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Postharvest Loss Control: Synergistic Plants Extract Inhibition of Ten Microbial Yam Rot Organisms

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Abstract

Nine microorganisms were isolated from four varieties of *Dioscorea rotundata* (gbongu, ogoja, Amula, Hembamkwase); four bacteria, *Erwinia carotovora*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella oxytoca* and five fungi, *Rhizopus stolonifera*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Penicillium marneffei*, from five local government areas of Benue State (Vandeikya, Ukum, Katsina Ala, Guma and Logo). Pathogenicity test on the isolates confirmed them to be the cause of rot. The nine plants extract (*Ceiba pentandra*, *Jatropha tanjorensis*, *Azadirachta indica*, *Moringa oleifera*, *Carica papaya*, *Mangifera indica*, *Daniella oliveri*, *Terminalia catapa* and *Passiflora edulis*) synergistically added inhibited all the four bacteria isolates completely. The five fungi were inhibited by 60% (c) - 80% (b); *Rhizopus stolonifera*, *Aspergillus* flavus, *Fusarium oxysporum;* and *Aspergillus niger*, *Penicillium marneffei,* respectively.

Keywords

Plant Extracts, Synergistic, Microorganisms, Inhibition, Yam Rot

1. Introduction

Africa is the highest producer of yam in the world. West Africa is the largest producer of yam in Africa and Nigeria, the highest in West Africa [1] [2] [3]. Benue state in the north central zone of Nigeria is the leading producer or at least among the leading producers of yam in the world. This is because of its fa-

vorable climatic condition like whether as well as soil [4] [5] [6]; unfortunately it is plague with postharvest losses. Postharvest losses are caused by high temperature, sprouting, nematodes, rodents, insects and microorganisms. The most devastating is that caused by microorganism [5]. Our earlier studies as well as other researchers show that bacteria and fungi are responsible for the highest losses [5] [6] [7] [8]. Research has shown that these loss causing organisms exist in an associative growth with each organism causing its characteristic damage which is evident in tissue damage and coloration [7] [8].

Pesticides and plant extracts can be used to prevent or at least reduce postharvest losses in yam [9] [10] [11]. Crops treated with pesticides have longer shelf life and prevent infestation by insects and microorganisms; unfortunately pesticides are also plague with toxicity, resistance of micro-organisms, environmental pollution and health hazards to users as lack of cleanliness may generate ill health; as a result, the crops treated with pesticides are not readily accepted in the international markets [12]. Unlike pesticides, no serious report has been recorded against Microbial resistance to plant extract [13].

Plant extracts are known to inhibit partially or completely only selected organisms [7] [8] [11]. Most single plant extracts are often unable to partially or completely inhibit all isolates [11]. Synergistic effect of some plants recorded better inhibition of 60% to 80% on some micro-organisms [9]. This is in agreement with what most African countries and perhaps other nations were using for their health care [13].

The aim of this work is to use plants which are known to be food to mankind and are at the same time used for medicinal purposes for humans and livestock, for preservation of food and indeed control of postharvest losses. It is hoped that with careful blending and changes in concentration of extracts applied to the organisms, complete or higher percentage of inhibition of all microorganisms may be achieved. This study reports our findings on synergistic effects using nine plants (*Ceiba pentandra*, *Jatropha tanjorensis*, *Azadarichta indica*, *Moringa oleifera*, *Carica papaya*, *Mangifera indica*, *Daniella oliveri*, *Terminalia catapa and Passiflora edulis*) and nine microorganisms; four bacteria (*Erwinia carotovora*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella oxytoca*) and five fungi (*Rhizopus stolonifera, Aspergillus niger*, *Aspergillus flavus*, *fusarium oxysporum*, *Penicillium marneffei*).

2. Materials and Methods

2.1. Source of Materials

Sixty cut or bruised tubers and sixty healthy tubers of *Discorea rotundata*; 6 tubers of each of the varaieties (Gbongu, Ogoja, Amula and Hembamkwase) were purchased from farmers in Vandeikya, Ukum, Katsina Ala, Guma and Logo Local government area of Benue State, amounting to 15 tubers of each variety per local government. The samples were labeled, dated, and packaged in cellophane bags at collection sites. These were transported to the Microbiology Laboratory

of Benue State University, Makurdi; where they were kept and some were analyzed on appearance of visible sign of rot, while others were used for pathogenicity test.

