https://dx.doi.org/10.4314/ijs.v26i2.17

ANTIMICROBIAL PROPERTY OF *DISTEMONANTHUS, ZANTHOXYLUM, MORINDA,* AND *MORINGA* SPECIES ON POTATO (*Solanum tuberosum*) POSTHARVEST BACTERIAL TUBER ROT

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(Received: 3rd June, 2024; Accepted: 19th July, 2024)

ABSTRACT

Potato (*Solanum tuberosum*) is a very important crop globally with a high value for human nutrition. Its productivity in Nigeria is hindered by bacterial rot disease, which is commonly managed with synthetic pesticides despite its detriment to humans and the environment. This study investigated the antimicrobial potential of four botanicals (*Distemonanthus benthamianus, Zanthoxylum zanthoxyloides* (*Zz*), *Morinda lucida,* and *Moringa oleifera* (*Mo*) *in vitro* against four postharvest potato tuber rot-causing bacteria. One hundred rotted tubers were collected from four markets viz: Bodija, Sabo, and Apata, Ibadan (Oyo State), and Garki (Abuja). Three concentrations (500, 1000, and 1500mg/ml) of methanol extract of each of the botanicals were evaluated against the rot bacteria. The antimicrobial property of the most effective explant *in vitro* was evaluated *in vivo* against the rot bacteria. Four bacteria (*Erwinia carotovora* (*Ec*), *Clavibacter* species, *Bacillus subtillis*, and *Pseudomonas syringe* pv. *Phaseolicola*) were isolated and identified to be responsible for the potato rot. The severity of rot induced by the bacteria ranged between 39.77 and 46.34%. *Distemonanthus benthamianus* extract (at 500-1500mg/ml) significantly ($p<0.05$) produced the highest antibacterial property *in vitro* against the four bacterial isolates, followed by *Zz* whereas the other explants showed no bacteriotoxic property except *Mo* against *Ec* only at 1500mg/ml. *Distemonanthus benthamianus,* next to Erythromycin (antibiotics), produced higher inhibition zones (5.0±0-9.3±3.1mm) on all the bacteria isolates than all other explants and significantly inhibited rot development in potato *in vivo*. The high antimicrobial potential of *D. benthamianus* against postharvest bacterial tuber rot of potato suggests a safe biopesticide for postharvest potato disease management

Keywords: *Distemonanthus benthamianus,* potato, plant extract, postharvest, *Zanthoxylum zanthoxyloides.*

INTRODUCTION

Potato (*Solanum tuberosum*) is next to wheat and rice globally in terms of important food crops for human consumption (Devaux *et al.*, 2020). It is an important source of carbohydrates, proteins, vitamins C, B6, and potassium (Camire *et al.,* 2009). Its global production is about 374.8 million tons on 17.8 million ha of land (FAO, 2021). The largest annual production (95.6 million tons) was recorded in China. Africa's total production has been put at about 27.2 million tons, with Egypt as the highest producer with about 6.2 million tons. Nigeria produces about 1.2 million tons annually on a land area of about 0.3 million ha (FAO, 2022). Potato is one of the staple tuber crops in Nigeria being produced mainly in Jos, Plateau State. It is eaten boiled, fried, and in stews. It is used industrially to brew alcoholic beverages while potato starch is used as thickeners and binders of soups (Yazid *et al.,* 2018).

Potato production is hindered by insect pests and disease infections. One of the most important constraints is postharvest spoilage of tubers. Many fungi such as *Fusarium* spp., *Phytophthora infestans*, *Aspergillus niger*, and bacterial species including *Erwinia carotovora var. carotovora, Ralstonia solanacearum* and *Streptomyces scabies* cause rotting of potato tubers (Kluepfel *et al.,* 2000). Some of the important bacterial diseases of potato are brown rot (caused by *R. solanacearum*), common scab (*S. scabies*), ring rot (*Clavibacter michiganensis* subsp. *sepodonicus*), black leg (*Pectobacterium carotovorum* subsp. *atrosepticum*) and soft rot (*P. carotovorum* subsp. *carotovorum*) (Abu-Obeid *et al.*, 2017). However, bacterial soft rot is one of the most damaging potato diseases causing a great reduction in yield and quality in the field and during transit. This disease is prevalent in tropical and subtropical regions (Bhat *et al.*, 2010a). Soft rot diseases are caused by *Bacillus*, *Pseudomonas*, *Enterobacter cloacae, Erwinia,* and other bacteria

(Agrios, 2005; Schroeder *et al.*, 2009). Soft rot caused by *P. carotovorum* subsp. *Carotovorum,* also known as *E. carotovora* subsp. *carotovora* has a wide host range in tropical regions infecting vegetable species including cabbage, cauliflower, lettuce, onion, pepper, carrot, and potato (Bhat *et al.*, 2010b; Mansfield *et al.*, 2012).

Amienyo and Ataga (2007) have reported the control tuber rot of potato using the synthetic pesticides Dichloro nitroanline against *Rhizopus* soft rot. However, chemical pesticides cause environmental pollution and adversely affect a variety of non-target organisms (Kwon-Ndung *et al.,* 2022; Ogunsola and Ogunsola, 2023). To tackle the growing challenge of pesticide hazards researchers are exploring safer alternatives like plant extracts (Borges *et al.*, 2021). These biopesticides have proven effective against diseases in various crops (Nurmansyah *et al.*, 2022; Akinbode, 2013; Ogunsola & Ogunsola, 2023).

