BACTERIAL SOFT ROT OF TUBERS INDUCED BY *ERWINIA SPP.* (JONES)

BY

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DECLARATION

I declare that the work in the thesis entitled "**BACTERIAL SOFT ROT OF TUBERS INDUCED BY *ERWINIA SPP.* (JONES)**" has been performed by me in the department of Crop Protection under the supervision of Professor A.D. Akpa, Dr(Mrs) O. Alabi, and Dr M.D Alegbejo. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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_____ _____

Professor A.D. Akpa Date
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DEDICATION

This thesis is dedicated to my Elder brother, **Mr Lawrence Aku** and the entire descendants of my late grandfather **Mr Okayi Aku**.

CERTIFICATION

This thesis titled **"BACTERIAL SOFT OF TUBERS INDUCED BY *ERWINIA SPP.* *(*JONES)"** BY AMODU, Umoru Simeon meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for the contribution to knowledge and literary presentation.

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ABSTRACT

The incidence of bacterial soft rot was investigated in three markets in Zaria area namely: Samaru, Wusasa and Sabon Gari markets. Examination of symptoms on tubers (both wounded and unwounded), were done on tubers stored under different environmental conditions in order to determine the factor(s) that might predispose them to attack by the soft rot pathogen. High populations of *Erwinia spp.* were isolated from the samples collected from various markets, which was an indication of high inoculum level from the various soils of origin or storehouses. Tubers without symptoms were frequently contaminated, usually at eye, lenticels and heel end, suggesting contamination from mother tuber to progeny. 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. Two methods were used, namely *in vitro* methods and on the tubers both whole and sliced. Lemon grass had the greatest inhibitory effect and it's statistically comparable with garlic, *Aloe vera*, borax and neem extracts. Eucalyptus had the least inhibitory effect and statistically similar to ginger extract. Two methods (Tuber slice assay and point titration assay) were used to screen yam (*Dioscorea rotundata*), sweet potato (*Ipomoea batatas*), Irish potato (*Solanum tuberosum*), cocoyam (*Xanthosoma sagittifolium*), cassava (*Manihot esculenta*) and carrot (*Daucus carota*) for susceptibility to soft rot bacteria. Irish potato ranked the most susceptible followed by carrot, sweet potato, yam, cocoyam and cassava. The two methods examined were considered to be effective for screening tubers for bacterial soft rot. However, with the single site titration assay, it is possible to determine not only the

extent of tissue maceration but also the bacterial population necessary for initiation of lesions. Tuber Slice assay on the other hand, has the advantage of being faster than the single site titration assay.

CHAPTER ONE

1.0 INTRODUCTION

1.1 General

In sub-Saharan Africa where population is ever increasing with low technological growth, drudgery is always associated with most works (Ofori and Hahn, 1991). This of course, necessitates the consumption of more energy-giving food like roots and tubers. Roots and tubers as the name implies are plants that develop starchy roots, tubers, stems, bulbs or corms that acts as a food store for the plant. Nutritionally, they are composed mainly of water and starch with small amount of protein, fibre, minerals and vitamins (mainly A and C). These crops include yam, cassava, Irish potato, cocoyam and sweet potatoes. Most of these tubers were in the recent past regarded as prestigious items of food, meant only for the affluent (Best, 1996).

Following the failure of earlier emphasis on cereals to bridge the food production gap, serious attention is currently being given to the development and promotion of traditionally starchy stable foods in many Africa countries including Nigeria (CICRTCR, 2000; Ofori and Hahn, 1991). Yam has a historic role in the socio-cultural life of many Africans, so much so that the new produce is seen as a source of celebration, especially in South-eastern and middle belt of Nigeria; and currently, presentation of specified number of tubers is a condition for the settlement of bride price in most West Africa countries (Dorosh, 1988).

Root and tubers deserve particular attention because many developing world's poorest and most food insecure households look to these crops as contributing, if not the principal source of food, nutrition, and cash income. Among other things, farmhouse-holds see the value of these crops in their ability to produce more yields per hectare with low doses of fertilizer and chemical inputs than other food commodities and also their ability to generate yields under condition where other crops may fail (Scott *et al.*, 2000).

Apart from the importance of these crops in food security and food self-sufficiency, they contribute significantly to the national economies and rural income and provide employment for most rural women in sub-Saharan Africa (Committee on Inter-centre Root and Tuber Crops Research (CICRTCR, 2000).

Consultative Group on International Agricultural Research (CGIAR, 2000) reported that farmers in developing countries harvested 439 million metric tons of the major roots and tubers with an estimated value of more then US $41 billion, nearly one fourth, the value of the major cereals. Sweet potato will play a supplementary role to cassava as food security and cash, while both potato and yam can help eliminate poverty and improve food security in their respective areas of greatest concentration, making these crops the most world wide demand food crops of the tropics (Anon, 1975; Albracht and Valleys, 1977; Nweke, 1992; Scott *et al*., 2000).

The population growth rates in many developing countries are still relatively high (1.5% per annum) and such will account for over 97% of the increase in the world population by 2020 (Pinstrup *et al.*, 1999; United Nations, 1999). Sub-Saharan Africa has the highest projected growth rates in population averaging over 2.4%. It is worthy to note therefore, that in the same sub-region roots and tubers play a greater role in local food system than they do in many parts of the world (Nweke, 1992; Scott, 1994). The linkages among agricultural production, income generation, and poverty eradication present another dimension to the potential contribution of these crops to the global food system and important implication for maximizing their potential in the years ahead (Maldonado *et al,* 1998).

Root and tubers mean different things to different people in different regions of the world and at different levels of economic well-being. These crops have myriad and complex parts to play in feeding the world in the coming decades. These include food security, raw materials for industries, reducing poverty, feed for animals and source of income to the farmer and government. CGIAR and CICRTCR Vision 2020 project that roots and tubers will be integrated into emerging markets through the efficient and economically sound production of a diversified range of high quality, competitive products for food, feed and industry. These crops' adaptation to marginal environment, their contribution to household food security and their great flexibility in mixed farming system make them an important component of a targeted strategy that seeks to improve the welfare of the rural poor and to link small holder farmers with this growth market.

Nigeria produces 40% of the total production of roots and tubers of the 80-90% in Africa. Nigeria leads in the production of cassava and yam. In 1994 Nigeria produced 47,000 mt of yam, cassava, and cocoyam compared with 19,700 mt of cereal (FAO, CBN, 1994). The various yields of these crops are yam 10-25 mt/ha. Cassava 40 mt/ha, Potato 25-40 mt/ha, sweet potato 15-30 mt/ha and coco yam 20-25 mt/ha (CBN, FAO, 1994).

Erwinia spp. induces soft rot of numerous crops both during the vegetative cycle and on stored tubers and is therefore considered the most threatening bacteria on potato and other tubers world-wide. Control of this disease with available chemicals has so far not been effective (Goto, 1992; Lowesz, 1992).

1.2 Purpose of the Study

Root and tuber are the target crops in the new millennium that mean many things to very many people of the world. These crops will mean the difference between subsistence and achieving a leg up on the economic ladder; in others, they will mean the difference between survival and starvation (CICRTCR, 2000). These great potential crops have alarming rates of post harvest loss due to soft rot induced by *Erwinia spp*. and hitherto, there is no effective strategies to control the disease (Carolina *et al.,* 2000). The aim of this research therefore is to initiate a control strategy using some natural plant products that are safe to man and environment-friendly to manage bacterial soft rot.

1.3 Objectives

a) To determine the incidence of bacterial soft rot of potato in markets in the Zaria area.

b) To determine the relative susceptibility of tubers of potatoes, sweet potatoes, yam, cassava, carrot and cocoyam to the soft- rot bacteria.

c) To evaluate some plant extracts for the control of bacterial soft rot.

CHAPTER TWO

2.0 LITERATURE REVIEW

Bacterial soft rot of tubers induced by *Erwinia spp* occurs in most parts of the world where tuber, roots, bulb and fleshy vegetables are grown. Jones (1901) made the first authoritative report of bacterial soft rot in the United States of America.

The typical soft rot is induced mainly by a number of species coming under the genus *Erwinia*. The main species are *Erwinia carotovora* subsp. *carotovora, Erwinia carotovora* sub sp. *atroseptica*, and *Erwinia chrysanthemi*. Recent reports of many workers agree with the above-mentioned species of *Erwinia* as causal agents of soft rot (Robert, 1994; Schober and Zadoks, 1999; Helias *et al.,* 2000).

All the three bacterial species can cause tuber soft rot. *E. c.* subsp. *atroseptica* dominate the cool temperate regions, while *E. chrysanthemi* occupy intermediate to high temperature regions and *E. c.* subsp. *carotovora* dominate both tropic and temperate regions of the world (Perombelon, 1979; Helias *et al.*, 2000). According to Erinle (1975) and Irwin (1975), the most frequently isolated bacteria from rotten plant materials recognized as causal agent of soft rot are E*rwinia* species. The same bacteria are also responsible for black leg disease of potato.

