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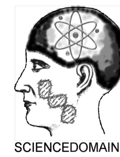

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Evaluation of Some Plant Extracts for the Control of Bacterial Soft Rot of Tubers

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The use of protective chemicals on ware tubers can render it unfit for consumption as tubers undergo little processes before consumption. Hitherto, there is no effective control measure to manage this disease (*Pectobactrium* ssp); therefore, selection of some plant extracts for the management the disease will be the preferred option. The objective of the study therefore was to evaluate some plant materials for the management of tuber soft rot bacteria induced by *pectobacterium ssp.* Aqueous extracts of neem (*Azadirachta indica* A. Juss), Eucalyptus leaves (*Eucalyptus citrodorus (L*)), Lemon grass (*Cymbopogon citratus* (Stapf)), Garlic bulb (*Allium sativum (*linn)), Ginger (*Zingiber officinale*) (Roscoe)) and *Aloe vera* were evaluated for the management of bacterial soft rot of some tubers (cocoyam, Irish potato, sweet cassava, sweet potato and white) induced by *Erwinia spp.* Two methods involving in vitro and tuber assays were used. Lemon grass, garlic, *Aloe vera*, neem extracts and borax salt had the greatest inhibitory effect on *Pectobacterium* spp., and therefore recommended for the management of tuber soft rot bacteria.

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1. INTRODUCTION

All over the world, current agricultural practices are moving away from the use of synthetic chemicals due to their adverse effects on ecosystem. The adoption of practices and use of plant materials that are environment friendly constitutes a vital component of sustainable agriculture. Nigeria is endowed with abundant plant materials, which may be employed to fight plant pests and diseases. This approach has recently been the pre-occupation of many crop protectionists in Nigeria. *Pectobacteriu* spp. George et al. [1] formally known as *Erwinia* spp. induces soft rot on these crops both during the vegetation cycle and on the stored tubers and therefore considered the most threatening bacterium on potatoes and other tubers worldwide [2,3]. The bacteria are ubiquitous plant pathogen with a wide host range (tubers, vegetables and horticultural crops) [4]. The typical soft rot is induced mainly by a number of species coming under the genus *Pectobacterium.* The main species are *Pectobacterium carotovorum* subsp. *carotovora*, *Pectobacterium atroseptica* subsp, *atroseptica* and *Pectobacterium chrysanthemi* subsp *chrysanthemi*. These species produce pectolytic enzymes that hydrolyze pectin between individual plant cells. This causes cells to separate, a disease plant pathologist term bacterial soft rot [4]. The disease (*pectobacterium ssp.*) is a major factor that limits production and transportation of roots and tubers as the disease is a threat both in the field, transits and store houses [4]. The use of protective chemicals on ware tubers is not advisable, it can render roots and tubers unfit for consumption as they undergo little processes before consumption. Hitherto, there is no effective control measure to manage this disease; therefore, selection of some plant extracts for the management the disease will be the preferred option. The objective of the study therefore was to evaluate some plant materials for the management of tuber soft rot bacteria induced by *pectobacterium ssp.*

2. MATERIALS AND METHODS

2.1 Experimental Materials

The plant materials used were Neem seeds (*Azadirachta indica*) (A) Juss, Eucalyptus leaves (*Eucalyptus citrodorus* L), Lemon grass (*Cymbopogon citratus*) and *Aloe vera* (Liliaceae sub species aloinae) and Borax salt. The tubers used were Irish potato (*Solanum tuberosum) (L),* sweet potato (*Ipomoea batatas) (L*) Poir), white yam (*Dioscorea rotundata*) (L), cocoyam (*Xanthosoma sagittifolium*) (L) Schott, sweet cassava (*Manihot esculenta* Crantz). Borax salt was obtained from National Research Institute for Chemical Technology (NARICT) Zaria. Neem fruits, Eucalyptus leaves, Lemon grass, and *Aloe vera* were collected from the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria. Ginger and garlic bulbs, as well as the various tubers were purchased from Samaru Market.

2.2 Preparation of Plants Extracts

Five grams of each of the plant material was weighed and pounded separately in a porcelain mortar to form a paste. This was then mixed with 100ml of water and allowed to stand for 6 h., after which they were filtered through Whatman No. 1 filter papers before filter–sterilizing them by means of Millipore filters (Millipore filter corporation, Bedford, Massachusetts USA).