The antimicrobial potentials of several plants have been reported. For instance, *Z. zanthoxyloides* (Fig. 1) of the family Rutaceae commonly known as Senegal prickly-ash or "artar" root or "Orin-ata" (spicy chewing stick) by the Yorubas in Nigeria, is used in traditional remedies for toothaches, malaria, and diarrhea (Singh and Singh, 2005). The pharmacological studies of *Distemonanthus benthamianus* (commonly called "Ayan" by the Yorubas in Nigeria and of the family *Fabaceae*) have revealed its antimicrobial, anti-tumor, and anti-oxidative properties (Adeniyi *et al.,* 2010). Similarly, the leaf extract of *Morinda lucida* of the family *Rubiaceae* (commonly known as Brimston tree or "Oruwo" in the southwest region of Nigeria) is widely used to treat hypertension and malaria (Soladoye, 2005; Osuntokun, 2015), while that of *Moringa oleifera* (Lam) ("drum stick tree") is known to have antimicrobial, antioxidant, anticancer, cardiovascular, hepatoprotective and anti-ulcer activities (Prabhu *et al.,* 2011).

However, the antimicrobial capacity of the *D. benthamianus, Z. zanthoxyloides, M. lucida,* and *M. oleifera* in the management of bacteria causing postharvest rot of potato are not well reported. While previous researches focused on fungal potato rot in this region (Ogunsola and Aduramigba-module, 2014), bacterial rot remains understudied. Most West African farmers still rely on harmful synthetic pesticides (Ogunsola and Ogunsola, 2023). This study was therefore conducted to identify the bacteria causing postharvest potato tuber rot and investigate the effectiveness of the four botanical extracts (*D. benthamianus, Z. zanthoxyloides, M. lucida,* and *M. oleifera*) in controlling these diseases.

MATERIALS AND METHODS

Potato tubers were obtained from four markets viz: Bodija, Sabo, and Apata in Ibadan, Oyo State (Southwestern), and Garki in Abuja (Northcentral) Nigeria. Thirty (25 rotted and 5 healthy) potato tubers were collected from each market making a total of 120 tubers (100 infected and 20 healthy). Four botanicals were evaluated (Figure 1). *D. benthamianus* stems and *Z. zanthoxyloides* roots obtained from the Apata market in Ibadan were authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State. *Morinda lucida* and *M. oleifera* leaves were obtained from the field genebank of the National Center for Genetic Research and Biotechnology (NACGRAB) Ibadan, Oyo State.

Isolation and identification of bacteria from infected potato tubers

The rotten potato samples were washed under running tap water and pieces of necrotic tissues were surface sterilized with 0.5% Sodium Hypochlorite (NaOCl) for 2 mins, and rinsed with sterile distilled water (SDW) before plating on Nutrient Agar (NA). The inoculation was followed by incubation at 37^0C for 24 hrs after which the plates were examined for bacterial growth. Bacterial isolates were sub-cultured on NA to obtain pure culture for identification. The isolates were identified using macroscopic and microscopic examination of growth features and biochemical reaction tests (Table 1) described by Okigbo (2003) as below:

Gram staining reaction: A thin smear was prepared from the pure isolate culture by placing a loopful of SDW on the centre of a clean slide and the selected bacteria colony was emulsified and air dried. The smear was fixed by passing it through the flame three times and flooded with crystal violet for 1 min, after which it was rinsed with

SDW and flooded with Lugol's iodine solution for a minute and washed off with distilled water. The slide was subsequently flooded with ethanol (70%) until there is no more colour and immediately washed with water to prevent excessive discoloration. This was followed by counter staining with Safranin for 1 min after which it was washed off with distilled water and then blotted for observation. Microscopic examination then revealed the presence of either Gram-positive (appearing purple) or Gram-negative bacteria (appearing pink).

Oxidase test: A piece of filter paper was soaked with a few drops of oxidase reagent (tetramethyl-p-phenylene diamine dihydrochloride). A bacterial colony picked with a sterile wire loop was then smeared on the filter paper and observed for the development of a blue-purple colour within 10 seconds which indicates a positive result.

Anaerobic growth: Peptone (2 g/l), NaCl (5 g/l), KH_2PO_4 (0.3 g/l), agar (3 g/l) and bromothymol blue (3 g/l) were dissolved in distilled water and the pH was adjusted to 7.1. Then 5 ml of the medium was dispensed in test tubes and sterilized at 121°C for 20 minutes. 10% aqueous solution of filter sterilized glucose was added to the medium. Two tubes were inoculated with each of the test organism, one tubes was covered with a layer of sterile melted Vaseline to a depth of about 5 mm and the tubes were incubated at 27°C for 48hours. A colour change from blue to yellow in both tubes is recorded as positive for anaerobic growth (Schaad *et al.*, 2001).