Plant leaves of *Ceiba pentandra*, *Jatropha tanjorensis*, *Azadarichta indica*, *Moringa Oleifera, Carica* papaya, *Mangifera indica*, *Daniella oliveri*, *Terminalia catapa* and *Passiflora edulis* used for the plants extract were collected along Gboko road, Makurdi.

2.2. Isolation of Microorganisms

Isolation of bacteria and fungi from rotten yam tubers was carried out by the methods of [7] [11].

2.2.1. Sample Preparation

The methods of [7] [11] were used without any modifications. The samples were individually washed in clean water to remove surface dirt and air dried. The skin was cut open to reveal the rotten tissues and about 5 mm pieces of the tissues were then cut into a beaker. A 10% sodium hypochlorite solution was added and held for 2 minutes. This was drained and washed three times with sterile water. They were then dried in a laminar air flow cabinet for 5 minutes on Whatman No.1 filter paper.

2.2.2. Culture Media Preparation

Nutrient Agar (HIMEDIA, India) for bacteria isolation and potato dextrose agar (Micro Master, India) for fungi isolation were prepared according to manufactures directions respectively. Biochemical test media and reagent were also prepared according to standard methods [7].

2.2.3. Direct Seeding

The method of [7] was used without any modification. The prepared pieces of yam samples were seeded, 3 pieces each, onto the prepared nutrient agar and potato dextrose agar plates respectively, incubated for 48 hours for the bacteria and seven days for the fungi.

2.2.4. Isolates Identification

The distinct colonies of organisms were sub-cultured severally until a single uniform growth was achieved. The bacteria were identified by the method of [14] and the fungi by the method of [15] [16].

2.2.5. Test for Pathogenicity

The methods of [7] [11] were used with little modifications; the isolates were tested to establish that tuber rot was due to their growth and activity in the tissues. The healthy yam tubers were washed in running tap water to remove surface dirt and soil. They were allowed to air dry, then their surfaces sterilized by cleaning with 70% ethanol solution. The outer layer was cut at a portion of the yam (but not allowed to fall off), 5 mm deep hole was made into the exposed tissues using a sterile 5 mm cork borer. A 3 mm cork borer was used to lift the

organisms and placed in the hole made for each category. The skin was replaced and sealed with a masking tape. This was kept in a safe chamber and monitored for nine days. Tubers were cut open transversely, tissue damage and pigmentation were compared with the initial parent rot, width and depth of rot was measured. Re-isolation of the seeded organisms was carried out. The test was carried out in triplicate and average measurement of width and depth of rot were recorded.

2.2.6. Plants Extract Preparation.

The methods of the Tiv traditional health care in Benue, Nigeria, using plant extracts (unpublished work) and [17] [18] were used with little modifications; plant leaves were blended in a ratio of 1:1. This forms the nucleus of the work. Fresh leaves of nine plants (*Ceiba pentandra*, *Jatropha tanjorensis*, *Azadarichta indica*, *Moringa Oleifera, Carica* papaya, *Mangifera indica*, *Daniella oliveri*, *Terminalia catapa* and *Passiflora edulis*) were collected, washed in clean water, put to drain in a clean plastic basket for 10 minutes. They were weighed (20.0 g each) using an electronic digital weighing balance (OHAUS model Scout pro SPU601). The leaves were cut to fine shredding with a kitchen knife and blended using a warring blender (Okapi, Marshall) into fine paste. The mix (50.0 g) was weighed into a sterilized 500 mL conical flask, 200 mL of sterile water was added and stirred with a magnetic stirrer for 10 minutes (Stuart, SB 162), it was plugged with cotton wool and kept to extract for 12 hours at room temperature. This was filtered with Whatman No 1 filter paper, the residue was washed with 10 mL of water four times. The combined volume was centrifuge (Centurion 2000 series) at 3500 rpm for 3 minutes. The supernatant was decanted into a sterile bottle, properly corked and stored in the refrigerator for antimicrobial inhibition test.

