KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

FACULTY OF AGRICULTURE

DEPARTMENT OF HORTICULTURE

POSTHARVEST MANAGEMENT OF CROWN ROT DISEASE OF BANANAS (*Musa spp.* (AAA)) USING EXTRACTS FROM *Ocimum gratissimum*, *Alstonia boonei* AND *Garcinia kola*.

A THESIS SUBMITTED TO THE SCHOOL OF RESEARCH AND GRADUATES STUDIES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE (MSC) POSTHARVEST PHYSIOLOGY DEGREE.

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MAY, 2008

DECLARATION

I, Michael Appiah-Sarpong hereby declare that this piece of work is solely from my own investigations and it has never been presented to this University or elsewhere for the award of degree or certificate of any kind. Works by other authors which served as sources of information has been duly and fully acknowledged by reference to the authors.

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DEDICATION

This work is dedicated to my parents Mr. And Mrs. Sarpong Darko whose immense help, care, spiritually and financially have made me who I am today. I also dedicate this work to my brothers and sisters.

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ABSTRACT

A study was conducted to assess the potential of *Ocimum gratissimum, Alstonia boonei* and *Garcinia kola* heckle to control the crown rot disease in bananas. The botanicals were compared with chemical fungicides namely Citrex and Tecto.

Colletotrichum musae, Botryodiplodia theobrome, Fusarium moniliforme and *Fusarium oxysporum* were isolated from diseased crown rot tissue from fruits collected from some parts of Ashanti region. Each of the isolates singly or in combination were able to cause crown rot disease when inoculated into healthy bananas crowns.

In a bioassay crude extract of leaves of *Ocimum gratissimum* showed consistent fungicidal activity against all four fungal isolates by producing zones of inhibition against them at 100% and 75% concentration. *Garcinia kola* showed fungicidal activity against *Colletotrichum musae, Fusarium moniliforme* and *Fusarium oxysporum* at 100% and 75% concentration while *Alstonia boonei* did not show any fungicidal activity against the fungal isolates.

Crown of dehanded unripe export Cavendish bananas were inoculated with the four isolated fungal and the various treatments namely *Ocimum gratissimum*, Tecto, Citrex and Sterile distilled water were applied to the inoculated bananas. The bananas were stored at 15°C – 17°C and 95% R/H in a cold room for two weeks. The bananas were then assessed for crown rot diseases. *Ocimum gratissimum* could not control the crown rot disease by post inoculation treatment when compared to the Control (sterile distilled water) but Tecto and Citrex were able to offer some form of

protection. Fruit qualities were also assessed in terms of total titratable acidity, pH, total soluble solids and peel colour. Fruit qualities were not affected by the various treatments. However, in an evaluation test, volunteers accepted bananas treated with Tecto and Citrex but bananas treated with *Ocimum gratissimum* poor since it affected flavour and peel colour adversely.

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1. PDA: Potato Dextrose Agar
2. CGIAR: Consultative Group on International Agriculture Research
3. GEPC: Ghana Export Promotion Council
4. FAO: Food and Agriculture Organization

1.0 INTRODUCTION

Banana (*Musa* spp) is an important food crop for man, especially in the tropics. It is predominantly a tropical crop and it is a staple food wherever they are grown. Banana is believed to have been grown in Africa since ancient times but it is thought to have been taken from Indonesia to Madagascar in the fifth century A.D. spreading into the heart of the continent and then to West Africa (FAO, 1991).

Bananas when ripe, are sweet and easily digestible and are eaten raw as a snack or dessert fruit but in the unripe state, they are cooked to provide a starchy food nutritionally similar to potato (FAO, 1991). Ripe banana contains 77% moisture, 1.5% protein, 1.0% fat, 20.6% carbohydrate, 0.9% fibre and 0.8% ash in addition to vitamin A and fair amount of vitamin C (FAO, 1991). Large quantities of beer are also made from them throughout their growing areas. It can also be used to prepare Banana Yogurt Lite and Banana Pops (FAO, 1991).

It remains amongst the largest commodity of export from the developing countries and it ranks fifth in export value among the category of tropical crops of major export. In tropical America and the Caribbean, they are of great socio-economic and nutritional significance and they generate considerable export earnings and employment (FAO, 1971).

Banana together with plantains, are known to constitute the fourth most global food commodity after rice, wheat and maize in terms of gross value of production (CGIAR, 1992). In 1989, Africa produced a reported total of 23.8 million tonnes of bananas, about 34.7 % of the world's total production. Ghana exported 3,232.64

metric tonnes to the European Union, which earned the country US$ 3, 250, 020 (GEPC, 2002).

Despite all these importance, banana has a problem of postharvest diseases among which is the crown rot. Crown rot is the result of fungal invasion of the hand tissue to which fruit fingers are attached. The fresh wound, where the crown of the hand is severed from the stalk is invaded by various fungi which cause the disease. The crown rot disease is caused by a complex of fungal organisms including *Fusarium spp, Botryodiplodia theobromae, Venticillium theobromae, Thievaviopsis paradoxa, Nigrospora spherical* and many other fungal species. The fungi are present on the fruits and flowers when placed in the dehanding tank and form part of the flora of the water used to remove latex. These fungi remain in the tissue near the surface of the crown. The fungi usually form a clearly visible layer of mould on the crown surface. Stover and Simmonds (1987) reported that crown rot together with anthracnose (which is caused by *Colletotrichum musae*) constitute the major postharvest problems in the banana industry. Crown rot problem is often seasonal with the highest incidence occurring during the hot humid weathers of April to September (Stover and Simmonds 1987).

The most effective control of Crown rot has been through the use of synthetic chemicals. These chemicals tend to leave residues on the produce which create potential health hazard for the consumer apart from polluting the environment (Rosenow *et al.*, 1996). Recently, more effort has been concentrated on the use of eco-friendly methods of disease control such as the use of biodegradable natural products especially from medicinal plants (Prithiviray and Singh, 1995). Botanicals

are mostly less harmful in the environment than synthetic pesticides because they degrade quickly (Dufour, 2001). Most botanical pesticides also tend to be less toxic to mammals, including man, than non-chemical pesticides although some might be toxic (FAO, 2000). Therefore, the application of natural plant extracts as an alternative to synthetic chemical treatment are

being explored. They can be as simple as pureed plant leaves, extracts of plant parts, or chemicals purified from plants.

This project is aimed at exploring ways to control crown rot in bananas using natural plant extracts.

The Specific objectives were:

1) to establish the efficacy of three botanical extracts from *Ocimum gratissimum* *Alstonia boonei*, and *Garcinia kola* in controlling crown rot disease of banana.
2) to determine which extract will best control Crown rot without affecting fruit quality compared with two other fungicides namely Tecto which is synthetic and Citrex which is an organic fungicide and
3) to assess biochemical changes on/in banana fruits following treatment with botanical fungicides

2.0 LITERATURE REVIEW

2.1 Taxonomy

Bananas belong to the Musaceae, known simply as the banana family. It is a relatively small family with only 5 genera and at most 150 species. The family is distinguished by being composed of fairly large, often treelike, tropical herbaceous plants with flowers subtended by distinct bracts. Bananas are unique among fruit crops since they are monocotyledons and not dicotyledons (Rieger, 2006).

2.2 Maturity

Fruits are harvested when about 75% mature, as angles are becoming less prominent and fruits on upper hands are light-green in color. At this stage, desiccated styles on tips of fruit can be easily rubbed off and this occurs at 75-80 days after opening of the first hand. Sometimes harvest may be delayed up to 100-110 days after opening of the first hand (Rieger, 2006).

2.3 Harvesting

Entire bunches are cut from pseudostems by hand, and carried on the shoulder or back to a nearby tram line for longer distance transport. A portion of bare stalk is left and used as a handle for transporting to the packinghouse (Rieger, 2006).

2.4 Fruit Ripening

During ripening, the banana fruit undergoes many physical and chemical changes after harvest that determines the quality of the fruit purchased by the consumer. Brady (1987) reported that fruit ripening is the result of a complex of changes, many of them probably occurring independently of each other. Fruit ripening quality is an

important postharvest selection criterion. Traditionally, the stage of ripening of banana, have been closely linked with the changes in peel colour (Loesecke, 1950); Palmer, 1971) and the matching of the peel colour against a set of standard colour plates to assess the ripeness of the fruit. Sometimes, high temperatures and low relative humidity could cause fruits to retain their green peel colour even though ripening has already commenced internally, creating a situation whereby the peel colour does not reflect the internal changes.

2.5 Factors that Affect Ripening of Banana

2.5.1 Temperature

In general, temperatures lower than 11.5 ^{0}C lead to symptoms of cold storage, but the critical temperature can vary with the cultivar, relative humidity and the length of exposure to cold (John and Marchal, 1995). Beyond 25 ^{0}C, fruit quality is altered because of modifications to metabolism during ripening. When temperatures go above 35 ^{0}C, fruit with a soft pulp but a green peel develop and this causes the pulp to soften faster than the colouring of the peel. Ripening is effectively blocked when temperature exceed 48 ^{0}C (John and Marchal, 1995).

2.5.2 Relative Humidity

The lower the relative humidity, the greater the loss of water and the shorter the duration of the preclimacteric (George *et al.*, 1982). A reduction of relative humidity does not alter the rate of respiration, but slows changes in weight relationships between pulp and peel, in the peel colour, in pulp softening and in the soluble sugars content (Broughton and Wu, 1979).

2.6 Changes that Occur During Ripening

Some of the major changes that occur are:

2.6.1. Peel and Pulp Colour

The disappearance or loss of peel green colour and the corresponding increase in yellowing of the peel during ripening are the obvious manifestations in banana. The loss of green colour is due to degradation of the chlorophyll structure. External changes in peel colour during ripening often reflect changes in chemistry (Wainwright and Hughes, 1989, 1990).

2.6.2. Conversion of Starch into Sugar

The most striking post-harvest chemical change which occurs during the postharvest ripening of banana is the hydrolysis of starch and the accumulation of sugars that is sucrose, glucose and fructose (Loesecke, 1950; Palmer, 1971), which are responsible for the sweetening of the fruit (as it ripens). In Cavendish bananas, the breakdown of starch and the synthesis of sugars are usually completed at full ripeness (Marriott *et al.*, 1981). Kwaa, (1995) reported that at senescence accumulated sugars are used up without replacement.

2.6.3. Pulp to Peel Ratio

Pulp to peel ratio is a good and consistent index of ripening of banana and it increases in response to ripeness (Dadzie and Orchard, 1997). Changes in pulp to peel ratios during ripening of banana indicate differential changes in moisture content of the peel and pulp. The increase in pulp to peel ratio during ripening is related to sugar concentration in the two tissues. During ripening, there is a rapid increase in the sugar concentration in the pulp compared to the peel thus contributing

to a differential change in osmotic pressure (Dadzie and Orchard, 1997). The peel loses water both by transpiration to the atmosphere and also to the pulp by osmosis, thereby, contributing to an increase in the fresh weight of the pulp as the fruit ripens. This results in an increase in the pulp to peel ratio during ripening Dadzie and Orchard, 1997).

2.6.4. Pulp Firmness

Under normal storage conditions, desert banana, cooking banana and plantain, undergo significant textural transformations as they pass through the ripening process. The crisp, hard and green fruit turns into a yellow fruit with tender and soft internal pulp at the optimal ripening stage, and becomes mushy as it advances towards senescence. The loss of firmness during ripening leads to lower quality and higher incidence of mechanical damage during handling and transportation.

Loss of pulp firmness during ripening varies with cultivar/hybrid. Pulp firmness is often inversely related to ripening, implying that, as ripening progressed, pulp firmness declined (Smith *et al.*, 1989). Generally, the triploid cultivars are usually firmer than the tetraploid hybrids (Dadzie, 1994). Loss of firmness or softening during ripening has been associated with two or three processes. The first is the breakdown of starch to form sugar. The second is the breakdown of the cell walls or reduction in the middle lamella cohesion due to solubilisation of pectic substances. The third is the movement of water from the peel to the pulp during ripening due to the process of osmosis (Palmer, 1971; Smith *et al.*, 1989).

2.6.5. Total Soluble Solids Content

During ripening of banana, the total soluble solids content increases. However, the magnitude of increase is dependent on cultivar. In most ripe fruits, including banana, sugar forms the main component of soluble solids, since the amount of sugar in fruits usually increases as the fruit matures and ripens (Dadzie and Orchard, 1997). Soluble solids content varies between cultivar and between stages of ripeness. In some hybrids, soluble solids contents increase to a peak and then decline (the drop in total soluble solids may be due to the conversion of sugar in the pulp to alcohol), while in others, total soluble solids continue to increase with ripening (Dadzie and Orchard, 1997)

2.6.6. Pulp pH and Total Titratable Acidity

Pulp pH and total titrable acidity are important post-harvest quality attributes in the assessment of fruit ripening quality. In most banana cultivars, there is a rapid decline in pulp pH in response to increasing ripeness (Dadzie and Orchard, 1997). However, the magnitude of decline is cultivar dependent. In general, when fruits are harvested at matured green stage, the pulp pH is high but as ripening progresses pH drops.

Organic acids decline during ripening as they are respired or converted to sugar (Wills *et al.*, 1989). Organic acids are important in giving a desired sugar-to-acid balance which results in the pleasant taste during ripening. Acidity, measured as titratable acidity in the pulp tissues of most banana cultivars/hybrids, shows large increases during ripening or as ripening progresses (Dadzie and Orchard, 1997). In Cavendish bananas, citric and malic acids are the most significant organic acids detected in green fruit (Inaba and Nakamura, 1988) and as ripening proceeds, the

malic acid content rises from 1.8 to 6.2 meq/100g fresh weight (Satyan and Parwardhan, 1984).

2.6.7. Peel and Pulp Moisture and Dry Matter Content

Peel and pulp moisture and dry matter content are important postharvest parameters in the evaluation of the ripening quality of banana. During ripening, the moisture content of the peel decreases whereas that of the pulp increases; this is because the peel loses water both to the atmosphere and to the pulp. In most cultivars/hybrids, the dry matter content of the peel and pulp during ripening does not change significantly (Dadzie and Orchard, 1997).

2.6.8. Changes in Respiration Rate and Ethylene Production

During ripening of banana, there is a tremendous increase in the amount of ethylene produced. This increase is usually accompanied by an increase in respiration rate of the fruit (a phenomenon which is called the climacteric). The rate of respiration and ethylene production usually depends on storage temperature, age of fruit and cultivar/hybrid (Kader, 1987).

2.7 Postharvest Diseases of Banana

Fungi are overwhelmingly important in postharvest diseases of fruits. Postharvest diseases can cause serious losses of fruits both in terms of quantity and quality and that fruits affected by disease have little or no market value. Postharvest pathogens can be divided into two groups based on their ability to gain entrance through the fruit skin into the fruit. One group is able to bypass the protective skin only through wounds. The other group has the ability to form special morphological structures

called appressoria that permit the fungus to penetrate the fruit cuticle and epidermis. The banana fruit is known to have many postharvest diseases. Some of them are crown rot, anthracnose, squirter rot, and finger rot (Kader, 1987).

