Utilization of *Moringa oleifera* extracts in postharvest disease management of yam (*Dioscorea rotundata*)

RESEARCH ARTICLE

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ABSTRACT

Yams are utilized as food as well as livestock feed all over the globe. Approximately more than one billion people are facing the challenge of hunger in developing nations and availability of yams could form part of the solutions to the hunger problem. It is on record that of all losses caused by plant diseases postharvest losses are the most costly. The two objectives of this research were to monitor the spoilage of yam tubers after harvest, isolate the pathogens responsible for spoilage and establish the efficacy of the extract of *Moringa oleifera* leaves, seed and bark. The pathogenic microorganisms were isolated and identified using standard procedures. Samples of *Moringa oleifera* collected from the Wesley University ethnobotanical garden and were dried under shade for 21 days. 10 grams of each plant part was pulverized and poured into 100ml of hot water of 1000C kept in pyrex beaker of 200ml and allowed to release its active ingredient without application of heat for a period of 1 hour. The extract was thereafter decanted. The efficacy of the extracts were tested on nutrient agar and potatoes dextrose agar. This was validated in-vivo by the application of the extract to naturally infected yam tubers. The results showed that *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Botrydiplodia theobromae*, *Rhizopus stolonifer* and *Mucor mucedo* were fungi identified in the work. The bacterial pathogens isolated were *Bacillus* sp; *Erwinia* sp, *Micrococcus* sp and *Staphylococcus* sp. The pathogenicity test conducted verified Koch's postulates and the moringa leaves extract had the highest zone of inhibition (22mm) which was followed by the bark and least in the seeds. The moringa extracts investigated in this research were promising and the efficacy of the leaves and bark have been established. The moringa leaves and bark extracts could be used as biocontrol and antimicrobial agents against postharvest tuber spoilage in yam.

Research Objective

Monitor yam spoilage, isolate pathogens, and establish efficacy of *Moringa oleifera* extracts

Key Pathogens

Aspergillus species, *Rhizopus stolonifer*, bacterial species including *Erwinia* and *Bacillus*

Main Finding

Moringa leaves extract showed highest inhibition (22mm), followed by bark, then seeds

Keywords: postharvest, spoilage, antimicrobial, *Moringa oleifera* disease management.

INTRODUCTION

Yams are a global food and livestock feed. In the tropics, fresh roots are boiled, fried, or roasted as a carbohydrate staple. A significant challenge is postharvest losses, which FAO (2024) reports as 20-40% for root and tuber crops in Sub-Saharan Africa, with yam losses reaching up to 60% in some regions (Stathers et al., 2024). Nigeria, producing 71% of global yam output (28.1 million tonnes annually), faces economic impacts exceeding $2.3 billion yearly from these losses (Kleih et al., 2012). This food security issue stems largely from inadequate storage and preservation, primarily due to microbial spoilage (FAO, 2023; IITA, 2009). According to Arya (2010), postharvest plant disease losses are the most costly. Yam and sweet potato are critical food sources in the tropics, alongside cassava, cocoyam, rice, maize, wheat, sorghum, millet, and various fruits, legumes, and vegetables.

However, postharvest deterioration from microbial invasion is the primary cause of loss in yam production and a major hindrance to long-term tuber storage (Ikotun, 1983; Ikotun, 1989; Okigbo et al., 2009). Pathogenic organisms typically enter tubers through areas separated from stems at harvest, broken root tips, or other natural openings and cracks sustained during harvesting, transit, or storage (Okigbo et al., 2009).

01

Global Food Security Challenge

Postharvest yam losses significantly contribute to food insecurity and economic hardship, particularly in developing nations.

02

Postharvest Loss Problem

Microbial invasion causes extensive deterioration and hinders long-term yam storage, representing the most costly plant disease losses.

03

Pathogen Entry Points

Organisms enter through harvest separation points, broken root tips, or surface cracks from harvesting, transit, or storage.

Nigeria is the world's largest yam producer, yielding 35.017 million MT annually (Kleih et al., 2012). Microbial-induced postharvest deterioration and rot are the single most important factor impeding commercial yam production in Nigeria (Ikotun, 1983; Ikotun, 1989), in addition to insufficient research and capacity building (Taiga, 2011; Onyeka et al., 2011). Annually, rot leads to 7 million MT of yam losses. Tuber rot can be soft, wet, or dry, occurring pre- or post-harvest due to soil-borne pathogen infections (Taiga, 2011).

Recent studies on plant extracts offer new opportunities for tuber disease control. While *Allium sativum* and *Psidium guineensis* are commonly used against microbial isolates, their application against fungi associated with potato and yam tuber rot is less frequent.