2.3 Preparation of Bacterial Inoculums

A small portion of tissue at the advancing margin of the diseased tubers was removed with a sterile scalpel and dropped in McCartney bottles containing 9ml of sterile distilled water (SDW). These were allowed to stand for 5 minutes. The content was vigorously agitated and plated on Cuppels and Kelman's medium [5] and incubated for 24h. at a temperature of *ca.* 27ºC Cuppels and Kelman's medium contains 30 g sodium polypectate, 0.6g $Cacl_20$, 2g $NaNo_3$, 1.5 mg Crystal violet, 3 g Agar, 1liter of distilled water. After 24h. incubation, wells were formed on the medium showing the presence of *Erwinia carotovora* subsp. *carotovora* [5]. The pathogen was picked from edges of the wells with a heat-sterilized platinum wire loop and sub-cultured on sucrose peptone Agar (SPA) following the method described by Collins and Lyne [6], until pure cultures were obtained Sucrose peptone Agar (SPA) contains 20g sucrose, 20g agar, 5g peptone, 0.5 g K_2HP04, 1 liter of distilled water. A loopful of bacterial growth was taken from 24-h old cultures and suspend in 9 ml of SDW from which a serial dilution of up to 10^{-7} was made and used as stock. Confirmation of the identity of the pathogen in the culture was done through the ability of the pathogen to induce rot in potato tuber within 24h.

2.3 Evaluation of Plant Extract

Two methods (Single site titration assay and Tuber slice assay) were used to evaluate the plants extracts for the control of bacterial soft rot

2.3.1 Single site titration assay

Tubers free of any obvious mechanical damage or disease were washed and then immersed for 5 min. in 0.5 % sodium hypochlorite (Clorox), rinsed with SDW, sprayed with 95% ethanol and allowed to dry at room temperature. The tubers were then bruised by sterilized knife at one site per tuber following the method described by Buelow et al. [7]. Prior to inoculation, the tubers were immersed in the extracts for 10 min., after which they were allowed to dry for 15-20 min. When this had been done, 0.1ml of the bacterial suspension adjusted to ca. 5.0x 10^7cfu/ml was then introduced at the bruised site. Sterile polythene bags containing moistened filter papers and little SDW were arranged for each treatment. These were incubated at *ca.* 27ºC for 48h. Tubers immersed in SDW were used as control. The experiment was laid out using a Completely Randomized Design (CRD) with three replications. After 48 hrs tubers were Tuber sliced vertically through the Single site of inoculation and the width of decayed tissue was measured. Data collected were analyzed statistically using analysis of variance (ANOVA) and means separated by means of Student Newman Kuels (SNK) Test.

2.3.2 Tuber slice assay

Tubers were cut 20mm thick slices into following the procedure described by Dobias [8] and Lojkowska and Kelman [9]. Surfaces of the tuber slices were disinfected as described above. Directly after slicing, a nick was made in the centre of each of the tuber slices. Treatment of the tuber slices with the plants extracts was done as described above. Eight sterile Petri-dishes containing filter papers moistened with SDW were arranged for the Tuber slices. Two Tuber slices were placed in each Petri-dish. One drop of bacterial suspension 0.1m containing 5.0x10^7cfu/ml was used to inoculate each slice. The control Petri dishes were treated with SDW. These were incubated at *ca.* 27ºC for 48h. After 48 hrs the width of

decay tissue were measured. The experiment was laid out using a CRD with three replications on a laboratory bench. Data collected were analyses statistically using analysis of variance and means separated by means of SNK.

2.4 Testing Sensitivity of the Organism to the Extract

The plants extracts were prepared as described above. Eight sterile Petri dishes were arranged for the set up. SPA was prepared in a flask and allowed to cool to about 45°C. Before the introduction of the medium, 0.2ml of the aqueous plant extract was introduced into the Petri-dishes with the aid of a micropipette, after which 20ml of molten SPA was poured into the Petri dishes. Immediately after this was done, 0.1 ml of bacterial suspension adjusted to *ca* $5.0x10^7$cfu/ml was then introduced into the medium with the aid of yellow-tip micropipette. The contents of the Petri dishes were mixed thoroughly by swirling five times each in clock wise, anti clock wise and north – south directions. The plates were then allowed to solidify and incubated immediately at *ca* 27ºC. The experiment was laid out using a CRD with three replications the experiment was repeated twice. Colony counts (cfu) were done per plate after 48h. Data collected were analyzed statistically using analysis of variance (ANOVA) and means were separated by means of SNK.

