes of postharvest losses of yams are high temperature, sprouting, nematodes, rodents, insects and microorganisms; the greatest culprit being microorganisms [1-3]. The yam tuber naturally has an outer cover, microorganisms cannot penetrate easily, but it is easily wounded by rodents, nematodes and people during weeding, harvesting and postharvest handling. Such wounds facilitate the penetration and development of rot microorganisms [2, 4, 5]. Organisms responsible for tuber rot include bacteria and fungi; they vary from place to place or may vary with time even on the same field [2, 3]. In most cases the activities of these organisms become pronounced during storage [4].
In many African countries, Nigeria inclusive, post harvest tuber losses can reach 50%. Food losses imply a decrease in the edible food or nutritional quality of the food that was originally meant for consumption by humans either by quality or quantity [6, 7]. This damage can be stopped or at least contained by the use of pesticides and plant extracts [6 - 10]. Pesticides partly or completely prevent damage of crops by insects and microorganisms; pesticides, however, are reported to be toxic, resisted by microorganisms, cause environmental pollution, and are often viewed negatively, locally and internationally [7, 11].

Aim

To isolate and identify the various microorganisms responsible for post- harvest losses of water yam and white yam tubers and to employ plant extracts for their control.

Study setting

2 cultivars of yam i.e. 2 in 1: water yam 3 varieties and white yam 4 varieties, totaling 7 varieties; from 5 Local Government Areas.
10 botanicals (plants leaves) + 1 synergistic mix; totaling 11.
4 solvents for extracting the active ingredients in single and synergic plant mix respectively.
1 control for each set of treatment; hence, we have 1 x 7 x 5 x 11 x 4 = 1,540.
Replication: 3.
Experimental design is therefore 1,540 x 3 = 4,620 in complete random block.

Key findings

- Six (6) rot microorganisms (bacteria and fungi) were isolated from Water yam
- Nine (9) rot causing microorganisms were isolated from White yam.
- Inhibition studies using ten plant extracts synergistically resulted in complete inhibition of all bacteria isolates and three (3) of fungi isolates; the remaining 2 fungi also showed susceptibility.

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Methods

Microbial Isolation
- Sample preparation was by the method of Okigbo and Emeka [12]
- Media Preparation by the method of Cheesbrough [13]
- Inoculation by the method of Okigbo and Emeka [12]

Tests and Identification of Isolates:
a) Test for identification by Cheesbrough [13]
b) Identification of bacteria by the method of Krieg [6]; of fungi by Barnett and Hunter, Sutton [7, 8].

Pathogenicity Test by the method of Okigbo and Emeka [12]
Cold Aqueous Synergistic Plants Extract preparation by the methods of Asare and Oseni [14]
Synergistic Plant Extract Incorporation and Inhibition test by the method of Amadioha and Obi [9]

Results

Similar isolates from the yams were grouped and given codes for onwards identification. Four bacteria and five fungi groups were coded bringing the total numbers of coded groups of the isolates to 9 organism types as shown in Table 1

Results of identification analysis obtained, along with standard identification guides employed, identified the bacteria isolates as *Serratia marcescens*, *Erwinia carotovora*, *Pseudomonas aeruginosa* and *Klebsiella oxytoca*. The fungi were identified and confirmed as *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Penicillium marneffei*, respectively; as shown in Table 2.

Table 1: Isolates codes/Identified organisms

Isolates codes	Identified organisms
Bacteria:	
$B_2$2	*Serratia marcescens*
$B_1$6	*Erwinia carotovora*
$B_1$10	*Pseudomonas aeruginosa*
Ban	*Klebsiella oxytoca*
Fungi:	
BH	*Aspergillus niger*
PW	*Fusarium oxysporum*
FWPh	*Rhizopus stolonifer*
LG	*Aspergillus flavus*
GWE	*Penicillium marneffei*

Table 2: Microorganisms isolated from Water Yam

Microorganisms	Water Yam varieties		
	Azawele wele	Kor	Banada
Bacteria:			
Pseudomonas aeruginosa	+	+	+
Erwinia carotovora	+	-	+
Fungi :			
Aspergillus niger	+	+	+
Aspergillus flavus	+	+	+
Rhizopus stolonifer	+	+	+
Penicillium marneffei	+	+	+

Key:
+ = present
- = absent

Table 3: Microorganisms isolated from White Yam

Microorganisms	White yam Varieties			
	Amula	**Ogoja**	**Hembam**	**Gbongu kwase**
Bacteria:				
Erwinia carotovora	-	+	+	+
Pseudomonas aeruginosa	+	+	+	+
Serratia marcescens	-	+	-	+
Klebsiella oxytoca	+	+	+	-
Fungi:				
Rhizopus stolonifer	+	+	+	+
Aspergillus niger	+	+	+	+
Aspergillus flavus	+	+	+	+
Fusarium oxysporum	+	+	+	+
Penicillium marneffei	+	+	+	+