Unlike synthetic fungicides that pose environmental and health risks, *Moringa oleifera* extracts contain natural antimicrobial compounds including glucosinolates, phenolic compounds, and flavonoids that demonstrate broad-spectrum activity against postharvest pathogens (Arshad et al., 2025). Recent studies show *Moringa* extracts achieve comparable efficacy to synthetic treatments while being biodegradable, non-toxic to humans, and accessible to smallholder farmers (Abdelwanis et al., 2024).

Moringa is a shrub plant, an angiosperm, dicot and perennial. It is also called drumstick tree, horseradish tree or ben tree. It is a small to medium-sized (usually growing to 10 or 12m in height), evergreen or deciduous tree. It is valued mainly for its edible fruits, leaves, flowers, roots and seed oil. *Moringa oleifera* can easily adapt to varied ecosystems and farming systems and so known for its resistance to drought and diseases (Odee, 1998).

Objective of the Study

The objectives of the study include the following: 1. to monitor spoilage of stored and yam tubers. 2. isolation and identification of microorganisms associated with the spoilage of yam tubers. 3. determination of the efficacy of aqueous *Moringa oleifera* leaves, seeds and bark extracts against pathogenic microorganisms responsible for tubers spoilage.

LITERATURE REVIEW

The world production of yam was estimated at 28.1 million tons in 2003. 96% of this came from West Africa, the main producers being; Nigeria with 71% of world production, Cote d'Ivoire 8.1% Benin 4.3% and Ghana 3.5% in the humid tropical countries of West African yams are one of the most highly regarded food products and are closely integrated into the social, cultural, economic and religious aspects of life. The ritual, ceremony and superstition often surrounding yam cultivation and utilization in West Africa is a strong indication of the antiquity of use of this crop. Nigeria, the world's largest yam producer, considers it to be a man's property and traditional ceremonies still accompany yam production indicating the highs status given to the plant. There are many varieties of yam species widespread throughout the humid tropics but the edible yams are derived mainly from about ten. The most economically important species are: Botany and Agronomy of Yam Yam is the name given to several plant species in the genus *Dioscorea* including *Dioscorea alata* (white yam). *Dioscorea bulbifera* (potato yam). *Dioscorea cayenensis* (yellow yam). *Dioscorea esculenta* (Asiatic yam) and *Dioscorea batatas* (Chinese yam) that are grown for their edible tubers.

Global Production

Nigeria dominates world yam production at 71%, with West Africa accounting for 96% of global output.

Yam Species

Multiple *Dioscorea* species including *alata*, *bulbifera*, *cayenensis*, *esculenta*, and *batatas* grown for edible tubers.

Cultural Significance

Deeply integrated into West African social, cultural, economic, and religious life with traditional ceremonies.

Botany and Agronomy of Yam

Yams are mainly grown in tropical and subtropical climates and they do not grow well at temperatures below 220C (71.60F) and are killed by frost. The optimum temperature for the growth of yams is between 25 and 300C (77-860F). They grow optimally in well-draining fertile soils with a pH between 5.5 and 6.5 in full sun or part shade. Very wet soils should be avoided as this promotes tuber rot. Propagation Yams are propagated negatively from small tubers. Land should be prepared for planting by plowing and harrowing. Tubers should be planted in trenches to a depth of 15cm (6 in) allowing at least 30cm (12 in) between individual plants and 1.5cm (5ft) between rows. the soil is often mounded around plants or ridged to aid drainage. It is common practice to stake plants with a 2-4m (6.6-13.2 ft) support to allow them to climb and ensure that all parts of the plant receive adequate sunlight.

General care and maintenance

Yams require 100cm of water distributed evenly throughout the growing season. Yam plants should be mulched after planting to prevent plants from drying out. Failure to mulch the plants will result in drastic decreases in yield.

Harvesting

Yams can be harvested at any time after the leaves have started to yellow. The soil should be carefully dug around the tuber and the tuber cut from the vine. Harvesting is best carried out on sunny, dry days to prevent tuber rot. Yam tubers are harvested in Nigeria mostly between June and September and most of which are stored in different storage facilities depending on the cultural and traditional values as well as the technological advancement of the people of such area (Amusa, 2000) until consumption or replanting.

Yam Diseases

Yam anthracnose disease

Anthracnose disease of yam has had a considerable impact on yam production world-wide. This is caused mostly by the fungus *Colletotrichum ploeosporioides* Jackson and Nwhoof. IITA (1993) reported that *Glomerela cingulate* (isolate number IMI W3725) was the yam anthracnose indicing pathogen in southwestern Nigeria (IITA, 1989-1993). *G. cingulate* is the perfect state of *C. gloeosporioides*, the form that is usually found causing fields anthracnose disease (Simon, 1993).

