ISOLATION AND CHARACTERISATION OF MICROORGANISMS

ASSOCIATED WITH ROT DISEASES OF FRUIT, STEM AND LEAF OF

Carica papaya L.

BY

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CUGP100271

A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF

BIOLOGICAL SCIENCES, COLLEGE OF SCIENCE AND TECHNOLOGY,

COVENANT UNIVERSITY, CANAANLAND, OTA, OGUN STATE,

NIGERIA.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD

OF MASTERS OF SCIENCE (M. Sc) DEGREE IN MICROBIOLOGY.

MAY, 2012.

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MAY, 2012.

…………………..

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Prof. Louis O. Egwari

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i

This is to certify that ONIHA, Margaret Ikhiwili, of the Department of Biological Sciences,

Covenant University, Ota carried out a research project under my supervision for the award of

Masters of Science (M. Sc), Microbiology.

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Prof. Louis O. Egwari

Date

(Supervisor and H.O.D. Biological Sciences)

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This project is dedicated to Almighty God, the Lord of lords for his unlimited love, grace,

protection and mercies bestowed upon me and for making this work a reality. To HIM be all the

glory, honour and praises forever more.

iii

I, Oniha, Margaret Ikhiwili (Matric Number:CUGP100271) declares that this project report is

based on the study undertaken by me in the Department of Biological Sciences (Microbiology),

School of Natural and Applied Sciences, College of Science and Technology, Covenant

University, Ota under the supervision of Prof. Louis O. Egwari. This project has not been

submitted anywhere else for any degree award. The ideas and reviews are products of research

conducted by me. All ideas of other authors and researchers used in this thesis have been duly

acknowledged.

ONIHA MARGARET IKHIWILI

Researcher

.................................................

Signature and Date

iv

My utmost acknowledgement goes to God the Almighty Father, for His divine love and

protection, for seeing me through the successful completion of this project and for helping me

surmount all the challenges faced throughout my Masters of Science programme.

My sincere gratitude goes to my supervisor, Prof. Louis O. Egwari for his mentorship and

assistance throughout the duration of my project work and not forgetting his support towards

impacting more knowledge and skills in me through the training programme I attended at

Nigerian Institute Of Science Laboratory Technology (NIST) in Ibadan, Nigeria.

My heartfelt gratitude goes to my mother, Zonal Chief Nursing Officer (Mrs) Oniha and my

sister Bernadette for their unending love, moral, spiritual and financial support and for being

there for me through thick and thin. May God in his infinite mercies continually bless you both

in Jesus name, Amen.

Special appreciation with gratitude extends to Dr (Mrs.) Angela O. Eni for her wonderful love,

time, understanding, support, belief in me as well as assisting me in all my areas of need right

from the day I stepped into Covenant University. May God reward you bountifully.

I say a big thank you to Mr Obinna Nwinyi, , Dr (Mrs) A.A. Ajayi, Mr Conrad Omohinmi, Dr

(Mrs) Ogunlana, Dr (Mrs) G. Olasehinde, Mrs Adekeye, Mrs Apata, Mr Oladipupo Adeyemi and

to other members of staff of the Dept. Biological Sciences of Covenant University, Ota for their

assistance, support and positive contribution towards the successful completion of my project.

My sincere appreciation goes to Prof. Adedotun A. Adekunle of the Dept. of Botany and

Microbiology of the University of Lagos, for his fatherly concern and support in obtaining my

Masters degree.

Special thanks to my friend and colleague Mrs. Abosede M. Ebabhi of Dept. of Botany and

Microbiology of the University of Lagos.

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Todimu, Phillip, Ehi, Cynthia, Olamide, Onyinye, just to mention a few. Thank you for your

friendship and for making my stay in Covenant University worthwhile and memorable.

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Fruit, stem and leaf rot is a major constraint to Carica papaya production. Twenty-one papaya

plant samples showing soft rot symptoms and comprising of fruits, stems and leaves were

collected from the Covenant University Papaya Research Plantation for microbial investigation.

The back, first layer after the back, inner layer and the seeds from four papaya fruits, as well as

stems (4) and leaves (4), showing symptoms of sooty rot and white fluffy rot, were also analyzed.

Following sample preparation and serial dilutions, three dilutions of each sample were plated out

in duplicates on nutrient agar, potato dextrose agar and papaya fruit agar using the direct

inoculation, streak, pour plate and spread plate methods. Pure cultures of each observed

microorganism was purified using sub-culturing techniques, then cultural, microscopic and

biochemical characteristics were used for identification of specific isolates. Koch’s postulates for

the determination of the microbial etiology of diseases were used to determine the causal agent

of the soft rot of papaya fruit. A total of ten microorganisms, Aspergillus niger, Aspergillus

flavus, Aspergillus fumigatus, Mucor spp, Penicillium spp, Alternaria spp, Colletotrichum

gloeosporioides, Bacillus spp, Staphylococcus spp and Pseudomonas spp, belonging to five

fungi and three bacteria genera were isolated. Papaya fruit agar supported the growth of A. niger,

A. fumigatus, A. flavus, Mucor spp, Bacillus spp and Staphylococcus spp but not Pseudomonas

spp. The most frequently isolated bacteria from the soft rot samples was Staphylococcus spp and

was isolated from 85.7%, 42.9% and 71.4% of the fruits, stems and leaves respectively, while

Pseudomonas spp was the least frequently isolated bacteria from all the plant parts. The most

frequently isolated fungi from the soft rot fruits was Mucor spp while A. niger was the most

frequently isolated from the stem (57.1 %) and leaf (42.9%). Colletotrichum gloeosporioides was

isolated from the fruits, but not from the soft rot infected stems and leaves. The most frequently

isolated bacteria from the sooty rot and white fluffy rot samples were Pseudomonas spp (75%)

and Bacillus spp (62.5%) respectively, while Penicillium spp was the most frequently isolated

fungus both from the sooty rot (56.3%) and white fluffy rot (43.8%) samples. Pathogenicity tests

presumptively identified Mucor spp as the most important pathogen associated with soft rot of

papaya fruit.

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1.0

INTRODUCTION

Fruits and vegetables are very important and have high dietary and nutritional qualities (Barth et

al., 2009). Studies have evaluated the association of fruit and vegetable consumption with the

reduction of risk of specific diseases (Hung et al., 2004). Their consumption has dramatically

increased by more than 30% during the past few decades (Barth et al., 2009). Fresh fruit and

vegetable consumption increased by 25.8% and 32.6%, respectively, and far exceeded the

increases observed for processed fruit and vegetable products. It is also estimated that about 20%

of all fruits and vegetables produced is lost each year due to spoilage (Barth et al., 2009). Raven,

et al., (2005) reported that 20 new human fungal pathogens are documented each year. It is

estimated that about 20-25% of the harvested fruits are decayed by pathogens during postharvest handling even in developed countries (Droby, 2006; Zhu, 2006).

Deterioration of foods generally is attributed to two main causes which are natural degradation

due to activities of enzymes and growth of microorganisms (bacteria, molds and yeasts). These

microorganisms can result in useful products through their activities particularly during

fermentation of foods such as wine and cheese. The negative effects of these microbial activities

result in decay, rotting of food and food poisoning hence the basis of microbial food spoilage

occurs when these microorganisms release their own enzymes into the foods and absorb the

nutrients thereby changing the physical and chemical states of the foods thus lowering the

nutritional value. Bacteria and fungi may also produce waste products which act as poisons or

toxins, thus causing the renowned ill-effects (Bakri et al., 2010).

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tropical and subtropical regions of the world being one of the most nutritious and cheapest fruits

grown and consumed in Nigeria (Baiyewu, 1994). It is usually grown as compound fruit crop or

semi-wild fruit crop from discarded seeds (Kuthe et al., 1974). Papaya (Carica papaya L.)

belongs to the family Caricaceae which incorporates 35 latex-containing species in six genera,

Carica, Cylicomorpha, Jarilla , Jacaratia, Horovitzia and Vasconcellea (Badillo, 2002) with the

genus Carica, consisting of only one species. Although opinions differ on the origin of C.

papaya, it is likely that C. papaya originates from the lowlands of eastern Central America, from

Mexico to Panama (Nakasone et al., 1998). It is a fast growing, short-lived, woody, large

herbaceous plant having latex vessels in all its fruits with an upright unbranched stem covered all

over with leaf scars (Agnew, 1968; Dick, 2003). It generally only branches when injured (Garret,

1995). The stem is hollow with a light green to a tan brown colour bearing leaves that are large

and deeply palmately lobed with the older leaves abscising as new leaves emerge (Morton,

1987). The papaya fruit is melon-like, oval to nearly round, elongated club–shaped and is rich in

latex when unripe with a green coloured skin. As it ripens, it becomes light or deep yellow

externally and the thick wall of succulent flesh becomes aromatic yellow-orange or various

shades of red. The fruit is then juicy, sweetish and some types are quite musky (Morton, 1987).

The fruit consist largely of water, sugar, vitamins A and C, protein and ash (Baiyewu, 1994). The

fruit can be freshly eaten or cooked and can also be used in the preparation of jellies, juice and

jams. Papaya has a mild laxative action and the seeds are used medicinally against worms and

ulcer (Baiyewu, 1994). The green fruits, leaves and flowers may also be used as cooked

vegetable (El Moussaoui et al., 2001).

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their use in the production of chewing gums, tenderizing meat and drug preparation for various

digestive ailments and for the treatment of gangrenous wounds as well as in the textile and

cosmetics industries (Kochhar, 1986; Villegas, 1997). Biochemically, its leaves and fruits are

good sources of several proteins and alkaloids with application to pharmaceutical, food,

industries, e.t.c. (El Moussaoui et al., 2001). The seed is used to expel worms and the flower may

be taken in an infusion to induce menstruation (Duke, 1984 and Oduola et al., 1986). Increasing

interest in medicinal herbs has increased scientific scrutiny of their therapeutic potentials and

safety thereby providing physicians with data to help patients make wise decisions about their

use (“O”Hara et al., 1998).

Papaya fruits are beset with problems of field and storage rot. Gutpa and Pathak (1986) identified

22 different fungi in post-harvest decay of papaya fruits which include Aspergillus niger,

Aspergillus flavus, Rhizopus oryzae, Fusarium moniliforme. Bacterial pathogens involved with

rot of papaya include the species of soft-rotting Erwinia, Pseudomonas, Xanthomonas,

Cytophaga and Bacillus (Liao et al., 1987; Lund, 1983). These microorganisms, under the

influence of environmental factors (include temperature, hydrogen ion concentration (pH),

oxygen, moisture content) pose a serious threat to papaya fruit production. Besides the losses in

income to the papaya fruit marketers, the rotten fruits could also cause a health hazard to

consumers. Krogh (1992) had earlier reported that most microbes infecting plant tissues often

produced secondary metabolites in their hosts, which are known to be hazardous to animals

including man. Some of these metabolites include the ergot alkaloids on cereals by Clavisep spp,

fumonisin on maize by Fusarium spp, aflatoxins and ochratoxins on several plants produced by

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703,800 metric tons (FAO, 2010), it’s plentiful and sold all year round as well as being globally

consumed by people for multiple benefits hence, the need for preservation and to boost its

production. This can be achieved among other things by preventing the occurrence of these

diseases through studying the ecology of the microbes.