Spore formation: A drop of SDW was placed on a clean slide, and a bacterial colony taken with a sterile wire loop were placed in the solution and air dry. The slide was flooded with a 5 % (w/v) aqueous solution of malachite green and stained for 10 minutes. This was followed by a thorough wash under running water and drying. The slide was counter-stained by flooding with a 0.5% (w/v) aqueous solution of safranin for 15 seconds, rinsed thoroughly with water, and blotted dry. The cells were observed at 40x magnification, with bacterial cells staining red and spores staining green (Schaad *et al.*, 2001).

Fluorescence pigment on King's B: Kings B medium was prepared following the manufacturer's instruction and then autoclaved. Bacteria isolates were streaked on the medium and incubated for 24 - 48 hours at 27°C. Plates were viewed under long wavelength (366 nm) ultraviolet lamp for fluorescence. Blue fluorescence and less pigment indicates the presence of pathovar of *P. syringae* (Schaad *et al.*, 2001).

Modified Sucrose peptone (MSP) medium: Modified sucrose peptone agar with added antimicrobial compounds and bromothymol blue was used. The medium composition include 20 g sucrose, 5 g peptone, 0.5g K_2HPO_4, 25 mg $MgSO_4.7H_2O$ and 20 g per liter of agar. The medium's pH was adjusted to 7.2, autoclaved and allowed to cool before adding a mixture of filter sterilized antibiotics solution. Yellow and doomed shaped and blue fluorescent pigment on this medium indicated the presence of *P. syringae* pv. *Phaseolicola* (Schaad *et al.*, 2001).

Triple sugar iron agar (TSI) test: The ability for the bacteria to ferment glucose, lactose and sucrose and to produce hydrogen sulphide was tested. A straight inoculating needle was used to pick a pure bacteria colony of 18 - 24 hours old. Each bacterium colony was inoculated in TSI agar by first stabbing through the center of the medium to the bottom of the tube, and then streaking the surface of the agar slant. The tubes were incubated at 37 °C for 18 - 24 hours and then examined for colour change in slant and butt. The blackening in the tube indicated the production of hydrogen sulphide (H_2S) and cracks in the medium indicated gas production (Cheesbrough, 2000)

Sulphide-indole-motility (SIM) test: This test was carried out to detect the motility, sulphide and indole production of each isolate. The semi-solid SIM medium was used. The isolates were stab-inoculated aseptically and incubated at 37°C for 24 hours. Motility was indicated by the spreading of the organism outside the line of stab, indole production was by the presence of a red-pink ring at the interphase after kovac's reagent was added and sulphide production by the changing of the original yellow colour media to black (Cheesbrough, 2000).

Modified Sucrose peptone (MSP) medium: Sucrose (20 g), peptone (5 g), K_2HPO_4 (0.5 g), $MgSO_4.7H_2O$ (25mg), and Agar (20 g) were dissolved in 1 litre of distilled water the pH adjusted to 7.2-7.4. The solution was autoclaved, cooled to 45°C and the following filter sterilized stock solutions were added: 2.0 ml of cycloheximide (100 mg/ml 75% methanol), 8.0 ml cephalexin (10 mg/ml distilled water), 1 ml **v**ancomycin (10 mg/ml distilled water) and 1.0 ml of bromothymol blue (15mg/ml 95% ethanol). Yellow and doomed shaped (levan positive) and blue fluorescent pigment on this medium shows the presence of *P. syringae* pv. *Phaseolicola* (Schaad et al., 2001)

Citrate test: Simmons citrate agar was used. A sterile inoculating loop was used to pick colonies from the 24-hour old culture and citrate slant aseptically before incubating at 35-37oC for 48 h to 7 days and observed for colour change. A change from green to deep blue is indicative of positive citrate utilization while the absence of a colour change indicates negative citrate utilization.

Catalase test: A drop of 3% hydrogen peroxide solution was placed on a clean grease-free slide, and a bacterial colony, taken with a sterile wire loop, was placed in the solution. The organism was mixed in the drop of hydrogen peroxide and observed for immediate and vigorous budding. The formation of bubbles (the release of oxygen) indicated a positive test and scanty or no bubbling implied negative result. Catalase-positive culture produces O_2 and bubble at ones (Cheesbrough, 2000).

Starch hydrolysis test: Starch agar, which is a differential nutritive medium was used. The bacteria isolates were inoculated onto a starch plate and incubated at 30°C until growth is observed (i.e. up to 48 hours). The Petri plates were then flooded with an iodine solution. If no enzyme is present, and therefore no hydrolysis, the amylose, and iodine react together to form a blue color. Depending on the concentration of the iodine used, iodine turns blue, purple, or black in the presence of starch. Appearance of purple-black colouration on the medium and a clear halo around the colonies indicates positive result.

Casein hydrolysis: A sterile inoculating needle was used to pick a pure bacteria colony of 18 - 24 hours old. The bacteria were inoculated by streaking a zig-zag line on the surface of the Skim milk agar plate. The plates were incubated at 37°C for up to 7 days and observed for a clear zone of casein hydrolysis along the bacterial growth line. Afterward, the plate was flooded with a 10% trichloroacetate solution. A positive test is indicated by the formation of a clear transparent zone of hydrolysis (casein hydrolysis) around the bacterial colony. A negative test is indicated by no clear zone of hydrolysis around the bacterial growth line (colonies).