Yam Mosaic Virus Disease

This disease is caused by an aphid-transited *potyvirus* that infects several species of *Dioscorea*, particularly *D. alata D. cayenensis*, *D. rotundata* and *D. trifida*. The symptoms observed in each host can be vein banding, curling mottling, green-spotting, flecking etc. Yam mosaic virus (YMV) is considered to cause the mot several losses in yams. It is known that the most economically important virus disease of yam so far characterized are caused by members of the *potyvirus* group, but there is inadequate information on the number and variability of these viruses.

Dry Rot

The symptoms through vary with varying coloration depending on the invading pathogen, the infected tissues become hard and dry. When tubes are infected with *Penicilium oxalicum* and *P. cyclopium*, the tubers turn brown, become hard and dry maintaining their integrity except when the tissues were invaded by *S. marcescens*. Such invaded tissues become covered with the greenish mycelia of the fungus. When tubers were infected with *Aspergillus niger* and *A. tamari*, such tissues subsequently turn brown with yellowish margin. *Rosellinia bounodes* and *Botryodiplodia theobromae*, has been reported to cause dry black rot. The infected tubers first turned grey and then black such tubers become pulverulent, breaking into small dry particles.

Soft rot

Infected tissues become soft ramified by the fungal mycelium. The causal fungi quickly ramified the tissue which turn brown and become soft and at times we due to a rapid collapse of the cell walls. Fungi associated with this type of rot are *Rhizopus spp*, *Mucor circinelloides*. *S. rolsii* and *Rhizoctonia solani* (Green et al., 1995) and *Armillariella mellea*.

Wet rot

Wet rot is characterized by the oozing of whitish fluid out of the tissue when pressed. This symptom is usually associated with a bacterium, *Erwinia carotovora* pv *carotovora*.

Effect of Diseases Associated with Yam and Their Management

Yam diseases control has been extensively studied, and several measures has been recommended. These include the use of crop rotation, fallowing and planting of healthy materials and the destruction of infected crop cultivars (Nwakiti, 1982). For soil borne diseases such as nematodes and selerotium diseases, the site on which yam plants are to be cultivated are often recommended for soil testing for the presence of the pathogen (Amusa, 2000). Nematode can be controlled by the use of crop rotation and the use of nematicides such as carbofuran granular at the planting (Amusa, 2000). Dipping of seed spaces in Nemacuron before planting also eliminate the inoculum of the pathogen from the planting materials. Early plowing and thorough disking which exposes the sclerotia to early germination and exhaustion before planning has also been recommended (Arene, 1980).

Planting of yam setts with disease-free material has been found very effective in reducing nematode problems. Yam setts are often treated with a suspension of Fernasan D (thiram) or two handfuls of wood ash in 4 litres of water (Osai, 1993), after which the yam setts are spread under shade for the cut surface to dry before planting. Use of virus-free planting materials and meristem culture has been recommended in the case of controlling viral diseases (Mantell, 1980).

For post-harvest losses, minimizing physical damage of tubers during post-harvest operations has been recommended and is being practiced. Treatment of yam tubers with fungicide such as benlate and Captan has been found to be effective in reducing fungal yam rot. Due to toxicity of many chemicals, the use of Tecto (Thiabendazole) locallymade fry gins or wood ash before storage which are known to have little or no mammalan toxicity have also been recommended. Fungal infected yam tuber treated with Tecto at the concentration of between 0.6 and 1.0kg/500 of yam tubers had significant reductions in weight loss compared with the control. Thiabendazole applications have been reported to stimulate sprouting of yam minisetts (Amusa and Ayinla, 1997). The boring beetle attack on shoot and tubers can be controlled by granular application of diazinon and carbofuran. While treatments of the yam tubers with insecticide dust (Actellic 2% Dust; aipirimiphos-methyl) will reduced fungal infections and also ameliorate physical damages acquired during harvest resulting in significantly fewer fungal seasons (Morse et al, 2000).Moreover, yam farmers in south western Nigeria have been processing over 1/2rd of the harvested yam tubers into chips or cubes which can be stored between 6 months and one year (Amusa, 2001) as a means of reducing post-harvest losses associated with yam storage. It has been reported that the most effective and desirable means of controlling field yam disease is by the selection and planting of resistant cultivars (Nwakiti et al., 1987).

The use of anthracnose-resistant cultivars (e.g TDA 291, TDA 297) bred and released by the international Institute of tropical Agriculture (IITA) has been advocated (IITA, 1993).