1.1

STATEMENT OF PROBLEM

Papaya is a useful plant with nutritional, medicinal and health benefits. In spite of all these

benefits, the plant is besieged by lots of pathogens both in the field and post-harvest diseases and

these diseases result in yield losses thus making its valuable components unavailable. The

papaya farm in Covenant University is particularly useful as a source of raw materials for the

production of some patented products. This farm is however, infested with pathogens on fruits,

stems, and leaves resulting in huge yield losses making the desired raw materials unavailable.

1.2

RESEARCH OBJECTIVES:

•

To isolate the microorganisms associated with the rot diseases of fruits, stems and leaves

of papaya

•

To identify and characterize the isolated microorganisms.

•

To determine the pathogenicity of the isolated microorganisms for papaya.

4

LITERATURE REVIEW

2.1

INTRODUCTION

Carica papaya L. commonly called papaya or pawpaw is a well-known plant with edible fruit

hence an important fruit crop. It belongs to the genus Carica and is one of native plants of

Central America; however, it has been planted widely in most tropical and subtropical countries.

Generally, the name of Carica papaya varies in different countries, for instance, papaya in

Malaysia and Thailand, papaw/paw paw in Australia; in Europe, papaya is also named “tree

melon” e.t.c. (Morton, 2006; Papaya, 2008). Recent years, attention of papaya is given due to the

nutritional and medical benefits of papaya, and most studies are concentrated on two parts,

namely: papaya fruit and papaya latex. Papaya latex is released from laticifers in both female and

hermaphrodite plants. Papaya latex contains at least four cysteine endopeptidases and other

constituents including hydrolase inhibitors and lipase, which has been widely applied in food

industries, pharmacy, and a direct treatment of pediatrics burns in Gambia (Azarkan et al., 2006;

Chen et al., 2005; Maria et al., 2006; Morcelle et al., 2006; Nitsawang et al., 2006; Starley et al.,

1999).

TAXONOMY

KINGDOM: Plantae

PHYLUM: Trachaeophyta

CLASS: Angiospermae

SUB-CLASS: Eudicots

SUPER-CLASS: Rosids

ORDER: Brassicales

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GENUS: Carica

SPECIES: C. papaya

2.2

ORIGIN AND DISTRIBUTION

The papaya is believed to be native to southern Mexico and neighboring Central America. The

origin of Carica papaya differs in opinion in the tropical America (Garrett et al., 1995). It is

likely that C. papaya originated from the lowlands of eastern Central America, from Mexico to

Panama (Nakasone et al., 1998). It is now present in every tropical and subtropical country

although it was first cultivated in Mexico several centuries before the emergence of the

Mesoamerican classic cultures. Papaya was first described in 1526 by the Spanish chronicler

Oviedo, who found it first on Panamanian and Colombian coasts with its fruit being rapidly

propagated in the tropics, most likely due to the abundant and highly viable seeds. The crop

adapted quite well to tropical areas with fertile soils and abundant rainfall. The history of

papaya’s spread was initiated approximately in 1500, when the Spanish conquerors carried its

seeds to Panama and Dominican Republic. During the following century, Spanish and

Portuguese sailors took the seeds to the Philippines, Malaysia and India. For 1600, the fruit had

been produced in warm regions of South and Central America, Southern Mexico, the Antilles,

Bahamas, Bermuda and Florida. In the same century, papaya seeds were taken from India to

Naples in Italy. The crop reached Hawaii between 1800 and 1820. Until 1900, papaya seeds were

taken to Florida, probably from Bahamas' plantations. The Solo variety has been cultivated in

Hawaii since 1911, probably brought from Barbados and Jamaica. The first seeds of the Maradol

variety were introduced into Mexico in 1978, through CONAFRUT, in Xalapa, Veracruz

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the Caribbean and South-east Asia (Philippines) during Spanish exploration in the 16th century

from where it was further distributed to India, the pacific and Africa (Villegas, 1997). Papaya

was introduced to Hawaii in the early 1800s by the Spanish explorer Don Francisco Marin and

became an export crop of Hawaii in 1984 (Fitch, 2005).Today, papaya is widely distributed

throughout the tropical and warmer subtropical areas of the World (Villegas, 1997) and has

become naturalized in many areas (Morton, 1987). The classification of papaya has undergone

many changes over the years. The genus Carica was previously classified under various plant

families, including Passifloraceae, Cucurbitaceae, Bixaceae, and Papayaceae. Today, it is

presently placed under Caricaceae, a plant family incorporating 35 latex-containing species in six

genera, Carica, Cylicomorpha, Jarilla , Jacaratia, Horovitzia and Vasconcellea (Badillo, 2002).

Carica, today consists of only one species with Vasconcellea now consisting of 21 species which

were formerly classified under the genus Carica (Badillo, 2002). It is widely believed that

papaya originated from the Caribbean coast of Central America, ranging from Argentina and

Chile to southern Mexico (Manshardt, 1992) through natural hybridization between Carica

peltata and another wild species (Purseglove, 1968)

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Region Area

Harvested (Ha)

Production (Mt)

Africa

128,807

1,344,230

Asia and the Pacific

157,203

2,063,352

Australia

403

5,027

Caribbean

9,179

179,060

Central America

28,966

1,057,024

North America

500

16,240

South America

65,546

2,120,370

Source: FAOSTAT, 2006.

Figure 2.0: Papaya major producing countries

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BOTANICAL DESCRIPTION

The papaya is a short-lived, fast-growing, woody, large, perennial, succulent, herb up to 10m in

height with self-supporting stems (Dick, 2003). It lives for about 5-10 years, although

commercial plantations are usually replanted sooner (Chay-Prove et al., 2000). Papayas normally

grow as single-stemmed plant but may become multi-stemmed when damaged. It has a crown of

soft, large, deeply palmately lobed leaves (7-9 main lobes) emerging from the apex of the trunk

on nearly horizontal petioles 25 to 100cm long and form a loose open crown (Villegas, 1997).

This soft, hollow, cylindrical trunk ranges from 30 to 40cm in diameter at the base thinning to

about 5cm diameter at the crown and are usually destroyed when they reach heights that make

harvesting of fruit difficult (Villegas ,1997). The hollow green or deep purple trunk is fleshy and

cylindrical with prominent leaf scars while the smooth bark has a greyish brown colour. The

leaves are dark green to yellow-green, vary from 25 to 75cm in diameter and have prominent

yellowish ribs and veins. The older leaves abscise as new leaves emerge, producing a palm-like

form to the plant (Morton, 1987). The bundle of leaves is visibly marked by the off-white nerves

embedded and reticulated veins; the underneath surface is pale green-yellow and opaque with the

petioles round and yellow-green, with sporadic purple or violet stains, fistulous form, fragile, 25100cm in length and 0.5-1.5cm thick (De La Cruz et al., 2003). Colleters (short-lived

multicellular stalked globules) are present at leaf bases and along vein margins and their

secretions may aid in protection against desiccation and/or protection against insect predators

(Rouse et al., 1999). The life of a leaf is 2.5 to 8 months and new leaves arise at the rate of 1.5 to

4 per week. All parts of the plant contain white latex with the plants being self pollinated (Jari,

2009).

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The flowers and fruits arise from buds on the trunk at the base of the leaves (Dick, 2003).The

five-petalled flowers are fleshy, waxy and slightly fragrant and are borne on inflorescences

which appear on the axils of the leaves, maturing into the large 15–45cm long, 10–30cm

diameter fruits. In nature, papaya plants are dioecious with some plants bearing only shortstalked female flowers or bisexual (perfect) flowers also on short stalks, while others may bear

only male flowers, clustered on panicles. Others at certain seasons produce short-stalked male

flowers, at other times perfect flowers. In addition, some plants may produce more than one type

of flower and exhibit different degrees of maleness or femaleness. The functional gender of

flowers can be reversed temporarily, depending on environmental conditions, such as day length,

soil moisture availability particularly temperature. Male or bisexual plants may change

completely to female plants after being beheaded. Certain varieties have a propensity for

producing certain types of flowers. For example, the Solo variety has flowers of both sexes 66%

of the time, so two out of three plants will produce fruit, even if planted singly. New flowers are

formed continuously, thus, a single hermaphrodite plant will have flowers and fruits in all stages

of development. The species (papaya) is polygamous and can be classified into three sex types:

male (staminate), hermaphroditic (bisexual) and female (pistillate) (Lemos et al., 2002; ChayProve et al., 2000). Hermaphrodites are the commercial standard, producing a pear- shaped fruit.

The percentage composition of typical papaya is: seed (8.5 %), skin (12 %) and pulp (79.5 %).

The short female flowers usually white or cream in colour, are held close against the stem as

single flowers or in clusters of 2-3 and of 3-4cm long (Chay- Prove et al. 2000). Male flowers

are smaller, numerous of 15-20 small flowers, borne on 60-90cm long pendulous inflorescences

(Nakasone et al., 1998).The white staminate flowers are funnel-shaped, with five corolla, and ten

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are elongated and of low quality; the pistillate flowers are larger, with five fleshy petals connate

towards the base, no stamens, a large cylindrical or globose superior ovary, and five sessile fanshaped stigmas (Bruce et al., 2008).

Fruit:

Papaya fruit is a berry with a thin, smooth exocarp (peel) and thick, fleshy mesocarp. Fruit may

be globose, ovoid, obovoid, and pyriform, resembles a melon, 15-50cm long, and 0.25-10kg in

weight (OECD, 2003). It is commonly spherical or cylindrical in form, round or obscurely fiveangled in transverse section to the walls of which are attached the numerous round wrinkled and

blackish seeds, the size of small peas, enclosed by a thin gelatinous aril (Morton, 1987). It has a

thin, smooth skin on the exterior, of orange-yellow to deep orange in color, while the flesh, is

concolorous with the skin. Female plants produce medium to large round-shaped fruit of good

quality and a large seed cavity. Bisexual plants produce small to medium elongated fruit of good

quality and a smaller seed cavity and is affected by environmental factors, particularly

temperature, that modify floral morphology during early development of the inflorescence

(Nakasone et al., 1998). Male plants with bisexual flowers may produce a few, elongated, poor

quality fruit. Fruits are ready to harvest five to six months after flowering, which occurs five to

eight months after seed germination (Chay-Prove et al., 2000). Ripe papaya fruit has smooth,

thin yellow-orange coloured skin. Depending on the cultivar, flesh thickness varies from 1.5 to

4cm (Nakasone et al., 1998) and flesh colour may be pale yellowish to red (Villegas, 1997;

Nakasone et al., 1998).