4. RESULTS

The effect of plant extracts on the population of *Pectobacterium species in vitro* using plate count methods is shown in Table 1. All the plant extracts significantly reduced the population of the bacteria compared with the control. Population reduction was however, the greatest with neem extract, which is also not significantly different from those of garlic, ginger and lemon extracts. Eucalyptus had the least inhibitory effect on the bacterial population though not significantly different from treatment with *Aloe-vera* extract. The trend of results was similar for experiments I and II. In comparison with the control and all the plant extracts, borax salt had inhibitory effect similar to that of garlic, ginger, neem and lemon extracts.

The effect of plant extracts on the inhibition of rot induced by *Erwinia ssp.* using single site titration assay is outlined in Table 2. Among the plant extracts used on potato, lemon grass extract had the greatest inhibitory effect, but it is however not significantly different from the effects of garlic, *Aloe vera*, and neem extract. Eucalyptus and ginger extract had the least inhibitory effect on rot induction. In comparison to the control and the plant extracts, borax salt had the greatest inhibitory effect. However, its effect was not significantly different from that of ginger, garlic, lemon and neem. However, all the plant extracts significantly reduced the population of the bacteria compared to the control. Similar trends were observed on other tested tubers, in the tuber slice assay there was considerable variation among the tubers (Table 3). All the plant extracts significantly reduced the population of the bacteria compared to the untreated control. On potato, garlic had the greatest inhibitory effect but was statistically similar to the effects of neem extract. This was followed by lemon grass extract, whose inhibitory effect was not significantly different from those of *Aloe Vera* and ginger. Eucalyptus had the least inhibitory effect, but its effect was comparable to that of ginger extract. Borax salt had inhibitory effect comparable to *Aloe vera*, and lemon.

On yam and sweet potato, garlic extract had the greatest inhibitory effect though its effect was not significantly different from those of Borax, lemon, *Aloe vera* and neem extract. These were followed by ginger extract. Apart from the control, Eucalyptus had the least

inhibitory effect. This result was similar on all the other tubers except on cassava where lemon grass and neem extracts had similar inhibitory effects.

Table 1. Effect of plant extracts on the population of *Erwinia ssp. in vitro* using dilution plate count method

Treatment	Mean bacterial count (cfu)/ml*	
	Experiment 1	Experiment II
Eucalyptus	$2.5x10^{7b}$	$2.4x10^{7b}$
Neem	$7.0x10^{6c}$	$7.8x10^{6c}$
Lemon	$1.2x10^{6c}$	$1.1x10^{6c}$
Borax	$7.1x10^{6c}$	$7.0x10^{6c}$
Aloe vera	$2.3x10^{7b}$	$2.3x10^{7b}$
Garlic	$8.0x10^{6c}$	$7.0x10^{6c}$
Ginger	$1.1x10^{7c}$	$1.2x10^{7c}$
Control	$7.7x10^{8a}$	$7.2x10^{8a}$

Means followed by the same letter are not significantly different at P = 0.05 using SNK test

Table 2. Inhibition of rot by plant extracts (diameter of rot/mm) in the single site titration assay

Plant extracts	Potato	Yam	Sweet potato	Cassava	Cocoyam
Eucalyptus	3.80^{b}	3.83^{b}	3.73^{b}	3.25^{b}	2.63^{b}
Neem	1.42^{c}	1.42^{c}	1.00^{c}	1.17^{c}	0.50^{c}
Lemon	0.95^{c}	0.67^{c}	0.95^{c}	0.42^{c}	0.58^{c}
Borax	0.67^{c}	0.50^{c}	0.33^{c}	0.58^{c}	0.17^{c}
Aloe vera	1.17^{c}	0.83^{c}	0.92^{c}	0.92^{c}	0.50^{c}
Garlic	1.00^{c}	0.75^{c}	0.67^{c}	0.58^{c}	0.33^{c}
Ginger	3.42^{b}	3.00^{b}	2.97^{b}	2.83^{b}	2.12^{b}
Control	18.83^{a}	9.17^{a}	11.00^{a}	8.50^{a}	8.50^{a}

Means followed by the same letter are not significantly different at P = 0.05 using SNK test.