2.8 Disease under Study

2.8.1 Crown Rot

Crown rot is a disease which occurs in all banana growing regions of the world and is one of the most important postharvest diseases of banana (Dadzie and Orchard 1997). It is potentially the most serious postharvest problem in the banana industry (Jeger *et al.*, 1995). The disease is largely a consequence of an important technological change in which hands are cut from stems and packaged in fiberboard cartons (Kader *et al., 1985*). It is characteristically a disease complex caused by several fungi, sometimes in association with other micro-organisms such as bacteria (Ogawa, 1970). A number of these fungi have been isolated from decaying crowns and the frequency of isolation varies between regions. Two or more of these fungi may attack the crown simultaneously or successively and cause tissue rotting. Different organisms predominate according to locality, time of year and other factors (Dadzie and Orchard 1997).

In its natural state, the tough skin of banana protects the fruit against fungal diseases. When the hands are cut from the stalk, the massive open wound becomes an ideal weak spot for crown rot fungi to enter and grow. Fungal spores on the fruit in the field are carried along (after harvesting the bunch) to the packing house. Spores follow the fruit right into the delatexing baths, where they are drawn deeply into the weak spot, the wound on the crown tissue (due to dehanding), (Stover and

Simmonds, 1987). Greene and Goos (1963) reported that when hands are cut from stalks, fungal spores can be drawn into crown tissues within 3 minutes to a depth of 5-7 cm, and may germinate and develop as deep-seated infections beyond the reach of chemical treatments.

Blackening of the crown tissues occurs initially at the cut surface, but the rot may spread into the crown during transportation (Jeger *et al.*, 1995), and rotting is most noticeable if transit times exceed 7 days, with a high incidence of the disease causing premature ripening. When the disease is severe, fingers drop from the crown when suspended. The severity of crown rot is highly unpredictable and it is not known why some hands in a box are affected and others not, and why one shipment is seriously affected whilst a prior or subsequent shipment from the same farm is not (Stover and Simmonds, 1987).

In Central America and the West Indies some of the fungi which have been isolated are *Colletotrichum musae* (Berk and Curt) var Arx, *Fusarium spp,* particularly *F. pallidoroseum* (Cooke) Sacc, *F. moliniforme* Sheld, and to a lesser extent *Verticillium theobromae* (Turc) Mason and Hughes, and *Botryodiplodia theobromae* Pat (Green and Goos, 1963; Griffee, 1976). *Ceratocystis paradoxa* (Dade) Moreau and *Fusarium spp* are the most commonly associated with crown rots in South America. In Mexico, these fungi may be found in association with *Botryodiplodia theobromae* Pat.; *Nigrospora sphaerica* (Sacc.) Mason; *Cladosporium cladosporioides* (Fresen.) de Vries; *Pestalotia leprogena* Speg.; *Chaetomium globosum* Kunze ex Fr.;*Trichoderma viride* Pers. ex Fr. and *Verticillium theobromae* (Turc) Mason and Hughes (Gonzalez and Zenteno, 1988). Sepiah and Nik-Mohd

(1987) reported that in Asia, *C. musae* (Berk and Curt) var Arx.; *Fusarium spp* and *B. theobromae* Pat are frequently isolated from rotting crowns whiles in Malaysia these fungi may be found in association with *Curvularia spp* and *Gliomastrix musicola.* Symptoms of crown rot are characterised by Softening and blackening of tissues at the cut crown surface and White, grey or pink mould may form on the surface of the cut crown (Meredith, 1965; Lukezic *et al.*, 1967; 1971; Ogawa, 1971; Snowdon, 1990; Ploetz *et al.*, 1994).

2.9 Postharvest Fungicide Application

Manners (1984) reported that chemicals were used to control plant diseases long before their causal organisms were known, and that many fungal diseases (both pre- and post-harvest) can be controlled by the application of chemicals. Although synthetic chemical fungicides are being used successfully for the control of various diseases, indiscriminate use of them has led to development of fungicide-resistant fungi and more importantly environmental health hazards, posing potential risk to animals and human beings (Lyon *et al.*, 1995).

These chemicals are known or suspected to be reproductive toxicants. They can have many negative influences on health, including disruption of the endocrine system, carcinogenicity and immune system suppression. Pesticide exposure may also affect male reproductive function and has been linked to miscarriage in women. Example includes the fungicide benomyl and vinclozolin (Annon, 2005). FAO, (1990) reported that benomyl had been withdrawn from all postharvest uses in the United States of American.

2.10 Essential Oil

Essential oils such as those made from basil, fenugreek, cumin, neem, mint, clove, and eucalyptus may be effective against a number of fungal pathogens. For instance, solutions of cumin or clove oil completely inhibit sugarcane rot, and basil oil can inhibit growth of soil borne pathogens (Quarles, 2006). The plant oils described here are complex mixtures of natural substances made by plants. Botanical oils are derived from various parts of the plants, such as flowers, fruits, leaves, and wood (Annon, 1999). The oils can be used as pesticides or fungicides to kill certain animals and fungi. Sometimes the chemicals in the oil, as well as the oil itself, are registered as pesticide or fungicide active ingredients. It is also fairly common for two or more oils to be used in the same commercial product. Fungicides made from botanical oils are derived from plants that are known to have fungistatic properties (Annon, 1999).

2.11 Plants used for Study

2.11.1 *Garcinia kola*

2.11.1.1 Ecology, Distribution and Description

Garcinia Kola belongs to the family Guttiferae (FAO, 1986). It is commonly known as bitter kola or false kola nut. It is a large spreading forest tree with a dense and heavy crown. It grows up to about 27m tall and 1.5m in girth. The back of the tree is greenish brown, thick and smooth. The leaves are up to 15cm long and 7.0cm wide. They are paired at the ends of the twigs, broadly elliptic, very shortly acuminate and shiny above. The leaves also have about 10 pairs of lateral nerves and very obscure

venation in between them. The mid-rib is prominent below and the petioles thickened. The tree flowers twice in a year; December to March and May to August. The female flowers are 1.4cm in diameter. It is globose, yellow and fleshly. They are separated from the male ones which are composed of four sepals, four greenish-white petals and stamens in bundles of four. The fruits are 7cm in diameter, reddish yellow in color and smooth. The fruit contains 3-4 pale yellow seeds in an orange–yellow pulp (Irvine 1961). *Garcinia kola* abounds in West and Central Africa in countries like Ghana, Togo, Zaire and Nigeria (FAO, 1986).

2.11.1.2 Uses

Adu-Tutu *et al.*, (1979) reported that *Garcinia kola* is used as chewing stick in Ghana.

2.11.1.3 Chemical Constituents

The stem bark has been reported to contain flavonoids and glycosides (Iwu and Igboko 1982).

2.11.2 *Alstonia boonei*

2.11.2.1 Ecology Distribution and Description

Alstonia boonei belongs to the family Apocynaceae. It is a large deciduous tree which can grow to about 45 meters high with a diameter of 1.2 meters. The back of the tree is grayish green and it is rough. When the bark is slashed in a rough angular way, a yellow milky latex exudes. The branches are in whorls with the leaves also in whorls of 5-8 and are simple subsessile to petiolate. The mid-rib is prominent below. The inflorescence is terminal and compound with 2-3 tiers of pseudo-unbels (FAO,

1986). The tree flowers between Octobers to March in a year. The fruits are formed by two pendent green follicles measuring up to 60 centimeters long (Voorhoeve, 1965). *Alstonia boonei* abounds in countries like Ghana, Cameroon, Sudan, Cote d'ivor and Nigeria.(FAO, 1986).

2.11.2.2 Chemical Constituent

Adesina (1982) in a pharmacological evaluation of the plant extract found it to be alcoholic with sedative properties.

2.11.2.3 Major Uses

It is used to treat breast pains by bathing the affected parts with extracts from the young leaves (FAO, 1986).

2.11.3 *Ocimum gratissimum*

2.11.3.1 Ecology, Distribution and Description

The plant *ocimum gratissimum* belongs to the family labiatae (Dssanayaka, 1981). It is an erect, small shrub about one metre in height with unequal sided base and serrated leave margins. The leaves are opposite, simple and do not have stipules (FAO, 1986). The flowers are creamish to yellow in color and are borne on compact racemous inflorescence and are hermaphrodite. The fruit size is small and it is a 4 lobed capsule. The leaves of the plant have a strong aromatic odour when crushed. The plant can grow in a variety of soils (FAO, 1986). *Ocimum gratissimum* is found in both the forest and savanna regions of West Africa (Sofowora, 1982).

2.11.3.2 Chemical Composition

The name of the active compound in the leaves is called thymol (Owusu-Ansah, 1996).

2.11.3.3 Uses

Kokware (1976) reported that the strongly scented leaves of the plant are rubbed between the palms and sniffed as a treatment for blocked nostrils. The leaves are also used to cure abdominal pains and cough.

2.12 Bioassay Technique

A number of techniques exist for screening the effectiveness of natural products for their bio-activeness against micro organism. Some of them are simple, rapid, reliable and convenient and they require little material to perform them whiles others requires special skills and sophisticated equipments and materials to perform them. Bioassay technique can be done by pouring a standard medium in which the microorganisms will grow into a Petri dish. Spore suspension of the targeted organism is then streaked gently across the set medium (Moses, 2005). For seeded plates, solution of the plant extract is introduced into wells which have been created with sterile cork borer. The plant extract can also be loaded unto sterile filter paper which has been cut into small disk shape (Moses, 2005). The paper containing the plant extract is put on the set medium containing the microorganisms. The plates are incubated for and the diameters of growth inhibition zones are measured. The diameter inhibition zone is a measure of fungicidal activity against organisms (Moses, 2005).

2.13 Minimum Inhibition Concentration.

Minimum inhibition concentrations are considered the 'gold standard' for determining the susceptibility of organisms to antimicrobials and are, therefore, used to judge the performance of all other methods of susceptibility testing (Andrews, 2001). Minimum inhibition concentrations are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the *in-vitro* activity of new antimicrobials. The minimum inhibition concentration is defined as the lowest concentration of a drug or substance that will inhibit the visible growth of an organism after overnight incubation (Andrews, 2001).

3.0 MATERIALS AND METHODS

The experiments were carried out at the Plant Pathology Section at Crops Research Institute, Fumesua and it involved isolation of disease causing organism, testing of plant extracts in an *in-vitro* analysis, application of various treatments on bananas for an *in-vivo* analysis, and an evaluation test to assessment of the effect of the various treatment on internal quality of treated bananas.

3.1 Isolation of Disease Causing Organisms

3.1.1 Medium Preparation

Medium used for isolating the disease causing organisms was Potato dextrose agar (PDA). The medium was prepared according to the manufacturer's instructions (Merck KGaA, Damstadt, Germany). The medium was autoclaved at 121 ^{0}C for five minutes and then cooled to about 50 ^{0}C. The prepared media was then poured into sterile plastic Petri dishes (4.5 mm internal diameter).

3.1.2 Preparation of Tissues and Isolation of Disease Causing Organisms

It involved the isolation of the micro organisms associated with the crown rot disease for use in pathogenicity tests. Bananas showing signs of the crown rot disease were obtained from four different locations namely Bompata, Bomso, Adum and Nkawie. A brief discussion with the seller showed that bananas bought in Kumasi were from Effiduase and the ones bought at Nkawie were brought from Toase. This was done in November 2005. Tissues from rotten crowns of bananas were taken and cut into smaller pieces. The cut pieces of crown tissues were surface sterilized in 5 % Sodium hypochlorite for 5 minutes with moderate agitation. The sodium hypochlorite solution was poured away and sterile distill water was used to washed the tissues

three times to remove the hypochlorite. The tissues were allowed to air dry in a sterile laminar flow. The dried tissues were then plated on the PDA (three tissues on a medium) in petri dishes. Para film was used to seal the Petri dishes to prevent any risk of contamination. The plated dishes were then incubated at 28^0 C for 24 hours under an initial 12 hour ultraviolet alternating light regime. When fungal tissues started growing, they were each sub cultured on to new PDA and incubated again. This process was repeated several times until pure cultures were obtained. Single spores were used to produce pure cultures of isolates.

3.1.3 Characterization and Identification of Fungi

Cultural characteristics such as pigment production and morphological features such as texture of colonies of each of the isolates were documented using 7 to 30 days old cultures. Microscopic characteristics of hyphae and conidia produced on PDA were also documented. Documented features of the isolates were depended on in identifying the fungal isolates following standard morphological and pictorial descriptions as given by Mathur and Kongsdal (2001).

3.2 Pathogenicity Test

3.2.1 Preparation of spores suspension

A plate of *Fusarium moniliforme* (14day old cultures) was flooded with sterile distilled water using a Gilson pipette fitted with a sterile D 1000 diamond tip. The surface was agitated with the tip of a sterile diamond tip. The mixture of conidia and hyphae fragments in a suspension were filtered through sterile muslin cloth into a sterile test tube to get a clear suspension containing the *Fusarium moniliforme* spores. A sample of the suspension was taken with the pipette and put on a slide. The

slide was viewed under a microscope to check for the presence of spores. Spore suspensions were prepared from *Colletotrichum musae, Botryodiplodia theobromae and Fusarium oxysporum* by the same process.

Mature green Cavendish bananas were obtained from Volta River Estates Limited at Akrade in the eastern region of Ghana. The bananas were washed under flowing tap water to remove all the latex. The crown part of the banana was then washed with 2 % sodium hypochlorite and washed over with sterile distilled water. A Gilson pipette fitted with a sterile D1000 Diamond tip was used to draw 300µl of the spore suspension at a concentration of 10^5 from each of the four isolates and used to inoculate the crown of the banana hands. The treatments were made up of the following spore suspension;

Table 1: Pathogenicity Test Treatments

Inoculum	
B. theobromae	*C. musae + F. moniliforme*
C. musae	*F. oxysporum + F. moniliforme*
F. oxysporum	*B. theobromae + C. musae + F. moniliforme*
F. moniliforme	*B. theobromae + C. musae + F. oxysporum*
B. theobromae + C. musae	*C. musae + F. oxysporum + F. moniliforme*
B. theobromae + F. moniliforme	*B. theobromae + F. oxysporum + F. moniliforme*
B. theobromae + F. oxysporum + F. moniliforme	*B. theobromae +Fusarium oxysporum*
C. musae + F. oxysporum +F. moniliforme + B. theobromae	Control (Sterile distilled water)
C. musae + F. oxysporum	

The inoculated bananas were then incubated at 28 ^{0}C for 4 days. After the incubation period the crowns that showed symptoms of the disease were cut into pieces. The

pieces were then surfaced sterilized in 5 % hypochlorite for 5 minutes. The sterile tissues were washed three times with sterile distilled water. The sterile tissues were air dried. The dried tissues were then plated on potato dextrose agar and incubated for seven days at 28°C. The isolates obtained were then identified in the same manner as described.