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TYPES OF PAPAYA

There are two types of papayas namely: Hawaiian and Mexican. The Hawaiian varieties are the

papayas commonly found in supermarkets with pear-shaped fruit generally weighing about 0.5kg

and have yellow skin when ripe. The flesh is bright orange or pinkish, depending on variety, with

small black seeds clustered in the center. Hawaiian papayas are easier to harvest because the

plants seldom grow taller than 243.8cm. Mexican papayas are much larger than the Hawaiian

types and may weigh up to 4.54kg and be more than 38.1cm long with its flesh being yellow,

orange or pink. The flavor though delicious is less intense than that of the Hawaiian papaya and

is slightly easier to grow than Hawaiian papayas.

Varieties Of Papaya Plant

There are many strains and varieties of papaya fruit having great variation in size, form and

colour. Due to its complex genetic make-up, there are few, if any, true cultivars of papaya which

are as uniform in horticultural characters as the cultivars of other herbaceous crops (Malo, 2001).

Hawaiian papaya referred to as Solo comes closer to deserving cultivar rank than any other type,

originally from Barbados (W. I.), Solo owes its constancy in character expression to a high

degree of natural self pollination of its bisexual flowers. This, in addition to continuous selection

of pear-shaped fruit produced by bi-sexual plants, has maintained Solo relatively unchanged over

the years. Improved selections, such as Sunrise Solo, have resulted from rigorous breeding work.

(Malo, 2001). The mountain papaya (C. candamarcencis ), is native to Andean regions from

Venezuela to Chile at elevation between (1,800-3,000m), it is stout, tall, bearing a small, yellow,

conical, five-angled fruit of sweet flavor. It is cultivated in climates too cold for the papaya,

including northern Chile where it thrives mainly in and around the towns of Coquimbo and La

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there and elsewhere.

Table 2.1 Fruit characteristics of papaya cultivars in major producing countries.

Cultivar

Country of origin

Fruit Characteristics

Kamiya

Hawaii

Small to medium-size, blocky shape and very

short neck. A deep yellow-orange skin with

firm, juicy, and very sweet flesh.

Mexican Red

Mexico

Rose-fleshed, with lighter flavor than Mexican

yellow. Medium to very large size and

generally not as sweet as Hawaiian types.

Mexican Yellow

Mexico

Has a very sweet, yellow-fleshed papaya of

medium to large fruit.

Maradol Roja

Cuba

Small to medium-sized fruit with very sweet

taste.

Vista Solo

USA

Medium to large fruit, yellow skin, orange to

yellow-orange flesh, producing high quality

fruit.

Waimanalo

Hawaii

Round fruit, short neck, smooth, glossy skin,

star-shaped cavity. Thick, firm flesh of orangeyellow colour with high flavor and quality.

Sunrise solo

Hawaii

Pear-shaped, slight neck, smooth skin, firm

flesh, reddish-orange, sweet, sugar content

high. Quality similar to Solo. Seed cavity not as

deeply indented as other Solo strains.

Sunset

Hawaii

Solo type, small to medium-sized, pear-shaped

fruit. Skin and flesh of orange-red with a very

sweet taste. Dwarf, high yielding plant.

Source: De Los Santos et al., 2000

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GROWTH HABIT AND SOIL

Papayas have exacting climate requirements for vigorous growth and fruit production. They are

well adapted to many soil types, must have warmth throughout the year and will be damaged by

light frosts. Brief exposure to 00C is damaging and prolonged cold without overhead sprinkling

will kill the plants. Cold, wet soil is almost always lethal and cool temperatures will also alter

fruit flavor. Papayas make excellent container and greenhouse specimens where soil moisture

and temperature can be moderated. They are best planted in mounds or against the foundation of

a building where water can be controlled (CRFG, 1998). It does not tolerate flooding even for

short duration and are easily killed by excess moisture but thrives well with adequate distribution

of rainfall throughout the year without flash floods, water-logging and strong winds (Agrolink,

2002). The soil needs to be moist in hot weather and dry in cold weather. Papayas do not tolerate

salty water or soil. Watering is the most critical aspect in raising papayas and when injured by

frost is particularly susceptible to root rot. The fast-growing papaya requires regular applications

of nitrogen fertilizers but the exact rates have not been established. They can take fairly hot

organic fertilizer such as chicken manure if used with deep irrigation after warm weather has

started. Phosphorus deficiency causes dark green foliage with a reddish-purple discoloration of

leaf veins and stalks. Papayas do not need to be pruned, but some growers pinch the seedlings or

cut back established plants to encourage multiple trunks. Optimum pH ranges from 5.5 to 6.7.

Overly acid soils are corrected by working in lime at the rate of 1-2 tons/acre (2.4-4.8 tons/ha)

(Morton, 1987). Usually weeding is performed manually or mechanically-aided, deep soil

disturbances can damage the root system. Black polyethylene film is used on the surface to avoid

weed growth (Infoagro, 2002). After extraction from ripe papaya, seeds are dried and dusted

with fungicides before planting. Potting soil can be sterilized by mixing 50-50 with vermiculite

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about two weeks, but may take three to five weeks. Gibberellic acid can be used to speed up

germination in some seasons. Seedlings usually begin flowering 9-12 months after they

germinate.

2.6

POLLINATION:

When papaya plant is inadequately pollinated, it will bear light fruits lacking uniformity in size

and shape. Therefore, hand-pollination is advisable in commercial plantations that are not

entirely bisexual. Bags are tied over bisexual blossoms for several days to assure that they are

self-pollinated. The progeny of self-pollinated bisexual flowers are 67% bisexual, the rest being

female. To cross-pollinate, 1 or 2 stamens from a bisexual flower are placed on the pistil of a

female flower about to open and a bag is tied over the flower for a few days. Most of such crosspollinated blooms should set fruit. Resulting seeds will produce 1/2 female and 1/2 bisexual

plants. By another method, all but the apical female flower buds are removed from a stalk and

the apical bud is bagged 1-2 days before opening. At full opening, the stigma is dusted with

pollen from a selected male bloom and the bag quickly resealed and it remains so for 7 days.

Plants from female flowers crossed with male flowers are 50-50 male and female. Bisexual

flowers pollinated by males give rise to 1/3 female, 1/3 bisexual and 1/3 male plants. South

African growers have long been urged to practice hand-pollination in order to maintain a selected

strain and, in breeding, to incorporate factors such as purple stem, yellow flowers and reddish

flesh so that the improved selection will be distinguishable from ordinary strains with non-purple

stems, white flowers and yellow flesh (Morton, 1987).

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PROPAGATION:

Seedling papaya do not transplant well hence planted in large containers so the seedlings will

have to be transplanted only once, when they go into the ground through careful handling,

making sure not to damage the root ball. In order to prevent damping off, the potting mix is

drenched with a fungicide containing benomyl or captan and plants are set a little high to allow

for settling. Plastic mulch will help keep the soil warm and dry in wet winter areas, but be

removed as soon as the weather becomes warm. Planting of at least three or four plants is done to

ensure females or hermaphroditic ones are planted and reproduction of the characteristics of a

preferred strain by air-layering has been successfully practiced on a small scale. All offshoots

except the lowest ones are girdled and layered after the parent plant has produced the first crop

of fruit. Later, when the parent has grown too tall for convenient harvesting the top is cut off and

new buds in the crown are pricked off until offshoots from the trunk appear and develop over a

period of 4 to 6 weeks. These are layered, removed and the trunk cut off above the originally

retained lowest sprout, which is then allowed to grow as the main stem. Thereafter the layering

of offshoots may be continued until the plant is exhausted.

2.8

HARVESTING:

Papayas are ready to harvest when most of the skin is yellow-green. After several days of

ripening at room temperature, they will be almost fully yellow and slightly soft to the touch.

Dark green fruit will not ripen properly off the tree, even though it may turn yellow on the

outside. Mature fruit can be stored at 7.20 C for about 3 weeks. Papayas are often sliced and

eaten by themselves or served with a myriad of other foods. Green papayas should not be eaten

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vegetable.

2.9

NUTRITIONAL VALUE

Papaya fruit is rich in carbohydrates (42.28% starch and 15.15% sugar in pulp), but is deficient

of protein and fat (Bari et al., 2006; Oyoyede, 2005). USDA National Nutrient database recorded

an orange-fleshed papaya (per 100 g) contained 39 kcal (163 kj) energy, 88.8g H 2 O, 0.61g

protein, 0.14g fat, 9.81g total carbohydrate, 1.8g fiber, 0.61g ash thus a very important food

source in some developing countries. A green papaya fruit as reported by Duke (1996), which

per 100g provides 26 calories, 92.1g H 2 O, 1.0g protein, 0.1g fat, 6.2g total carbohydrate, 0.9g

fiber and 0.6g ash. Vitamins C and A as well as certain minerals (potassium, copper and

magnesium) are rich in papaya fruit. Additionally, Wall (2006) stated that papaya fruits of

Hawaii cultivar (100g) contained 9% of DRI (dietary reference intake) for copper and 6-8% of

DRI for magnesium. Papaya fruit also contains high levels of vitamin C (51.2mg/100g), vitamin

A precursors including β-carotene (232.3μg/100g), and β-cryptoxanthin (594.3μg/100g), as well

as magnesium (19.2-32.7mg/100g) (Wall, 2006). The papaya seeds contain protein (24.3%),

fatty oil (25.3%), total carbohydrate (32.5%) and significantly high level of unsaturated fatty

acids. The papaya leaves (per 100g), contain 74 calories, 77.5g H 2 O, 7.0g. protein, 2.0g fat,

11.3g total carbohydrate, 1.8g fiber, 2.2g ash, 344mg calcium, 142mg phosphorus, 0.8mg iron,

16mg sodium, 652mg potassium, 11,565ug β-carotene equivalent, 0.09mg thiamine, 0.48mg

riboflavin, 2.1mg niacin, and 140mg ascorbic acid, as well 136mg vitamin E (Duke, 1996).

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ECONOMIC IMPORTANCE

The ripe fruit is usually eaten raw, without skin or seeds. The unripe green fruit of papaya can be

eaten cooked, usually in curries, stews and in salads when combined with lettuce. In Brazil it is

served as a dessert, sliced, with the addition of a little sugar and whipped cream .The fruit has a

relatively high amount of pectin hence the production of jellies, pickles, preserves, pies, and

sherbets. When used as a breakfast fruit it is cut in halves longitudinally, and after the seeds are

removed, served with the addition of lemon juice, salt and pepper, or sugar, according to taste

(Morton, 1987). Green papaya is used in south East Asian cooking, both raw and cooked. The

black seeds are edible and have a sharp, spicy taste and are sometimes ground serving as a

substitute for black pepper. In some parts of Asia, the young leaves of papaya are steamed and

eaten like spinach while in other parts, papaya leaves are made into tea as a preventive for

malaria, though there is no real scientific evidence for the effectiveness of this treatment

(Morton, 1987).