2.1 Diseases of Tubers

Pests and diseases during growth and post harvest storage have a major influence on productivity of tubers and cultivation. Microorganisms are found in close association with plants, living either within or around it and some, frequently or occasionally causing food crops to loose palatability, altering nutrient quality and bringing about changes leading to spoilage and loss of the crops. Losses resulting from the activity of microorganisms represent a major part of the total losses in stored foods (Martin, 1976; Akem and Asiedu, 1994; CARDI, 1992). Rot-inducing organisms commonly isolated include *Rhizopus nigricans, Fusarium*

sp., *Aspergillus niger, Clostridium sp, Pseudomonas sp. Bacillus* and *Erwinia* spp. were reported on yam, onions and potato (Anon, 1979; Erinle, 1975; Irwin, 1975; Okonkwo, *et al.,* 1995). Bacterial soft rot of tubers induced by *Erwinia carotovora subsp. carotovora* is a major spoilage disease during harvest and storage (Sturz and Malteson, 1996, Schober and Zadoks, 1996; Helias *et al.*, 2000). The disease is a constant hazard to roots and tuber in all the growing regions of the world. Soft rot Erwinias tend to out-compete other bacteria in tuber rot because of their ability to produce large quantities of a wide range of cell wall-degrading enzymes (Perombelon, 2002).

2.2 Economic Importance of the Disease

The most important disease responsible for heavy crop losses of root and tubers is the soft rot. It is frequently referred to as field and market disease because it causes yield loss in the field, in transit, and in the market stores (Okonkwo *et al*., 1995). In storage the bacteria causes severe rot of tubers, some time resulting to total yield loss. Schober and Zadoks (1999) reported that the bacteria could cause up to 60% yield loss. In the store the damage could be alarming; within 3-5 days, a whole fruit or tuber may be converted into soft, watery, decayed mass. Robert and Blanchette (1994) established that *Erwinia spp.* can degrade lignified cell walls of wood known to be resistant to aggressive brown and white rot fungi of *Eusideroxylon zwageri*, the world's most durable timbers (Daniel and Nilson, 1989; Singh *et al*., 1990; Singh and Butchan 1991). It is the major cause of deterioration in totem poles, house fronts (facial board), and other carved wooden object that are exposed to outside moist environment (Robert and Blanchette, 1994). Apeyuan (2000) reported that the pathogen could damage all plants storage organs. Losses due to *Erwinia spp.* infection are described as follows: Various losses -Losses due to increased evaporation, respiration following infection; Quality loss - Loss due to flavour or smell produced following the attack of soft rot pathogen; Food loss -Loss of food value due to decaying; Monetary loss - Loss due to lower market value; Seed loss - Loss of viable seed of tubers for subsequent planting, and Loss of goodwill - Loss of reputation due to association of poor quality produce with a source of country (Ajibola, 1999). The extent of loss varies widely from country to country and is influenced by the climates as well as the condition of storage (Perombelon and Salmond, 1995).

2.3 Symptoms of Soft rot

Latent infection of tuber by *Erwinia spp.* may show different symptoms such as non-emergence of 'seeds', chlorosis, wilting and haulm desiccation depending on the cultivar and the organ affected and the environmental condition (Perombelon and Kelman, 1987). Under wet conditions *Erwinia* situated mostly in xylem vessels, penetrate and macerate the adjoining parenchymatous tissues, resulting in a black leg of the stem. Desiccation and wilting, resulting from absence of water flow caused by bacteria and /or gum-like materials and tyloses, happen predominantly under dry conditions (Perombelon and Salmond, 1995). On the tubers or fruit, it appears first on the tissue as a small water soaked lesion, which enlarge rapidly in diameter and depth. The affected area become soft and mushy while its surface becomes discoloured and somewhat depressed or it may appear wrinkled or blistered. The margin of the lesion usually are well defined at first but later become obscure, the tissues within the affected region disintegrate into a mushy mass of disorganized plant and bacterial cells. Frequently cracks develop and the slimy mass exudes to the surface, accompanied by foul smell or sulphurous odour which is characteristic of the disease; where upon exposure to the air, it may turn gray or dark brown. If however the rot developed in a dry atmosphere where there is rapid loss of water by evaporation, the infected tissue dry up quickly and may remain attached to the healthy tissue or may slough off. The ability of *E.spp.* to degrade the peptic substance of plant cell walls brings about extensive maceration of plant tissue leading to the mass soft decay (Handa *et al..,* 1989; Zttenbiao *et al.,* 1989). This involves the break down of the middle lamella of the plant parenchyma, which has been attributed to enzyme action (Zttenbiao *et al.*, 1989). Robert and Blanchette (1994) identified endo-β-1-4 arabinase and endo- β-1-4- xylanases as being produced by *Erwinia spp.* and are capable of hydrolyzing the carbohydrate constituent of higher plant cell walls. Robert and Blanchette. (1994) reports that these enzymes were responsible for the degradation of the ligno-cellulose complex in wood. Other enzymes include cellulase, protease (Handa *et al.*, 1989; Zttenbiao *et al.*, 1989), pectinmethylesterase (Handa *et al.,* 1989), pectinase, and polygalacturonase (Maher and Kelman, 1981) and polygalacturonic acid transeliminase (Chatterjee *et al.*, 1981). The symptoms develop rapidly under high temperature and high relative humidity.

2.4 Host Range

The host range of bacterial soft rot is wide and it cut across the storage organs of different plant families. Mostly common on plants that have fleshly storage tissues e.g. root, tuber, fruits such as tomato, egg plant succulent stem, stalk or leaves such as lettuce and cabbage (Erinle, 1981; Apeyuan, 2000). *Erwinia spp.* causes rot on stored tubers as well as on a wide range of other plants as tomatoes, pepper and onions (Hadas *et al.,* 2001; Erinle, 1975). In Yugoslavia, Arsenijevic (1978) reported it on sunflower, cactus, cabbage, pineapple, pepper, tomato, carrot and cauliflower. Tsuyama (1978) reported it on tobacco. Robert and Blanchette (1994) established that *Erwinia spp.* can degrade lignified cell walls of wood known to be resistant to aggressive brown and white rot fungi, *Eusideroxoylon zwageri*, the world's most durable timber.

2.5 Epidemiology

Erwinia spp., are facultative anaerobe, can survive variable environmental conditions. Unlike other bacterial species, they do not have spores to carry them over long unfavourable periods. However they survive in infected plant materials in the field, storage, in infected plant debris, soil, and the pupae of seed corn maggots (Burr and Scroth, 1977; De Boer *et al.,* 1979). Gudmasted and Secor (1983) also found *Erwinia spp.* over-wintering in naturally infected agricultural soils. The bacteria were also found in rhizosphere of a few weeds and crop plants and also irrigation water (Marti *et al.,* 1989; Person and Hegart, 1989; Helias *et al*., 2000). Romdhani and El Mahajous (1997) confirmed this when they noticed that over 90% of imported seed potato showed latent infection by bacteria.

Insects and wind have been involved in the spread of *Erwinia spp.*. Many workers reported whitefly as vector of the pathogen (Brewer *et al.*, 1980). Seed corn maggot (*Hylemyia cilicrura*) greatly facilitates the dissemination of the pathogen in the field and in stores. The bodies of the insects' larvae may become contaminated with bacteria when they crawl about in the infected soil debris. When such insects attacks

healthy plants storage organ by burrowing holes into them, they do not only transmit the bacteria, but they put the bacteria into the plants where they can surely cause the disease (Helias *et al*., 2000). DeJaczko *et al.* (1983) isolated the pathogen from *Drosophila melanogaster*. An appreciable level was found in both the gut and faecal materials. Brewer *et al.* (1980) observed that *Erwinia spp.* survived both internally and externally on *D. melanogaster,* and *D. buskin*. Internal survival was found to be longer. The importance of insects in the dissemination of soft rot pathogen is also affirmed by Graham *et al.* (1976), Kloepper (1979), Kloepper,(1981), Tarr (1981), Fucikousky *et al.* (1978). Other organisms implicated in the spread are nematodes (Chantotanao and Jesen, 1969) and earthworms (Perombelon and Lowesz, 1970). Wind aerosols disperse the bacteria (Perombelon, 1978; Graham and Harrison, 1976; Graham *et al.,* 1977).

2.6 Factors Affecting Infection and Bacterial Multiplication

The entry of the pathogen is through the natural opening or wounds and injury produced at weeding, harvesting and in transit or by insect attack. Erinle (1975) reported that the bacteria enter the storage organs through wounds and lenticels. Penetration by microorganisms appears to be at maximum during the early stages of tuber formation, before lenticels suberized (Thyner *et al*., 1997).

Multiplication of the soft rot bacteria is determined by three factors: the population of the host plants, the population of the pathogen and the environmental conditions (Goto, 1992). Temperature and humidity are important factors in disease development and severity. Moisture availability is considered to be one of the most important determinants for bacterial growth and development (Colhoun, 1973). Both bacterial multiplication and pathogenicity are temperature and moisture dependent (Mildenhall, *et al.,* 1981., Dealto and Surico, 1982; Goto, 1992; Membre and Burlot, 1994).