However these screening procedures are very cumbersome and time consuming. Screening for resistance varieties with the use of toxic metabolite of *Colletotrichum spp* has been found effective, reliable and comparable to the conventional screening methods.

Use of Botanicals in Management of Diseases

Numerous in vitro studies have validated the efficacy of plant-derived pesticides in many branches of agriculture. Comparatively however, fewer in vivo works have been done on stored products caused by moulds and storage bacteria. These investigators however inferred that plant materials were fungitoxic and prophylactic against rots organisms. Many rot inducing organisms of cassava including *Aspergillus niger* were inhibited in vitro by plant-based fungicides from *A. meleguata* and *A. indica.* It was also reported that *Allium sativum* exhibited the toxic effects on all the organisms assayed in a study while *O. gratissiumum* retarded the *mycelial* growth of the mycoflora by 64%.

USE OF MORINGA EXTRACTS IN THE MANAGEMENT OF DISEASES

Moringa oleifera is the most widely cultivated species of a monogeneric family the Moringa cease, which has become naturalized in many locations in the tropics. It is native to the southern foothills of the highways in the North-west India and grown mainly in semi-arid tropical and subtropical areas of North Eastern and South Western Africa particularly in Nigeria. It is a perennial softwood tree which grows best in dry sandy soil with deep roots and timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses. All parts of the Moringa tree are edible and have long been consumed by humans. *M. Olifera* can easily adapt to varied ecosystems and farming systems and so known for its resistance to drought and diseases.

Traditional Medicine

Various parts used therapeutically for rheumatism, venomous bites, cardiac stimulants, cholera, scurvy, respiratory ailments, and tumors.

Antimicrobial Properties

Leaf juice has antibacterial and antimalarial properties. Seed extracts show anti-fungal activities against dermatophytes.

Multiple Applications

Used for water purification, animal feed, medicine, food, fuel wood, and as natural coagulant for turbid water clarification.

The many uses for Moringa include: ally cropping (biomass production), animal forage (leave and treated seed-cake), and biogas (from leaves), domestic cleaning agent (crushed leaves). Blue dye (wood), fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey- and sugar cane juice clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, biopesticide (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum), water purification (powdered seeds). Moringa has been proven to be useful sources of food, medicinal products, fuel wood, renewable polymer products, animal and acquculuture feeds.

Drumstick tree continues to have an important role in traditional Asian and West African medicine. Some of the earliest studies on the therapeutic effects of *Moringa oleifera* extracts are that of Kohler et al. In Nigeria, it is locally used as tonic and aphrodisiac, and in the treatment of intestinal worms and asthma. It is the most used because it is the most widely distributed, popularly known and most utilized of the Moringa species. In traditional medicine various parts of the tree are used therapeutically, including for treatment of rheumatism, venomous bites, and as cardiac and circulatory stimulants, cholera, scurvy, respiratory ailments, tumours and they are also applied externally to cure inflammatory swellings. Juice extracted from the leaves has antibacterial and antimalarial properties. Moringa seed oil (30-40% yield), also known as Ben oil, is a sweet non-sticking, non-drying oil that rests rancidity.

Moringa oleifera has been used extensively in traditional medicine for the treatment of several ailments, promotes digestion, skin diseases, diarrhea, as stimulant in paralytic afflictions, epilepsy and hysteria. Various parts of the plant have been shown to be useful, such as the roots have been experimentally shown to have anti-inflammatory action, the leave, stem bark and seeds have been reported to have therapeutic properties. The seed powder of *Moringa oleifera* works as a natural coagulant which clarifies very turbid water. Studied on crude ethanol extract of dried *Moringa oleifera* seeds have been suggested to have anti-tumor promoting activity and also wound healing property, while invitro anti-fungal activities against dermatophytes such as *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Microsporum canis* has been reported, indicating that extracts could be used for future development of anti-skin disease agent. In Nigeria, *Moringa oleifera* is an edible plant mostly found in the Northern part of the country. Despite much of the information on the excellent medicinal value of the plant, a lot more work is required to further determine the scope of its value as an alternative, cheap and effective antimicrobial agent in addition to its other uses.

MATERIALS AND METHODS

Study Area

The experiment was conducted in the Microbiology laboratory of Wesley University, Ondo.

Source and collection of materials

Diseased and healthy sweet potato and yam tubers were obtained from Moferere market, Ondo West Local Government Area, Ondo State. *Moringa* leaves, seeds, and barks were collected from Wesley University's ethnobotanical garden. Distilled water, laboratory gloves, spatula, sodium hypochlorite, foil paper, and cotton wool were sourced from the Microbiology laboratory store at Wesley University, Ondo.