3.3 Extracting the Essential Plant Crude Oil from *Alstonia boonei, Garcinia kola* and *Ocimum gratissimum.*

Plant materials for the work were obtained from the Kwame Nkrumah University of Science and Technology botanical garden and were authenticated by Amissah (2005) at the Botanical gardens. The samples were air dried for 5days. The woody samples (*Garcinia kola and Alstonia boonei*) were pounded into coarse particles and the dried leaves of the *Ocimum gratissimum* were also crushed. Samples were then put in thimbles and placed in the Soxhlet extractor. The Soxhlet extractor was then mounted on a round bottom flask which is on a heating mantle. A condenser was clamped on top of the Soxhlet. The solvent for extraction (ethanol 95 %) was poured down on the thimbles through the Soxhlet and was allowed to reflux down into the round bottom flask. The condenser on top of the Soxhlet was connected to a tap. The tap was turned on for water to flow through the condenser. After this the heating mantle was turned on and the essential oils extracted.

Table 2a: Extraction for *in-vitro* analysis

Amount of each sample and solvent used in the extraction and its output are as follows.

Botanical	Amount of sample (g)	Amount of ethanol (ml)	Output (ml)
Alstonia boonei	508	1000	60
Ocimum gratissimum	400	1000	48
Garcinia kola	508	1000	60

Table 2b: Extraction for *in-vivo* analysis

Botanical	Amount of sample (g)	Amount of ethanol (ml)	Output (ml
Ocimum gratissimum	1300	3300	150

3.4 Bioassay

A Gilson pipette P 200 fitted with a sterile D1000 Diamond tip was used to draw 200µl of the spore suspension of each isolate onto a prepared solidified Potato dextrose agar in pltes. Spore suspensions were prepared from 14-day old cultures for *Fusarium oxysporum, Fusarium moniliforme, Colletotrichum musae* and 30-day old culture for *Botryodiplodia theobromae*. A sterile L-shaped glass rod was used to spread the spore suspension evenly on the Potato dextrose agar. The spores were allowed to dry on the Potato dextrose agar for ten minutes. A sterile cork borer (5mm in diametrer) was used to create three wells per plate in a triangular fashion on the plates with the sporesform. Different concentrations of the extracted botanicals were prepared at 100%, 75%, 50% and 25% µl (v/ v) for the Bioassay studies.

A Gilson pipette 200 fitted with a sterile D1000 Diamond tip was used to draw 50µl of crude extract of *Ocimum gratissimum* at 100% concentration and used to fill each of the three wells created in the Potato dextrose agar. This procedure was repeated at 75%, 50% and 25% concentration respectively. The petri dishes were then sealed with paraffin and incubated at 28^0 C for 24 hours. This process was repeated at 100%, 75%, 50% and 25% concentration for the other plant extracts (*Alstonia boonei* and *Garcinia kola*). This procedure was replicated three times. The petri dishes were allowed to stand on the bench for 10 minutes for the plant extracts to diffuse into the solidified agar. The plates were incubated at 28 ^{0}C and examined over days for zones of inhibition (Moses, 2005).

3.5 Inoculation of Banana and Application of various Treatments

Five bunches of matured freshly harvested Cavendish bananas (cultivar M 52) at an unripe stage, were obtained from Volta River Estates Limited. The bananas were then cut into clusters of hands and washed under running water for five minutes to remove the exuding latex from the surface of the crown of the banana. Thirty-six banana hands of uniform size and without any defect were selected. The banana hands were allowed to air dry. The surface of the crown were inoculated with 300µl each of prepared spore suspension from the four isolates namely, *Colletotrichum musae, Botryodiplodia theobromae Fusarium moniliforme and Fusarium oxysporum* at a concentration of $2x10^4$. Treatments were made up of three different fungicides namely Tecto (thiabendazole), Citrex, crude extract of *Ocimum gratissimum* and a control (without fungicides or extract). The bananas were divided into four groups.

200ml of Tecto was poured into a hand pump and used to spray the crown of the bananas in group one at a concentration of 10ml per 5liters of water (manufacturer's instructions). Crowns of bananas in group two were sprayed with 200ml of Citrex at a concentration of 10ml per 5 liters of water. While bananas in group three were sprayed with 200ml of crude extract of *Ocimum gratissimum* at 75% concentration. Bananas in group four were not sprayed with anything. The various treatments were replicated three times.

All the treated banana hands were allowed to air dry and were packed into transparent polythene bags with each treatment in a different bag. The air in the polythene bags were removed with the help of a vacuum machine and the polythene bags sealed with celotape and labeled. The bagged bananas were then stored at 15 ^{0}C for two weeks after which they were taken out for normal ripening (Dadzie and Ochard, 1997).

Plate 1. Packaged Bananas in a storage cold room

3.6 Experimental Design

A 4 factor (Tecto, Citrex, *Ocimum gratissimum* and Control) experimental design in a Completely Randomized Design was used. The data was analyzed using an analysis of variance and Duncans Multiple Range Test was used to separate treatment mean ($P \leq 0.05$).

3.7 Temperature and Relative Humidity Measurements

An HM 34 Temperature/ Humidity meter; model Vaisala (LCD digital thermometer displayed in °C and % R/H), was used to measure the relative humidity and temperature at the laboratory where the experiment was conducted. Temperature and humidity were measured thrice daily at 6am, 12noon and 6pm and the average recorded.

3.8 Biochemical and Quality Assessment of Treated Banana.

This stage involved the determination of pH, Total Soluble Solids and Total Titratable Acidity in the bananas and the assessment of peel colour and crown rots as ripening progressed.

3.8.1 Parameters for Assessment

3.8.2 Crown Rot Assessment

The level of crown rot was evaluated visually at color stage six using the scale as shown in table 3.

Table 3: Score used for Crown Rot Assessment

SCORE	STAGE OF INFECTION
0	No Disease (No Symptoms)
1	Decay to some or the entire wound
2	Up to one third of crown tissue decayed
3	Between one third and two thirds of crown tissue decayed
4	Between two thirds and all of crown tissue decayed
5	Decay present in up to half of pedicel
6	Decay present in half to all of pedicel
7	Extensive decay in all the pedicel
8	Decay developing in fingers

Source: Jones (1991)

3.8.3 pH

Seventy grams (70g) of banana pulp was weighed and placed in a blender. It was blended for 3minutes and filtered through sterile muslin cloth. A pH electrode was placed in the filtrate. Two minutes were allowed for the reading to stabilize and the pH value of each sampled filtrate was taken. This process was repeated three times (Dadzie, 1998).

3.8.4 Total Soluble Solids (^{0}Brix)

A drop of each of the blended sample was used in a Bellingham and Stanley (0-30 %) refractometer and the values expressed in ^{0}Brix.

3.8.5 Total Titratable Acidity

Seventy grams (70g) of pulp tissue was weighed into a kitchen blender and blended for 3 minutes. 25 ml of the filtrate was transferred into a 125 ml conical flask. 25 ml of distilled water and 5 drops of phenolphthalein indicator was added. A 25 ml burette was filled with 0.1 N sodium hydroxide (NaOH) and adjusted to the zero mark after eliminating air bubbles. It was then Titrated with 0.1 N sodium hydroxide until the indicator just turn pink/red and the titre volume was recorded. The results were expressed (e.g. as milliequivalent per 100 g sample) in terms of the predominant acid present in banana (Josylin, 1970).

3.8.6 Peel Colour

The CSIRO (1972) colour chart for determining the ripening stages of banana fruits was used to determine the peel colour changes as shown in table 4.

Table 4: Ripening stages of banana used in scoring for peel colour

STAGE	PEEL COLOUR
1	Green
2	Green with a trace of yellow
3	More green than yellow
4	More yellow than green
5	Yellow with a green tip
6	Full yellow
7	Yellow, lightly flecked with brown
8	Yellow, with increasing brown areas

Source: CSIRO (1972)

3.9 Sensory Evaluation

Ten untrained panelist were asked to assess treated fruits on the basis of texture, flavor, Taste, Sweetness, color and overall acceptability using the hedonic scale developed by Dadzie (1994) as shown in Table 5a and 5b.

Table 5a: Hedonic scale

SCALE	TEXTURE	FLAVOR	TASTE
5	Very firm	Excellent	Excellent
4	Firm	very acceptable	Like very much
3	Soft	Good	Good
2	Very soft	Fair	Fair
1	Too soft	Poor	Poor

Source: Dadzie (1998)

Table 5b: Hedonic scale

SCALE	SWEETNESS	PEEL COLOUR	OVERALL ACCEPTABILITY
1	Too sweet	Excellent	Excellent
2	Very sweet	very acceptable	Very good
3	Sweet	Good	Good
4	Slightly sweet	Fair	Fair
5	Unsweet	Poor	Poor

Source: Dadzie (1998)

4.0 RESULTS

The results of the study are shown in figure 1 to figure 8, Tables 6 to 8e and plate 2a to 4h showing fungal isolates, characteristics of isolates, bioassay results and efficacy of treatments on crown rot disease control and quality of bananas after application of treatments.

4.1 Fungi isolation

The fungi suspected to cause crown rot disease complex were isolated from banana samples collected from three suburbs in the Kumasi Metropolitan area and one from Nkawie. In all, four species of fungi were isolated from banana samples collected from the three localities in Kumasi and in Nkawie. The fungi were *Botryodiplodia theobromae*, *Fusarium moniliforme*, *Fusarium oxysporum* and *Colletotrichum musae*.

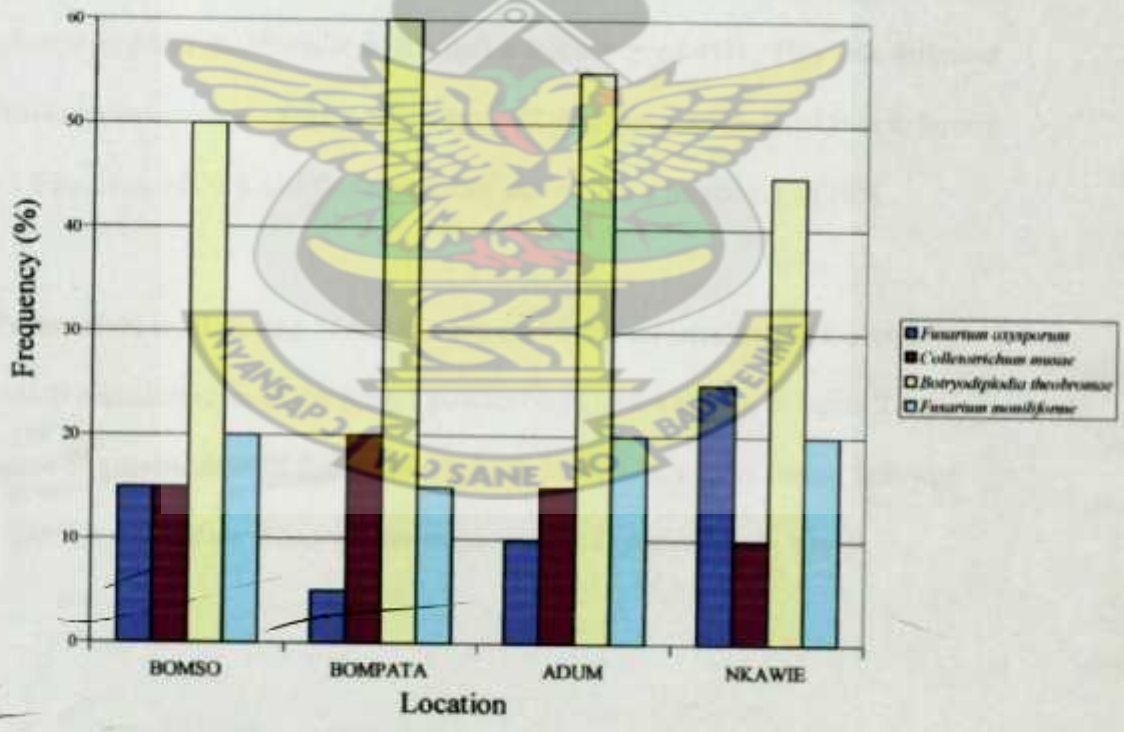


Figure 1: Frequency of fungal isolates against location.

From the twenty bananas obtained from Bomso, Kumasi, *Botryodiplodia theobromae* was the most frequently isolated (50%). This was followed by *Fusarium moniliforme* with a frequency of 20%. *Fusarium oxysporum* and Colletotrichum *musae* followed with a frequency of 20% each. The twenty bananas obtained from Bompata had *Botryodiplodia theobromae* with the highest frequency of 60%. This was followed by *Colletotrichum musae* with a frequency of 20%. *Fusarium moniliforme* followed with a frequency of 15% and *Fusarium oxysporum* had a frequency of 5%.

Out of the twenty bananas obtained from Adum, Kumasi, also had *Botryodiplodia theobromae* with the highest frequency of isolation (55%). This was followed by *Fusarium moniliforme* with a frequency of 20%. *Colletotrichum musae* followed with a frequency of 15% and *Fusarium oxysporum* had a frequency of 10%. Finally from the twenty bananas obtained from Nkawie, the most occurring fungus isolated was *Botryodiplodia theobromae* which had a frequency of 45%. This was followed by *Fusarium oxysporum* with a frequency of 25%. *Fusarium moniliforme* followed with a frequency of 20% and *Colletotrichum musae* had a frequency of 10%.

4.2 Parameters used during Identification and Characterization of Isolates

1. Growth characteristics of the fungal isolates on PDA are shown in figure 2. Diameter of growth showed that *Botryodiplodia theobromae* grew fastest followed by *Fusarium oxysporum, Fusarium moniliforme* and *Colletotrichum musae*.

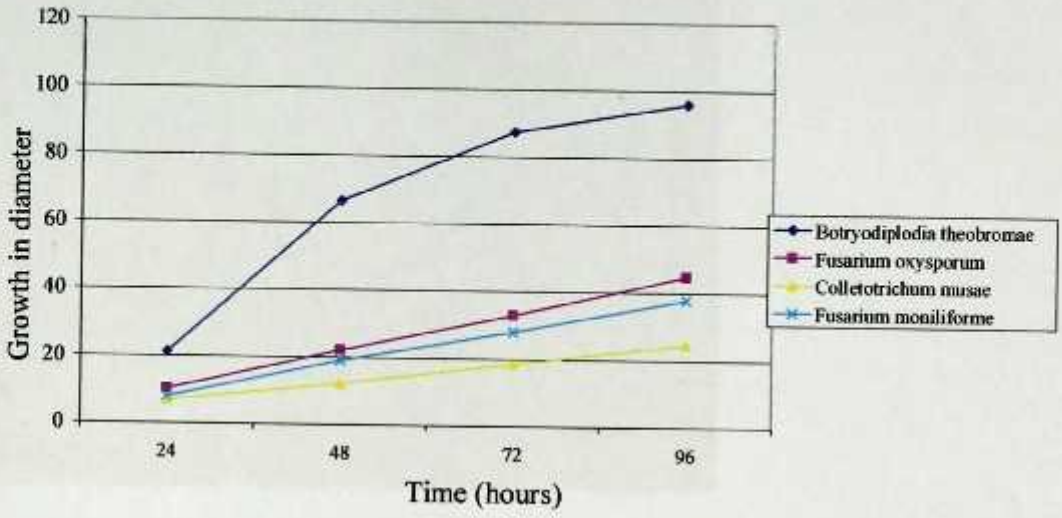


Figure 2: A graph of growth in diameter of fungal isolates against time.