In potato, the minimum inoculum density needed for infection is temperature dependent and free water is required for disease development. Pathogenicity of soft rot *Erwinia* is directly related to water availability

in the crop tissue (Schober and Zadoks., 1999). The susceptibility of the tissues increases with water potentials (Perombelon and Salmond, 1995). Schober and Zadoks (1999) reported that temperature and water availability during tuber production; harvesting and storage are the most important factors that affect the survival, growth and infection by soft rot bacteria.

Erinle (1975) discovered the range of temperature requirement of *Erwinia spp.* as being between 20^0-38^0C. High temperature has been reported to favour the growth and expression of soft rot of *Erwinia carotovora sub sp carotovora* (Adeniji, 1970; Perombelon, 1979).

Okonkwo *et al*. (1995) confirmed that addition of more than 100 kg nitrogen per hectare to the soil lead in certain cases to an increase in the incidence of tuber soft rot.

2.7 Control

Partial control of the disease is possible by taking care to avoid wounding of tuber, bulb and root crops at the time of weeding operation, harvest, transportation and storage. Care should be taken to ensure that only the recommended doses of nitrogen fertilizer are applied to the crops.

Crop rotation with cereal, which is not susceptible, should be practiced (Okonkwo *et al*., 1995; Abeygunawardena, 1969). Harvesting of properly matured tubers decreases susceptibility to the disease (Okonkwo *et al*., 1995; Bartz, 1981).

The use of chemicals like mercuric chloride, formaldehyde solution, 1.2% sodium hypochlorite solution, 12% hydroxymercurinitrophenol and 2% hydromercurichloride phenol in 37.5l of water apparently

eliminated the soft rot pathogen from the surface of the tuber, but the use of such tuber has been seriously questioned in may areas of the world and also both of these treatments proved to be more liable to cause injury to the tuber (Walker, 1969). In essence no effective and commercially practical methods for chemical control of the bacterial infection have been developed (Carolina *et al*., 2000).

The use of genetic engineering is still at an experimental stage even though success has been reported. The introduction of resistance directly into plants via expression of genes encoding proteins with antibacterial activity has been reported (During, 1996; Shah 1997; Mougues *et al.*, 1998). Several proteins and peptides with antibacterial activity have been tested in the defense system of different organism (Jolles and Jolles, 1984; Hultmark, 1993; Bowman, 1987) including plants (Molina *et al.,* 1993, Broekaert *et al*., 1995, Garcia-Olmedo *et al.*, 1995). Recent reports have shown enhanced bacterial resistant in plants transformed with genes encoding some of these proteins and peptides (Carmona *et al.,* 1993, During *et al.*, 1993, Jay *et al.,* 1993, Allefs *et al.*, 1996, Molina *et al*, 1993 and Arce *et al.,* 1999).

Furthermore, introduction of the gene encoding the bacteriophage T4 lyso zyme in potato plant (During *et al*, 1993), the chicken lysozyme in tobacco plants (Trudel *et al*., 1992), and the human lysozyme in tobacco plants (Nakajima *et al*., 1997) has been reported. The technology behind all these genetic methods are high and expensive and beyond the reach of average farmers.

Storage under cool, dry condition has been found to be the most successful and many researchers have suggested the use of this method (Erinle, 1975; Tamini and Hadwan, 1978; Erinle, 1981; Dookem and Autrey, 1991). It is however; unfortunate that the measure is at present beyond the reach of the peasant farmer or small scale wholesale or retailer.

Presently no effective strategies to control bacterial soft rot in potatoes tubers are available and all commercial varieties of tubers especially potato are susceptible to infection by the soft rot pathogen (Bourne *et al.*, 1981; Carolin *et al.*, 2000).

2.7.1 The Use of Plant Extracts and Local Materials for the Control of Plant Diseases.

Ecologically oriented plant protection is critical to develop and apply economically sustainable pest and disease management techniques to avoid the use of synthetic pesticides (Herren, 1994). Such an approach would address both the environmental and economic aspects, such as sustainability. One of such approaches is the use of plant extracts for the control of plant diseases, which has recently been the preoccupation of many crop protectionists in Nigeria.

Ginger (*Zingiber officinales* Roscoe) is cultivated as an annual crop, which is propagated vegetatively, and largely produced in some parts of northern Nigeria. Ginger posses a warm pungent taste, pleasant odour and peppery substance called Zingerone. Vijaya Lakshmi *et al.* (1997) reported on the effectiveness of Ginger extract for the control of American boll worm (Helicoverpa armigera) and whitefly (*Bemisia tabaci*).

Aloe vera a succulent plant (*Aloe bandenis* M), a member of the lily and onion family, also related to garlic of which there are more than three hundred varieties but of which only a few had medicinal properties. It is generally accepted that the most potent was Aloe *bandenis* M (Amodioha, 1999; Ahmed and Beg, 2001). *Aloe vera* contains catheric anthraglylcosides as its active principles. These are mainly anthraguinone, lignin, salicylic acid and other components. The important components are anthrax quinine, aloin and Emodin which acts as painkillers; they also function as antibacterial and antivirals (Amodioha, 1999). It can provides 20 out of the 22 amino acids needed by the body and more importantly, it provides 8 out of the essential amino acid which the body can not synthesize (Ahmed and Beg, 2001).

The major species of *Eucalyptus* are *Eucalyptus globules, E. citrodarus,* and *E. camadulensi* (Family: Myrtaceae). The leaves contain 0.5-3.5% volatile oil, teanis, polyphenolic acid, caffeic, ferulic, gentisic, flavonoids, quercetin, quercetinin, tutin, hyperoside and eucalypin. (Fordham and Codd, 1972) Eucalyptus oil and eucalyptol reportedly have antiseptic (antibacterial properties), strongly antibacterial properties against several strains of *Streptococcus*. Medicinally, it is used to affect the permeability of the blood capillaries to several other medicines (Brieskon and Schlicht., 1976). *Eucalyptus camadulensis*'s leaves have been found to be effective against *Callosobrucus maculatus* on stored cowpea (Dike and Mshelia, 1997). Leaves and oil are reportedly used for wound, burns, ulcer, and cancer treatment. In addition, aqueous extracts of the leaves are used for treating aching joint, bacterial dysentery, ring warms, pulmonary tuberculoses. (Xiao and Chen, 1988)

The pesticidal activities of garlic as a repellant and antifeedant have been reported (Oparaeke and Dike, 1996). The anti-fungal ability of garlic extracts against several rhizosphere and rhizoplane fungi (*in vitro*) has been demonstrated (Murthy and Amonkar 1974; Ahmed and Agnihotri, 1977; Agrawal, 1978; Shashikanth *et al.*, 1981; Obagwu, 1997). In the Philippines, Pordesimo and Ilag (1976) reported that crude juice extract from fresh garlic bulb was toxic (*in vitro*) in varying degrees to *Colletotrichum gloesporoides*. The conidial germination of *C. gloesporoides* and *C. capsici* was also inhibited by garlic extract (Gupta *et al.*, 1981). The bactericidal activity of garlic derives from allylpropyl disulphide, dially disulphide, and two or more sulphur containing compounds (Bhatnagar *et al.,* 1948).

Tiwani *et al.* (1966) tried the insecticidal, fumigant, and repellant properties of lemon grass oil against housefly and mosquito with much success. He therefore concluded that products of this grass are quit promising insecticide. The rootstock of lemon grass is aromatic and repels moths (Walt and Breyer, 1962). Sharaby (1989) further confirmed the insecticidal property when he tried the oil. He reported 1-2% concentration destroyed egg masses and newly hatched larvae of *Spodoptera exigna*, a cotton pest. Dike and Mbah (1992) reported that trials with 5g, 10g and 20g lemon grass stem and leaves powder mixed with

100g of cowpea controlled *Callosobruchus maculatus* with adult mortality of 85.5%, 97.5% and 100% after four weeks of treatment respectively. Jija (1999) used it to control *Callosobruchus maculatus* on stored cowpea.

Lemon grass contains 80% citral, citronella, graniol methyl-heptone, n-dectyl-aldehyde and linalol, all of which are active ingredients used in the manufacture of insecticide (Poucher, 1994).

Various parts of neem have been used over the years in experiments to demonstrate its pesticidal properties (Emechebe, 1996; Obagwu, 1997; Emechebe and Alabi, 1997; Stoll, 1998; Aliyu, 1999). Although with respect to its efficacy in disease control, parts of neem have been reported to be highly effective in controlling a wide range of fugal disease. Emechebe (1996) found foliar applications of aqueous neem seed extracts to be effective in controlling bacterial blight of cowpea. The active principle of neem is azadirachtin, which is said to be more concentrated in the seed. Almost every part of the tree is bitter, and has found application in natural pest control. The bitter principle of neem are said to be nimbidin (Bhatnagar *et al.*, 1948).