Preparation of culture media

Nutrient agar

Twenty-eight grams of nutrient agar powder were dissolved in 100 liters of distilled water, then autoclaved at 121°C for 15 minutes. The solution was cooled in a 45°C water bath before dispensing into petri dishes, allowing it to solidify prior to microorganism introduction.

Potato dextrose agar

Thirty-six point nine grams of potato dextrose agar were weighed, and 1 liter of distilled water was added and stirred to dissolve. The mixture was autoclaved at 121°C for 15 minutes. It was then cooled in a 45°C water bath before dispensing into petri dishes, allowing it to solidify before microorganism introduction.

Preparation of *Moringa* extracts

Selected *Moringa* parts (leaves, seeds, and bark) were thoroughly washed with sterile distilled water to remove dust, then air-dried at room temperature (27°C) for three weeks. The air-dried seeds and barks were ground into powder using a mortar and pestle, while the leaves were milled into powder with a blender. Ten grams of each powdered plant part were collected.

Aqueous extraction

Ten grams of each pulverized plant part were weighed and transferred to a conical flask. One hundred milliliters of distilled water were added, and the mixture was boiled for 15 minutes in a 100°C water bath, then filtered while hot.

Isolation of Pathogens

Isolation from Yam Tubers

Pathogens were isolated from rotted sweet potato and yam tubers following the method described by Okigbo (2005). Tubers were surface sterilized with cotton wool and 70% ethanol. A small portion of the diseased area was then picked with sterile forceps and spread on prepared media. Inoculated nutrient agar plates for bacteria were incubated at 37°C for 24-48 hours, while potato dextrose agar plates for fungi were incubated at room temperature for 2-5 days. Plates were observed daily for cell growth, and distinct colonies were examined upon establishment.

Sub-culturing

Distinct colonies were sub-cultured onto fresh media. A sterile inoculating loop was used to streak each colony onto PDA plates for fungi, incubated at 25°C for 2-5 days. For bacteria, desired colonies were streaked on nutrient agar plates and incubated at 37°C for 24 hours to obtain pure cultures.

Preservation of Isolates

All fungal isolates were preserved and maintained on PDA slants, and bacterial isolates on nutrient agar slants, both stored at 4°C in a refrigerator.

Identification of Isolates

Fungi and bacteria isolates were identified after characterization. Colony and structural characteristics were compared with known taxa in standard manuals (e.g., Bergey's Manual) for identification and confirmation.

Identification of Bacteria Isolates

Bacteria isolates were identified based on existing taxa in standard manuals like Bergey's Manual of Determinative Bacteriology (Goszczynska et al., 2000; Bucaman and Gibbons, 2004). Characterization involved four levels:

1. Colony Features: Close examination recorded extent of growth, color, nature of colony edge, and elevation.

2. Microscopic Examination: Isolates were observed under a binocular microscope to record reaction to general dyes (Gram stain) and specific dyes (indicating spores, flagella). Cell shape and arrangement were also noted.

3. Biochemical Reaction Tests: Tests included catalase activity (bubble production for positive/negative reaction), starch hydrolysis, M.R.V.P, and H_2S production.

4. Sugar Utilization Tests: Determined the isolates' ability to utilize sucrose, glucose, maltose, and lactose, indicated by acid and/or gas production.

Fungi isolates

The colony features of each fungi isolates will be studied and documented including; texture, extent of growth, colour, and presence of visible mycelia, pigmentation, form/shape and edge of colonies. Following this, slide mounts of each isolates will be made and stained with lactophenol/cotton blue-dye. The slide mount will be examined (viewed) under a low power microscope (x20) and the structures will be noted including the presence of sporogiophores, conidiophores etc, their direction of growth, branching, septation, shape and colour of conidia and spores etc, these characteristics would be recorded and later compared with those in the fungi manual.

Pathogenicity test

The identified isolates were tested on their ability or otherwise to initiate disease symptoms on a healthy yam tuber and sweet potato tuber. Small healthy sweet potato and yam tubers were selected and labeled accordingly. With the aid of a flamed forceps, tiny holes were bored on the surface of the tubers and a core sample of the isolate from the pure culture was inserted into the holes and then closed back with sweet potato or the yam flesh. The inoculated points were marked with the aid of a permanent marker. Similar holes were made on an adjacent position in the sweet potato and yam tubers and closed back again with the flesh but without any pathogen to serve as control. All the inoculated yam and sweet potato samples were kept at room temperature (28-300C) and watched over a period or 7-14 days for soft rot symptoms such as wetting, softening, discoloration (darkening) and offensive odor. At the end, microorganisms were re-isolated from the points of each inoculation. Test on the control of the pathogen were based on the ability of the plant extracts to inhibit the growth of the pathogen in the culture plate.