2. Shape of conidia or spores of fungal isolates was the principal character depended on in identification as shown in plate 2a-2d for *Fusarium oxysporum*, for ***Fusarium moniliforme***, for *Botryodiplodia theobromae* and for *Collettotricum musae*.

Plate 2a: *Fusarium oxysporum* spores under the microscope (X 40)

Plate 2b: *Fusarium moniliforme* spores under the microscope (X 40)

Plate 2c: *Botryodiplodia theobromae* spores under the microscope (X 40)

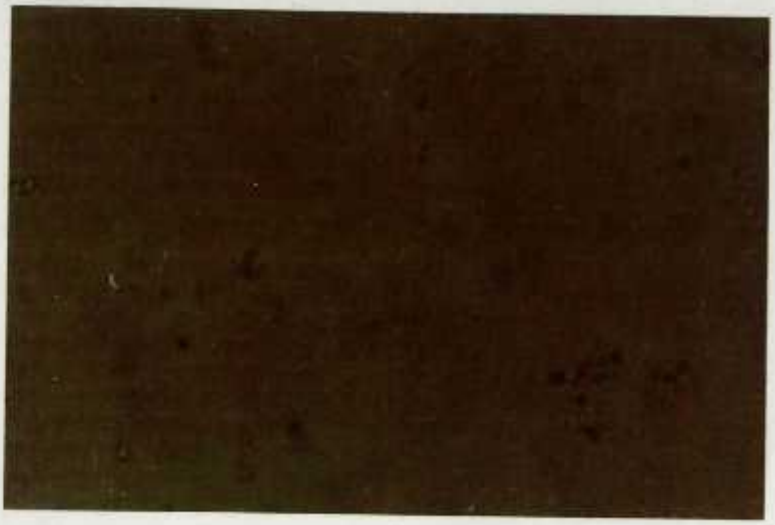


Plate 2d: *Colletotrichum musae* spores under the microscope (X 40)

3. Pigment production or colour of colony also helped in identification of isolates (See Plate 3a to3d.

Plate 3a. Mycelium colour of isolated fungi *Fusarium oxysporum*

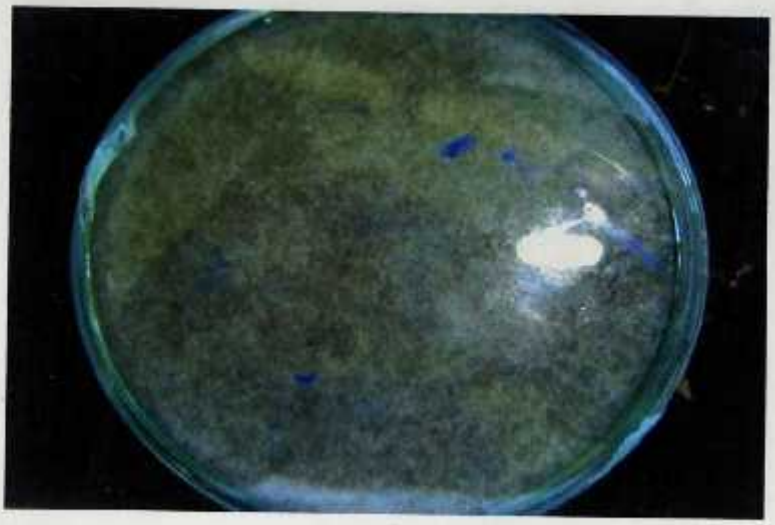


Plate 3b. Mycelium colour of isolated fungi *Botryodiplodia theobromae*

Plate 3c. Mycelium colour of isolated fungi *Fusarium moniliforme*

Plate 3d. Mycelium colour of isolated fungi *Colletotrichum musae*

4.3 Result from Pathogenicity Test

Pathogenicity test showing the mean crown rot disease when bananas was inoculated with singly or in combination of two or more fungal species (See Table 6). Combination of all four fungal isolates gave the highest score of 8, while the individual fungal isolates gave the lowest score of 4 for the crown rot ratings.

Table 6: Results from the pathogenicity test showing mean crown rot ratings

Inoculum	Mean crown rot rating
Botryodiplodia theobromae	4.00
Colletotrichum musae	4.00
Fusarium oxysporum	4.00
Fusarium moniliforme	4.00
Botryodiplodia theobromae + Colletotrichum musae	4.00
Botryodiplodia theobromae + Fusarium oxysporum	4.00
Botryodiplodia theobromae + Fusarium moniliforme	5.00
Colletotrichum musae + Fusarium oxysporum	4.00
Colletotrichum musae + Fusarium moniliforme	4.00
Fusarium oxysporum + Fusarium moniliforme	4.00
B. theobromae + C. musae + F. moniliforme	5.00
B. theobromae + C. musae + F. oxysporum	6.00
C. musae + F. oxysporum + F. moniliforme	4.00
C. musae + F. oxysporum +F. moniliforme + B. theobromae	8.00
B. theobromae + F. oxysporum + F. moniliforme	4.00
Control (Sterile distilled water)	1.00

4.4 Bioassay results

Results from bioassay study of *Garcinia kola*, *Ocimum gratissimum* and *Alstonia boonei* on *C. musae*, *F. oxysporum*, *F. moniliforme* and *B. theobromae* are shown in tables 7a to 7d and plate 4a to 4g. It shows the zones of inhibition created by the botanicals on the fungal isolates at different concentrations.

Table 7a shows the measured zones of inhibition created by the different botanical extracts at 100% concentration. At 100% concentration, the crude extract from *Alstonia boonei* did not inhibit the growth of any of the four fungal isolates. The extract from *Garcinia kola* produced zones of inhibition on three of the fungal isolates namely *Fusarium moniliforme*, *Colletotrichum musae* and *Fusarium*

oxysporum but did not inhibit growth of *Botryodiplodia theobromae*. *Ocimum gratissimum* was able to give zones of inhibition on all the four fungal isolates and it also gave the highest inhibition zones compared with the other plant extracts.

Table 7a: Diameter of zones of inhibition formed on the potatoes dextrose agar by plant extracts at 100% concentration.

Isolates	**Extracts (100 %)**	**Zones of inhibition diameter(mm)***
Botryodiplodia theobromae	*Alstonia boonei*	0
	Ocimum gratissimum	7.66
	Garcinia kola	0
Fusarium oxysporum	*Alstonia boonei*	0
	Ocimum gratissimum	7.55
	Garcinia kola	2.66
Colletotrichum musae	*Alstonia boonei*	0
	Ocimum gratissimum	10.10
	Garcinia kola	1
Fusarium moniliforme	*Alstonia boonei*	0
	Ocimum gratissimum	7.66
	Garcinia kola	4

* Average of triplicate reading from each plate

Table 7a above shows the measured zones of inhibition created by the different botanical extracts at 100% concentration. At 100% concentration, the crude extract from *Alstonia boonei* did not inhibit the growth of any of the four fungal isolates. The extract from *Garcinia kola* produced zones of inhibition on three of the fungal isolates namely *Fusarium moniliforme*, *Colletotrichum musae* and *Fusarium oxysporum* but did not inhibit growth of *Botryodiplodia theobromae*. *Ocimum gratissimum* was able to give zones of inhibition on all the four fungal isolates and it also gave the highest inhibition zones compared with the other plant extracts.

Table 7b: Diameter of zones of inhibition formed on the potatoes dextrose agar by plant extracts at 75% concentration

Isolates	**Extracts (75 %)**	**Zones of inhibition diameter(mm) ***
Botryodiplodia theobromae	*Alstonia boonei*	0
	Ocimum gratissimum	7.00
	Garcinia kola	0
Fusarium oxysporum	*Alstonia boonei*	0
	Ocimum gratissimum	7.30
	Garcinia kola	3.03
Colletotrichum musae	*Alstonia boonei*	0
	Ocimum gratissimum	8.33
	Garcinia kola	1.83
Fusarium moniliforme	*Alstonia boonei*	0
	Ocimum gratissimum	6.83
	Garcinia kola	5.38

* Average of triplicate reading from each plate

Table 7b shows that at 75% concentration, the extract from *Garcinia kola* produced zones of inhibition on *Fusarium moniliforme*, *Colletotrichum musae* and *Fusarium oxysporum* but did not produce any zones of inhibition against *Botryodiplodia theobromae*. *Alstonia boonei* did not inhibit the growth of any of the isolates. *Ocimum gratissimum* showed the highest activity against all isolates again at 75% concentration.

Table 7c: Diameter of zones of inhibition formed on the potatoes dextrose agar by plant extracts at 50% concentration.

Isolates	Extracts (50 %)	Zones of inhibition diameter(mm) *
Botryodiplodia theobromae	*Alstonia boonei*	0
	Ocimum gratissimum	0
	Garcinia kola	0
Fusarium oxysporum	*Alstonia boonei*	0
	Ocimum gratissimum	0
	Garcinia kola	0
Colletotrichum musae	*Alstonia boonei*	0
	Ocimum gratissimum	4.10
	Garcinia kola	0
Fusarium moniliforme	*Alstonia boonei*	0
	Ocimum gratissimum	0
	Garcinia kola	0

*Average of triplicate reading from each plate.

The only activity at 50% was achieved by with *Ocimum gratissimum* against *Colletotrichum musae* (Table 7c).

Table 7d: Diameter of zones of inhibition formed on the potatoes dextrose agar by plant extracts at 25% concentration

Isolates	Extracts (25 %)	Zones of inhibition diameter(mm) *
Botryodiplodia theobromae	*Alstonia boonei*	0
	Ocimum gratissimum	0
	Garcinia kola	0
Fusarium oxysporum	*Alstonia boonei*	0
	Ocimum gratissimum	0
	Garcinia kola	0
Colletotrichum musae	*Alstonia boonei*	0
	Ocimum gratissimum	0
	Garcinia kola	0
Fusarium moniliforme	*Alstonia boonei*	0
	Ocimum gratissimum	0
	Garcinia kola	0

*Average of triplicate reading from each plate

At 25 % concentration, none of the plant extracts was able to inhibit the growth of any of the fungal isolates (Table 7d)

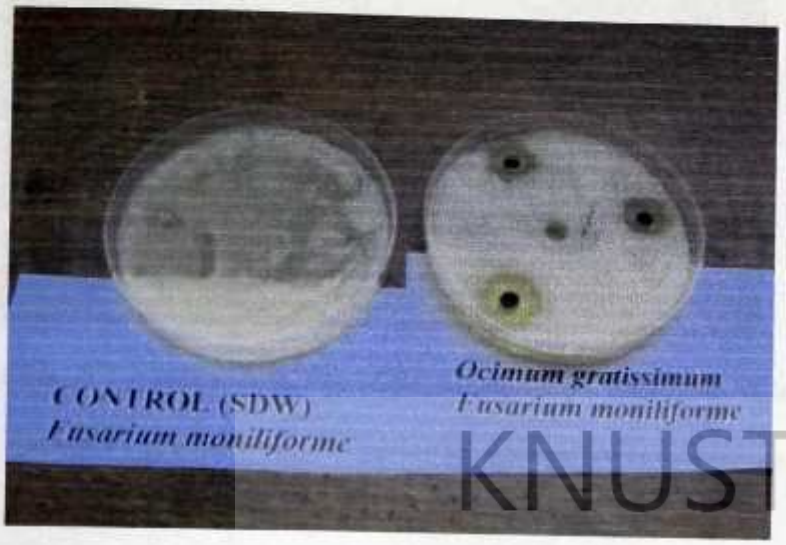


Plate 4a: A plate showing zones of inhibition caused by *Ocimum gratissimum* against *Fusarium moniliforme* on PDA compared to the control (sterile distilled water)on the left.

Plate 4b: A plate showing zones of inhibition caused by *Garcinia kola* on *Fusarium moniliforme* on PDA compared to a control (sterile distilled water) on the left.

Plate 4c: A plate showing zones of inhibition caused by *Garcinia kola* against *Fusarium oxysporum* on the PDA compared to the control (sterile distilled water) on the left.

Plate 4d: A plate showing zones of inhibition caused by *Ocimum gratissimum* on *Fusarium oxysporum* on a PDA compared to a control (sterile distilled water) on the left.

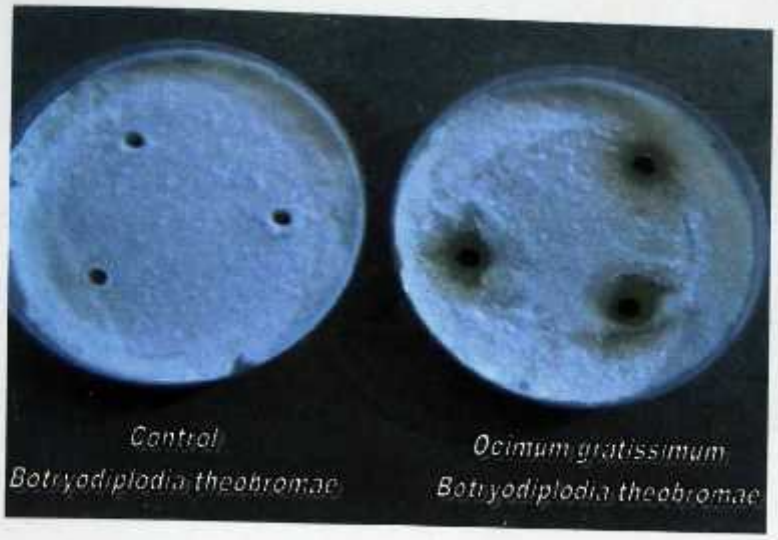


Plate 4e: Zones of inhibition caused by *Ocimum gratissimum* on *Botryodiplodia theobromae* on a PDA compared to the control (sterile distilled water) on the left.

Plate 4f: Zones of inhibition caused by *Ocimum gratissimum* on *Colletotrichum musae* on a PDA compared to a control (sterile distilled water) on the right.

Plate 4g: Zones of inhibition caused by *Garcinia kola* on *Colletotrichum musae* on a PDA compared to a control (sterile distilled water).