Gelatin hydrolysis: A heavy inoculum of an 18 to 24 hour old test bacteria was stab-inoculated into tubes containing nutrient gelatin medium. The inoculated tubes and an uninoculated control tube were incubated at 37°C for up to 1 week, and checked daily for gelatin liquefaction. The tubes were immersed in an ice bath for 15 to 30 minutes. Afterwards, they were tilted to observe if gelatin has been hydrolyzed. Hydrolyzed gelatin results in a liquid medium even after exposure to cold temperature (ice bath), while the uninoculated control medium will remain solid. For weak positive results, the inoculated nutrient gelatin tube was incubated longer until complete liquefaction was observed. The hydrolysis of gelatin indicates the secretion of gelatinase by the test organism into the medium (Aryal, 2022).

Pathogenicity of the isolated organisms on healthy whole tubers

Fresh, healthy potato tubers were washed under running tap water to remove soil and debris from the surface. The whole tubers were then surface-sterilized in a 0.5% sodium hypochlorite solution and rinsed with sterile distilled water. Each tuber was bored to a depth of 1 cm in the middle using a flame-sterilized cork borer with a 3 mm diameter. A 3 mm mycelial disc of the test pathogen was placed at the bottom of the hole, covered with the previously removed tuber piece, and sealed with vaseline to prevent contamination. The control setup consisted of tubers similarly bored and inoculated with sterile PDA discs of 3 mm and sealed with vaseline. All inoculated tubers were placed in polyethylene bags moistened inside with sterile cotton wool soaked in sterile distilled water

to maintain high humidity and incubated at 28 ± 2°C for 7 days (Terna *et al.*, 2015). At the end of the incubation period, the tubers were cut open at the point of inoculations and observed for rot development, rot disease was calculated by the method of Ayodele and Iwhiwhu (2011) as below:

Percentage area of rotted tissue =

$$\frac{\text{Area of rotted tissue}}{\text{total tuber surface area}} \times 100$$

with $\text{area of rotted tissue} = \frac{1}{3} \times \pi \times d \times h$

where d = diameter of lesion and h = depth of lesion and

$$\text{Total tuber surface area} = \frac{1}{3} \times \pi \times D \times H$$

where D= diameter of tuber and H = length of tuber)

Assessment of rot development was by destructive sampling. Measurement of the rotted area was expressed as a percentage of the total surface area of the yam tuber. Rot severity was ranked from 0 to 5, with 0 denoting no rotting, 1 very mild rotting (less than 5% of the tuber affected), 2 representing mild rotting (5% to 10%), 3 signifying moderate rotting (more than 10% to 25%), 4 indicating severe rotting (more than 25% to 50%), and 5 reflecting very severe rotting (more than 50% of the tuber affected). Re-isolation and identification of the fungi was done to establish Koch's postulates.

Preparation of plant extracts

Stems of *D. benthamianus*, roots of Z. *zanthoxyloides,* and leaves of *M. lucida* and *M. oleifera* were rinsed with sterile water, dried at 28 ± 2°C for fourteen days, and then powdered. Two hundred grams of each botanical was extracted in 1000 ml of 90% methanol at 28±2°C for 7 days. This was followed by filtering the mixture using sterile muslin cloth. The filtrates were concentrated to dryness in a vacuum with a rotary evaporator followed by keeping the extracts air-tight at 4°C before use (Gbadamosi and Oyedele, 2012). The extracts were then reconstituted with SDW at 500 mg, 1000 mg, and 1500 mg/ml concentrations as described by Oloyede *et al.* (2012).

***In vitro* assessment of the antimicrobial potentials of plant extracts against the growth of rot bacteria**

The agar-well dilution method was used. The experiment was set up in a 4 (extracts) by 3 (concentrations) by 4 (bacteria) factorial laid in a CRD with treatments replicated three times. Four isolated bacterial isolates (*E. carotovora, Clavibacter* sp. *Bacillus subtillis*, and *P. syringe pv. Phaseolicola*) from rotten potato samples were grown on sterile broth for 24 hours (Gbadamosi and Oyedele 2012). A sterile cotton swab was dipped into the suspension and used to streak the surface of the nutrient agar plates. The streaking was repeated 3 times to ensure even distribution of the pathogens. The inoculum was allowed to diffuse into the agar for 10 minutes. Five (5 mm diameter) wells were aseptically made using sterilized cork borer at equidistance to each other and 0.1ml (100ul) of each plant extract at 500mg, 1000mg, and 1500 mg/ml concentrations were carefully placed into each hole (in known direction). The fourth and fifth holes contained an equal volume of broad-spectrum antibiotics (Erythromycin) at 500 mg/L as positive control and SDW as negative control. The plates were incubated at 37°C for 18 – 24 hrs. The zone of inhibition of each well was determined by measuring the underside of the plate (in millimeters) using a meter rule (Oloyede *et al.,* 2012)

***In vivo* evaluation of plant extracts for antimicrobial activity against potato bacterial tuber rot**

The experiment utilized the 100 mg/ml concentration of *D. benthamianus* methanol extract that exhibited the highest antimicrobial efficacy *in vitro*. Clean, visually healthy potato tubers underwent surface sterilization, followed by the creation of a 1 cm deep hole using a 5 mm cork borer at the center of each tuber. The hole was then filled with 1 ml of the aforementioned extract. Subsequently, 5 mm mycelial discs of the four test bacterial rot pathogens were placed at the base of the hole. The tuber segment initially removed was replaced over the hole and sealed with vaseline to prevent external contamination. Control experiments involved potato tubers with holes filled with SDW. The tubers were individually enclosed in polyethylene bags and incubated (at 28±2°C) for 7 days before assessing

disease progression. Each treatment was replicated four times. Bacterial rot development was assessed by destructive sampling of tubers, following a methodology outlined by Sangoyomi (2004) and using the rot severity scale established by Ayodele and Iwhiwhu (2011) described above.