2.11

INDUSTRIAL IMPORTANCE:

Papayas can be used as a food, a cooking aid and in medicine. Industrially, the stem and bark are

also used in rope production. Papaya is frequently used as a hair conditioner, but should be used

in small amounts. Green papaya fruit and the tree's latex are both rich in an enzyme called

papain, a protease which is useful in tenderizing meat and other proteins. Its ability to break

down tough meat fibers was used for thousands of years by indigenous Americans and is

included as a component in powdered meat tenderizers. Papaya is marketed in tablet form to

remedy digestive problems.

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CHEMICAL COMPOUNDS

Carica papaya contains many biologically active compounds two of which are very important

namely chymopapain and papain, and aid in digestion. Papain also is used to treat arthritis with

the level of the compounds varying in the fruits, latex, leaves, and roots. In addition, plant parts

from male and female trees differ in the quantity of the two compounds. For example, phenolic

compounds tend to be higher in males than females. The quantity of fresh papaya latex and dry

latex (crude papain) also vary with the sex and the age of the plant. Female and hermaphrodite

plants yield more crude papain than male ones and older fruit yields more than younger fruit.

(Oderinde, 2002). However, the activity of the papain is higher in the extracts from the younger

fruit than the older fruit. Cultivars also vary in the quantity of the compounds. For example, the

primary and secondary volatile compounds in the fruit of one cultivar studied were linalool and

trans-linalool oxide, respectively. In another cultivar, the primary and secondary volatile

compounds were cis-linalool oxide and linalool, respectively. Recent years, papaya latex and its

commercial products have been widely applied in baking and beverage industries, pharmacy and

new chemicals synthesis. There are four major components including papain (EC. 3.4.22.2),

chymopapain (EC. 3.4.22.6), caricain (EC. 3.4.22.30), glycyl endopeptidase (EC. 3.4.22.25), and

papaya lipase (EC. 3.1.1.3).

2.13

TOXICITY AND ALLERGY

Besides the numerous benefits, consumption of papaya was also reported to cause adverse

reactions including toxicology and allergy due to the release of a latex fluid when not quite ripe,

which can cause irritation and provoke allergic reaction in some people. Some persons are

allergic to the flower powder, and fruit as well as the latex that may also result in irritation due to

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De clerck et al., (2003) stated papaya-borne allergy belonged to IgE-mediated immune system

disorder, which can result in oropharyngeal itching, angioedema, wheal, flare reaction and rhinoconjunctivitis. The papaya fruits, seeds, latex, and leaves also contains carpaine, an anthelmintic

alkaloid (a drug that removes parasitic worms from the body), which can be dangerous in high

doses. Papaya contains about 6% of the level of beta carotene found in carrots (Berrin, 1997).

Additionally, papaya fruit contains high level of carotenoids but excessive consumption of

papaya might cause carotenemia, a harmless yellowing of the palms and soles (Carica papaya,

2001; Duke, 1996; Papaya 2008). With the exception of infertility, there have been no adverse

reactions from the consumption of Carica papaya fruit, latex, or extracts. Cyangenic glucosides

present in the leaves and roots, which can form cyanide and can subsequently, introduce

undesirable effects on human health. Tannin is also rich in the papaya fruits and leaves, which is

a major antinutrient in plants both of which at high concentrations can cause adverse reactions.

In trials with rats, daily oral doses of benzene and alcohol extracts (20mg/kg body weight (BW)

for 30 days) did not affect body or reproductive organ weights or adversely affect liver or kidney

function. However, aqueous extracts (1mg/kg BW for 7 or 15 days) and benzene extracts given

orally to female rats caused infertility and irregular oestrous cycles. Male rats given ethanol seed

extract orally (10 or 50 mg/day for 30, 60, or 90 days) or intramuscularly (0.1 or 1.0 mg/day for

15 or 30 days) had decreased sperm motility, decreased testis mass and sperm count. Studies

with aqueous seed extracts also decreased fertility in male rats and returned to normal within 60

days after the treatments were discontinued in both gender of rats (Das, 1980). In addition to

decreasing infertility, papain in the latex might cause uterine contractions /abortions shortly after

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responsible for adhering the newly fertilized egg to the wall of the uterus (Rahmat et al., 2009).

2.14

HEALTH BENEFITS

2.14a Antioxidative benefits: Oxidative damage is related to high incidents of some

degenerative diseases including cancer, arthritis, arteriosclerosis, inflammation, aging and brain

dysfunction. Antioxidants are the substances that can prevent or retard the oxidation of easily

oxidisable materials such as fat, the functions of which are generally based on their abilities to

scavenge reactive free radicals in food (MacDonald-Wicks et al., 2006). Papaya fruit offers not

only the luscious taste but is a rich source of antioxidant nutrients such as carotenes, vitamin C

and flavonoids; the B vitamins, folate and pantothenic acid; the minerals (potassium and

magnesium); and fiber which promote the health of the cardiovascular system and also provide

protection against colon cancer. Vitamins B, C and E, carotenoids and phenolic compounds are

the most abundant antioxidants present in plant foods (Hernadez et al., 2007). Most studies

reported that papaya fruits and its leaves had high antioxidant capacity due to their high contents

of vitamin B (in leaves), vitamins C and E (in fruits), as well as carotenoids (Bari et al., 2006;

Lim et al., 2007; Setiawan et al., 2006). It has been reported that papaya fruits contained low

total phenolic compounds content.

2.14b Medical benefits

Besides antioxidative property, papaya plant is an important medicinal plant due to its specific

enzymes. Recent years, papain and other endopeptidases have shown several medical benefits,

such as defibrinating wounds and treatment of cuts, rashes, stings edemas and burns through

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made from fermented papaya flesh, and is applied as a gel-like paste. With the exception of

papain, other endopeptidases, such as leukopapain and chymopapain, also facilitate wound

cleaning, promoting growth and improving the quality of the scar. Papaya contains some specific

antimicrobial substrates including carpaine and aglycones (Starley et al., 1999). Oyoyede (2005)

reported the extracts of fruits and seeds have antibacterial activity against Staphylococcus

aureus, Bacillus cereus, Esherischia coli, Pseudomanas aeruginosa and Shigella flexneri.

Additionally, papaya fruits have several other applications, such as the relief of nervous pains

and elephantoid growth. Papaya injections have been known to treat ruptured discs. Women in

India, Bangladesh, Pakistan, Sri Lanka, and other countries have long used green papaya as a

folk remedy for contraception and abortion. Enslaved women in the West Indies were noted for

consuming papaya to prevent pregnancies and thus preventing their children from being born

into slavery (Morton, 1987). Papaya is a powerful defense against cancer and has effective

curative and healing properties, dissolves the protein coating that forms around cancer cells and

helps the immune system to destroy them more easily (Pandey et al., 2002). Both papain and

chymopapain are powerful proteolytic enzymes which help to break down proteins from foods

into amino acids which can be recombined to produce protein useable by humans. Proteolytic

enzymes can destroy the defense shields of viruses, tumors, allergens, yeasts, and various forms

of fungus which will make it easy for our natural immune system to destroy the alien invaders.

These enzymes also protect the body from inflammation and insufficient amino acids will lead to

accumulation of bacteria in the intestinal tract. Older people produce less of the enzymes needed

to effectively digest proteins into amino acid hence elderly people should also eat lots of papaya

to stay healthy. Fibrin, another substance found in papaya reduces the risk of blood clots and

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as well as preventing stroke. Proteolytic enzymes containing fibrin are helpful for those who sit

for long hours thus reducing the risk of blood clots in legs. People taking long plane journey and

those having desk jobs should take plenty of papaya. Carotenoids, notably beta carotene and

lycopene give papaya its orange pinkish colour. They are effective cancer-fighting agents.

Lycopene induces cancer cell death, increases anti-metastatic activity, and strengthens the

protective enzymes. In China, the intake of green tea and foods rich in lycopene provide

protection against prostate cancer (Otsuki et al., 2010). A compound called isothiocyanate is also

found in papaya which inhibits both the formation and development of cancer cells. In animal

experiments, isothiocyanates were found effective against cancers of the breast, lung, colon,

pancreas and prostate; as well as leukemia. Medical research in animals has confirmed that

papaya seeds have contraceptive effects in adult male langur monkeys, and possibly in adult

male humans (Lohiya et al., 2002). Unripe papaya is especially effective in large amounts or

high doses while ripe papaya is not teratogenic and will not cause miscarriage in small amounts.

Phytochemicals in papaya may suppress the effects of progesterone. (Oderinde, 2002).

Table 2.2 Chemical components of per 100 g orange-fleshed papaya fruit

Nutrient

Units

Value per 100 grams

Water

G

88.83

Energy

kcal

39

Energy

Kj

163

Protein

G

0.61

Total lipid (fat)

G

0.14

Ash

G

0.61

Proximate

23

G

9.81

Fiber, total dietary

G

1.8

Sugars, total

G

5.90

Calcium, Ca

mg

24

Magnesium, Mg

mg

10

Potassium, K

mg

257

Sodium, Na

mg

3

Zinc, Zn

mg

0.07

Vitamin C, total ascorbic acid

mg

61.8

Niacin

mg

0.338

Pantothenic acid

mg

0.218

Folate, total

mcg

38

Folate, food

mcg

38

Folate

mcg

38

Choline, total

mg

6.1

Vitamin A,IU

IU

1094

Vitamin A

mcg

55

Virtamin E (alpha-tocopherol)

mg

0.73

Vitamin K

mcg

2.6

Fatty acids, toital saturated

G

0.043

Fatty acids, total monosaturated

G

0.038

Fatty acids, total polysaturated

G

0.031

Minerals

Vitamins

Lipids

24

Carotene, beta

mcg

276

Cryptoxanthin, beta

mcg

761

Lutein+Zeaxanthin

mcg

75

Source: USDA Nutrient database (2007).

2.15

INSECT PESTS AND NEMATODES

Papaya plants are attacked by a number of insect pests including:

The papaya fruit fly (Toxotrypana curvicauda), lays eggs through the papaya fruit peel into the

fruit cavity where the larvae feed and eventually emerge from the ruined fruit. This fly is

commonly mistaken for a wasp due to its long abdomen and yellow and black markings. Fruits

infested with papaya fruit fly may show yellow areas and may drop from the tree prematurely.