4.5 Effect of Treatments on Crown rot Development under Cold Room Conditions (Before Fruit Ripening).

After storage for two weeks in a cold room at 15 ^{0}C, bananas treated with extract from *Ocimum gratissimum* had an average crown rot rating of 3.00 on the Jones scale while bananas treated with the fungicides Tecto and Citrex had mean crown rot rating of 1.00 and 1.66 respectively. The bananas which were used as control (no treatment) also had a crown rot rating of 3.00 (See Figure 3 and Plate 6a to 6d).

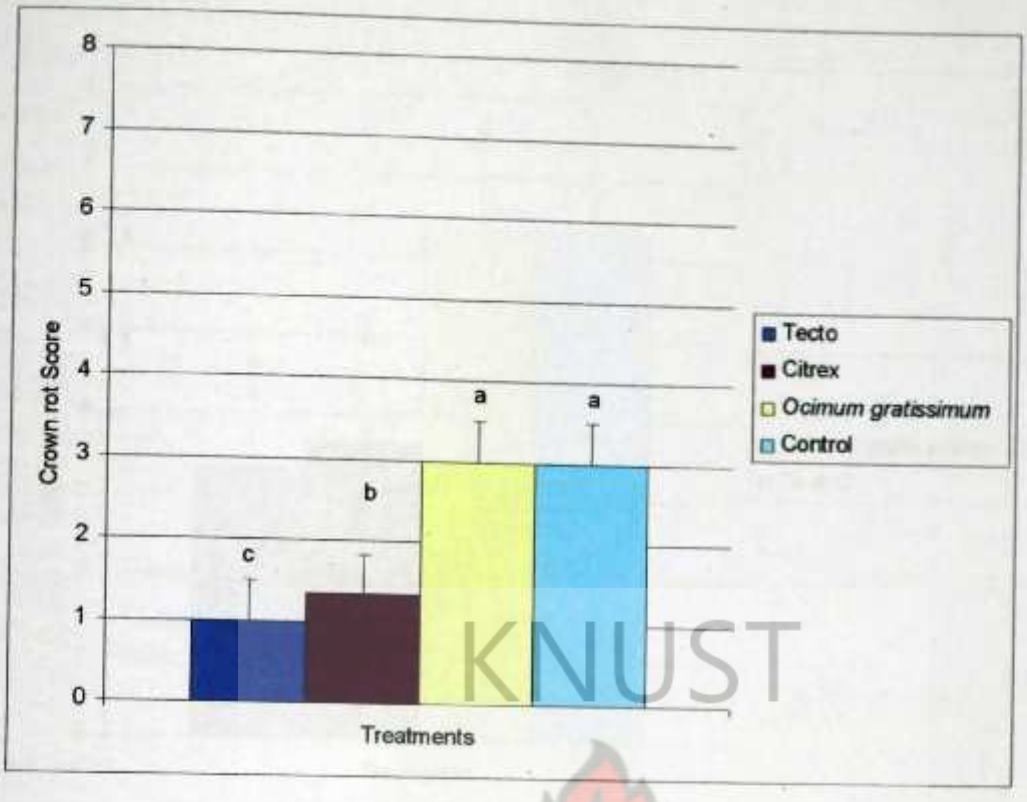


Figure 3: Crown rot scores of unripe bananas after cold room storage

4.6 Effect of Treatments on Bananas Crown Rot Development under Ambient Storage. (After Fruit had Ripen).

When the bananas were allowed to ripen at room temperature (after removal from cold storage room) a mean crown rot score of 6.33 was recorded using the Jones scale for the bananas treated with *Ocimum gratissimum*. Bananas treated with Tecto had a mean crown rot score of 3.33 while bananas treated with Citrex had a mean crown rot score of 3.67.The bananas which were used as control (without any treatment) gave a mean crown rot rating of 6.33 on the Jones scale (See Figure 4 and Plate 6e to 6h).

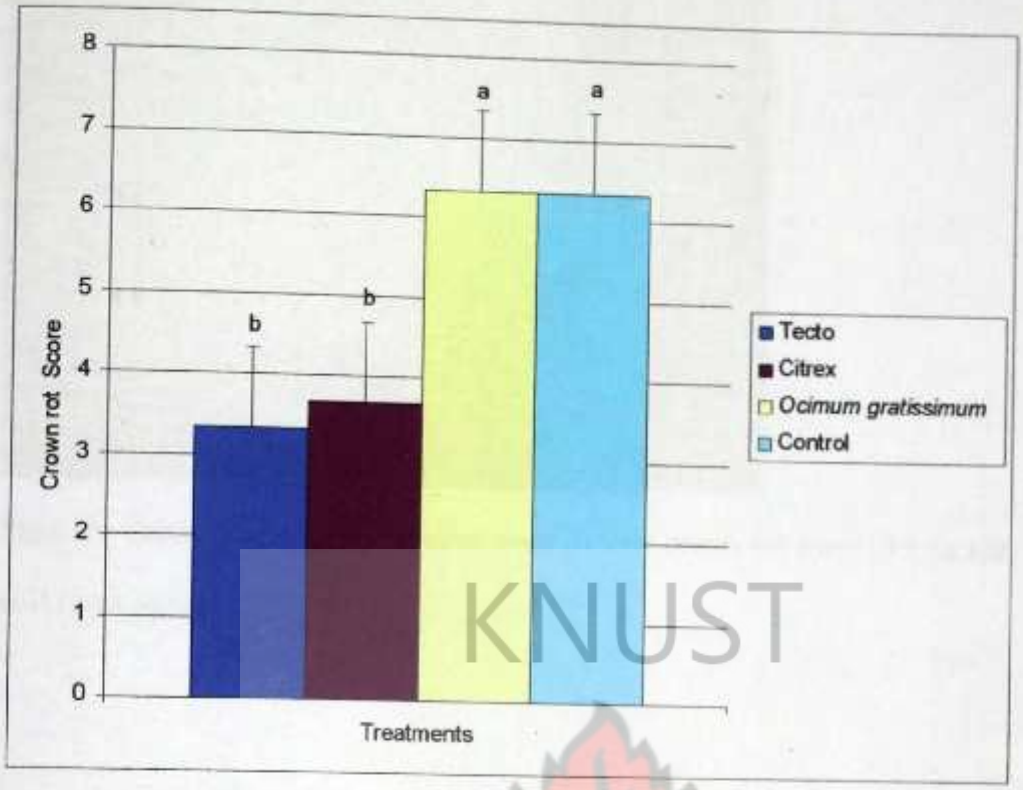


Figure 4: Crown rot scores at full ripening of bananas.

Plate 6a: Tecto treated banana (colour stage 1) with crown rot score of 1.00 after cold room storage.

Plate 6b: Citrex treated banana (colour stage 1) with crown rot score of 1.33 after cold room storage.

Plate 6c: *Ocimum gratissimum* treated banana (colour stage 1) with crown rot score of 3.00 after cold room storage.

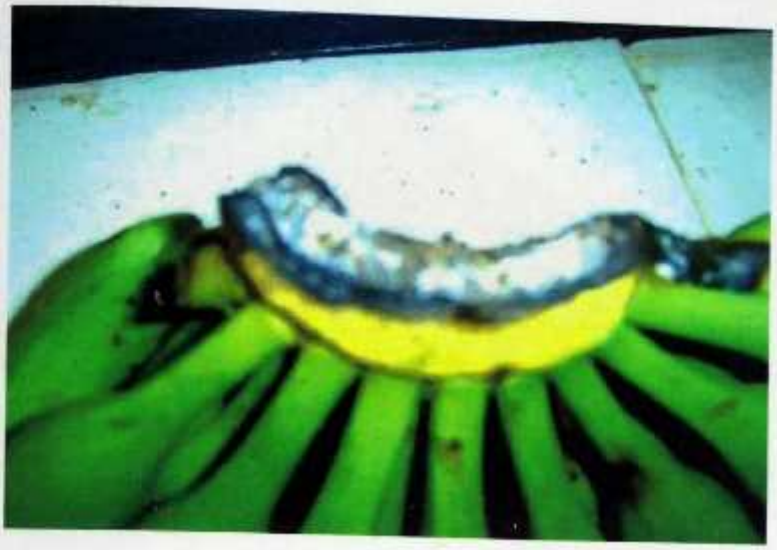


Plate 6d: Unripe banana (colour stage 1) without any treatment (control) with a crown rot score of 3.00 after cold room storage.

Plate 6e: Ripe banana which have been treated with Citrex with a crown rot score of 4.00

Plate 6f: Ripe banana treated with *Ocimum gratissimum* with a crown rot score of 7.00

Plate 6g: Ripe banana treated with Tecto with a crown rot score of 4.00

Plate 6h: Ripe banana without any treatment (control) with a crown rot score of 7.00

4.7 Effect of Treatments on Total Soluble Solids of Bananas

The mean total soluble solids of the treated banana showed an initial value of 1.00 ^{0}Brix for all the treatments. Total soluble solids started to increase as the bananas ripen for all the treatments reaching maximum at colour stage eight with a mean of 20.50 ^{0}Brix for the bananas treated with Citrax. The other treatments namely *Ocimum gratissimum*, Tecto and the control all gave a mean of 20.40 ^{0}Brix (See table 5).

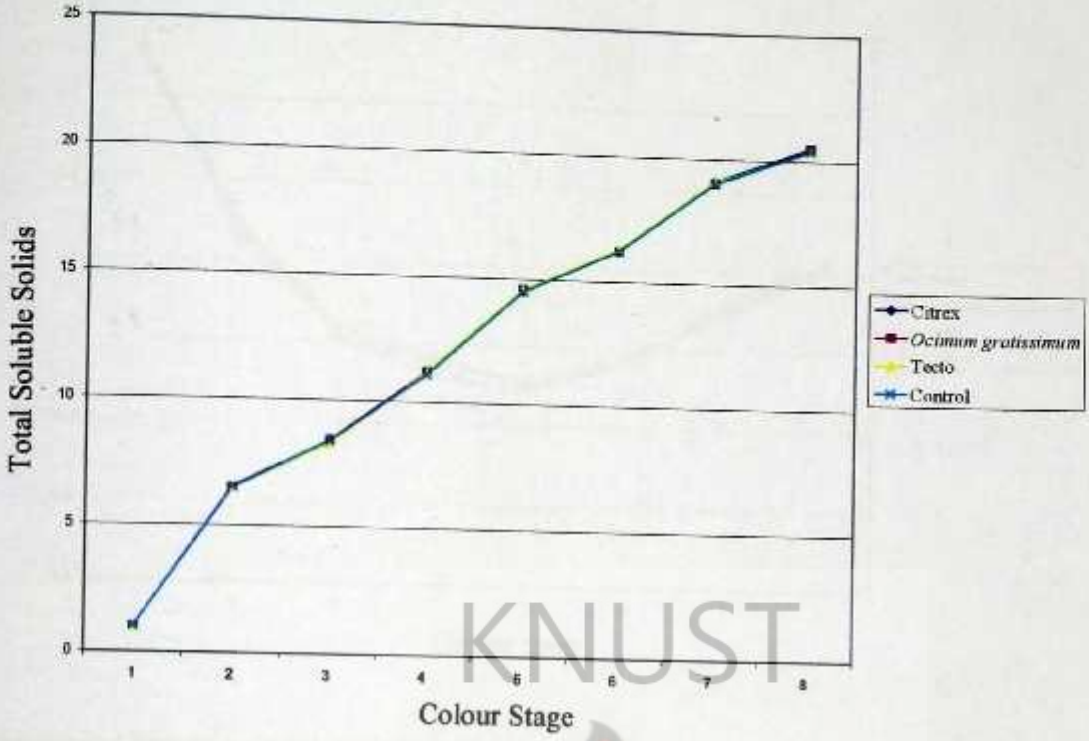


Figure 5: Total soluble solids in treated banana against Colour stage during ripening.

4.8 Effect of Treatments on Pulp pH of Bananas

Results from the study showed that Pulp pH of all the treatment showed initial high mean values of 5.73 for Citrex, 5.70 for Tecto, 5.71 for *Ocimum gratissimum* and 5.73 for the control at colour stage 1 (figure 6). Pulp pH for the various treatments started to decrease as ripening of bananas progressed reaching their lowest values of 4.95 for bananas treated with Citrex, 4.97 for bananas treated with Tecto, 4.94 for bananas treated with *Ocimum gratissimum* and 4.92 for the control (without any treatment) at colour stage 4. Pulp pH values started to increase after colour stage 4 reaching high values of 5.22 for bananas treated with Citrex, 5.23 for bananas treated with Tecto, 5.22 for bananas treated with *Ocimum gratissimum* and 5.20 for Control at colour stage 8.

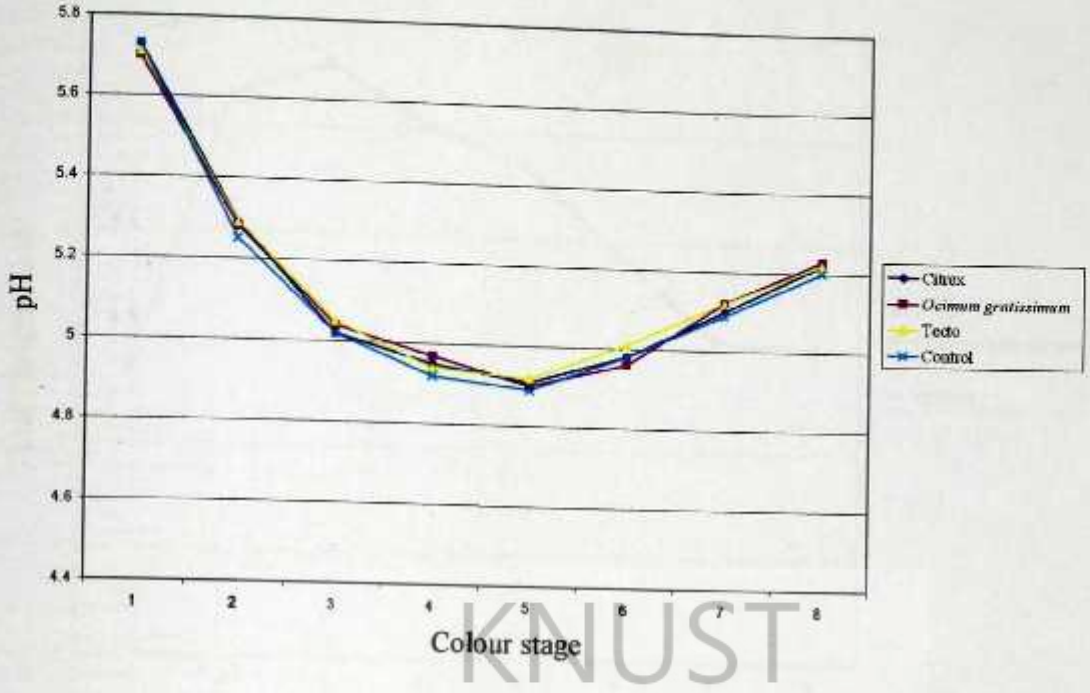


Figure 6: Changes pulp pH during ripening of treated bananas

4.9 Effect of Treatments on Total Titratable Acidity of Bananas

Results in (Figure 7) show an initial mean low total titratable acidity of 2.72 for Citrex, 2.70 for Tecto, 2.71 for Ocimum gratissimum and 2.70 for Control at colour stage 1. Total Titratable Acidity was highest at colour stage 3 with values of 5.61 for bananas treated with Citrex, 5.63 for Tecto, 5.60 for Ocimum gratissimum and 5.61 for Control. After colour stage 3, total titratable acidity started to decrease reaching low values of 3.00 for Citrex, 3.01 for Tecto, 2.99 for *Ocimum gratissimum* and 2.97 for Control.