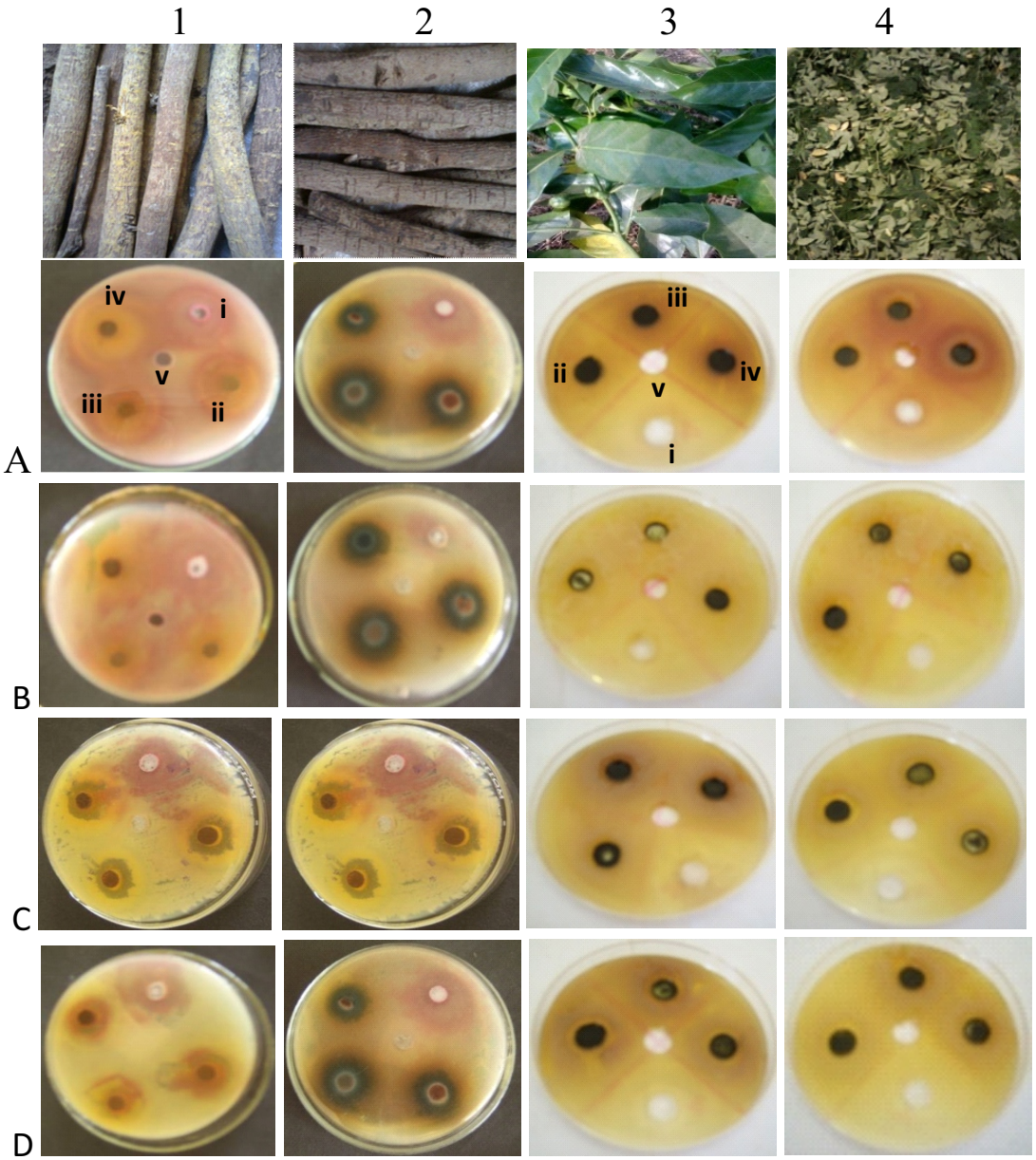

Figure 1: Antimicrobial effects of methanol extracts of (1) *D. benthamianus,* (2) *Z. zanthoxyloides,* (3) *M. lucida* and (4) *M. oleifera* against four bacteria isolates (A-D) incubated at 28 °C for 24 hours. (A) *E. carotovora* (B) *Clavibacter species,* (C) *B. subtilis*, (D). Treatments are: (i) positive control (antibiotics) (ii) 500 mg/ml (iii) 1000 mg/ml (iv) 1500 mg/ml (v) negative control.