Key:
+ = present
- = absent

Results cont'd

Cold aqueous synergistic Ten plants extract inhibition Test on Isolates as shown in Table 4, recorded better inhibition. There was complete inhibition (a) of all the four bacteria isolates at 2 mL extract incorporation; 10 mL extract incorporation in media recorded complete inhibition (a) of three out of the five fungi isolates (*Rhizopus stolonifer*, *Fusarium oxysporum* and *Penicillium marneffei* and high inhibition (b) of the other two (*Aspergillus niger* and *Aspergilus flavus*), respectively. Further extract quantity addition was no longer necessary because at 10 mL incorporation the media was too soft, again in treatment, there is room for repeated dosage.

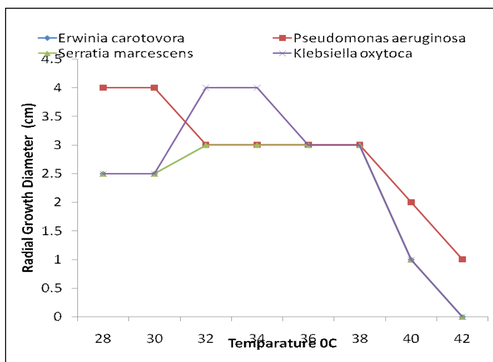
Table 4: Cold Aqueous Synergistic Ten plants extract inhibition Test on Isolates

Microorganism	Extract volume applied									
	1*ml*	2*ml*	3*ml*	4*ml*	5*ml*	6*ml*	7*ml*	8*ml*	9*ml*	10*ml*
Rhizopus stolonifer	-[f]	-[f]	-[f]	+[e]	+++[c]	+++[c]	+++[c]	+++[c]	++++[b]	+++++[a]
Aspergillus niger	-[f]	-[f]	+[e]	+[e]	+++[c]	+++[c]	++++[b]	++++[b]	++++[b]	++++[b]
Aspergillus flavus	-[f]	-[f]	-[f]	+[e]	+++[c]	++++[b]	++++[b]	++++[b]	++++[b]	++++[b]
Fusarium oxysporum	-[f]	-[f]	-[f]	+[e]	+++[c]	+++[c]	++++[b]	++++[b]	+++++[a]	+++++[a]
Penicillium marneffei	-[f]	-[f]	+[e]	++[d]	++++[b]	++++[b]	++++[b]	++++[b]	+++++[a]	+++++[a]
Erwinia carotovora	+[d]	+++++[a]	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
Pseudomonas aeruginosa	-[f]	+++++[a]	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
Serratia marcescens	-[f]	+++++[a]	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
Klebsiella oxytoca	+[d]	+++++[a]	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*

Summary of inhibition:
a = 9, b = 15, c = 9, d = 5, e = 6, f = 13
Key:
- = no inhibition
+ = mild inhibition (05 – 34% inhibition)
++ = moderate inhibition (35 – 54% inhibition)
+++ = good inhibition (55 – 74% inhibition)
++++ = high inhibition (75 – 99% inhibition)
+++++ = complete inhibition
NA = not applicable
a = complete inhibition, b = high inhibition, c = good inhibition,
d = moderate inhibition e = mild inhibition, f = no inhibition

Conclusions

Postharvest yam rot microorganisms were sensitive to the synergistic plant extract and the extract was observed to enhance the complete inhibition of bacteria more than the fungi at lower volumes. It was observed to be bactericidal, fungi static and fungicidal.



•The optimal growth temperature for *Erwinia carotovora* was in the range of 32 – 38 ^{O}C, with colony diameter of 3.0 mm; *Pseudomonas aeruginosa* recorded optimal growth temperature in the range of 28 – 30 ^{O}C and 4.0 mm colony diameter; *Serratia marcescens* grew optimally in the range of 32 – 38 ^{O}C, with a colony diameter of 3.0 mm and *Klebsiella oxytoca* recorded optimal growth temperature in the range of 32 - 34 ^{O}C with colony diameter of 4.0 mm as shown in Figure 1.

•The five fungi isolates (*Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus*, *Fussarium oxysporum* and *Penicillium marneffei*) recorded optimal growth temperature of 38 ^{O}C as shown in figure 2.

Figures 1 /2: Showing Optimum Growth Temperatures of Bacteria and Fungi isolates

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NRI | Natural Resources Institute

BSU: Benue State University Makurdi

CEFTER: Centre For Food Technology And Research

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