2.2.7. Plant Extract Incorporation

The method of [11] was used. The extract, 1.0 mL - 5.0 mL, each were pipette into sterile petri dishes and 20 mL of molten agar or potato dextrose agar held at 45°C was poured on it respectively and mixed thoroughly. These were allowed to solidify and used for the inhibition test.

2.2.8. Inhibition Test

The methods of [7] [11] were used with little modifications; the bacteria and fungi isolates were seeded individually on the plants extract incorporated nutrient agar and potato dextrose agar respectively, controls were also set up by seeding on media without extract addition and incubated at 37˚C for 48 hours for the bacteria and 7days for the fungi; using the optimum temperature obtained from earlier work [7] [8]. Various concentrations of the extract in the media was used by adding 1.0 mL to 5.0 mL to observe the effect of changes in concentration of the synergistic mixture on the microorganisms. The plates were observed for microbial activity. Growth diameter was measured and compared with the control to device a percentage growth width using a transparent plastic

ruler.

3. Results and Discussion

3.1. Results

Four bacteria species (*Erwinia carotovora*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Klebsiella oxytoca*) and five fungi species (*Rhizopus stolonifera*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Penicillium marneffei* were isolated from the rotten yam tubers (Table 1).

Pathogenicity test on the isolated microorganisms showed tissue rot, damage intensity and different pigment production by different microorganisms. Re-isolation of the seeded organisms from the rotten tissues confirms them to be responsible for the rot (Table 2).

Synergistic effect of nine plants extract mix on the isolates reveals that 1.0 mL addition of extracts in the culture media was able to inhibit mildly (e) the growth

Table 1. Microorganisms Isolated from Rotten *Dioscorea rotundata* (White Yams).

Bacteria	Fungi
Erwinia carotovora	*Rhizopus stolonifera*
Pseudomonas aeruginosa	*Aspergillus niger*
Serratia marcecens	*Aspergillus flavus*
Klebsiella oxytoca	*Fusarium oxysporum*
	Penicillium marneffei

Table 2. Pathogenicity Test/Average Triplicate Rot Measurements (cm) of Yam Tuber Varieties.

Microorganisms	Yam Varieties							
	Gbongu		Ogoja		Amula		Hembamkwase	
	Width	depth	Width	depth	Width	depth	Width	depth
Erwinia carotovora	1.2	2.3	1.2	2.9	1.0	4.8	1.8	3.2
Psendomona aeruginosa	2.1	3.0	1.4	2.9	1.2	2.4	1.5	3.0
Serratia marcescens	1.2	3.1	1.2	3.0	1.1	2.2	1.2	3.2
Klebsiella oxytoca	1.0	1.8	1.5	2.8	1.4	3.6	1.6	2.4
Rhizopus stolonifera	2.3	4.1	1.0	2.6	1.0	2.6	1.0	2.8
Aspergillus niger	1.7	3.0	2.0	3.3	2.2	3.2	1.4	3.0
Aspergillus flavus	1.8	2.6	2.1	3.2	1.8	2.4	2.3	3.2
Fusarium oxysporum	2.1	2.2	1.2	2.4	1.6	3.0	1.0	2.0
Penicillium maneffei	1.2	2.0	1.5	2.2	1.1	2.0	1.6	2.4
All bacteria	2.1	3.6	2.2	3.4	1.6	3.2	2.0	3.4
All fungi	2.0	3.3	2.7	3.3	1.8	2.2	2.2	3.6
All bacteria and all fungi	3.1	3.5	2.3	3.6	2.4	4.2	3.6	4.2

of only two bacteria (Erwinia *carotovora* and *Klebsiella oxytoca*) without affecting the other bacteria or fungi; 2.0 mL incorporation of the extract was able to inhibit all the four bacteria isolates completely, the fungi isolates were still not inhibited. Addition of 3.0 mL of extract showed mild inhibition (e) for *Aspergillus niger* and *Penicillium marneffei* without affecting the other three fungi; 4.0 mL incorporation of the extract showed mild inhibition (e) of *Rhizopus stolonifera* and *Fusarium oxytoca*, moderate inhibition (d) of *Aspergillius niger*, *Penicillium marneffei* and *Aspergillus flavus*. There was marked increase in the inhibition with the addition of 5.0 mL of the extract, showing good inhibition (c) for *Rhizopus stolonifera*, *Aspergillus flavus* and *Fusarium oxysporum*; and high inhibition (b) for *Aspergillus niger* and *Penicillium marneffei* (Table 3).