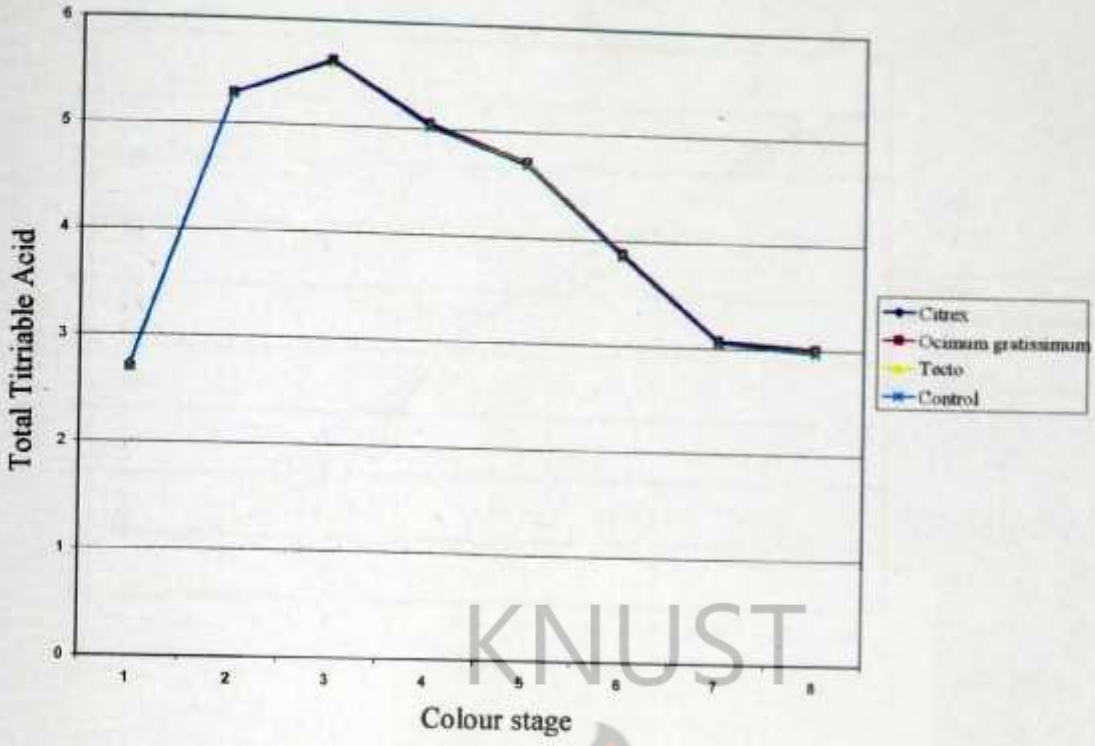


Figure 7: Total titriable acid in treated bananas during ripening.

4.10 Effect of Treatments on Ripening of Peel of Bananas

Results from the study showed that ripening of the treated bananas started on the third day (Figure 8). By the ninth day, colour development of the bananas was at colour stage 6. On the twelfth day of the study ripening of all the bananas were at colour stage 8.

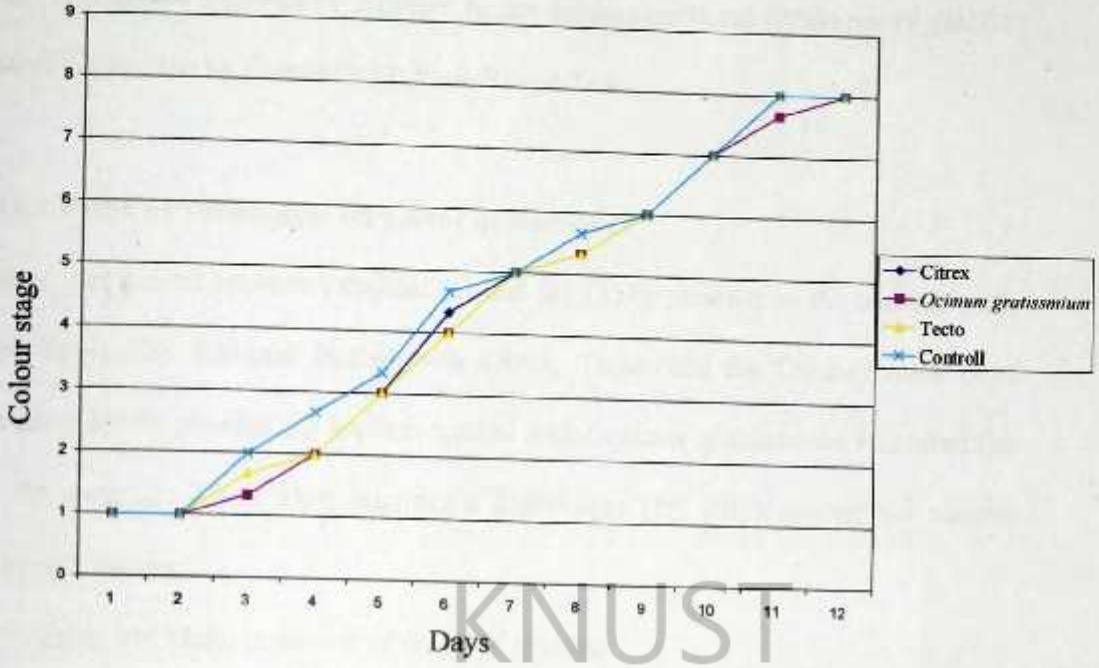


Figure 8: Changes in peel colour during ripening of treated bananas

4.11 Sensory Evaluation of Treated Bananas

4.11.1 Effect of Treatments on Taste of Bananas

Table 8a shows the data from bananas treatment with various botanicals and chemicals. Panelists for sensory analysis indicated that the treated bananas tasted excellent (5) on the hedonic scale. There were no significant differences ($P \leq 0.05$) among the various treatment means.

Table 8a: Mean treatment of taste of bananas

Treatment	Mean taste
Citrex	5.00a
Ocimum gratissimum	5.00a
Tecto	5.00a
Control	5.00a

CV 0.0%

Any two means followed by different letters shows significant difference (P≤ 0.05). Mean separation by Duncan's Multiple Range Test.

4.11.2 Effect of Treatments on Flavor of Bananas

Flavor was scored between excellent (5) and fair (2) by panelist on the hedonic scale (See Table 8b). Bananas treated with Citrex, Tecto, and the Control were rated excellent by the panelist but bananas treated with *Ocimum gratissimum* was rated fair by the panelist. There were significant differences (P≤ 0.05) among the various treatment means.

Table 8b: Mean treatment of flavor of bananas

Treatment	Mean Flavor
Citrex	5.00a
Control	5.00a
Tecto	5.00a
Ocimum gratissimum	2.58b

CV 15.3%

Any two means followed by different letters shows significant difference (P≤ 0.05). Mean separation by Duncan's Multiple Range.

4.11.3 Effect of Treatments on Texture of Bananas

Sensory analysis by panelist indicated that the Texture of the bananas were firm (4) on the hedonic scale. There were no significant differences (P 0.05) among the various treatment means (See Table 8c).

Table 8c: Mean treatment of Texture of bananas

Treatment	Mean Pulp texture
Citrax	4a
Ocimum gratissimum	4a
Tecto	4a
Control	4a

CV 0.0%

Any two means followed by different letters shows significant difference (P≤ 0.05).

Mean separation by Duncan's Multiple Range Test.

4.11.4 Effect of Treatments on Peel Colour of Banana

Peel colour of the bananas were scored between excellent (5) and fair (2) by the panelist used for the sensory analysis (Table 8d) on the hedonic scale. There were significant differences (P≤ 0.05) among the various treatment means.

Table 8d: Mean treatment of peel colour of bananas

Treatment	Mean Colour
Citrax	5.00a
Control	5.00a
Tecto	5.00a
Ocimum gratissimum	2.53b

CV 17.3%

Any two means followed by different letters shows significant difference (P≤ 0.05).

Mean separation by Duncan's Multiple Range Test.

4.11.5 Effect of Treatments on Overall Acceptability of Bananas

Panelist for the sensory analysis indicated that the overall acceptability of the bananas were between excellent (5) and fair (2) on the hedonic scale. There was significant differences ($P \leq 0.05$) among the various treatment means. Bananas treated with ocimum gratissimum were the least acceptable (Table 8e).

Table 8e: Mean treatment of overall acceptability of bananas

Treatment	Mean Overall acceptability
Citrex	5.00a
Control	5.00a
Tecto	5.00a
Ocimum gratissimum	2.14b

CV 4.2%

Any two means followed by different letters shows significant difference ($P \leq 0.05$).

Mean separation by Duncan's Multiple Range Test.

5.0 DISCUSSION

5.1 Isolated Fungi and Pathogenicity Test

Fungi isolated from diseased bananas were *Botryodiplodia theobromae, Fusarium moniliforme, Fusarium oxysporum* and *Colletotrichum musae*. Among the four fungal organisms *Botryodiplodia theobromae* was the most frequently isolated followed by *Colletotrichum musae* and *Fusarium moniliforme* respectively with *Fusarium oxysporum* having the smallest frequency of isolation. It can be concluded that *Botryodiplodia theobromae* is the prevalent fungus while *Fusarium oxysporum* is the least prevalent fungus that causes crown rot disease in the localities (Adum, Bompata, Nkawie and Bomso) that the bananas were obtained

Pathogenicity of the isolates was confirmed by inoculating pure spore suspension of the isolated fungi into the crown part of the banana fruit. These produced typical crown rot symptoms on the banana fruit. Even though it has been reported by Stover and Simmonds (1987) that the disease is a complex one, isolated fungal pathogens were able to each cause crown rot disease alone. Disease severity was, however, low when fungal isolates were inoculated individually. Disease severity increased when fungal isolates were inoculated in two or more species combinations respectively. Disease severity was highest when all the four isolated fungi were combined, as this produced extensive decay in the inoculated bananas (see Table 6).

5.2 Antifungal Activity of Botanical Extracts

5.2.1 Effect of *Garcinia kola, Ocimum gratissimum* and *Alstonia boonei* on Fungal Isolates

Crude extract from *Garcinia kola* was able to produce zones of inhibition against three of the four test isolates namely, *Fusarium moniliforme, Fusarium oxysporum* and *Colletotrichum musae* but it did not show any fungicidal activity against *Botryodiplodia theobromae.* The crude extract showed fungicidal activity at 100% and 75% concentration but at 50% and 25% concentration, the crude extract did not show any fungicidal activity against any of the test isolates (See Table 7a to 7d and plates 5b, 5c and 5g). It is possible that at 100% and 75% concentration, the active ingredient applied was high enough to inhibit growth of isolates. This collaborate the work of Gbedema (2002) that crude extract from *Garcinia kola* has fungicidal activity against *Candida albicans.* At 50% and 25% concentration, the amount of active ingredient in the extract was reduced such that it could not have any activity effect on the fungal isolates.

Ocimum gratissimum showed consistent fungicidal activity against all the four test isolates by producing zones of inhibition against them in the study (See Table 7a to 7d and Plates 5a, 5d, 5e and 5f. Crude extract of *Ocimum gratissimum* at concentrations of 100% and 75% inhibited growth of all the four fungal isolates (*B. theobromae, C. musae, F. oxysporum and F. moniliforme*). The amounts of active ingredient in the crude extract at these concentrations were sufficiently high enough to inhibit growth of the fungal organisms. However at 50% concentration, the amount of active ingredient in the crude extract was able to inhibit growth of *Colletotrichum musae* while at 25% concentration the crude extract was not able to

inhibit the growth of any of the fungal isolates. This collaborates work by Okigbo, and Ogbonnaya, (2006) who found out that extracts from *Ocimum gratissimum* could control *Aspergillus niger, A. flavus, Fusarium oxsporium, Botryodiplodia theobromae* and *Penicillium chrysogenum.*

Alstonia boonei did not produce any fungicidal effect on fungal isolate.

5.3 Effect of Treatments on Crown Rot Disease Control

In all, four treatments were tested for their potency to control crown rot disease. Results from the study showed that none of the treatments was able to completely control the crown rot disease.

Tecto and Citrex were able to limit the development of crown rot disease during storage at 15 ^{0}C and 95% relative humidity for two weeks. Bananas treated with Tecto and Citrex recorded a mean disease score of 1.00 and 1.33 on the Jones scale during storage. The temperature at 15^{0}C was far below the optimum for growth of the fungal isolates and that may account for the low crown rot severity at that temperature. However, when the bananas were allowed to ripen under room conditions with an average temperature of 28.4 ^{0}C and 68.95% relative humidity respectively, without ethylene applications, disease severity increased to 3.33 and 3.67 on the Jones scale. The increase in crown rot disease development during ripening under natural conditions could be attributed to increase in temperature which might have increased the rate of development of fungal isolates and low concentrations of chemicals used or the chemical effect not persistant enough to prevent growth of organisms over a long period of time.

Ocimum gratissimum could not control the crown rot disease. Bananas treated with *Ocimum gratissimum* recorded disease mean scores of 3.00 on the Jones scale during two weeks storage at 15 ^{0}C and 95% relative humidity and 6.33 when the bananas were allowed to ripen naturally. Explanation for this occurrence is in the diverse characteristic stability in the compound found in the crude extract (Awuah, 2006) as well as the diffusive effect of the active substance into the wound created. Awuah, (2006) reported that the active compounds in *ocimum gratissimum* is not reliably stable over long periods of application.

Bananas which were not given any treatment and were used as control also had crown rot disease score of 3.00 during two weeks storage at 15 ^{0}C and 95% relative humidity and 6.33 after storage. This happened because no treatment was applied to the bananas and this led to fungi pathogens having their way without any check. Comparatively, Tecto proved to be the most effective antifungal product in limiting the development of crown rot disease in this study. Significant differences ($P \leq 0.05$) exited among the treatments (See Appendix).