Data analysis

Data on rot severity and inhibition zone were transformed using Square root Transformation before analysis. Data were subjected to analysis of variance (ANOVA) with PROC GLM of Statistical Analysis System, version 9.2 (SAS, 2008) package. Means separation was carried out using Least Significant Difference Test (LSD) and Student Newman Keuls (SNK) at $p<0.01$.

Table 1: Morphological, microscopic and biochemical identification of bacteria isolated from post-harvest rotted potato.

Test	Suspected bacteria			
	Clavibacter species	*Erwinia species*	*Bacillus subtilis*	*Pseudomonas syringae*
Colour	Yellow	Red	Cream	Cream
Shape	Round	Round	Round	Round
Elevation	Convex	Convex	Convex	Convex
Gram reaction	+	-	+	-
Cell shape	Rods	Rods	Rods	Rods
Oxidase	-	-	+	+
Anaerobic	-	+	+	-
Aerobic	+	-	-	+
Spore formation	-	-	+	-
Motility	-	+	+	-
Fluorescence pigment on King's B	-	-	-	+
MSP*	na	na	na	+
Indole production	-	+	-	-
Gelatin hydrolysis	-	+	+	-
Glucose Utilization	+	+	+	+
Sucrose Utilization	+	+	+	-
Lactose Utilization	-	-	-	-
H_2S production	-	+	-	-
Gas production	-	+	-	-
Citrate	-	-	+	-
Catalase	+	+	+	+
Starch hydrolysis	-	-	+	-
Casein hydrolysis	-	-	+	-

* MSP, Modified Sucrose Peptone; na, not applicable

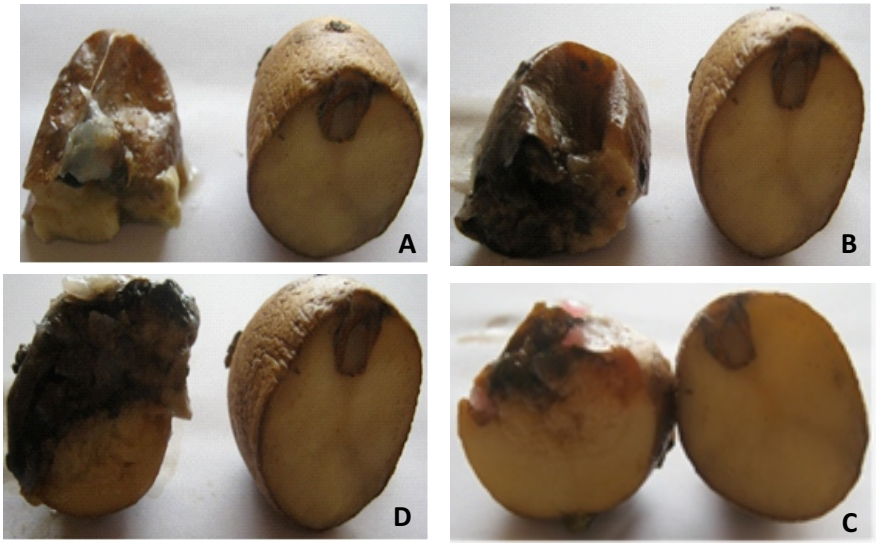

Figure 2: Inoculated and healthy potato tubers showing bacterial rot caused by (A) *E. carotovora* (B) *Clavibacter* species (C) *B. subtilis* and (D *P. syringe.*

RESULTS

The morphological characteristics of the bacteria isolated from the rotted potato samples are presented in Table 1. Four bacteria were isolated and identified biochemically as *E. carotovora Clavibacter* species *B. subtilis*, and *Pseudomonas syringae* pv. *phaseolicola* (Tables 1, 2, and 3). The pathogenicity test confirmed the ability of the four bacteria to induce rot disease, mostly soft rot, on healthy potato tubers (Figure 2). The tuber rot severity produced by *E. carotovora* (46.34%), *Clavibacter* species (45.94%), *B. subtilis* (39.77%), and *P. syringae pv phaseolicola* (41.46%) were significantly higher than the healthy control (Table 4) and all similarly produced severe rotting.

The results revealed a significant antimicrobial property of the plant extracts against the four bacteria (Table 2). The inhibition zones produced by the four plant extracts against the four bacterial isolates varied significantly (Table 3). The varied extract concentrations (500, 1000, and 1500 mg/ml) were not significant ($p<0.01$) on the rot bacteria except on *E. carotovora,* in which there is also a significant interactive effect between the extract and the concentration (Table 2). Extract concentration of 1500 of *M. oleifera* produced higher inhibition of *E. carotovora* than the lower rates (Table 3). The antibiotics (Erythromycin) produced significantly higher inhibition zones than the plant extracts for all the bacterial isolates except for *Clavibacter* species where *D. benthamianus* produced the same high inhibition as the antibiotics (Table 3).