The easiest control for this pest is to place a paper bag over individual fruit when they are small

and leave the bag on until harvest. Home gardeners often protect the fruit from attack by

covering with paper bags, but must be done early, soon after the flower parts have fallen, and the

bags must be replaced every 10 days or 2 weeks as the fruits develop. Rolled newspaper may be

utilized instead of bags and is more economical. (De La Cruz et al., 2003).

Fruit flies: There are also two other species of fruit flies recorded from papaya in East Africa,

namely Bactrocera invadens and Ceratitis rosa. These flies usually deposit their eggs in ripe

fruits, some lay eggs on green papaya, but most of the eggs die due to the latex secreted when

fruits are punctured by females while laying eggs (Mossler et al., 2002). Developing larvae cause

rotting of ripening fruits. Fruit flies are a major concern of papaya importing countries.

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papaya stem, usually found in, on, or near the stem amongst the flowers and fruits. It eats into the

fruit and the stem and makes way for the entrance of anthracnose. Damage can be prevented if

spraying is begun at the beginning of fruit set, or at least at the first sign of webs (De La Cruz et

al., 2003).

The papaya whitefly (Trialeuroides variabilis) is generally only a pest of the leaves, causing

leaves to drop and reducing fruit production. Female whiteflies lay yellow oval eggs, which

appear dusted, singly on the undersides of leaves. The nymphs go through three instars, the first

instar or crawler has well-developed legs and is the only mobile immature life stage. After

finding a suitable feeding site on the lower surface of a leaf, the crawlers insert their mouthparts,

begin feeding, and usually do not move again while in the nymphal stage. The subsequent instars

are flattened, oval, and scale-like. Whiteflies in the pupal stage are more convex, with large,

conspicuous red eyes. (Crane, 2005; Pena et al., 2007).

The papaya scale (Philephedra tuberculosa): Scale infestation results in 3 types of damage to

papaya plants. First, flower and leaf drop occur from severely infested young plants. Secondly,

when the infestation on seedlings or on young plants is localized near the apex, distortion of

apical leaves is induced. Thirdly, females attached to the fruit cause damage that makes fruits

unmarketable. Female scales produce up to 900 eggs over 3 to 4 weeks, eggs hatch after 12-17

days, and crawlers settle on leaves, stems and fruits. The papaya scale pass through two nymphal

(immature) stages.

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microscopic, worm-like organisms that feed on papaya plant roots, causing plants to decline in

vigor and making more plants more susceptible to toppling over because of the loss of roots.

Aphids: Aphids do not colonize papaya plants, but are a serious threat to papaya production due

to their ability to transmit diseases, in particular papaya rinsgpot virus (PRSV) and the papaya

mosaic virus. The aphids, Myzus persicae (Sulzer) and Lipaphis erysimi (Katenbach) infest

leaves of papaya plantings in south Florida. (Johnson et al., 2006)

The Spider mites

Some species of mites are known to cause damage to Carica papaya and they include:

•

Broad mite (Polyphagotarsonemus latus)

•

Spider mites (Tetranychus spp., Eutetranychus spp. and Oligonychus gossypii)

•

False spider mite (Brevipalpus phoenicis)

Broad mites attack mainly the terminal buds; feeding on the young leaves as they emerge from

the growing point. Affected leaves are hardened, distorted, thick, brittle with down, curled edges.

Severe infestations inhibit new stem growth, with consequent reduction in fruit production.

Broad mites are tiny (0.1-0.2mm long) and cannot be seen with the naked eye. They are even

difficult to detect with a hand lens. Therefore, an attack by broad mites is usually detected by

symptoms of damage. Their damage may be confused with injury caused by some herbicides

because in both cases, the leaves become claw-like with prominent veins but grey or bronze scar

tissue between the veins on the underside of the leaves distinguishes mite from herbicide

damage.

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Infested leaves show yellow patches on the upper surface, particularly between main veins and

midrib (Mossler et al., 2002). Feeding by mites causes scarring and discoloration of fruit, and

reduced fruit size affecting its market value. Infestations usually begin on the older leaves,

spreading to younger, growing plants with serious infestations occuring during long, dry periods.

The false spider mite (Brevipalpus phoenicis) usually feeds on the trunk below the level where

the bottom whorl of leaves is attached. The mites move upward on the trunk and outward onto

the leaves and fruits as the population increases, leaving a large, conspicuous, damaged area

behind them. The affected area becomes raised and blister-like, later, the affected tissue dries up,

dies and becomes discolored, forming a large, continuous callous area, light brown and scaly.

Damage by feeding on young papaya fruits is manifested by sunken areas. Sometimes, feeding

by the mite causes a copious outflow of a milky white liquid that mars the appearance of the

fruits. Under heavy mite infestations, the papaya stem, which normally remains green for a long

time, becomes brownish and corky in appearance, and has a spindly growth (Kessing et al.,

2002).

2.16

DISEASES OF PAPAYA

The principal diseases affecting papaya include papaya ringspot virus, anthracnose

(Colletotrichum gloeosporioides), powdery mildew (Oidium caricae), leaf spot (Corynespora

cassiicola), and blight (Phytophthora spp.) (Ploetz, 1998).

Papaya ringspot virus is one of the most important diseases of papaya. The earliest symptoms

are a yellow mottling of leaves and vein-clearing of leaves. As the disease progresses, the lobes

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on leaf petioles and the main stem. Fruit symptoms consist of dark circles or C-shaped markings

on the fruit peel. Seeds from small, pear-shaped Solo-type fruit tend to be more susceptible to

this virus than the larger, elongated, oval-shaped fruit found in many local markets.

Anthracnose caused by the fungus Colletotrichum gloeosporioides, is a serious post-harvest

disease of papaya which primarily attacks the maturing fruit. The pathogen initially infects

intact, non-wounded, immature green fruit in the field. However, symptom development

generally occurs after harvest, especially when the fruit is ripe. Disease symptoms begin as small

water-soaked spots on ripening fruits, as the spots develop they become sunken, turning brown

or black and may enlarge to 5cm in diameter. The fungus may produce a pink mass of spores in

the middle of these older spots. The pathogen can grow into the fruits, resulting in softening of

the tissue and an off flavor of the pulp. The environmental conditions that favor the pathogen are

high temperatures (optimal is 280C) and high humidity. Disease spores are spread by wind or

rain. Anthracnose can be controlled by following an adequate fungicide spray program beginning

at fruit set and continuing at regular intervals (usually every 10 to 14 days) while the plants are

producing fruit. Post-harvest application of the fungicide thiabendazole (1000 ppm spray or dip)

is effective in reducing the amount of anthracnose decay. Also, a postharvest hot water dip at

480C for 20 minutes will significantly reduce the amount of anthracnose. Although no known

cultivar of papaya offers complete resistance to anthracnose, the Hawaiian cultivar Sunrise is

more resistant than Kapoho cultivar (Pernezny et al., 2003).

Powdery mildew (Oidium caricae) is primarily a disease of the leaves in Florida. A superficial

white growth on the leaf surfaces leads to small, light yellow spots on the lower surfaces of the

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areas fall out of the leaves, giving them a shot-hole effect.

Phytophthora Blight: The fungus known as Phytophthora spp, is capable of causing dampingoff, root rot, stem rot, stem girdling, and fruit rot. Cool, wet environmental conditions with high

soil moisture favor disease development. Damping-off occurs in very young plants or in the field

shortly after transplanting and is characterized by rapid wilting and plant death. Spots on the

stems of established plants begin as water-soaked lesions, especially at fruit and branch scars.

These areas can enlarge and girdle the plant, resulting in wilt and death of the plant top. Root

infection can be severe and rapid. The first indication of major root infection often is rapid

browning and wilting of the plants, a foul odor, followed by total collapse within days. Fruit

infection is the most obvious aspect of the disease and potentially very important economically,

because of the possibility of carry-over to the market. Diseased fruit show water soaked spots,

then becomes covered with a characteristic mass of whitish fungal growth and the fruit

eventually shrivels and falls to the ground, where it serves as an important source of inoculum

for root rot (Pernezney et al., 2003).

Papaya apical necrosis is a viral infection with symptoms including a drooping and downward

cupping of the leaves, reduced leaf size, and browning of the leaf margins. At present there is no

control for this disease.

SOME ROT DISEASES ASSOCIATED WITH PAPAYA PLANT

Watery Soft Rot: Watery soft rot, caused by the fungus Rhizopus stolonifer, is a common postharvest disease of papayas. It is important only during fruit storage and transit hence rarely seen

in the field. When Rhizopus infects fruit already packed for market, the watery leakage causes an

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the entire fruit but leaves the cuticle intact. The fungus can grow through any break in the cuticle

and spread rapidly to adjacent fruits, often destroying the entire contents of a box within a few

days. The infected fruit is often covered by a coarse gray to black hairy fungal mass. The

affected fruits quickly become colonized by yeasts and bacteria and have a sour odor. Rhizopus

can enter the fruit tissue only through wounds and cannot penetrate uninjured fruit surfaces.

Therefore, wounding that occurs during harvesting, transporting, or postharvest handling plays

an important role in the development of the disease. The incidence of watery soft rot increases

during rainy weather, in part because of higher inoculum levels, higher humidity, and an increase

in the number of fruit lesions caused by other fungi. High humidity and temperatures of about

250C during storage or transit are optimum for Rhizopus soft rot development.(Bowcamp et al.,

1971)

Erwinia rot

A bacteria disease caused by Erwinia spp, starts with visible spots (lesions), angular, dark brown

to black, greasy and water-soaked appearance on the lower surface of the leaf. Yellowing,

wilting and death of the foliage are characteristized with this disease. The petioles of the affected

leaves show water-soaked spots as do the upper portion of the main stem. Leaves drop, and as

the disease progresses, the top of the plant rots and the tree dies. The giant African snail carries

the bacterium that causes this disease in healthy plants. Chemical control is not recommended for

this disease because there is no effective spray against systemic bacterial infections. Control of

the giant African snail is necessary to avoid the spread of this disease and this has been done

with the use of flat worm that controls the giant African snail.

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Wet fruit rot is caused by the fungus Phomopsis, and in its early stages resembles Rhizopus

watery soft rot. It occurs most frequently as a stem-end rot, although any part of the fruit can be

affected. Symptoms include a discoloration of the tissue around the stem end, which soon breaks

down and becomes colonized by a whitish-gray mold. The fungus grows rapidly, causing lesions

to expand very quickly and extend into the seed cavity. The cuticle over the infected area

remains intact, soft, mushy, and wet but, unlike tissue affected by Rhizopus watery soft rot, does

not usually leak liquids. Wounding of the fruits is required for infection. The disease usually

develops on fully ripened fruit and is rare on green fruits in the field. Control of wet fruit rot, like

the control of many other postharvest diseases of papaya, must begin in the field. Regular field

sprays with protective fungicides help to reduce inoculum levels and prevent infection through

wounds that might occur in the field. Dead leaves should be removed from trees because they

may become heavily infected with Phomopsis and interfere with spraying.