CHAPTER THREE

3.0 A MARKET SURVEY OF THE INCIDENCE OF BACTERIAL SOFT ROT OF POTATOES IN THE ZARIA AREA OF KADUNA STATE

3.1 Introduction

Zaria is one of the producing zones of Irish potatoes in Nigeria. It is produced under irrigation during the harmattan period when temperatures are sufficiently low (30.7 ^{o}C) (Okonkwo, 1995; Appendix 8.18). The produce is sold in both local market within Zaria and environs mostly by retailers.

3.2 Materials and Methods

3.2.1 Sampling Sites

The study covered three markets: namely Sabon-Gari, Wusasa and Samaru markets. In addition to sampling tubers, the following observations were also made in each market:

- The source of the potato tubers.
- Harvesting methods.
- Transportation system from the farm to the market and the container used to convey the produce.
- The structure of market stores or shops.

- The studies were carried out in May, 2005.

3.2.2 Sampling Procedure

There were about seven potatoes stores in each market containing approximately six bags of potatoes each. Samples were randomly taken from newly opened four bags and already displayed containers. A total of half-bag of potato tubers were taken from each market for observation and examination for soft rot symptom. Also from the sample taken, tubers were sorted into wounded and unwounded grades. The collected samples were incubated under different environmental conditions to see whether soft rot develops and what pathogen could be responsible for the rot.

3.2.3 Isolation of the Pathogen

Ten tubers showing symptoms of soft rot were selected and rotted tissues were taken from the edge of the lesions, rinsed in sterile distilled water (SDW) to eliminate the rot particles. Pooled portion of tissues were then macerated to form a paste in the mortar and loopful of it was suspended in 9ml of SDW with the aid of heat-sterilized platinum loop. The suspension was agitated and allowed to stand for 10-15 min., after which serial dilutions were made. Dilution of 10^{-5} was used for plating. Aliquots, 0.1ml, of the suspension were introduced into King's medium B agar (KB) (King *et al*., 1954; Helias *et al*., 2000; Yahiaoui *et al*., 2003) with the aid of a yellow tip micropipette. After this, the plates were allowed to solidify and were incubated immediately at 27^{o}C. Colony enumeration was done after 24 and 48 hr. This was done with samples collected from the various markets.

Five grams of lenticels and eyes were collected from the pooled portion of potato tissues in each area. *Erwinia spp were* isolated and enumerated on King's medium B agar as described above. The same procedure was followed for inter-lenticels.

Samples were also sorted into wounded and unwounded tubers and both were stored under air-tight polythene bags in ambient air on the laboratory bench to determine the incidence of soft rot pathogen and also the conditions that affects the development of rot during storage. The above procedure was followed to determine population of *Erwinia spp.* On sample collected from each market. The identity of the isolate was tested by its ability to rot potato within 24hrs.

3.3 Results and Discussion

Erwinia spp. occurred in quite high numbers (Table 3.1) even on the apparently healthy tubers. Latent infection of tubers from the field can lead to the development of rot symptoms in transit and in storage depending on the environmental condition (Perombelon and Kelman, 1987). The major source of primary inoculum of *Erwinia* in potato crops is infected tubers. One rotten tuber can contaminate over 100 kg of healthy tubers during grading and transportation (Elphinstone and Perombelon, 1986).

The high level of *Erwinia* in the lenticels and inter-lenticels, agree with the reports of (Perombelon, 1975) that tuber contamination usually occurs through lenticels, which constitute both an important point of entry and a site where the pathogen can remain latent. Susceptibility of lenticels to penetration by the pathogen appears to be at a maximum during the early stages of tuber formation, before lenticels suberized (Thyner, *et al*., 1997) and the fact that significant number of the pathogen was detected on the inter-lenticels suggests a very high initial inoculum level from the fields, or the store houses.

The high percentage of rot on the wounded tubers in polythene bags (Table 3.2) and under room temperature (Table 3.3) indicate the susceptibility imposed on the tubers during the lifting of tubers from the soil mechanically where the physiology of the crop may be altered to a state more conducive to infection and spread of bacterial soft rot (Perombelon and Salmond, 1995). This was so because growth of

and infection by pathogenic bacteria will occur only in plants or in wounded tissue where water is available (Schober and Zadoks, 1999). Farmers carry out most harvesting manually and wounding of tubers or bruising of tubers during transportation is inevitable (Okonkwo *et al*., 1995). The complete rot observed in storage under poor ventilation in polythene bags was as a result of high relative humidity and temperature build up and the favourable microclimate created in the bags. Interactions of the effects of temperature and relative humidity led to the high incidences observed (Schober and Zadoks, 1999).

Table 3.1. *Erwinia* population on potato tubers in Zaria area

Source of tubers	**Description**	***Erwinia spp.* Population (cfu/ml)**
Samaru rotted tubers	Packed in bags and displayed in baskets	2.0×10^9
Samaru lenticels/eye	Packed in bags and displayed in baskets	4.0×10^7
Samaru inter-lenticels	Packed in bags and displayed in baskets	1.0×10^7
Sabon Gari Rotted tubers	Spread on the floor of store rooms	4.5×10^9
Sabon Gari Lenticels/Eye	Spread on the floor of stone rooms	2.4×10^9
Sabon Gari Inter-lenticels	Displayed in variable containers.	1.2×10^8
Wusasa rotted tubers	Displayed in variable containers	3.2×10^9
Wusasa lenticels/Eye	Displayed in variable containers	3×10^8
Wusasa inter-lenticels	Displayed in variable containers	1×10^7

Cfu – Colony forming unit.

Table 3.2. Incidence of *Erwinia spp.* following storage in polythene bags

Treatment*	Soft Rot Incidence	
	One week	**2 weeks**
Samaru unwounded	40%	90%
Samaru wounded	70%	100%
Wusasa unwounded	50%	90%
Wusasa wounded	85%	100%
Sabon Gari unwounded	40%	80%
Sabon Gari wounded	75%	100%

* 10 tubers per sample

Table 3.3. Incidence of *Erwinia spp.* following storage under room temperature

Treatment*	Soft Rot Incidence	
	One week	**2 weeks**
Samaru unwounded	30%	0%
Samaru wounded	60%	30%
Wusasa unwounded	40%	0%
Wusasa wounded	50%	60%
Sabon Gari unwounded	10%	0%
Sabon Gari wounded	60%	70%

- 10 tubers per sample

There was high incidence of rot in wounded samples collected from various areas after one-month storage of potato tubers under room temperature (Fig. 3.1). The highest incidence of rots were observed on wounded samples during the first two weeks followed by a progressive decline in subsequent weeks. At the

fourth week, the rot incidence was reduced to zero percent. Similar trend also was observed on the intact tuber samples, except that the rot incidence was reduced to zero at the third week.

These phenomena observed could be due to some factors that mediate the rot development. The highest percentage of rot observed on wounded tubers during the first week of storage could be due to the freshness of wound and water films on the wounds which favour the entry, growth and infections of the tubers by the pathogenic bacteria. The water films on the fresh wounds might have persisted for some days and is considered to be one of the most important factors aiding bacterial growth and development (Colhoun, 1973). In addition, the depth of wounds could also play significant role that may lead to differential rots in different areas (Bain and Perombelon, 1988).

The rots of the intact tubers might due to the high moisture content of the fresh tubers (Perombelon and Salmond, 1995). Also there is a possibility that the pathogen can be transmitted from infected tubers to non-infected ones through contact with rotten tubers (Elphinstone and Perombelon, 1986).