3.2. Discussion

The study confirms the isolate as microorganisms responsible for microbial yam tuber losses in most part of Benue State, Nigeria as they were previously isolated [7] [8]. The isolation of *Aspergillus flavus* and absence of *Botryodiplodia theobromae* from rotten *Dioscorea rotundata* in the present work as against the previous work [7] is an indication that presence of the organisms varies with daily climatic changes.

Pathogenicity test confirms that the isolates were responsible for the yam tuber rot as earlier reported [7] [8] and indeed in agreement with other reports [4] [8].

The complete inhibition of the four bacteria (*Erwinia carotovora*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Klebsiella oxytoca*) by the synergistic plants extract (*Ceiba pentandra*, *Jatropha tanjorensis*, Azadarichta *indica*, *Moringa Oleifera, Carica* papaya, *Mangifera indica*, *Daniella oliveri*, *Terminalia catapa* and *Passiflora edulis*) has not yet been reported by other workers, using

Table 3. Synergistic Activity of Plant Extracts Incorporation on Isolates.

Microorganism	Extract volume applied				
	1 ml	2 ml	3 ml	4 ml	5 ml
Bacteria					
Erwinia carotovora	$+^{e}$	$+++++^{a}$	NA	NA	NA
Pseudomonas aeruginosa	$-^{f}$	$+++++^{a}$	NA	NA	NA
Serratia marcescens	$-^{f}$	$+++++^{a}$	NA	NA	NA
Klebsiella oxytoca	$+^{e}$	$+++++^{a}$	NA	NA	NA
Fungi					
Rhizopus stolonifera	$-^{f}$	$-^{f}$	$-^{f}$	$+^{e}$	$+++^{c}$
Aspergillus niger	$-^{f}$	$-^{f}$	$+^{e}$	$++^{d}$	$++++^{b}$
Aspergillus flavus	$-^{f}$	$-^{f}$	$-^{f}$	$++^{d}$	$+++^{c}$
Fusarium oxysporum	$-^{f}$	$-^{f}$	$-^{f}$	$+^{e}$	$+++^{c}$
Penicillium marneffei	$-^{f}$	$-^{f}$	$+^{e}$	$++^{d}$	$++++^{b}$

synergistic or single plant extracts. Single bacteria have been inhibited by synergistic plant extracts as well as single plant extract [7] [8] [11]. In earlier work [7] [8], it was only *Erwinia* carotovora, that was inhibited completely (a), by a single plant extract (*Terminelia catapa*); *Pseudomonas aeruginosa* and *Klebsiella oxytoca* recorded good inhibition (b) with *Jatropha tanjorensis* plant extract. Synergistic extract appear to enhance the complete inhibition of bacteria more than the fungi. The synergistic mixture comprising of plants earlier used was not able to inhibit completely *Rhizopus stolonifera* earlier completely inhibited (a) by single plant extract, *Passiflora edulis* [8]; most probably because the concentration of the individual plants in the synergistic mixture was not sufficient to give complete inhibition; more so that inhibition increases with increase in concentration of the plant extract. All the fungi studied showed lower inhibition with synergistic extract compared with single extract and showed a gradual increase in inhibition with increase in concentration of the synergistic extract. The result is in agreement with the findings of [19]. Fungi spore formation time delayed on 5.0 mL extract media from 3 days to 7 days in the isolates, which suggest most strongly that higher concentrations shall be more effective and may inhibit it completely.

4. Conclusion

The plants in the study are all edible by man and livestock without any recorded danger on health. There is need to research further on the use of synergistic plants extract for their application on tubers and perhaps crops in general to prevent microbial rot and poison for shelf life elongation and the economic benefits that may follow.

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