Alternaria Fruit Spot

Alternaria fruit spots are depressed, circular to oval lesions that eventually become black as a

result of pathogen sporulation. Lesions are restricted to the surface of the fruit and do not cause

extensive rotting of the flesh. However, lesions from multiple infection sites can coalesce as they

expand and eventually cover the entire fruit surface. Alternaria fruit spot rarely develops on

fruits kept or ripened at room temperature but, fruits that are kept in cold storage (<100C) for 10

to 14 days will suffer chilling injury and a high incidence of Alternaria. The fungicides

chlorothalonil or mancozeb will significantly reduce postharvest Alternaria development if

sprayed biweekly during fruits growth. However, a preharvest spray program alone does not

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also will reduce the disease.

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3.0

MATERIALS AND METHODS

3.1.1

CULTURE MEDIA

The media used in this study include: Nutrient Agar (Oxoid-Exp: Dec, 2014), McConkey agar

(Oxoid-Exp: Dec, 2014) Potato Dextrose Agar (Oxoid-Exp: Oct, 2014) and Papaya Fruit Agar

(Prepared).

3.1.2. REAGENTS AND INDICATORS

Reagents include: Ethanol, hydrogen peroxide, crystal violet, lugol’s iodine, safranin, 70%

ethanol, malachite green, lactophenol, chloramphenicol, phenol red indicator and methyl red

indicator.

3.2

THE PAPAYA FARM

This is situated inside the premises of Covenant University Canaanland, Ota, Ogun State. It

consists of numerous papaya plants bearing both typical hermaphrodite species. The plants

produce different types of fruit varieties ranging from oval to pyriform ones with the pyriform

(pear-shaped) fruit being the most abundant. The plants are as tall as 192cm and about 50cm in

width.

3.2.1

RAISING OF NURSERY

The nursery is located on the left hand side of the farm and is the site for raising young papaya

seedlings in clean transparent polythene bags. The seedlings were raised in the open as well as in

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the farm plantation for continuous growth and observation.

3.2.2

TAGGING AND MAINTENANCE OF THE NURSERY AND FARM

Twenty-five of the plants were selected for observation of the emergence period of fruit

production as well as observation made on improved plant growth. Measurements were taken of

the top region, middle region and bottom regions of the stem. Maintenance was done by

continuous watering of the plants using watering cans manually. In the nursery, seedlings were

also raised in the flat-boxes (wooden boxes perforated at the base for raising young seedlings)

and were maintained by persistent watering and constant weeding.

3.2.3

COLLECTION OF SAMPLES

Infected and non-infected fruits, stems and leaves samples were collected in sterile sample bags

and universal bottles labelled and taken to the Microbiology laboratory of the Department of

Biological Sciences, Covenant University for isolation of the potential pathogenic agents.

3.2.4

DOCUMENTATION OF DISEASE SYMPTOMS:

Field diagnosis of the disease was based on visual observation of the symptoms on papaya

plants. Observations were made on the fruits, leaves and stems. Symptoms observed were

described and the distribution of the diseased plants in the field was also noted. The reason for

being selective in the area of collection of samples is due predominantly to the abundance of

papaya plants in the area affording us the opportunity of selecting the fruits, stems and leaves not

affected by microbes. The plant material used in this study included the papaya varieties of the

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were collected from the field for isolation of the potential pathogenic agents.

A) Stems: Symptoms include wetness of the stem region as well as white fluffy rot and sooty

rot features.

B) Leaves: Presence of yellow colouration, drooping, dark brown colouration of leaves, also

some with white fluffy rot and sooty rot symptoms found all over the entire leaves

present on their stems.

C) Fruits: Presence of depressed, dark brown coloured, sunken water soaked lesions, black

sunken, dry depressions with white concentric regions, as well as white fluffy rot and

sooty rot symptoms covering the entire skin of fruits and the same symptoms found on

the stems and leaves.

3.2.5

PROCESSING OF SAMPLES FOR CULTURING

A) Rinse Method: The infected fruits were rinsed with sterile distilled water upon

collection. The rinsed fruits were then cultured onto different media for isolation of the

microbes. Different regions which include immediate layer, inner layer, after wash of

both ripe and unripe fruits, water from rinsing the seeds and others.

B) Swab Method: Sterile swab sticks were employed in collecting samples from the

infected regions of the fruits, stems and leaves that had soot, white rot and dark-brown

sunken soft regions .The inoculated swabs were then used to make an inoculum pool on

the surface of well dried agar plate and spread out by streaking.

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order to obtain immediate layer of the fruit and inner pulp containing seeds from which

samples were taken of these regions for culture.

D) Preparation of Dilution: Dilutions of 10-1 to 10-9 were prepared. First 1g of the sample

was dispensed in 9ml of sterile distilled water, mixed and allowed to settle. From this

tube, 10-1 dilution was prepared by pipetting 1ml into another test-tube containing 9ml of

sterile distilled water and homogenized. This gave a dilution of 10-2. This dilution was

prepared to obtain the other dilutions.

3.2.6

CULTURE TECHNIQUE

A) Direct Inoculation: The samples were plated out directly onto the media after cutting out

small portions of infected parts using sterile scalpel. These cut out portions were

asceptically placed either onto or into the media for isolation.

B) Streak Method: Inoculating loop was sterilized first with ethanol and then flamed until

red-hot in spirit lamp. The sterilized loop was then used to pick up the samples from the

infected fruit and streaked across the agar plate in 4 planes on the different media.

C) Pour Plate Method: Sterile distilled water (9ml) was dispensed into 10 labelled sterile

test-tubes. The scooped infected fruit portions (1gm) were added to 9ml of sterile distilled

water in a test-tube. After thorough agitation, 1ml was drawn with a sterile pipette into

another test-tube containing 9ml of sterile distilled water. This serial dilution was

repeated to produce dilutions of 10-1 through 10-9. Aliquots of 2.0ml from tubes 10-3, 10-4

and 10-5 were asceptically transferred into 18ml of molten agar in universal containers,

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aerobically at 37 0C for 18-72 hours.

3.3

STERILIZATION OF GLASSWARES

Dry heat was the method employed with the hot air oven being the equipment used. The

glasswares include: pipettes, test-tubes, McCartney bottles and Durham tubes. They were

sterilized in the hot air oven at 1600C for 1 hour. Pipettes were dried and kept in a canister before

sterilization. Test-tubes were wrapped in aluminum foil, scalpels were wrapped in aluminum foil

while the beakers, measuring cylinders and conical flasks were plugged with cotton wool before

sterilization.

3.3.1

PREPARATION OF MEDIA

NUTRIENT AGAR AND POTATO DEXTROSE AGAR

Media were prepared by weighing approximate amount of the powder and dissolved in 1 litre of

distilled water in a conical flask. The flasks were then plugged with cotton wool wrapped with

aluminium foil and sealed firmly with masking tape. The media were then homogenized by

boiling before sterilizing in the autoclave at 1210C for 15minutes.The sterile media were allowed

to cool to about 450C before being poured into sterile petri-dishes and allowed to set.

3.3.2

PREPARATION OF PAPAYA FRUIT AGAR MEDIA

Papaya fruit agar was prepared by first washing the healthy papaya fruits with sterile distilled

water and sterilized with 70% ethanol. After which the skin was peeled and the clean pulp

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conical flasks and steamed at 1000C in the autoclave, upon cooling the mixture was sifted using

muslin cloth and filtered. Agar agar (2% wt/v) was added to the filtered papaya fruit extract and

autoclaved at 1210C for 15minutes.

3.3.3

ISOLATION OF MICROORGANISMS

Nutrient Agar was used for the isolation of bacteria while the Potato Dextrose Agar was used for

isolating fungi. Papaya agar was used as a general purpose media for bacteria and fungi. Media

used were prepared according to manufacturer’s specification. The Nutrient Agar plates were

incubated aerobically at 370C for 24 hours. The Potato Dextrose Agar plates were incubated at

280C for 3-5 days for mould isolates and 370C for 24 hours for yeast isolates. Papaya agar plates

were incubated for bacteria and fungi as specified. Potato Dextrose Agar was fortified with 2ml

of chloramphenicol to prevent bacterial growth. The samples from the fruit rot were directly

streaked out onto the NA and PDA plates. The rotten parts of the infected fruits were also picked

using sterile swab sticks and were used to seed NA and PDA plates and spread out by streaking.

The inoculating loop was flamed after each streaking in order to allow for the reduction in

population and growth of distinct colonies on plates as well as for easy identification. For the

leaf and stem rot samples, 1g of each of the samples was weighed into sterile test-tubes to which

9ml of sterile distilled water was added and serially diluted. From an appropriate dilution of 10-4,

1ml was pipetted into both NA and PDA plates which were labelled properly and incubated

appropriately. The workbench was disinfected with 70% ethanol. PDA plates were incubated on

the workbench at room temperature (25-280C) for 5 days. NA plates were incubated making use

of the incubator at 370C for 24-48 hours.

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PROCEDURE FOR SUBCULTURING

Pure isolates were obtained by selecting discrete colonies and having them subcultured onto

petri-dishes containing freshly prepared PDA and NA media. The fungal isolates were sub

cultured onto PDA plates using a 5mm cork borer while the bacterial isolates were streaked out

onto NA plates respectively.

3.3.5

PRESERVATION OF ISOLATES

Pure isolates were inoculated onto freshly prepared NA and PDA slants in McCartney bottles for

bacteria and fungi preservation respectively. NA cultures were incubated for 24 hours in an

incubator at 370C and then stored in the refrigerator at 40C. PDA cultures were incubated for 24

hours on the workbench (280C) and stored in the refrigerator at 40C.

3.3.6. IDENTIFICATION AND CHARACTERISATION OF ISOLATED STRAINS

3.3.6.1.

GRAM’S STAINING

The pure bacterial isolates were stained according to Gram’s techniques as described by Baker

(1967). This consisted of the following steps:

A thin smear was prepared on clean glass slide, air dried, and heat fixed by placing the slide

gently over the flame of the spirit lamp.

The smear was stained with crystal violet for 1 minute, and then rinsed with tap water.

The smear was then covered with Lugol’s iodine for 60 seconds and washed off under gentle

running tap water.

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and then counterstained with safranin for 30 seconds. It was again rinsed with tap water and the

slide blotted dry with a piece of filter paper. The stained cells were examined with the oil

immersion objective lens of the light microscope. The gram positive organism is characterized

by a purple colour while a gram negative organism takes on a pink colour as well as the shape of

the cells were also examined.