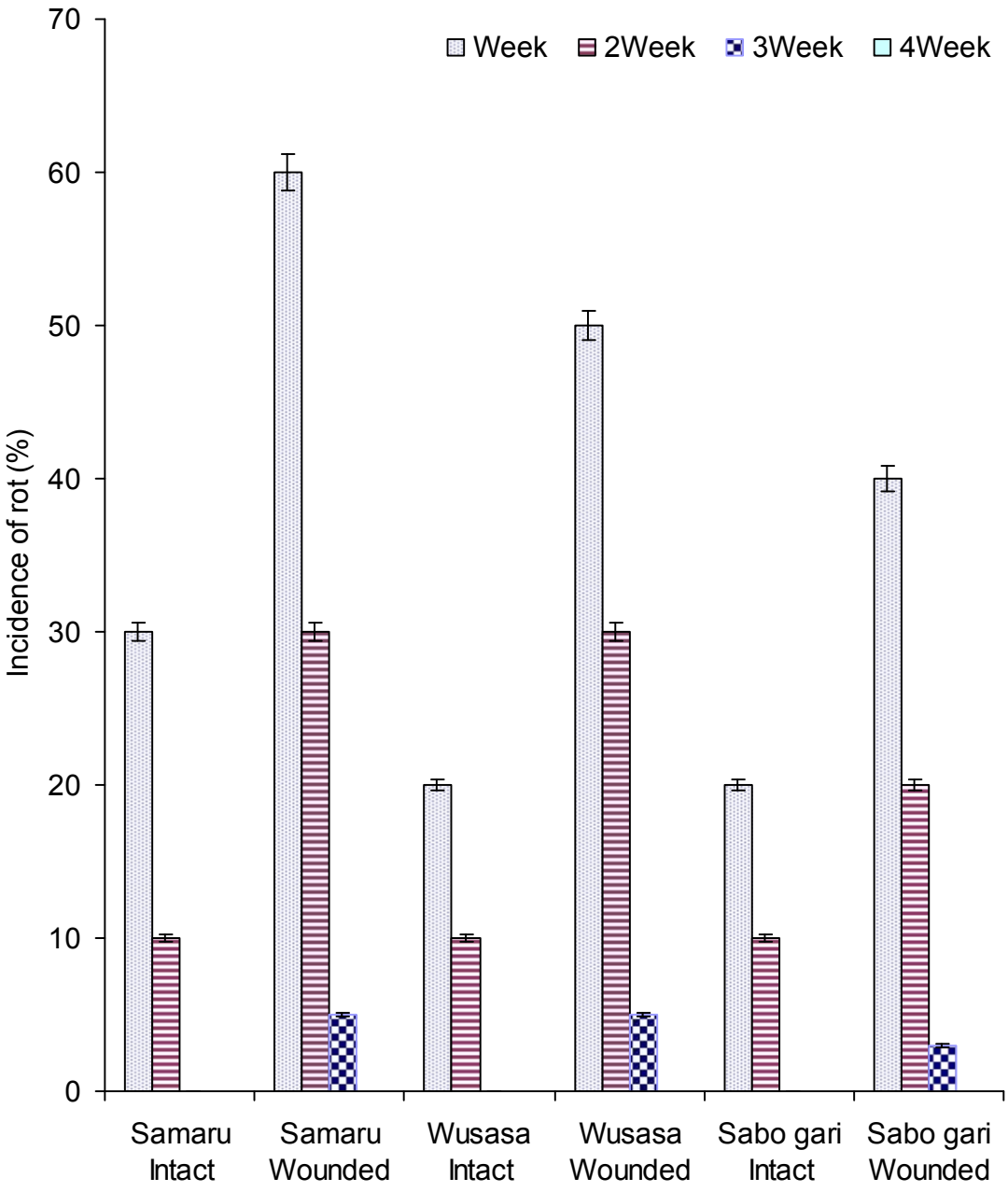


Fig.3.1: Rot incidence after one month storage under ambient temperature

The general reduction of rot on both wounded and intact tubers could be as a result of wound healing on the wounded tubers and curing effects on intact tubers. Similar observations have been reported by other authors (Bownman *et al*, 1984 Knoweles *et al*., 1982). Potato tubers are known to consist of about 80% water, which gradually reduce with time and this result in weight loss. The ambient temperature ensure increased rate of evaporation with decrease in relative humidity (Booth and Shaw, 1981). The bacterial populations will not increase under these circumstances but they can survive latently. In general, however, the drier the tuber surface, the fewer the numbers of saprophytic bacterial flora as a whole and correspondingly so were the numbers of Erwinias (Hayward, 1974; Erinle, 1975). During storage under ambient temperature a high death rate combined with a low growth rate of the soft rot bacteria might result in a decrease of bacterial populations below minimum densities needed for infection.

Since potato is produced during the harmattan months (November to February) under irrigation, the bulk of potatoes will be highly contaminated with the soft rot pathogen from the irrigation water (Okonkwo, *et al*., 1975, Helias *et al*., 2000). This may account for significant proportion of the pathogen isolated from the lenticels and the inter-lenticels and the progressive decline following storage. Also harvesting of the crop is normally done in the months of April and May which coincides with high relative humidity (*ca*.70-79%) (Appendix 8.18), which is favourable for growth of the pathogen. Inadequate supply of clean seeds may be another contributing factor as infected seed tubers are a major source of inoculum (Perombelon, 1974). Most markets in Zaria have poor store structures constructed with zinc roofing sheets, which create favourable conditions for soft rot pathogen to develop explosively. Poor ventilation causes heat build-up and high relative humidity in potato stores, thus increasing the activity of microorganisms which cause rotting of tubers. Poor ventilation of farmers' store in Nigeria leads to high storage losses experienced by farmers have been reported by (Okonkwo *et al*., 1986).

On the floor of the store, tubers are usually spread 4-5 layers (8-14 cm) thick. Potato tuber is a living botanical organ and respires. A good storage condition ensures rapid removal of the products of respiration, which could cause tuber spoilage during storage. . It has been reported that farmers lose as high as 40% of their stored seed within 3 months of storage as a result of poor storage conditions (Okonkwo *et al*., 1988).

On the other hand market stores are never disinfected before bringing in another consignment of the commodity. Consequently, even if the tubers are free of the pathogen from the field and in transit, the tubers will be contaminated during the temporary storage in the market stores.

CHAPTER FOUR

4.0 EVALUATION OF SOME PLANT EXTRACTS FOR THE CONTROL OF BACTERIAL SOFT ROT OF TUBERS

4.1 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,

4.2 Material and Methods

4.2.1 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 (X*anthosoma* 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.

4.2.2 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).

4.2.3 Preparation of bacterial inoculum

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 (Cuppels and Kelman, 1974) and incubated for 24 h. at a temperature of *ca.* 27^{o}C Cuppels and Kelman's medium contains 30g sodium polypectate, 0.6g $Cacl_20$, 2g $NaNo_3$, 1.5mg Crystal violet, 3g Agar, 1liter of distilled water. After 24 h. incubation, wells were formed on the medium showing the presence of *Erwinia carotovora* subsp. *carotovora* (Cuppels and Kelman, 1974). 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 (1977), until pure cultures were obtained Sucrose peptone Agar (SPA) contains 20g sucrose, 20g agar, 5g peptone, 0.5g 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 24 h.

4.2.4 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

4.2.4.1 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*. (1986). 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. 5.0 x 10^7 cfu/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.* 27^{o}C for 48 h. 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 siteof 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.

4.2.4.2 Tuber Slice Assay

Tubers were cut 20mm thick slices into following the procedure described by Dobias (1976) and Lojkowska and Kelman (1994). 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 5.0x 10^7 cfu/ml was used to inoculate each slice. The control Petri dishes were treated with SDW. These were incubated at *ca.* 27^{o}C for 48 h. 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.

4.2.5 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^{o}C. Before the introduction of the medium, 0.2 ml of the aqueous plant extract was introduced into the Petri-dishes with the aid of a micropipette, after which 20 ml of molten SPA was poured into the Petri dishes. Immediately after this was done, 0.1 ml of bacterial suspension adjusted to *ca* 5.0 x 10^{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* 27^{o}C. The experiment was laid out using a CRD with three replications the experiment was repeated twice. Colony counts (cfu) were done per plate after 48 h. Data collected were analyzed statistically using analysis of variance (ANOVA) and means were separated by means of SNK.

4.3 Results and Discussion

The effect of plant extracts on the population of *Erwinia species in vitro* using plate count methods is shown in Table 4.1. All the plant extracts significantly reduced the population of the bacteria compared with the control. Population reduction was however, 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.

Table 4.1. 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	$2.5 \times 10^{7\,b}$	$2.4 \times 10^{7\,b}$
Neem	$7.0 \times 10^{6\,c}$	$7.8 \times 10^{6\,c}$
Lemon	$1.2 \times 10^{6\,c}$	$1.1 \times 10^{6\,c}$
Borax	$7.1 \times 10^{6\,c}$	7.0×10^{6c}
Aloe vera	$2.3 \times 10^{7\,b}$	$2.3 \times 10^{7\,b}$
Garlic	$8.0 \times 10^{6\,c}$	7.0×10^{6c}
Ginger	1.1×10^{7c}	$1.2 \times 10^{7\,c}$
Control	$7.7 \times 10^{7\,a}$	7.2×10^{7a}

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

The effect of plant extracts on the inhibition of rot induced by *Erwinia ssp.* using single site titration assay is outlined in Table 4.2. 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 was observed on other tested tubers, in the tuber slice assay there was considerable variation among the tubers (Table 4. 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. The variability of results

Table 4.2. 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 4.3 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

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 (Lojkowska and Kelman, 1994).

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 (Poucher, 1974; Jija 1999; Oparaeke, 2004). 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 (Poucher, 1974; Oparaeke, 2004).

Also confirmed was the antimicrobial activity of garlic against a broad spectrum of fungal and bacterial organisms as reported by other authors (Ahmed and Agnihotri, 1977; Agrawal, 1978; Shashikanth, 1981; Gondwe *et al*., 1996; Shenge, 2002). The antimicrobial properties of garlic have been attributed to the presence of an essential oil that contains allyl disulphide ($C_6H_2S_2$) diallyl disulphide ($C_6H_{10}S_2$) and two or more sulphur-containing compounds (Shenge 2002; Obagwu, 2003).

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. 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 (Amodioha, 1999; Ahmed, and Beg, 2001).