Distemonanthus benthamianus produced high inhibition zones against all the bacterial isolates next to that by the antibiotics at 500 – 1500 mg/ml concentration. It showed a significantly higher inhibition than other plant extracts against *Clavibacter* species. Meanwhile, *Z. zanthoxyloides* also produced high bacterial inhibition *in vitro,* next to *D. benthamianus,* and showed significantly higher inhibition zones (12 ± 2.0 - 14.7 ± 5.0) than other plant extracts against *E. carotovora,* similar to that of the antibiotics. *Morinda lucida* and *M. oleifera* were not generally effective against the bacterial isolates. *Morinda lucida* extract did not show any significant inhibition of all the bacteria while *M. oleifera* inhibited the growth of *E. carotovora* only at 1500 mg/ml.

Table 2: Analysis of variance of the antimicrobial effect of plant extracts on potato rot-causing bacteria.

Source of variation	d.f.	*Erwinia carotovora*	*Clavibacter species*	*Bacillus subtilis*	*Pseudomonas syringae*
Extract (E)	3	277.73**	151.58**	36.58**	80.04**
Conc (C)	2	14.78**	0.86ns	0.65ns	3.69ns
E x C	6	26.70**	0.75ns	0.83ns	2.68ns
Error	24	3.06	1.33	1.97	2.16

E, plant extract; C, extract concentration

** significant at $p < 0.01$; ns, not significant

Table 3: Inhibition zones (mm) caused by the antimicrobial activity of plant extracts onpotato rot causing bacterial isolates at 24 hours of incubation.

Plant extract	[a]Conc. (mg/ml)	*Erwinia carotovora*	*Clavibacter species*	*Bacillus subtilis*	*Pseudomonas syringae*
D. benthamianus	500	8.7±1.2b	7.7±1.2a	5.7±0.6b	8.0±2.0bc
	1000	9.0±0b	9.3±3.1a	6.0±1.0b	8.7±1.5b
	1500	8.7±1.2b	8.7±2.1a	5.0±0bc	7.0±1.7bc
Z. zanthoxyloides	500	14.7±5.0a	0±0b	6.3±3.2b	7.7±2.1bc
	1000	12.3±1.5ab	0±0b	6.0±1.0b	8.3±1.5bc
	1500	12±2.0ab	0±0b	7.0±1.7b	5.0±1.0cd
M. lucida	500	0±0c	1.0±0b	2.5±0.5bc	2.7±0.6de
	1000	0±0c	1.0±0b	3.0±0bc	2.8±0.3de
	1500	0±0c	2.0±1.0b	3.0±0bc	3.0±0de
M. oleifera	500	1.0±0c	0±0b	1.7±1.0bc	1.8±1.0de
	1000	2.3±1.2c	0±0b	2.3±1.5bc	1.7±1.1de
	1500	11.0±ab	0±0b	3.0±1/0bc	2.2±1.8de
[b] + control (E)	0.5	14.7±2.5a	8.0±0a	18.3±5.7a	14±1.7a
[c] - control	-	0±0c	0±0b	0±0c	0±0e

[a]Conc, extract concentration, [b]+ control (E), positive control erythromycin (antibiotics), [c]– control, negative control (sterile distilled water)
Mean values with the same letter along each column are not different according to student Newman Keuls ($p<0.05$)

In vivo experiment showed significant reduction in rot developed by the bacterial infected potato treated with *D. benthamianus* extract at 100 mg/ml concentration (Table 5). This result supported the high antimicrobial potential of *D. benthamianus* observed *in vitro*. This extract significantly ($p<0.01$) reduced the severity of rot development caused by *Clavibacter* species from 45.9±23.3 to 1.8±0.6 and inhibited tuber rot formation by the other three bacteria (Table 5).

Table 4: Rot disease severity on potato artificially inoculated with bacterial isolates at 7th day.

Bacteria	Rot severity	Severity rank
Erwinia carotovora	46.3±21.5a	4
Clavibacter sp.	45.9± 13.4a	4
Bacillus subtilis	39.8± 17.7a	4
Pseudomonas syringae	41.5± 9.4a	4
- control	0±0b	0

Table 5: Antimicrobial effects of *D. benthamianus* extract on the development of potato rot caused by four bacterial pathogens at 7 days after inoculation.

Extract treatment	*Erwinia carotovora*	*Clavibacter species*	*Bacillus subtilis*	*Pseudomonas syringae*
[a] - control	46.3±21.5a	45.9±23.3a	39.8±10.8a	41.5±9.4a
D. benthamianus	0b	1.8±0.6b	0b	0b

[a] - control, negative control (without any extract)

DISCUSSION

This study investigated the antimicrobial potential of *D. benthamianus, Z. zanthoxyloides M. lucida,* and *M. oleifera in vitro* against four bacteria: *E. carotovora, Clavibacter* species, *B. subtillis*, and *P. syringe* pv. *Phaseolicola* which are responsible for causing postharvest tuber rot diseases in potato. The pathogenicity test confirmed the ability of the isolated bacteria pathogens to induce soft rot on potato tubers. Most of the bacteria have been previously reported on potato tuber rot (Kluepfel *et al.,* 2000; Agrios, 2005; Abu-Obeid *et al*., 2017)

The findings revealed an antimicrobial property of the extracts against all the four bacteria. The variation in the inhibition zones produced by the four plant extracts against the bacterial isolates implied different levels of antimicrobial potentials of the botanicals. The results also indicated that each of the three levels of extract concentrations (500, 1000, and 1500 mg/ml) produced similar effect on the rot bacteria except on *E. carotovora,* in which there is also a significant interactive effect between the extract and the concentration. This implies that inhibition zones produced against the bacterial isolates did not vary with the three levels of extract concentrations except for *E. carotovora* in which the highest concentration (1500 mg/ml) of *M. oleifera* produced the highest inhibition.