In vitro test

Eight bacteria and fungi isolates were used to carry out the test. 15-20mls of molten PDA and nutrient agar were dispensed in Petri dishes and were allowed to solidify. With the aid of a swab stick three replicates of each isolates were made on petri dishes containing the solidified agar medium and they were labeled accordingly. An 8mm diameter of work borer was used to create holes at different portions on the petri dish. Three holes contained *Moringa* leaf, seed and bark extracts respectively and the forth contained the control which was distilled water. The plates were arranged on laboratory desk following complete randomized design. The petri plates containing the bacteria isolates were incubated at 370C for 5-7 days during which measurement of growth was done using a meter rule at an interval of 24 hours.

Identification of organisms

Gram staining technique

A smear of the inoculum was prepared on a glass slide and was passed through flame slightly to heat fix it, the smear was then stained with crystal violet (primary stain which imparts its colour on the cells), after 60 seconds, the crystal violet was washed off, and the smear was flooded with iodine, after 30 seconds, the iodine was washed off under slow running tap and the washed with ethanol (decolorizing agent which removes the purple from the cell of some species), the ethanol is then rinsed off under slow running tap and the smear was counter stained with safranin (secondary stain; a basic red dye), after 30 second the safranin was washed off under slow running tap, blotted dry and examined under the microscope. The Gram positive bacteria retained the purple dye, while those that lose the purple colour (turned pink) were classified as Gram negative.

Biochemical test carried out on pure isolates

Catalase test

Grease-free slides were cleaned and flamed wire loop was used to pick 24hours old culture of organism unto the slide. This was emulsified with a loop full of hydrogen perioxide. The results of reaction were recorded. Formation of immediate and sustained bubbles resulting from effervescence was recorded as positive reactions.

Starch hydrolysis

Twenty-eight grams of nutrient agar powder and 10g of starch was weighed and dissolved in 1000ml of distilled water. It was autoclaved at 1210C for 15 minutes. It was allowed to cool and then poured into petri dish and allowed to set. The plate was streaked with 24hours old culture once across the surface, inverted and incubated at 370C for 48hours. It was then test with the reagents by addition of gram's iodine to the growth area with the aid of dropper pipette. Clear zone was recorded as positive while blue-black colouration was noted as negative reaction.

Sugar fermentation test

Fermentation test media included fermentable sugar (mannitol) for energy production, non-fermentation sources of nitrogen and other nutritional requirements (as a basal medium), a pH indicator (phenol red), and durham tubes (vertically inverted) that collects gas and 24hours old culture of organisms.

The basal medium (peptone water) was prepared, adding 1.0g of the desired sugar and a few drops of phenol red. 5.0ml was dispensed into labeled test tubes with inverted Durham tubes, corked, and autoclaved at 1210C for 15 minutes. After cooling, the sugar media were inoculated with a loopful of 24-hour old culture and incubated at 370C for 72 hours with daily examination. A yellow color change of phenol red indicated acid formation (fermentation), and gas production was noted by gas collection in the inverted Durham tubes.

The methyl red and vogues-proskauer test

The methyl red (MR) and Voges-Proskauer (VP) tests differentiate between *coli* and *acrogens*. Coliform organisms rapidly ferment dextrose, causing a pH drop. Sterile MR-VP broths were aseptically inoculated in duplicate with a 24-hour old broth culture and incubated for 48 hours at 370C. After incubation, methyl red indicator detected high acid concentrations for the MR test, while Barritt's reagent detected acetylmethylcarbinol for the VP test. A red coloration indicated a positive MR result; yellow indicated negative. For VP, a deep rose color after acetylmethylcarbinol addition indicated a positive result; absence of rose color indicated negative.

Hydrogen Sulphide Test (H2S)

Triple Sugar Iron Agar slant was prepared by dispensing 17g of medium into 1 liter of distilled water, heating to dissolve, and then dispensing 10-20ml into test tubes. These were autoclaved and solidified in a slanted position. A 24-hour old nutrient broth culture was stabbed into the butt and streaked along the slope, then incubated at 360C for 24-48 hours. Observations recorded included acid production (pH change), CO_2 production, and blackening due to H_2S production. Blackening indicated a positive result; yellow indicated negative.