3.3.6.2

SPORE STAINING

The procedure for this is as follows:

A thin smear was prepared on a clean glass slide, air dried and heat fixed. The smear was flooded

with Methylene blue for 5 minutes. The smear was counter stained with carbon fuschin for 30

seconds. This was then rinsed again with tap water and blotted with filter paper. The stained cells

were then examined under the oil immersion objective. The spores were coloured green and

vegetative cells red.

3.3.6.3

MOTILITY TEST

The stabbing technique was used to carry out this test. Test-tubes containing sterilized Sim Agar

were prepared. Sterilized inoculating needle was used to pick up isolates from their pure cultures.

Each test-tube was stabbed with the needle rubbed with each isolate in the middle. The test-tubes

were then incubated at 370C for 24hours. After 24 hours, the tubes were observed for the motility

of the isolates. A motile isolate usually grows away from the point where the medium was

stabbed.

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BIOCHEMICAL TESTS

3.4.1. CATALASE TEST

This demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from

hydrogen peroxide (H 2 O 2 ). A suspension of the bacterium colony was made on a clean glass

slide with about 0.2ml of sterile water and followed by the addition of 0.5ml of hydrogen

peroxide over it. The production of gas bubbles indicated a positive reaction.

2 H 2 O 2 →2 H 2 O + O 2

3.4.2. CITRATE UTILIZATION TEST

This test demonstrates the use of citrate as a sole source of carbon by alkalinization of the

medium by the bacteria. The inocula were inoculated into slants of sterilized Simmon’s citrate

agar and incubated at 370 C for 72 hours. Positive result changes the green colour of the agar to

blue colour, indicating the presence of citrate utilizing bacteria.

3.4.3

COAGULASE TEST

This test demonstrates the ability of bacteria to produce coagulase as a defence mechanism by

clotting the areas of plasma around it thereby enabling them to resist phagocytosis. Isolated

cultures were emulsified in drops of saline, one serving as real and the other as the control. Few

drops of plasma were dropped onto the real bacterial suspension. Immediate coarse clumping of

the mixture within 5-10 seconds indicates positive coagulase test.

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INDOLE TEST

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to

indole. Isolated cultures were inoculated into peptone broth medium, incubated at 370C for 48

hours after which 5 drops of Kovac’s reagent was added to the culture. A red ring indicates

indole production.

3.4.5. VOGES PROSKAUER TEST

This reaction depends on the production of dextrose of acetyl methyl carbinol or acetoin, which

is oxidized by the addition of alkaline to diacetyl, which gives a pink colour (Baker, 1967).

Cultures were inoculated into test-tubes of 5ml Methyl Red-Voges Proskauer medium incubated

at 370C for 2 days. Drops of 10% potassium hydroxide were then added and the test-tube left for

1 hour. A positive reaction was indicated by the development of rose pink colour.

3.4.6. UREASE TEST

Bacteria particularly those growing in an environment exposed to urine may decompose by

means of enzyme urease.

NH 2 CO.NH 2 + H 2 O→2NH 3 +CO 2

The occurrence of this enzyme can be tested for by growing the organism in the presence of urea.

These isolates were inoculated into slants of urea medium and incubated at 370C for 24 hours.

Urease positive cultures produced a red-pink colour due to changes in the colour of the indicator.

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The methyl-red test was employed to detect the production of acid during fermentation of

glucose such that the pH of a culture was sustained below a value of 4.5 as shown by the change

in colour of the methyl-red indicator which was added at the end of the period of incubation.

Cultures were inoculated into 5ml of MR-VP medium and incubated at 370C for 3 days.5 drops

of methyl red indicator was then added. A bright red colouration indicated a positive reaction

while a yellow colouration indicated a negative reaction.

3.4.8. OXIDASE TEST

Oxidase test was employed to detect organisms that use O 2 as an electron acceptor during the

oxidation of reduced cytochrome c to form water and oxidized cytochrome c. A fresh 1%

solution of dimethyl-paraphenylene diamine dihydrochloride was prepared the same day. A few

drops were poured onto the surface of the agar plate so as to cover it and later decanted. Positive

colonies turned purple within 10 seconds.

3.4.9. HYDROGEN SULPHIDE PRODUCTION

This test is used to identify Enterobacteriaceae whereby Hydrogen sulphide is produced when a

sulphur containing amino acid is decomposed by the enzymatic action of the bacterium. Innocula

were kept in sterilized McCartney bottles containing nutrient broth. A lead acetate paper strip

was inserted in the neck of the tube above the medium and was incubated at 370C for 24 hours. A

blackening of the lower part of the paper strip indicates hydrogen-sulphide production.

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The sugar solutions were 1% of glucose, lactose, sucrose, maltose, arabinose, galactose and

fructose. The medium used was peptone broth. The different sugars were prepared and sterilized

with that of glucose and fructose at 1150C for 10 minutes while the other sugars at 1210C for 15

minutes. Phenol red was used as indicator for acid production. 10ml of the medium was

dispensed into test tubes containing inverted Durham tubes to detect the presence of gas by the

isolates. Tubes of each of the carbon sources were inoculated with an organism and incubated at

370C for 4 to 7 days with daily observation for changes in colour of the indicator. The

uninoculated tubes served as Control.

3.5.0

IDENTIFICATION OF MOULD ISOLATES

The fungal growths that appeared were primarily identified using cultural and morphological

features. The mould isolates were identified by staining with Lactophenol cotton blue. It allows

for the identification of various fungal structures such as presence or absence or rhizoids,

hyphae, spores as well as other additional structures. And the procedure for this is as follows:

• Two drops of lactophenol cotton blue reagent was placed on a clean, grease-free glass

slide.

• A small tuft of the fungus was obtained using sterile inoculating needle and transferred to

the glass slide.

• A cover slip was placed over the preparation and examined under the microscope using

magnification of X400.

• The Fungal structures of importance were observed.

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Hyphae: Septate or Non-septate

Mycelium: Coloured or Non-coloured

Spores: Types of asexual, Nature of spores

Presence of special structures: such as stolon, rhizoids and foot cells.

Pathogenicity test: To ascertain pathogenicity of the various fungi isolated, freshly harvested

matured uninfected fruits were surfaced sterilized with 70% alcohol and rinsed in sterile distilled

water according to Chukwuka et al (2010). With a 5mm diameter sterile cork borer, 2cm long

cylindrical cores were removed from each fruit; discs of 7-day old cultures of each isolate were

removed from agar plates and placed in the bored holes on each fruit. Vaseline jelly was smeared

to completely seal each hole (Ebele, 2010). After one week of incubation at room temperature,

(28 ± 20C) the inoculated fruits were incised and observed for disease development. The fungal

colonies that appeared were primarily identified using cultural and morphological features

(Barnett and Hunter, 1972). Rot symptoms developed with different fungal isolates were

compared to the natural original rot (Ebele, 2010).

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RESULTS

The results in fig.4.1.shows that the most isolated bacteria from the soft rot papaya samples were

Bacillus spp and Staphylococcus spp with 85.7% each while Mucor spp was the fungal genus

most isolated (fig.4.2). From the results in fig 4.3, the bacterial isolate most occurring from the

white fluffy rot samples was Bacillus spp (37.5%) and Penicillium spp (37.5%) was the most

occurring fungus as obtained in fig 4.4. Pseudomonas spp was the most isolated bacteria (62.5%)

as shown in fig.4.5 while in fig 4.6 Penicillium spp was observed to be the most isolated fungi

with 50%.

In figs 4.7 and 4.8, bacterial and fungal isolates with the highest frequency of occurrence from

the soft rot were Staphylococcus spp (66.7%) and Aspergillus niger (52.4%) respectively while

for the sooty rot Pseudomonas spp (75%) was the most isolated bacteria and Penicillium spp

(56.3%) was the fungal isolate with the highest frequency of occurrence. Results from figs. 4.7

and 4.8 showed Bacillus spp (60%) and Aspergillus fumigatus (37.5%) to be the most occurring

bacterial and fungal isolates respectively from the white fluffy rot. The results of figs 4.7 and 4.8,

also showed the least occurring bacterial and fungal isolates from all three main types of rot

diseases to give the following values respectively, Pseudomonas spp (23.8%), Penicillium spp

(4.8%) from soft rot for the bacterial and fungal isolates respectively; Staphylococcus spp

(43.8%), Aspergillus fumigatus (25%) from the sooty rot for the bacterial and fungal isolates

respectively while Staphylococcus spp with Pseudomonas spp (43.8%) as well as Mucor spp

(31.3%) from the white fluffy rot as the bacterial and fungal isolates respectively. The most

isolated bacteria genus from all the thirty-three samples collected for microbial investigation was

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thirty-three samples was Mucor spp with 60.6% (figs 4.9 and 4.10).The Papaya fruit agar

supported the growth of similar microorganisms with Staphylococcus spp and Mucor spp being

the most isolated bacteria and fungi genera giving 83.3% and 91.7% respectively. Results in

fig.4.17 indicates Mucor spp (60%) to be the most reisolated followed closely by Aspergillus spp

(53.3%) while the least reisolated were Aspergillus fumigatus (26.7%) and Colletotrichum

gloeosporioides (26.7%) from the reisolation procedure following pathogenicity test.

Table 4.0:

Sample Types and Distribution

SAMPLE TYPE

TOTAL NO COLLECTED

Fruits

11

Leaves

11

Stems

11

Total

33

48

Samples

Cultural

Microscopic

Organisms

characteristics

characteristics

Identified

F1

Black fluffy growth Thick septate hyphae with Aspergillus niger

with white edges

conidia borne in chains

from the sterigmata

F2

Lemon green powdery Green conidiospores with Aspergillus flavus

growth

septate hyphae

F3

Grey-green

growth

fluffy Septate

with Aspergillus

fumigatus

borne on

hyphae

conidiospores

conidia

F4

White, heavy, wooly, Thick non-septate hyphae Mucor spp

fluffy growth covering with dark sporangiospores

entire plate

F5

Velvet,

flat,compact, Septate hyphae with erect Collectotrichum

grayish

white

in conidiophores, cylindrical gloeosporioides

concentric manner

shaped conidia

F6

Flat

white

cottony Erect conidiophores, septate Alternaria spp

growth on plate

hyphae with cylindrical

conidia

F7

Blue-green

growth on plate

fluffy Blue-green conidiospores Penicillium spp

borne in multilink chains

KEY:

F1:Fungal isolate 1, F2:Fungal isolate 2, F3:Fungal isolate3, F4:Fungal isolate 4,

F5:Fungal isolate 5.