Distemonanthus benthamianus was the most effective of the four botanicals at 500 – 1500 mg/ml concentration. The *Z. zanthoxyloides* was next to *D. benthamianus* in antimicrobial capacity. However, *Morinda lucida* and *M. oleifera* did not show antimicrobial property against the potato rot-causing bacteria except the growth inhibition of *E. carotovora* by *M. oleifera*. This contradicts the report of Owolabi *et al*., (2022) where *M. lucida* produced significant antimicrobial properties against *Pseudomonas aeruginosa* and *B. subtilis*. Similarly, despite the reported high antimicrobial property of *M. oleifera* on plant diseases (Oniha *et al.* 2021), its methanolic extract did not show any antimicrobial property against the bacteria except *E. carotovora* (only at 1500 mg/ml) with high inhibition zone as high as that produced by the antibiotics (Erythromycin).

In vivo experiment confirmed the high antimicrobial potential of *D. benthamianus* extract on the four bacterial rot. Among the four plant extracts used, *D. benthamianus* followed by *Z. zanthoxyloides* showed significant antimicrobial potential *in vitro* on the test bacterial isolates. These results showed that some locally occurring plants used in folklore human medicine similarly possess antimicrobial effects on plant pathogenic bacteria (Olufolaji and Ojo, 2005). For instance, jute (*Corchorus capsularis* L.) leaf extracts showed pronounced inhibitory effects on *E. carotovora* subsp. *carotovora* both in *in vitro* and storage experiments (Rahman *et al*., 2012).

The antibacterial properties of *D. benthamianus* and *Z. zanthoxyloides* on the test bacteria might have been enhanced by the active ingredients of the botanicals. The antifungal potential of *D. benthamianus* surpasses that of four other Nigerian chewing sticks tested against *Candida albicans* and various fungi (Adekunle and Odukoya, 2006). This efficacy is attributed to secondary metabolites such as saponins, flavonoids, and alkaloids (Adekunle and Odukoya, 2006), which are influenced by factors such as plant age, extracting solvent, extraction method, and harvest time (Okigbo, 2003). Methanolic extraction likely contributed to the effectiveness of these plant extracts. Variations in bacteriostatic activity between *M. lucida* and *M. oleifera* extracts could stem from differences in the water solubility of the active ingredient or the activity of inhibitors to the bacteriostatic capacity. The differing efficacy between *D. benthamianus* and *Z. zanthoxyloides* suggests that botanicals may contain some secondary metabolites with variable antimicrobial effects on pathogens. Studies have shown varied levels and types of antimicrobial compounds in different botanicals against phytopathogens (Uchegbu et al., 2016). Phytoconstituent analysis of *Zanthoxylum* species has revealed terpenoids, alkaloids (benzophenanthridines, furoquinolines, aporphines), aromatic and aliphatic amides, coumarins, and lignans, with reported antibacterial activities of its phenolic acids and alkaloids (Adesina, 2006).

Distemonanthus benthamianus exhibited inhibition of all investigated bacteria at concentrations of 500, 1000, and 1500 mg/ml, making it the most effective botanical. The antimicrobial actions of

these botanicals likely stem from the combined effects of phytochemicals against rot-causing pathogens (Ogunsola and Ogunsola, 2023). This botanical could be utilized as a safe, eco-friendly biopesticide for enhanced postharvest protection of potato tubers.

CONCLUSION

Distemonanthus benthamianus emerged as the most potent inhibitor, effectively suppressing the growth of all four rot bacteria at 500 to 1500 mg/ml concentrations both *in vitro* and *in vivo*. Conversely, M. lucida failed to exhibit any antibacterial activity. These findings underscore the potential of harnessing this naturally occurring antimicrobial botanical as a robust strategy for managing rot diseases. The extract from *D. benthamianus* presents a cost-effective and environmentally friendly alternative to synthetic chemicals for controlling postharvest tuber rot in potatoes. However, further investigations are warranted to assess its efficacy in bulk tuber storage conditions and to evaluate any potential impacts on potato quality, particularly concerning taste and industrial applications.

ACKNOWLEDGMENTS

The assistance on disease diagnostics from Dr S. A. Kazeem of the Post-Entry Quarantine, Surveillance and Diagnostic Station, Nigeria Agricultural Quarantine Service (NAQS), Moore Plantation, Ibadan, and Dr. Oluwole Oguntade of the Germplasm Health Unit, International Institute of Tropical Agriculture (IITA) Ibadan Nigeria, are appreciated

FUNDING

There was no financial support to the authors for this study

CONFLICT OF INTEREST

There is no conflict of interest

AUTHORS CONTRIBUTIONS

Both authors contributed equally to the conceptualization, design, data collection, data analysis, and writing, and also approved the final manuscript

DECLARATION

This manuscript is only submitted to the Ife Journal of Science and is not under review elsewhere or being considered for publication by any other journal.

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