RESULTS

Effect of microorganisms on the spoilage and weight of stored yam tubers

Sweet potato and yam tubers experienced weight loss during storage due to water loss (transpiration) and biomass loss from microbial activities. Microbial degradation significantly contributed to spoilage, turning tuber tissue into powder. Samples of *Dioscorea rotundata* showed substantial weight loss, ranging from 17.4% to 84.2% and 24.0% to 72.0% respectively. Tables 1 illustrate varying weight loss levels in yam tubers over two consecutive months.

Table 1: Percentage weight loss in yam tuber over a period of 2 months

Samples	Initial weight (g)	1 month after storage (g)	2 months after storage (g)	%weight loss 1	%weight loss 2
YMT 1	310	227	109	26.8	64.8
YMT 2	480	295	154	38.5	67.9
YMT 3	250	190	107	24.0	57.2
YMT 4	1150	800	458	30.4	60.2
YMT 5	105	70	34	33.3	67.6
YMT6	75	50	21	33.3	72.0
YMT7	100	67	31	33.0	69.0
YMT8	80	52	24	35.0	70.0
YMT9	215	145	85	32.6	60.5

Source: Author's computation

Cultural and biochemical characteristics of bacteria pathogens isolated

Tables 2 represent the identification and preliminary confirmatory tests for the bacteria isolated from sweet potato and yam tubers based on Bergey's manual of determinative bacteriology. Identification was done in three levels; colony features, microscopic examination and biochemical reaction tests.

In Characterization and identification, *Erwinia* sp was found to be rod shaped cream in colour, slightly raised colonies, a Gram negative, rod after staining. Smear culture with a drop of hydrogen peroxide (H_2O_2) produced bubbles indicating positive for catalase tests. It utilized glucose similar to what was reported by Bradbury.

Table 2: Morphological and microscopic characteristics of fungi isolated from *Dioscorea rotundata*

Isolates	Colonial morphology on PDA	Microscopic characteristics	Probable organism
YMT 1	Creamy threat-like colonies	Round sporangia with many mycelia	*Mucor mucedo*
YMT 3	Yellowish or slightly brown	Conidiophores arising from long branched, thick-walled, mostly brownish sometimes branched footcells, Condiia are large and mostly globose	*Aspergillus niger*
YMT 4	Creamy thread-like colonies	Round sporangia with mycelia	*Mucor mucedo*
YMT 5	Yellowish or slightly brown	Conidiophores arising from long branched, thick-walled, mostly brownish sometimes branched footcells, Conidia are large and mostly globose	*Aspergillus niger*
YMT 9	Colonies showed typical green surface pigmentation with a suede-like surface consisting of dense conidiophores Texture was powdery and the colour on the reverse side was yellow	Septate hyphae with thin-walled conidiophore stipes are short, smooth-walled and had conical shaped terminal, vesicles conidia were produced in basipetal succession forming long chains and globose to subglobose	*Aspergillus fumigatus*

Table 3: Antimicrobial activity of *Moringa* Plant extracts against bacterial isolate (in vitro)

	Plate 1 Zones of Inhibition			Control (Distilled	Plate 2 Zones of Inhibition			Control (Distilled	Plate 3 Zones of Inhibition			Control (Distilled
Extracts	Leaf (mm)	Seed (mm)	Bark (mm)	Water) (mm)	Leaf (mm)	Seed (mm)	Bark (mm)	Water) (mm)	Leaf (mm)	Seed (mm)	Bark (mm)	Water) (mm)
YMT 6 Day 2	12	15	14	8	12	14	14	8	12	14	14	8
YMT 8 Day 2	18	8	8	8	18	8	8	8	18	8	8	8

Morphological and microscopic characteristics of fungal isolated

Some morphological and microscopic characteristics of fungal isolated from sweet potato and yam tubers are revealed in Tables 3

Culture and sensitivity test of selected isolates

Those cells that were inhibited failed to resist the action of the corresponding plant extracts against them so they became sensitive to the extracts. Antimicrobial sensitivity testing revealed that the extract that was most effective against the microorganisms causing rot on tuber was the *Moringa* leaves extract. The other two extracts, *Moringa* seeds and *Moringa* back extracts were found to be satisfactory.