5.4 Effect of Treatments on Total Soluble Solids (Brix) of Bananas

The presence of sugars in the banana fruit is due to conversion of starch into reducing sugars during ripening of the banana fruit. Dadzie and Orchard, (1997) working with Cavendish Banana, reported that total soluble solids start increasing from colour stage two as banana start ripening and might decline after colour stage six due to the conversion of sugars to alcohol or there will not be a decline in total soluble solids up to colour stage eight. In this study, total soluble solids(in all treatments) started increasing after colour stage one peaking at colour stage eight

with no decline. It can therefore be said that the various treatments did not have any effect on the total soluble solids of the bananas during ripening. No significant differences existed among means of treatments ($P \leq 0.05$).

5.5 Effect of Treatments on Total Titrable Acidity of Bananas

Total titrable acidity refers to the concentration of free hydrogen ions in the fruit. Acidity measured as titrable acidity in the pulp tissues of most banana cultivars shows a large increase as ripening starts. For example malic acid content rises from 1.8 to 6.2 meq/100 g fresh weights during ripening (Satyan and Parwardhan, 1984), However, Wills *et al.*, (1989), observed that loss of acidity during ripening is often rapid. In this study, titrable acidity in all the treatments rose, peaked at colour stage 2 and then declined. No significant differences were observed among treatments ($P \leq 0.05$).

5.6 Effect of Treatments on Pulp pH of Bananas

Pulp pH is used to represent the amount of acidity or alkalinity in the banana. The results showed an increase in pH towards acidity as ripening progress as observed by Dadzie and Orchard (1997) who reported that in most bananas, there is an increase in pulp pH as fruits ripen. There was a decrease in pH acidity after colour stage 5. It might be due to organic acids being respired or converted to sugars as ripening ends. This confirms Wills *et al.*, (1989), report that organic acids are respired or converted to sugars as ripening nears completion. This leads to a decrease in pH acidity as ripening nears its peak. Results from the study showed that Pulp pH of the fruits were not affected by the various treatments as there were no significant differences ($P \leq 0.05$) among treatment means (see figure 6).

5.7 Effect of Treatments, Relative humidity and Temperature on ripening of Bananas

During the study, bananas treated with *ocimum gratissimum*, Tecto, Citrex and the control were allowed to ripen under room conditions without the application of ethylene. With mean temperatures below 35 ^{0}C and a mean relative humidity of 72.1%, ripening of the bananas were not blocked as observed by Dadzie and Orchard, (1997) who reported that ripening of bananas is effectively blocked when temperature reaches 48 ^{0}C. No significant differences ($P \leq 0.05$) existed among the treatment means.

5.8 Sensory Evaluation of Bananas

5.8.1 Effect of Treatment on Taste

Taste is due to sensations felt on the tongue (Tan, 2000). During the study, panelists indicated that the taste of the treated bananas was excellent (score 5) for the various treatments used. This indicated that treatments did not diffuse into the pulp of the bananas and so did not affect the taste of the bananas. No significant differences ($P \leq 0.05$) existed among the treatment means.

5.8.2 Effect of Treatment on Flavour

Ripening bananas are known to emit aroma which is caused by volatile compounds like amyl esters, butyl esters, aldehydes and ketones (Thompson and Burden 1995). Aroma is normally used to represent flavour (Tan, 2000). Panelists indicated excellent (score 5) for bananas treated with Citrex, Tecto and the control but for the bananas treated with *Ocimum gratissimum*, panelists indicated poor (score 2) due to the strong smell which is usually found in the leaves of the *Ocimum gratissimum* as

noted by Kokware (1976). The strong scent from the *Ocimum gratissimum* leaves suppressed the aromatic flavour usually associated with ripe bananas. Significant differences ($P \leq 0.05$) existed among the treatment (See Appendix).

5.8.3 Effect of Treatments on Texture (Pulp firmness)

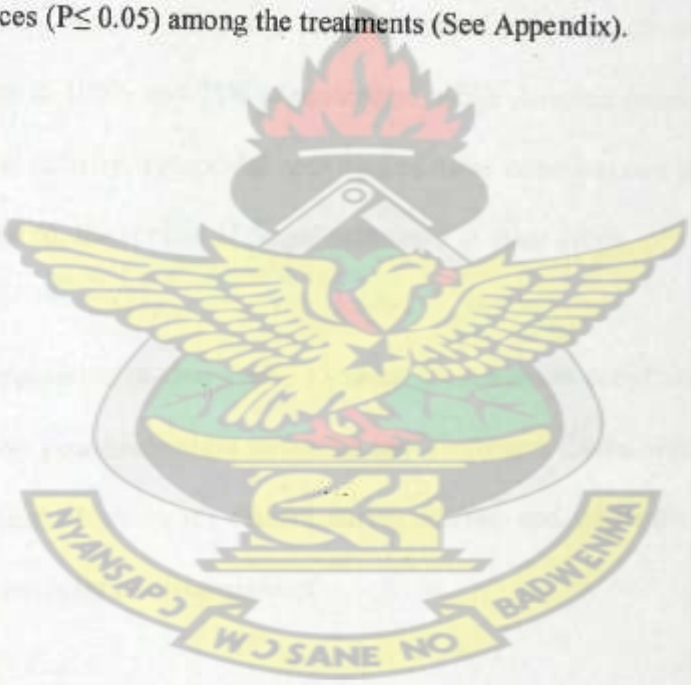
Texture is used to represent the extent of softness of the banana pulp. The texture of banana is a composite attribute resulting from a combination of several factors such as water turgor and structural components of tissues and cells. Dadzie and Ochard (1997) reported that the normally firm pulp at harvest becomes tender and soft at the eating stage. When the treated bananas were exposed to room conditions (without ethylene application) for them to ripen, panelists rated all the treated bananas as firm (score 4 on the Jones scale). This was due to the fact that temperatures which pertained at ripening in the room did not exceed 35 ^{0}C. Dadzie and Orchard (1997) reported that the texture of ripening bananas is affected when bananas are exposed to temperatures above 35 ^{0}C. The treatment did not affect pulp firmness.

5.8.4 Effect of Treatments on Peel Colour of Bananas

During the study, panelists indicated that the peel colour of the treated bananas were excellent (score 5) for those treated with Tecto, Citrex and the Control. Panelists indicated poor (score 2) for bananas treated with *Ocimum gratissimum* because the green pigment in the leaves of *Ocimum gratissimum* stained the golden yellow colour of the ripe banana making it unattractive. Significant differences ($P \leq 0.05$) existed among the treatment means. *Ocimum gratissimum* is therefore not a good product to be considered for the control of crown rot if even it was effective.

5.8.5 Effect of Treatments on Overall Acceptability

The overall acceptability was based on the combination of the following factors; texture, taste, flavour, and peel colour (appearance). During the study, panelists indicated excellent (score 5) for bananas treated with Tecto, Citrex and the Control but for bananas treated with *Ocimum gratissimum*, panelists indicated poor (score 2). The poor flavor and the poor peel colour of the banana fruits treated with *Ocimum gratissimum* were responsible for the poor acceptance. These were due to the strong smell associated with the leaves of *Ocimum gratissimum* and poor peel colour due to the green pigment in the leaves of *Ocimum gratissimum* which stained the golden yellow colour of the banana peel thus making the bananas unattractive. There were significant differences ($P \leq 0.05$) among the treatments (See Appendix).



6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

In this study, the following fungal organisms: *Colletotrichum musae, Botryodiplodia theobromae, Fusarium moniliforme and Fusarium oxysporum* were isolated from banana fruits with infected crowns from four different localities in the Ashanti region. Pathogenicity test with the isolated fungi revealed that each fungus was able to cause crown rot disease when inoculated singly into the crown or when combined inoculations were carried out.

The study also showed that *Ocimum gratissimum* has fungicidal properties as it was able to inhibit growth of all the fungal isolates used in the experiment at 100% and 75% concentration in bioassay. *Garcinia kola* showed fungicidal activity against three test organisms at 100% and 75% concentration while *Alstonia boonei* did not show any fungicidal activity. Fungicidal activities of these botanical can be studied further and exploited for the control of fungal pathogens in other crops.

However, in a comparative *in-vivo* study, *Ocimum gratissimum* could not control crown rot disease by post-inoculation treatment but Tecto and Citrex were able to limit the development of crown rot disease during storage and when the bananas were allowed to ripen under room conditions.

Fruit qualities which were assessed in terms of Total titratable acidity, pH, Total soluble solids and peel colour were not affected by the various treatments applied. In an evaluation test, volunteers rated bananas treated with Tecto and Citrex as excellent but rated bananas treated with *Ocimum gratissimum* poor since *Ocimum gratissimum* affected flavour and peel colour of the bananas treated with it.

It can be concluded that the four fungal isolates *Fusarium moniliforme,fusarium oxysporium, Colletotrichum musae,* and *Botryodiplodia theobromae,* can cause crown rot disease of banana when inoculated singly or in combinations. *Ocimum gratissimum* and *Garcinia kola* have fungicidal properties because they were able to inhibit the growth of fungal isolates during bioassay study.

Fruit quality assessed in terms of pH, Total titrable acid, Total soluble acids and Peel colour were not affected by treatments used.

However, in an evaluation test, Tecto and Citrex treated bananas were accepted but bananas treated with Ocimum gratissimum were rated poor because treatment affected flavor and peel colour adversely.

Based on the above conclusion, the following recommendations are, therefore, made;

a) Though *Ocimumm gratissimum* and *Garcinia kola* did not control crown rot successfully, the fungicidal properties of the two botanicals can be studied further and stability of active components increased. It is possible that the fungicidal activities of the two botanicals can be exploited against pathogens of other plants.

b) Other modes of application, like dipping, should be tried in other research to see if extracts from *Ocimum gratissimum* could control the crown rot disease.

c) Other fungi toxic medicinal plants should be tried in further research to determine if they could control the crown rot diseases.

REFERNCES

Adesinia, S. L. (1982). Studies on some plants used as anticonvulsant in American and African traditional medicine.fitoterapi 1982 S/G 147-162.

Adu-Tutu, M., Afful Y, Asante-Appiah K, Lieberman D, Hall J. B, and Elvin-Lewis M.(1979). Chewing stick usagein Southern Ghana, Economic Botany, **33**, 320-328

Amissah, S. (2005). Technician. Botanical Garden, Kwame Nkrumah University of Science and Technology.

Andrews, M. J. (2001). Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy. **48,** Suppl. SI, 5-16

Anonymous (1999). Pesticides made with Essential Oil. http://www.beyondpesticides.org/infoservices/pesticidefactsheet/least toxic/essential.htm

Anonymous (2005). Natural Resources Defense Council: Health Hazards Of Pesticides. http://www.nrdc.org.com/health/kids/farm/chap1.aspect,

Awuah, R.T. (2006). Personal communication. Plant pathologist. Kwame Nkrumah University of Science and Technology.

Brady, C. J. (1987). Fruit ripening. Annu. Rev. Plant Physiol. **38**:155-178.

Broughton, W. J. and Wu, K. F. (1997). Storage conditions and ripening of two cultivar of banana. Scientia Hortic. **10**: 83-93.

CGIAR (1992). Future priorities and Strategies. Consultative Group on International Agriculture Researeh. Technical Advisory Committee. TAC Secretariat FAO,Rome, Italy.

CSIRO (1972). Banana ripening guide. Commonwealth Scientific and Industrial Research Organisation, Melbourne. Division of Food Research. Circular 8.

Dadzie, K. and Orchard, J.E. (1997). Routine postharvest screening of Banana/Plantain hybrids: Criteria and methods. INIBAP Technical Guidelines 2.

Dadzie, B. K. (1994). Six monthly reports for the INIBAP/FHIA/NRI (ODA Holdback) project on post-harvest cooking banana and plantain characterization (April – September, 1994).

Dsanayaka, F. (1981). *A revised handbook of flora of Ceylon*. Vol. 3. American publication Co. Ltd. New Delhi. Pp 100-103.

Dufour, R. (2001) Biointensive Integrated Pest Management (IPM Fundamentals of Sustainable Agriculture .http://www.attra.org/attra-pub/PDF/ipm.pdf.

FAO (1971). The World Banana Economy: Structure, Performance and Prospect. Commodity Bulletin Series No. 50, Rome Pp 1.

FAO (1986). Some medicinal forest plants of Africa and Latin America. Forestry paper **67**: 155-157.

FAO (1990). Pesticide Residues in Food. Plant Production and Protection Paper Report **102.** pp17.

FAO (1991). Postharvest and processing technologies of African staple food: A technical compendium. Agricultural Services Bulletin **89**, Pg 113 Rome.

FAO (1986). Some medicinal forest plants of Africa and Latin America. Forestry paper **67**: 155-157.

FAO (2000). Inter-Country Program for the development and Application of Integrated Pest Management in Vegetable growing. An Ecological Guide. www.communityipm.org.

Gbedema, S. Y. (2002). Antimicrobial properties of extracts of *Garcinia kola* stem wood and characterization of the isolated active Hydroxybiflavanoid constituent. Unpublished Msc thesis, Kwame Nkrumah University of Science

and Technology. Pp 39.

George, J.B., Marriott, J., Palmer, J.M. and Karikari, S.K. (1982) Sensitivity to water stress and ethylene to stored plantain fruits. *J. Exp. Bot.* **33**: 1194-1201

George, J. B. and Marriott, J. (1983). The effect of humidity in plantain ripening. Scientia Horticulturae **21**:37-43.

Ghana Export Promotion Council (2002). Report on Export performance.

Gonzalez, M. and Zenteno, M (1988). Action of the fungicide Tecto-60 on the fungi of banana crown rot. Rev. Mex. Mic. **4**: 59-67.

Griffee, P.J. (1976) Pathogenicity of some fungi isolated from diseased crowns of banana hands. *Phythopathol.* **Z 85**: 206-216.

Green, G. L. and Goos, R. D. (1963). Fungi associated with crown rot of boxed bananas. Phythopathology. **53**: 271-216.

Inaba, A. and Nakamura, R. (1988) Numerical expression for estimating the minimum ethylene exposure time necessary to induce ripening in banana fruit. *J. Am. Soc. Hortic. Sci.* 561-564

Irvine, F. R. (1961) *Woody Plants of Ghana*, Oxford Uni. Press. Page 146.

Iwu M. M and Igboko, O. A (1982). Flavonoids of Garcinia kola seeds, *Journal of Natural products*, **45**, 5, Pp. 650-651

Jeger, M.J., Eden-Green, S., Thresh, J.M., Johanson, A., Waller, J.M. and Brown, A.E. (1995) Banana diseases. In: Gowen, S. ed *Bananas and Plantains*. 1st ed pp 362-363. Chapman and Hall, London.

John, P. and Marchal, J. (1995) Ripening and biochemistry of the fruit. In: *Bananas and Plantains* (ed. Gowen, S.) 1st ed. Chapman and Hall, London. pp 434-467.

Jones, D.R. (1991) Chemical control of crown rot in Queensland bananas. *Australian*

Journal of Experimental Agriculture **31**: 693-698.