Table 3. Inhibition of rot by plant extracts (diameter of rot/mm) in the tuber slice assay

Plant extracts	Irish potato	Yam	Sweet potato	Cassava	Cocoyam
Eucalyptus	8.67^{b}	7.67^{b}	8.00^{b}	6.67^{b}	5.17^{b}
Neem	3.00^{de}	2.85^{d}	2.00^{d}	2.33^{cd}	1.33^{d}
Lemon	4.17^{cd}	2.00^{d}	2.33^{d}	2.33^{cd}	0.83^{d}
Borax	4.21^{cd}	1.00^{d}	2.50^{d}	1.17^{d}	0.50^{d}
Aloe vera	4.33^{cd}	0.33^{d}	2.00^{d}	1.33^{d}	0.67^{d}
Garlic	1.33^{e}	0.83^{d}	0.67^{d}	0.67^{d}	0.83^{d}
Ginger	6.67^{b}	5.50^{c}	5.50^{c}	4.33^{c}	3.50^{c}
Control	18.83^{a}	11.50^{a}	11.33^{a}	9.67^{a}	9.00^{a}

Means followed by the same letter are not significantly different at P = 0.05 using SNK

5. DISCUSSION

The variability of results observed under the tuber slice assay, could not be attributed to the various extracts used but rather was due to a number of factors that may modify the reaction of tubers following slicing. For example, the location in the tubers from which tuber slices were cut may influence the susceptibility of tuber slices to the rot pathogen. The location on tuber like the cortex and medullary tissue has different resistance to rot pathogen [9].

The experiment has shown the effectiveness of lemon grass (*C. citratus*) in reducing the population of the pathogen both *in vitro* and on the treated tubers. The insecticidal and antimicrobial activity of lemon grass against many insects and pathogens has been reported by other authors [10,11]. The antimicrobial properties could be attributed to the presence of 80% citral, citronella, geraniol, methyl-heptone, n-dectyl-aldehyde and linanol in the plants, all of which are active ingredients used in the manufacture of pesticides [11].

Also confirmed was the antimicrobial activity of garlic against a broad spectrum of fungal and bacterial organisms as reported by other authors [12-16]. The antimicrobial properties of garlic have been attributed to the presence of an essential oil that contains allyl disulphide (C_6H_2 S_2) diallyl disulphide (C_6 H_{10} S_2) and two or more sulphur-containing compounds [16,17].

The wide range of antimicrobial activity of *Aloe vera* has been confirmed by this work. It significantly reduced the population of the bacteria both *in vitro* and on the tested tubers compared to control. The active principles are mainly anthroguinone, lignin, salicylic acid, anthrax quinine, aloin and emodin, which acts as painkillers; they also function as antibacterial and antivirals [18,19].

Furthermore the experiment confirmed the antimicrobial activity of neem, which is in agreement with the findings of previous workers [20-24]. This is however contrary to the report of Shenge [16] that neem seed extract was not effective in reducing the population of (*Xanthomonas campestris* pv. *malvacearum*).

The antimicrobial activity of Eucalyptus leaves could be attributed to the presence of 0.5–3.5% volatile oil, tannins, polyphenolic acid, caffeic, ferulic, gentisic, flavonoids, quercetinin, tutin, hyperoside and eucalypin [25]. Eucalyptus oil and eucalyptol reportedly have strong antibacterial properties against several strains of *Streptococcus* [26].

Finally, the experiment has also shown the effectiveness of ginger in significantly reducing the population of the bacterial soft rot both *in vitro* and on tested tubers. This could be as a result of the Zingerone substance in the plant which is the active principle [27].

6. CONCLUSION

Using plant material to control pests may alleviate the burden of heavy reliance on synthetic pesticides. However, given the low mammalian toxicity of the plant materials, they may be appropriate for low impact structural pest management as part of a complete IPM approach. These practices are labor intensive, but are economically and ecologically sound since it does not require sophiscated technology and it is appropriate for resource poor farmers in the developing countries.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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