The experiment also confirmed the antimicrobial activity of neem, which is in agreement with the findings of previous workers (Emechebe, 1996; Obagwu, 1997; Emechebe and Alabi, 1997; Stoll, 1998; Aliyu, 1999). This is however contrary to the report of Shenge (2002) 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 (Lawrence, 1990). Eucalyptus oil and eucalyptol reportedly have strong antibacterial properties against several strains of *Streptococcus* (Brieskon and Schlicht., 1976).

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 Epidi *et al.* (2005)

CHAPTER FIVE

5.0 DETERMINATION OF THE RELATIVE SUSCEPTIBILITY OF TUBERS TO SOFT ROT PATHOGEN

5.1 Introduction

Tubers generally, vary in their susceptibility to soft rot induced by *Erwinia spp.*, because of the large number of environmental, cultural and genetic factors that influence its development and severity. It is therefore difficult to evaluate results obtained by different investigators in attempts to develop standardized procedures for evaluation of soft rot resistance of tubers (Lojkowska and Kelman, 1994). An important problem that impedes the breeding for soft rot resistance is the lack of generally accepted method for selection of resistance varieties. The objective of this study was to compare effectiveness of two methods for the evaluation of bacterial soft rot resistance in tubers.

5.2 Material and Methods

Materials were yam (white yam) cocoyam, cassava, sweet potato, potato and carrot; care was taken not to select injured or diseased samples. Two methods (single site titration assay and slice assay) were used to evaluate the susceptibility of the tubers to soft rot induced by *Erwinia ssp.*.

5.2.1 Single site titration assay

Single site titration assay was carried out as described earlier in Section 4.2.5.1. Data collected were analyzed statistically using analysis of variance (ANOVA) and means were separated by means of Student Newman Kuels Test. The experiment was repeated twice

5.2.2 Tuber slice assay

Tuber slice assay was done as described earlier in Section 4.2.5.2. Data collected were analyzed statistically using analysis of variance and means were separated by means of Student Newman Kuels Test. The experiment was repeated twice.

5.3 Results and Discussion

The ranking of tubers in order of their susceptibility to *Erwinia ssp.* using single site titration assay is shown in Fig. 5.1. In this method, there was little or no variation between experiments I and II and this lead to clear-cut ranking of tubers. Irish potato ranked the most susceptible followed by carrot, sweet potato, yam, cocoyam and cassava in decreasing order of susceptibility. Factors that may influence differential susceptibility of these tubers to soft rot erwinias may be numerous and includes amongst others, the production history of the various tubers such as the nutritional status of the tubers, like the use of nitrogen fertilizer and stress-imposed susceptibility during harvest and transportation (Lojkowska and Kelman, 1994; Okonkwo *et al*., 1995; Schober and Zadoks, 1999). The degree of suberization of the periderm also affects the susceptibility (Perombelon, 1979).

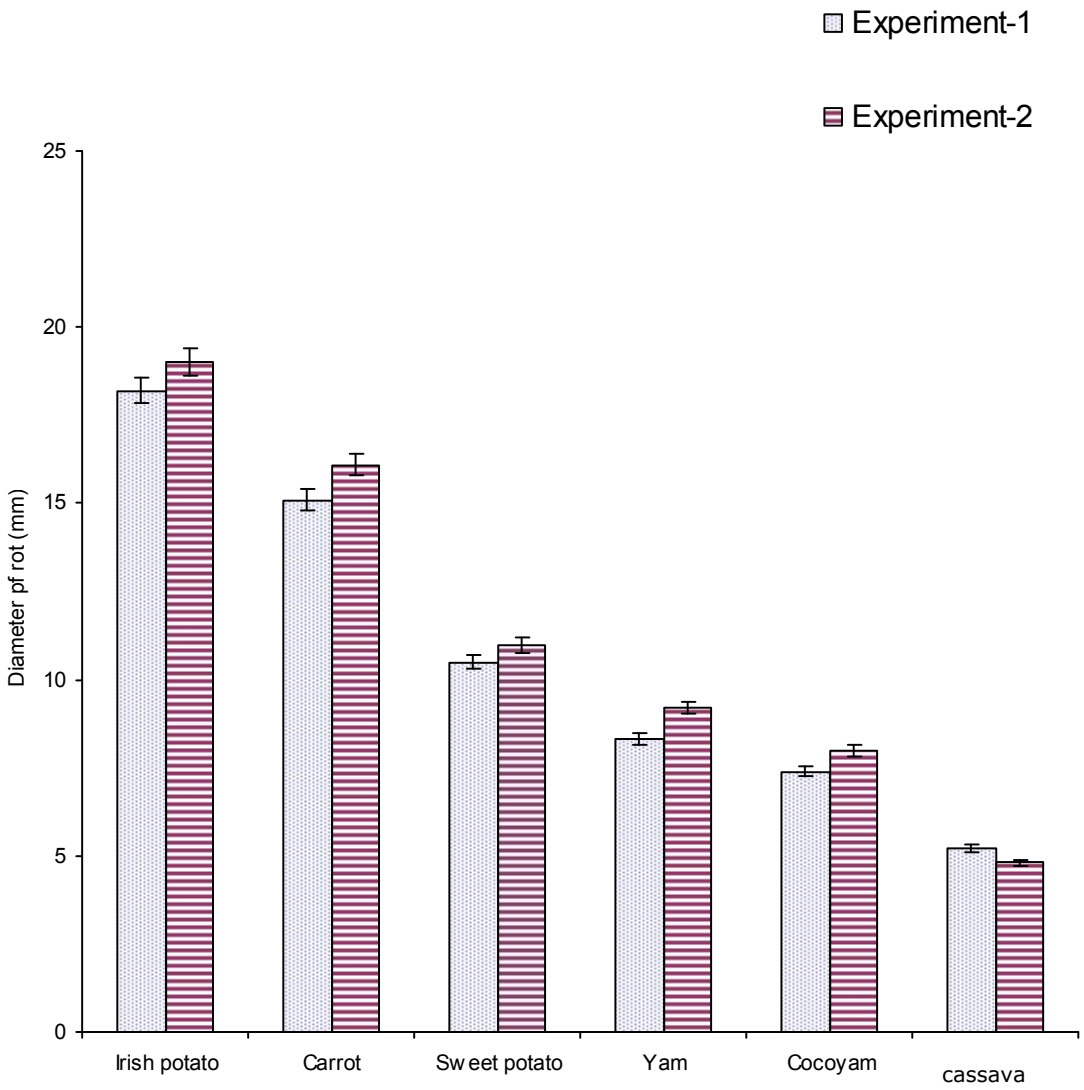


Fig 5.1: The relative susceptibilities of various tubers to soft rot pathogen using the single titration assay.

Cell size, shape and arrangement could also play some roles in the resistance of the various tubers for example; the cell type and arrangement noticed in carrot, sweet potato and cassava were the same in most respects. The only difference was in the shapes. In contrast, to other tubers' cell arrangements, which are parenchymatous, those of the Irish potato were collenchymatous which may account for its high susceptibility. Although, carrot had similar cell arrangement with other tubers but has smaller cell size and this may explain why it ranked second in susceptibility (Apeyuan, 2000).

The susceptibility or otherwise of other tubers may be related to water availability in the crop tissue. The susceptibility of plant tissue is reported (Colhoum, 1973; Perombelon and Salmond, 1995) to increase with water potential, high level of pectin methylation, increasing resistance of cell walls to enzyme degradation to soft rot pathogen which was identified in some tubers (Perombelon, 1992). This and many other chemical compositions in tissues of cassava and cocoyam may account for their high resistance to soft rot observed in this work.

The ranking of tubers in order of their susceptibility to E*rwinia ssp.* using slice assay is represented in Fig. 5.2. Again, Irish potato ranked the most susceptible followed by carrot, which was comparable to sweet potato. This was followed by yam, which was statistically similar to sweet potato. Cocoyam was the least susceptible and was statistically similar to cassava.

Direct comparison of the results obtained by the various screening methods used in this study is very difficult. This is because both methods involved different procedure and the test materials underwent different processes before inoculation which may influence the susceptibility or otherwise of the tubers. In

tuber slice assay however, great variability in ranking of tubers for susceptibility was evident both within and between the two experiments (1 and 11), with no clear distinction in the ranking of the various tubers. High variability of results associated with this assay has been reported previously (Laphood *et al*., 1984). As a result tubers differs in specific rank order from test to test using tuber slice assay (Lojkowska and Kelman, 1994). The high variability of the data obtained from the slice test in this work, may be attributed to a number of factors that may modify reaction of slices to the pathogen inoculated. For example the location in tubers from which slices were cut many influence the susceptibility or otherwise of the tubers. Also, location of inoculation on the slices could influence the result. Medullary tissue was believed to be significantly more susceptible than the cortex tissue (Bain and Perombelon, 1988; Lojkowska and Kelman, 1994). Because of the great variability of the results associated with the inoculation of slices the reliability of this approach can be questioned as a standardized method for evaluation of resistance.