Josylin, M. A. (1970). *Acidimetry. in Methods in Food Analysis* (M. A. Josylin, ed.). Academic Press, New York. Pp. 401-559

Kader, A. A. (1987). Respiration and gas exchange of vegetables. In ; *Post-harvest Physiology of Vegetables.* (ed. Weichmann, J). Marcel Dekker Inc., New York. Pp. 27-30.

Kader, A. A, Kasmire, R. F, Mitchell, F.G, Reid, M.S, Sommer, F. N, Thompson, J. (1985). *Postharvest Technology of Horticultural Crops.* The Regents of the University of California, Division of Agriculture and Natural Resources. Pp. 75-85.

Kokware, J. O. (1976). Medicinal Plants of East Africa. E. A. Literature Bureau. Nairobi.

Kwaa, O.M. (1995) Development of innovative techniques for the shelf-life extension of eggplant fruits. Unpublished M.Phil. Thesis. Dept. of Biochemistry, KNUST, Kumasi.

Loesecke, H. (1950) Banana. 2nd ed. Inter Science Publ, Inc, New York.

Lukezic, F. L., Kaiser, W. J. & Martinez, M. M. (1967). The incidence of crown rot of boxed bananas in relation to microbial populations of the crown. Canadian Jou. Bot. **45**: 413-421.

Lyon, G. D, Beblinski, T, Newton, A. C, (1995). Novel disease control compounds; the potential to immunize plants against infection. Plant pathology **44**: 407-27.

Manners, J. C. (1984) *Prineiples of Plant Pathology.* Cambridge University press. 2nd edition. Pp 229-243.

Marriot, J., Robinson, M and Karikari, S.K. (1981) Starch and sugar transformation During the ripening of plantains and bananas. *Jour. Sci. Food Agric.*

32:101-102.

Marthur, S.K. and Kongsdal, O (2001). Common *Laboratory Seed Health Testing Methods for Detecting Fungi*. Kandrups Bogtrykkeri. Denmark. Published by The International Seed Testing Association. Box 308, 8303 Bassersdolf. CH-Switzerland. Pp 164-255.

Medlicott, A. P., Semple, A. J., Thompson, A. J., Blackbourne, H. R. & Thompson, A. K. (1992). Measurement of colour changes in ripening bananas and mangoes by instrumental, chemical and visual assessments. Trop. Agric. (Trinidad) 69(2):161-166.

Meredith, D. S. (1965). Tip rot of banana fruits in Jamaica. *Verticillium theobromae* and *Fusarium* spp. Transactions of the British Mycological Society **48**:327-336.

Meredith, D. S. (1971). Transport and storage diseases of bananas: Biology and Control. Trop. Agric. (Trinidad) **48**(1):35-50.

Moses, E. (2005). Personal communication. Plant pathologist. Crops Research Institute,Ghana.

Ogawa, J. M. (1970). Post-harvest diseases of banana in China (Taiwan). FAO Plant Protection Bulletin **18**:31-42.

Okigbo, R. N. and Ogbonnaya, U. O. (2006) Antifungal effects of two tropical plant leaf extracts *(Ocimum gratissimum* and *Aframomum melegueta)* on postharvest yam (*Dioscorea* spp.) rot. African Journal of Biotechnology vol. 5 (9), pp. 727-731,

Owusu-Ansah, E. (1996). Biological activity, toxicological effects and bioactive chemical composition of an extract from the leaves of *Ocimum gratissimum*. Unpublished Msc thesis, Kwame Nkrumah University of Science and Technology. Pp 30.

Ploetz, R. C., Zentmyer, G. A., Nishijima, W. T., Rohrbach, K. G. and Ohr, H. D. (1994). Compendium of tropical fruit diseases. APS Press, USA. 88 pp.

Palmer, J. K. (1971). The banana. In; *the Biochemistry of Fruits and Their Products.* Vol.2 (ed. A.C. Hulme) Academic Press, London. Pp 65-105.

Prithiviraj B, and Singh U, (1995). Biological control of plant pathogens. Journal of Recent Advances in Applied Sciences **10**, 99-100.

Quarles W. (2006). Least-Toxic Controls of Plant Diseases. Integrated Pest Management. Bio-integral Resource Centre. www.bbg.org/gar2/topics/.../natural disease/leasttoxic.

Rieger, M. (2006). Banana and plantain – *Musa spp.* www.uga.edu/fruit/banana.

Rosenow S, Soltysiak U. and Verschuur G. (1996). *Organic farming(Sustainable Agriculture Put Into Practice.* Pp 8.

Satyan, S.H. and Parwardhan, M.V. (1984) Purification and regulatory properties of phosphoenolpyruvate carboxylase from banana fruits of 'Dwarf Cavendish'. *J. Food Sci. Technol.* **21**: 135-138

Smith, N.J.S., Tucker, G.A. and Jeger, J. (1989) Softening and cell wall changes in bananas and plantains. *Aspects of Applied Biology* **20**:57-65.

Snowdon, A. L. (1990). A colour atlas of post-harvest diseases and disorders of fruits and vegetables Vol. 1: General introduction and fruits. Wolfe Scientific Publications, London, UK. pp 302.

Sofowora, E. A. (1982). *Medicinal Plants and Traditional Medicine in Africa.* John Wiley and Sons Ltd, Toronto. Pp 71.

Sepiah, M and Nik-Mohd, N. A. M (1987). Effect of benomyl and prochloraz on postharvest diseases of banana. Asean Food journal **3**: 101-104.

Stover, R.H. and Simmonds, N.W. (1987) *Bananas. 3rd ed.* Longman, London.

Pp 301-304.

Tan, S. C. (2000). Determinants of eating quality in fruit and vegetables. Proccedings of the Nutrition Society of Austrilia **24**. pp183.

Thompson, A. K. and Burden, O. J. (1995). *Bananas and Plantains.* (ed. S. Gowen). Published by Chapman and Hall, 2-6 Boundary Row, London SE1 8HN. Pp 425.

Voorhoeve, A. G. (1965). Liberian high forest trees. Wageningen. Centrum voor land bouwpublikaties en land bouwdocunentate. Agric. Research report No. 652. pp 5.

Wainwright, H. & Hughes, P. (1989). Objective measurement of banana pulp colour. *International* Jou. Food Sci. Technol. **24**:553-558

Wainwright, H. & Hughes, P. (1990). Changes in banana pulp colour during ripening. Fruits **45**(1):25-28.

Wills, R. H. H., McGlasson, W. B., Graham, D., Lee, T. H. & Hall, E. G. (1989). *Postharvest: An Introduction to the Physiology and Handling of Fruit and Vegetables. 3rd edition. Blackwell Scientific Publications, Oxford, UK. pp176.*

Appendix

Frequencies of Isolation of fungi from bananas obtained from four areas

FUNGAL ISOLATE	FREQUENCY / PERCENTAGES			
	BOMSO	BOMPATA	ADUM	NKAWIE
Fusarium oxysporum	15	5	10	25
Colletotrichum musae	15	20	15	10
Botryodiplodia theobromae	50	60	55	45
Fusarium moniliforme	20	15	20	20
TOTAL	100	100	100	100

Growth rate of isolates on potatoes dextrose agar

Fungal isolate	**Diameter of growth rate (mm/hour)^{m}**			
	24hr	48hr	72hr	96hr
Botryodiplodia theobromae	21.25	66.50	87.5	96
Fusarium oxysporum	10.25	22.25	33.00	44.75
Colletotrichum musae	7.00	12.25	18.50	24.50
Fusarium moniliforme	8.00	19.00	28.00	37.75

Table of results for biochemical analysis

Mean Pulp Acidity

Treatments	Colour stage							
	1	2	3	4	5	6	7	8
Citrex	2.72	5.29	5.61	5.05	4.71	3.86	3.07	3.00
Ocimum gratissimum	2.70	5.30	5.63	5.06	4.72	3.88	3.08	3.01
Tecto	2.71	5.28	5.60	5.03	4.71	3.86	3.05	2.99
Control	2.70	5.28	5.61	5.02	4.69	3.85	3.05	2.97

Mean Total Soluble Solids

TreatmentS	Colour Stage							
	1	2	3	4	5	6	7	8
Citrex	1.00	6.50	8.30	11.20	14.50	16.20	19.10	20.50
Ocimum gratissimum	1.00	6.50	8.40	11.20	14.60	16.20	19.00	20.50
Tecto	1.00	6.50	8.30	11.20	14.60	16.30	19.10	20.40
Control	1.00	6.50	8.40	11.10	14.50	16.20	19.00	20.40

Mean pH

Treatments	Colour Stage							
	1	2	3	4	5	6	7	8
Citrex	5.73	5.29	5.02	4.95	4.91	4.98	5.10	5.22
Ocimum gratissimum	5.70	5.28	5.04	4.97	4.90	4.96	5.12	5.23
Tecto	5.71	5.29	5.05	4.94	4.92	5.01	5.12	5.22
Control	5.73	5.25	5.02	4.92	4.89	4.98	5.09	5.20

Table 10a: Mean treatment of taste of bananas

Treatment	Mean taste
Citrex	5.00a
Ocimum gratissimum	5.00a
Tecto	5.00a
Control	5.00a

CV 0.0%

Any two mean followed by different letters shows significant difference at ($P \leq 0.05$).

Mean separation by Duncan's Multiple Range.

Table 10b: Mean treatment of flavour of bananas

Treatment	Mean Flavour
Citrex	5.00a
Control	5.00a
Tecto	5.00a
Ocimum gratissimum	2.58b

CV 15.3%

Any two mean followed by different letters shows significant difference at ($P \leq 0.05$).

Mean separation by Duncan's Multiple Range.

Table 10c: Mean treatment of Texture of bananas

Treatment	Mean Pulp texture
Citrax	4a
Ocimum gratissimum	4a
Tecto	4a
Control	4a

CV 0.0%

Any two mean followed by different letters shows significant difference at ($P \leq 0.05$).

Mean separation by Duncan's Multiple Range.

Table 10d: Mean treatment of appearance of bananas

Treatment	Mean Colour
Citrax	5.00a
Control	5.00a
Tecto	5.00a
Ocimum gratissimum	2.53b

CV 17.3%

Any two mean followed by different letters shows significant difference at ($P \leq 0.05$)

Mean separation by Duncan's Multiple Range.

Table 10e: Mean treatment of overall acceptability of bananas

Treatment	Mean Overall acceptability
Citrex	5.00a
Control	5.00a
Tecto	5.00a
Ocimum gratissimum	2.14b

CV 4.2%

Any two mean followed by different letters shows significant difference at ($P \leq 0.05$). Mean separation by Duncan's Multiple Range.

Daily Mean temperature and Relative Humidity data taken during the period of the experiment.

Table 11: Temperature and Relative Humidity readings during the experiment

Day	Temperature	Relative Humidity
1	28.3	64.7
2	27.8	64.1
3	28.4	70.1
4	28.9	71.6
5	27.3	70.1
6	29.1	69.2
7	28.5	70.0
8	27.9	68.3
9	30.2	69.3
10	29.0	72.1
11	28.5	69.8
12	26.9	68.1

***** Analysis of Variance *****

Variate: Crown Rot Score before Ripening

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F. Cal	F. Probability
Treatment	3	9.00000	3.00000	36.00	<.001
Residual	8	0.66667	0.08333		
Total	11	9.66667			

Standard Errors of Differences of Means 0.2357

Co-efficient of Variation % 13.3

Lsd 0.5435

***** Analysis of Variance *****

Variate: Crown Rot Score after Ripening

Source of Variation	Degree of freedom	Sum of Squares	Mean Square	F. Cal	F. Probability
Treatment	3	24.2500	8.0833	24.25	<.001
Residual	8	2.6667	0.3333		
Total	11	26.9167			

Standard Errors of Differences of Means 0.471

Co-efficient of Variation % 11.7

Lsd 1.653

***** Analysis of variance *****

Variate: Colour

Source of Variation	Degree of freedom	Sum of Squares	Mean Square	F. Cal	F. Probability
Treatment	3	94.5000	31.5000	273.00	<.001
Residual	52	6.0000	0.1154		
Total	55	100.5000			

Standard Errors of Differences of Means 0.1284

Co-efficient of Variation % 8.0

***** Analysis of Variance *****

Variate: Flavour

Source of Variation	Degree of freedom	Sum of Squares	Mean Square	F. Cal	F. Probability
Treatment	3	68.8645	22.9548	53.97	<.001
Residual	52	22.1176	0.4253		
Total	55	90.9821			

Standard Errors of Differences of Means 0.0505

Co-efficient of Variation 15.3

***** Analysis of Variance *****

Variate: Taste

Source of Variation	Degree of freedom	Sum of Squares	Mean Square	F. Cal	F. Probability
Treatment	3	0	0	0	
Residual	52	0	0		
Total	55	0			

Standard Errors of Differences of Means 0.000

Co-efficient of Variation 0.0

***** Analysis of Variance *****

Variate: Texture

Source of Variation	Degree of freedom	Sum of Squares	Mean Square	F. Cal	F. Probability
Treatment	3	0	0	0	
Residual	52	0	0		
Total	55	0			

Standard Errors of Differences of Means 0.000

Co-efficient of Variation 0.0

***** Analysis of Variance *****

Variate: Overall Acceptability

Source of Variation	Degree of freedom	Sum of Squares	Mean Square	F. Cal	F. Probability
Treatment	3	85.71429	28.57143	866.67	<.001
Residual	52	1.71429	0.03297		
Total	55	87.42857			

Standard Errors of Differences of Means 0.0686

Co-efficient of Variation % 4.2

*****Analysis of Variance *****

Variate: Peel Colure

Source of Variation	Degree of freedom	Sum of Squares	Mean Square	F. Cal	F. Probability
Treatment	3	0.438	0.146	0.02	0.996
Residual	44	296.025	6.728		
Total	47	296.463			

Standard Errors of Differences of Means 1.059

Co-efficient of Variation % 59.4

****** Analysis of Variance *****

Variate: Pulp pH

Source of Variation	Degree of freedom	Sum of Squares	Mean Square	F. Cal	F. Probability
Treatment	3	0.00654	0.00218	0.03	0.991
Residual	92	5.79703	0.06301		
Total	95	5.80357			

Standard Errors of Differences of Means 0.0725

Co-efficient of Variation % 4.9

***** Analysis of Variance *****

Variate: Total Soluble Solids

Source of Variation	Degree of freedom	Sum of Squares	Mean Square	F. Cal	F. Probability
Treatment	3	2.47	0.82	0.02	0.996
Residual	92	3711.51	40.34		
Total	95	3713.98			

Standard Errors of Differences of Means 1.834

Co-efficient of Variation % 52.6

*****Analysis of Variance *****

Variate: Total Titratable Acid

Source of Variation	Degree of freedom	Sum of Squares	Mean Square	F. Cal	F. Probability
Treatment	3	0.007	0.002	0.00	1.000
Residual	92	111.415	1.211		
Total	95	111.422			

Standard Errors of Differences of Means 0.318

Co-efficient of Variation % 26.5