Table 4: Antimicrobial activity of *Moringa* Plant extracts against fungi isolates (*in vitro*)

Extracts	Plate 1 Zones of Inhibition Leaf (mm)	Seed (mm)	Bark (mm)	Control (Distilled Water) (mm)	Plate 2 Zones of Inhibition Leaf (mm)	Seed (mm)	Bark (mm)	Control (Distilled Water) (mm)	Plate 3 Zones of Inhibition Leaf (mm)	Seed (mm)	Bark (mm)	Control (Distilled Water) (mm)
YMT 3 Day 2	14	8	8	8	14	8	8	8	14	8	8	8
YMT 4 Day 2	8	12	8	8	8	12	8	8	8	12	8	8

DISCUSSION

This study corroborated diversified forms of fungal and bacterial species associated with rot of sweet potato and yam tubers. Fungi species isolated include: *Aspergilus fumigatus*. *Aspergillus flavus*, *Aspergillus niger*, *Botrydiplodia theobromae*, *Mucor mucedo* and *Rhizopus stolonifer*, and bacterial species include: *Bacillus species*, *Erwinia species*, *Enterococcus species*, *Micrococcus species* and *Staphylococcus species*. The *Aspergillus species* are one of the major causes of rot in sweet potato and yam tubers. The fungi: *Aspergilus fumigatus*. *Aspergillus flavus* isolated are known to be human pathogenic or opportunistic human pathogen organisms. They may even secrete substances that are harmful to man (Ajay et al, 2011).

22mm

Highest Inhibition

Moringa leaves extract showed maximum zone of inhibition

7M

Annual Loss

Million metric tons of yam lost annually in Nigeria

71%

Global Production

Nigeria's share of world yam production

Different types of spoilage were observed on sweet potato and yam tubers and it ranged from dry, soft to wet rot. Sweet potato and yam are perishable and are susceptible to a number of fungi and bacteria. Infection of sweet potato and yam tubers can cause the production of bitter flavor, tissue breakdown and surface blemishes. Common sources of infection are from *Rhizopus sp*, (soft rot) and *Botrydiplodia* (black rot).

Wastage from unprocessed yam tubers results from chemical reactions after an injury in the tubers, excessive sprouting, shrinkage and spoilage due to bacterial and fungal attack (Paneerselv and Abdul Jaleel, 2008). *Ipomoea batatas* and *Dioscorea rotundata* suffered massive weight loss during storage ranging from 17.4 to 84.2% and 24.0 to 72.0% respectively. Wet rot was observed on a yam tuber which started with dry rot to soft rot and led to depression of the yam tuber when it was pressed leading to the exudation of fluid out of the infected tissue. This symptom was caused by the bacterium, *Erwinia carotovora* (IITA, 1993). Result of the pathogeniety test confirmed that the organisms isolated were responsible for the disease they caused on sweet potato and yam tubers.

Antimicrobial activity test with aqueous extracts of *Moringa oleifera* leaves, seeds and barks demonstrated efficacy of antimicrobial potentials on test pathogenic organisms in vitro. This implied that *Moringa oleifera* commonly used in human medicine also possesses antimicrobial effects on plant pathogenic microorganism. The efficacy of these plant extracts is enhanced by the presence of active ingredients, Moringa leaves extract was the most effective of all the extracts tested causing inhibition of the growth of almost all the tested pathogens in vitro and this was followed by Moringa seed extract and Moringa bark extracts coming last.

Moringa bark extracts had the highest zone of inhibition, inhibiting the growth of a *Bacillus sp* with a diameter of 22mm. Moringa leaf and bark extracts have no inhibitory effect on *Mucor mucedo* while Moringa seed and bark extracts showed no zones of inhibition on *Aspergillus niger*, *Aspergillus fumigatus* and *Micrococcus sp*.

CONCLUSION AND RECOMMENDATIONS

Aqueous extracts of *Moringa oleifera* leaves could serve as bioprotective agent against microorganism causing spoilage in sweet potato and yam tubers. These have potentials as alternative to synthetic bactericide and fungicides. This method of phyto disease control is eco-friendly, cost effective, economically viable and not toxic to humans and animals. This method is also at the disposal of peasant farmers and marketers of sweet potato and yam tubers.

01

Biocontrol Agent Development

Moringa leaves and bark extracts should be developed as commercial biocontrol agents for postharvest yam storage.

02

Farmer Education

Train peasant farmers and marketers on preparation and application of Moringa extracts for yam preservation.

03

Research Extension

Further research needed to determine optimal concentrations and application methods for different storage conditions.

04

Sustainable Agriculture

Promote eco-friendly, cost-effective alternatives to synthetic fungicides for sustainable food security.

In order to prevent degradation of biodiversity and the adverse effect of the use of synthetic fungicides in control of microbial rot in sweet potato and yam tubers. It is therefore recommended based on this research work that leaves, seeds and bark extracts of *Moringa oleifera* are capable of inhibiting the growth of bacteria and fungi pathogens that cause tuber spoilage in sweet potato and yam in the store.

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CONFLICTS OF INTEREST

The author declares no conflict of interest

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