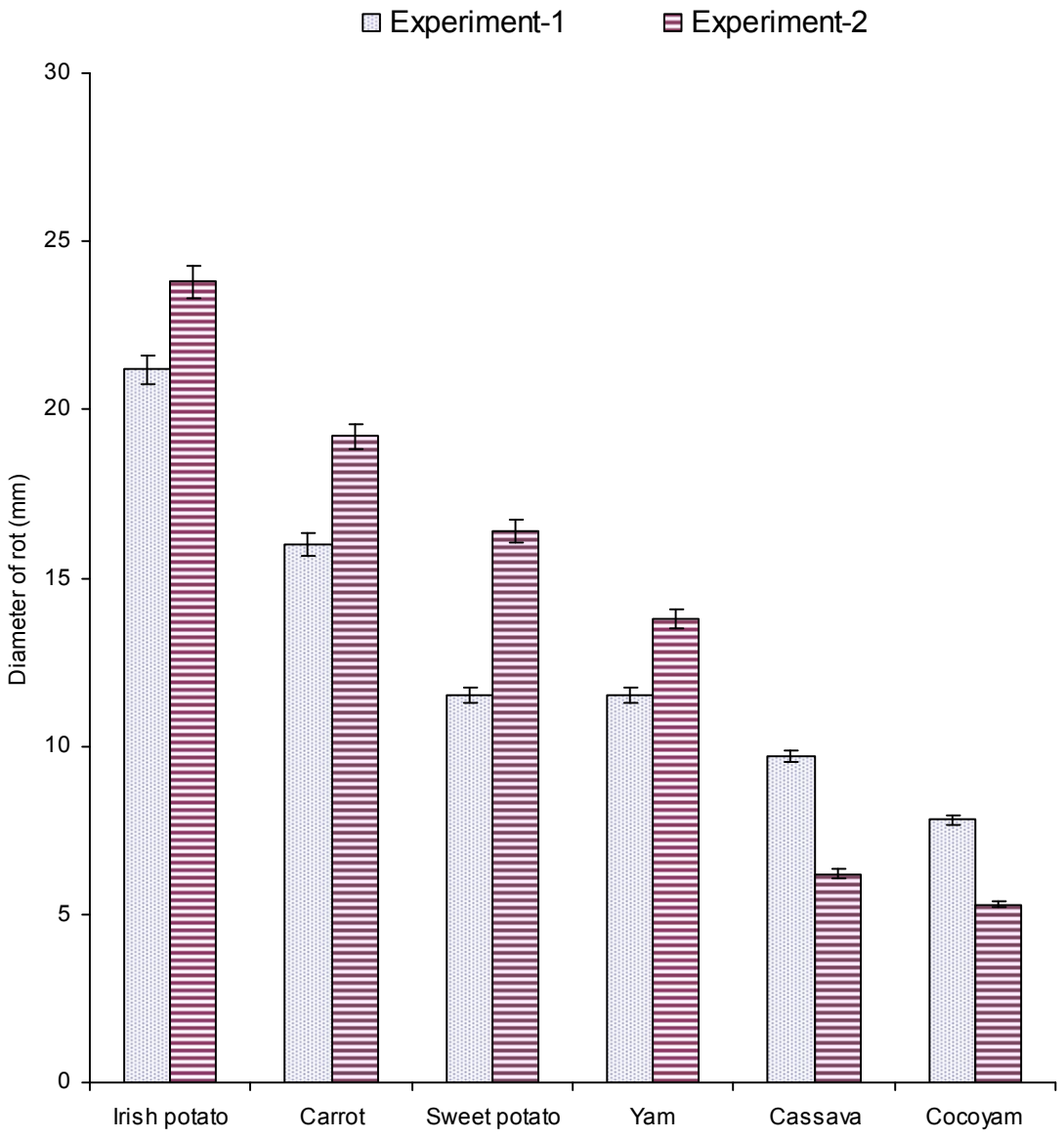


Fig 5.2: The relative susceptibilities of various tubers to soft rot pathogen using tuber slice assay.

However, to produce reliable result using this method, large numbers of tubers are required so that uniform parts of tubers (Medullary or cortex) could be inoculated. Relatively, this method is faster than the single site titration. However, the single site titration method is very useful if only limited numbers of tubers are available. This method could be recommended also when susceptibility of the tubers to different bacterial strains needs to be evaluated (Lojkowska and Kelman, 1994).

With the single site titration method it is possible to determine not only the extent of tissue maceration, but also the bacterial population necessary for initiation of lesions. However, this method is time-consuming and could not be recommended for screening large numbers of tubers. As a general rule, the whole tuber assay is preferable because the mechanism of resistance is the function of the whole system of tubers (Periderm, Medullary and Cortex).

CHAPTER SIX

6.0 SUMMARY AND CONCLUSION

Incidences of bacterial soft rot in the Zaria area was determined on potato tubers sampled from Samaru and Wusasa markets. *Erwinia* isolation was carried out on each sample collected from the rotted, wounded and intact tubers in each market area. The study revealed high incidence of 10^9 cfu/ml of bacteria population in the Zaria area. The fact that significant populations of the bacteria were detected on the apparently intact tubers suggests a high initial inoculum level from the fields where the potatoes were grown. Conditions that favours bacterial soft rot such as high percentage of wounded tubers was observed.

Potato tubers are more susceptible to infection by the pathogen within the first two weeks of harvest. As the wound healing and curing of the tubers progresses, correspondingly the bacterial population decreases below the minimum densities needed for infection. The possibility for the control of the tubers against the bacterial rot is most needed within the first two weeks after harvest when the crop stand the high risk of great loss.

Extracts of six plant materials viz: Lemon grass (*Cymbopogon citrates* stapt), garlic bulb (*Allium sativum* Linn), ginger (*Zingiber officinale* Roscoe), Aloe vera (*Aloe bandenis*. M) and Eucalyptus (Eucalyptus *citrodorus* L.) were evaluated for the management of bacterial soft rot.

In vitro studies were conducted to assess the effectiveness of plant extracts on the soft rot Erwinias. Extracts were evaluated at the concentration 5gm/100ml of water. Borax salt was used as checks alongside the control. Population reduction was however, greatest with neem extracts, which is also comparable with garlic, gingerer and lemon grass extract. Eucalyptus and Aloe vera had the least inhibitory effect on bacterial population.

In order to determine their effect on stored tubers, aqueous extract of the plants materials were assessed using two methods. In single site titration, the effects of the plants extracts were the same on all the tubers tested. Lemon grass had the greatest inhibitory effect and it was statistically comparable with garlic, Aloe vera, borax and neem extracts. Eucalyptus had the least inhibitory effect, which is comparable with ginger extracts but significantly greater than the control. In slice assay, similar results were obtained with little variation on some tubers; in potato and cassava, clear cut ranking could not be obtained between experiments I and II apparently due to the parts of the slice inoculated.

This work has demonstrated the effectiveness of lemon grass, garlic, Aloe vera and neem extracts in reducing bacterial soft rot both *in vitro* and on tested tubers. It is worthy of note that no safe and effective chemical control has been discovered to manage this disease in Nigeria. A part from the hazards associated with the indiscriminate use of chemical pesticides and the cost implication, the use of chemical pesticides is highly limited on tubers due to the peculiar nature of tubers, which are living botanical organ and which are directly consumed.

All these plant materials are relatively cheap and available all year round within the reach of many resource-poor farmers on whom the production of tubers in Nigeria largely depends. Since the washing of tubers before storage or sale is a common feature in Nigeria, the farmer can equally use the solution of extracts to rinse the washed tubers before storage especially the first two weeks after harvest, when tubers are highly exposed to predisposing factors. The methodology adopted for this work is simple and straightforward. It is therefore, hoped that the results of this study will be useful in improving potato production in Nigeria and in enhancing effective storage that will promote the availability of the crop all year round as against the seasonality syndrome due to poor storage.

In order to rank the tubers in order of their susceptibility to the soft rot bacteria, two methods were adopted: these were slice assay and single site titration assay. Tubers screened were yam (*D. rotundata*), sweet potato (*I. batatas*), Irish potato *(S. tuberosum*), cocoyam (*Xanthosoma* sp.) and cassava (*M. esculenta*). In

whole tuber assay, there was little no variation between experiments I and II and this led to clear cut ranking of the tubers. Irish potato ranked the most susceptible followed by carrot, sweet potato, yam cocoyam and cassava. In the tuber slice assay, these clear-cut ranking could not be obtained due to variability that is associated with this method such as the parts of slice inoculated and stressed imposed on tubers during slicing. However, to produce reliable results using this method, large numbers of tubers are required so that uniform parts of tubers would be inoculated. This method has the advantage of being faster than the whole tuber assay. However, with single site titration, it is possible to determine not only the extent of tissue maceration but also the bacterial population necessary for initiation of lesions. It is hoped that the findings of this work could serve as an answer to an important problem that impedes the breeding for soft rot resistance, which is the lack of a generally accepted screening method for selection of resistance clones or lines.

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8.0 APPENDICES

Appendix 8.1. One month storage under room temperature

Treatment (20 tubers)	Rot incidence at week			
	1	2	3	4
Samaru intact	30%	10%	0%	0%
Samaru wounded	60%	30%	5%	0%
Wusasa intact	20%	10%	0%	0%
Wusasa wounded	50%	30%	5%	0%
Sabon Gari intact	20%	10%	0%	0%
Sabon Gari intact	40%	20%	3%	0%

Appendix 8.2: ANOVA produce for experiment1 testing the sensitivity of the pathogen to the plant extracts

Source	DF	SS	MS	F	Pr > F
Model	9	11897.6042	1321.9560	63.49	0.0001
Error	14	291.5208	20.8229		
Total	23	12189.12500			
Rep	2	64.3125	32.1563	1.64	0.0247
Treat	7	11833.2917	1690.4702	81.18	0.001

Appendix 8.3: ANOVA procedure for experiment II. Testing for the sensitivity of the pathogen to the plant extract.

Source	DF	SS	MS	F	
Model	9	11789.5132	1311.8470	62.50	0.0001

Error	14	290.5106	19.59		
Total	23	11980.0238			
Rep	2	64.2515	32.1453	1.64	0.0246
Treat	7	11753.2857	1685.4602	81.17	0.0001

Appendix 8.4: ANOVA procedure for plant extracts on yam (Single site titration assay).

Source	AF	SS	MS	F	Pr > F.
Model	9	183.0990	20.3443	25.000	0.001
Error	14	11.3906	0.8136		
Total	23	194.4896			
Rep	2	0.5677	0.28389	0.35	0.7114
Treat	7	182.5313	26.0759	32.05	0.0001

Appendix 8.5: ANOVA procedure for plant extract on cassava (Single site titration assay).

Source	DF	SS	MS	F	Pr > F
Model	9	158.8672	17.6519	23.16	0.0001
Error	14	10.6719	0.76234		
Total	23	169.5391			
Rep	2	2.0781	1.0391	1.36	0.2878
Treat	7	156.7890	22.3984	29.38	0.0001

Appendix 8.6: ANOVA procedure for plants extracts on cocoyam (Single site titration assay).

Source	DF	SS	MS	F	Pr > F
Model	9	158.672	17.6519	23.16	0.0001
Error	14	10.6719	0.7623		
Total	23	169.53901			
Rep	2	2.0781	1.391	1.36	0.2878
Treat	7	156.7891	22.3984	29.38	0.0001

Appendix 8.7: ANOVA procedure for plant extract on sweet potato (Single site titration

assay).

Source	DF	SS	MS	F	Pr > F
Model	9	267.5765	29.7301	40.54	0.0001
Error	14	10.2681	0.733		
Total	23				
Rep	2	0.8852	0.4426	0.60	0.5608
Treat	7	266.6913	38.0988	51.95	0.0001

Appendix 8.8: ANOVA procedure for plant extracts on Irish potato (Single site titration assay).

Source	DF	SS	MS	F	PR>F
Model	9	793.4389	88.1599	199.79	0.0001
Error	14	6.1777	0.4413		
Total	23	799.6166			
Rep	2	0.0806	0.0403	0.09	0.9132
Treatment	7	793. 3582	113. 3369		0.0001

Appendix 8.9: ANOVA procedure for plant extracts on yam (Tuber slice assay).

Source	DF	SS	MS	F	PR>F
Model	9	312.7926	34.7547	30.63	0.0001
Error	14	15.8848	1.1346		
Total	23	328.6774			
Rep	2	2.7502	1.3751	1.21	0.3270
Treatment	7	310.0424	44.2918	39.04	0.0001

Appendix 8.10: ANOVA procedure for plant extracts on cassava (Tuber slice assay).

Source	DF	SS	MS	F	PR>F
Model	9	210.0104	23.3345	18.00	0.0001
Error	14	18.1458	1.2961		
Total	23	228.1563			
Rep	2	1.1875	0.5938	0.46	0.6416
Treatment	7	208,8229	29.8318	23.02	0.0001

Appendix 8.11: ANOVA procedure for plant extracts on cocoyam (Tuber slice assay).

Source	DF	SS	MS	F	PR>F
Model	9	194.9270	21.6586	35.41	0.0001
Error	14	8.5625	0.6116		
Total	23	203.4896			
Rep	2	2.2708	1.1354	1.86	0.1927
Treatment	7	192.6563	27.5223	45.00	0.0001

Appendix 8.12: ANOVA procedure for plant extracts on sweet potato (Tuber slice assay).

Source	DF	SS	MS	F	PR>F
Model	9	290.9792	32.3310	39.43	0.0001
Error	14	11.4792	0.8199		
Total	23	302.4583			
Rep	2	4.5208	2.2604	2.76	0.0978
Treatment	7	286.4585	40.9226	49.91	0.0001

Appendix 8.13: ANOVA procedure for plant extracts on Potato tuber slice assay.

Source	DF	SS	MS	F	PR>F
Model	9	557.7188	66.4132	48.88	0.0001
Error	14	19.0208	1.3586		
Total	23	616.7396			
Rep	2	2.6458	1.3229	0.97	0.4018
Treatment	7	595.0729	85.0104	62.57	0.0001

Appendix 8.14: ANOVA procedure for screening for susceptibility of tubers to soft rot bacteria (Single site titration assay)

Source	DF	SS	MS	F	PR>F
Model	6	262.7800	43.7967	338.63	0.0001
Error	5	0.6467	0.1293		
Total	11	263.4267			
Experiment	1	0.9633	0.9633	7.45	0.0413
Treatment	5	261.8167	52.3633	404.87	0.0001

Critical value of T= 2.57

LSD = 0.9245.

Appendix 8.15: Ranking of Tubers

Tubers	Means of width of rot
Yam	8.75^{d}
Cocoyam	7.70^{e}
Cassava	5.00^{f}
Carrot	15.60^{b}
Sweet potato	10.75^{c}
Irish potato	18.60^{a}

Appendix 8.16: ANOVA procedure for screening tubers for susceptibility to soft rot Bacteria (Tuber slice assay)

Source	DF	SS	MS	F	PR>F
Model	6	359.7500	59.9583	10.59	0.0102
Error	5	28.3167	5.6633		
Total	11	388.0667			

Experiment	1	4.0833	4.0833	0.72	0.4346
Treatment	5	355.6667	71.1333	12.56	0.0074

Critical value of T= 2.57

LSD = 6.1174

Appendix 8.17: Ranking of tubers

Tubers	Means of width of rot
Yam	12.65^{bcd}
Cocoyam	6.55^{d}
Cassava	7.95^{cd}
Carrot	17.60^{ab}
Sweet potato	13.95^{bc}
Irish potato	12.50^{a}

Appendix 8.18. Ten year summary of temperature and relative humidity in the Zaria area.

Month	1996			1997			1998			1999	
	Temp. (°C)		RH	Temp. (°C)		RH	Temp. (°C)		RH	Temp. (°C)	
	Min	Max		Min	Max		Min	Max		Min	Max
November	11.7	32.6	16.2	14.8	32.9	43.3	20.2	33.9	34.6	16.5	32.8
December	14.5	37.2	32.1	12.4	31.4	18.5	16.8	32.4	26.4	15.2	31.2
January	10.3	32.3	23.6	13.3	32.5	17.8	13.5	30.0	17.2	16.1	32.3
February	17.0	33.4	23.9	13.4	29.4	14.3	17.3	33.4	12.1	18.7	35.6
March	20.8	37.7	31.7	19.0	35.1	23.8	19.5	34.0	11.4	23.1	38.4
April	23.0	27.4	55.8	21.8	35.1	57.2	23.3	36.9	45.0	25.2	37.6
May	21.8	34.2	69.8	21.1	32.1	74.9	22.5	33.2	76.7	24.7	35.8

Appendix 8.18. Contd.

Month	2001			2002			2003			2004			2005		
	Temp. (° C)		RH	Temp. (° C)		RH	Temp. (° C)		RH	Temp. (° C)		RH	Temp. (° C)		RH
	Min	Max		Min	Max		Min	Max		Min	Max		Min	Max	
November	16.0	33.0	28.0	17.4	30.6	57.9	18.4	35.0	26.6	18.6	35.0	30.8	18.5	36.0	37.8
December	17.0	33.0	24.0	15.4	31.6	65.0	15.6	31.1	18.7	16.9	33.0	15.4	19.2	35.0	32
January	14.7	30.8	24.0	15.2	27.7	23.2	15.9	32.7	71.0	15.6	31.8	14.0	14.2	29.7	16.0
February	17.2	32.4	21.3	18.6	32.5	18.0	19.4	33.3	75.5	16.6	31.7	13.6	19.5	35.3	20.5
March	21.8	37.3	18.5	23.0	37.1	29.0	21.5	36.8	71.3	19.6	34.0	16.9	22.5	38.9	23.0
April	24.1	36.0	52.4	25.6	37.3	68.0	24.7	37.6	72.2	25.8	38.1	52.0	24.0	40.0	40.5
May	24.2	36.0	75.7	26.0	37.4	79.4	24.0	37.5	71.1	22.0	35.0	66.3	22.4	34.1	68.0