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Pigmentation

Colony

Motility

morphology

Orange,

Entire,

Yellow, Pink,

convex,

Creamy

smooth

Yellow,

Entire,

Cream, Pink

convex,

Gram

Spore

Shape

Organism

reaction

formation

of cell

Identified

+

-

-

-

+

+

Cocci in Staphylococcus

clusters

spp

Short

Bacillus spp

rods

smooth

Green,

Flat, smooth,

Creamy

shiny-surface

-

-

50

-

Short

Pseudomonas

rods

spp

BACTERIA ISOLATED FROM 33 INFECTED PAPAYA SAMPLES COLLECTED

FROM COVENANT UNIVERSITY PAPAYA RESEARCH PLANTATION

La

C

CI CO H 2 M

V

O

U

IN

Sugar Fermentation

b

T

T

P

XI

R

D

G

L

S

F

M

G

A

L

A

U

R

A

A

R

+

+

+

+

+

+

+

A

S

R

Co

Organism

identified

de

B1

+

+

-

-

-

-

+

-

-

Bacillus

subtilis

B2

+

-

-

-

+

-

+

-

-

+

+

+

+

+

+

+

Bacillus

cereus

B3

+

+

-

-

-

-

+

-

-

+

-

+

+

+

+

+

Staphyloc

occus spp

B4

+

+

-

-

-

-

+

+

-

+

+

+

+

+

+

+

Staphyloc

occus

aereus

B5

+

+

-

-

-

-

+

-

-

+

-

-

+

+

+

+

Pseudom

onas spp

KEY:

B1-B5: Bacterial isolate 1 to Bacterial isolate 5

+: Positive

-: Negative

MR: Methyl Red, CO: Coagulase, H 2 S: Hydrogen sulphide, OXI: Oxidase, UR: Urease,

IND: Indole, CIT: Citrate, AR: Arabinose, MA: Maltose, FR: Fructose, SU: Sucrose,

VP: Voges Proskauer, GL: Glucose, GA: Galactose, CAT: Catalase, LA: Lactose.

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PAPAYAS COLLECTED FROM COVENANT UNIVERSITY PAPAYA RESEARCH

PLANTATION

Table 4.3:

Bacterial Isolates From Sooty and White fluffy Rot Papaya Samples

SAMPLES

Bacillus spp

Staphylococcus spp

Pseudomonas spp

SR

-

+

+

ILR

+

-

+

AWR

+

-

+

WSR

-

-

+

ILU

+

-

-

WU

+

+

-

SU

+

-

+

AWU

+

-

+

SDU

+

-

+

KEY:

+ve: positive, -ve: negative, SU: Sooty ripe, AWU: After wash unripe, ILR: Inner layer

ripe, WU: Water from seeds unripe, SU: Sooty unripe, AWU: After wash unripe, SDU:

Seeds unripe, ILU: Inner layer unripe, IMLR: Immediate layer ripe, IMLU: Immediate

layer unripe.

52

SAMPLES

Fungal Isolates From Sooty and White fluffy Rot Papaya Samples

Aspergillus

Aspergillus

Aspergillus

Penicillium

Mucor

Alternaria

niger

flavus

fumigatus

spp

spp

spp

WSR

-

-

-

+

-

-

SR

+

-

-

+

+

-

ILR

-

-

+

-

-

-

IMLR

-

-

-

-

-

-

WSU

-

-

-

-

+

-

ILU

-

-

-

+

+

-

SU

-

-

-

+

-

-

AWU

-

-

-

+

+

-

SDU

-

+

-

+

-

-

IMLU

-

-

-

+

+

-

WU

-

-

+

+

-

-

KEY:

+ve: positive, -ve: negative, SU: Sooty ripe, AWU: After wash unripe, ILR: Inner layer

ripe, WU: Water from seeds unripe, SU: Sooty unripe, AWU: After wash unripe, SDU:

Seeds unripe, ILU: Inner layer unripe, IMLR: Immediate layer ripe, IMLU: Immediate

layer unripe.

53

Plant Part

Bacteria Isolates From Total of 33 Samples

Number

of Bacillus spp

Samples

Staphylococcus

Pseudomonas spp

spp

Fruit

7

+

+

+

Leaf (SFR)

7

+

+

+

Stem

7

+

+

+

Fruit

2

+

+

+

Leaf(STR)

2

+

+

+

Stem

2

+

+

+

Fruit

2

+

+

+

Leaf(WFR)

2

+

+

+

Stem

2

+

+

+

KEY:

+ve: Positive

-ve: Negative

SFR: Soft Rot

STR: Sooty Rot

WFR: White fluffy rot.

54

Plant Part

Fungal Isolates From A Total of 33 Samples

Number

AN

AFL

AFU

MS

CG

ALTS

PS

of

Samples

Fruit

7

+

-

+

+

+

+

+

Leaf (SFR)

7

+

+

+

+

-

+

+

Stem

7

+

+

+

+

-

-

-

Fruit

2

+

+

+

+

-

+

+

Leaf(STR)

2

+

-

+

-

-

-

+

Stem

2

+

-

+

-

-

-

+

Fruit

2

+

+

+

+

-

-

+

Leaf(WFR)

2

+

-

+

-

-

-

+

Stem

2

+

-

+

-

-

-

+

KEY:

+ve: Positive,

-ve: Negative,

SFR: Soft Rot,

STR: Sooty Rot,

AFL: Aspergillus flavus,

WFR: White fluffy Rot, AN:Aspergillus niger,

AFU: Aspergillus fumigatus,

CG: Colletotrichum gloeosporioides,

MS: Mucor spp,

ALTS: Alternaria spp,

55

PS: Penicillium spp.

21 papaya samples from the Covenant University Papaya Plantation.

56

papaya samples from the Covenant University Papaya Plantation.

57

fluffy rot of papaya samples from the Covenant University Papaya Plantation.

58

papaya samples from the Covenant University Papaya Plantation.

59

of papaya samples from the Covenant University Papaya Plantation.

60

papaya samples from the Covenant University Papaya Plantation.

61

and white fluffy rot from papaya samples from the Covenant University Papaya

Plantation.

62

rot and white fluffy rot from papaya samples from the Covenant University Papaya

Plantation.

63

samples from the Covenant University Papaya Plantation.

64

samples from the Covenant University Papaya Plantation.

65

samples from Covenant University Papaya Plantation using Papaya fruit agar.

66

rot of fruit samples using Papaya fruit agar.

67

68

69

respectively.

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71

72

73

74

75

76

77

78

79

80

81

82

83

84

85

PAPAYA FRUITS USING THE ORIGINAL FUNGAL ISOLATES.

Figure 4.18 : Frequency of occurrence (%) of fungi species isolated from the healthy

papaya fruit samples used for pathogenicity tests.

86

87

88

DISCUSSION

This study revealed the microorganisms associated with some rot diseases in papaya plant. The

presence and isolation of these microorganisms depict that they are the causal agents responsible

for the deterioration of such an economical and medicinal plant. In all the isolates produced from

the 33 samples collected from Covenant University Papaya Plantation, ten microorganisms

namely: Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Mucor spp, Penicillium

spp, Alternaria spp, Colletotrichum gloeosporioides, Bacillus spp, Staphylococcus spp and

Pseudomonas spp, belonging to five fungi and three bacteria genera were isolated and identified.

The Papaya fruit agar made from the pulp of papaya fruit supported the growth of A.niger, A.

fumigatus ,A. flavus, M.spp, B.spp and S.spp but not Pseudomonas spp. From the Papaya fruit

agar media used for culturing inocula from all the samples worked with, Staphylococcus spp and

Mucor spp were the most isolated bacteria and fungi genera giving 83.3% and 91.7%

respectively.

The most frequently isolated bacteria from the soft rot samples was S.spp and was isolated from

85.7%, 42.9% and 71.4% of the fruits, stems and leaves respectively, while P. spp was the least

frequently isolated bacteria from all the plant parts. The fungi most frequently occuring from the

soft rot fruits was M. spp while A. niger was the most frequently isolated fungi from the stem rot

(57.1 %) and leaf rot (42.9%). From the fruit samples, only C. gloeosporioides was isolated but

not from the stem and leaf samples of the soft rot. Bacterial and fungal isolates associated with

the soft rot symptoms were Staphylococcus spp (66.7%) and Aspergillus niger (52.4%)

respectively while the sooty rot had Pseudomonas spp (75%) and Penicillium spp (56.3%) as the

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bacterial and fungal isolates to be Bacillus spp (60%) and Aspergillus fumigatus (37.5%)

respectively (figs 4.7 and 4.8). The results of figs 4.7 and 4.8, also showed the least occurring

bacterial and fungal isolates from all three main types of rots: Pseudomonas spp (23.8%),

Penicillium spp (4.8%) from soft rot for the bacterial and fungal isolates respectively;

Staphylococcus spp (43.8%), Aspergillus fumigatus (25%) from the sooty rot for the bacterial

and fungal isolates respectively while Staphylococcus spp with Pseudomonas spp (43.8%) as

well as Mucor spp (31.3%) from the white fluffy rot as the bacterial and fungal isolates

respectively. The microbial load of symptomatic ripe fruits was higher than those of

symptomatic unripe fruits. Staphylococcus spp and Aspergillus niger were isolated from the

symptomatic ripe fruits but not from any of the symptomatic unripe fruit. The fewer organisms

isolated from unripe fruits may be associated with the high acidity of unripe fruits and the

profuse secretion of latex (Guptha et al., 1986; Liao et al., 1987). From all the thirty-three

samples collected for microbial investigation Bacillus spp was bacteria genus most isolated with

an occurrence of 97% while the highest occurring fungal isolate from the thirty-three samples

was Mucor spp with 60.6% (figs 4.9 and 4.10). Two species of Bacillus spp and Staphylococcus

spp were characterized based on their reactions to methyl red and vogues proskauer tests for

Bacillus spp while Staphylococcus spp were distinguished based on oxidase and urease tests thus

the bacteria species identified were: Bacillus subtilis, Bacillus cereus, Staphylococcus aureus

and Staphylococcus spp. This is consistent with the findings of previous studies (ICSMF, 1998;

Okonko et al., 2008).

Tables 4.5 and 4.6 showing the bacterial and fungal isolates from the sooty rot samples, revealed

that the soot is due to the presence of Pseudomonas spp, Penicillium spp, Aspergillus niger and

90

Penicillium spp for bacterial and fungal isolates respectively. Although the after wash of skins of

the fruits from the two types of symptoms revealed Pseudomonas spp being present on the skin

surface when cultured and even from the seeds.

A total of 15 healthy papaya fruits were used for the pathogenicity tests which involved the

reinoculation of the original fungal isolates into the healthy fruit samples. 10 healthy fruits

served as the real experiments while the remaining 5 samples served as the control experiments.

The results of the pathogenicity test conducted according to Chukwuka (2010) in fig.4.17

showed Mucor spp (60%) to be the most reisolated followed closely by Aspergillus niger

(53.3%) while the least reisolated were Aspergillus fumigatus (26.7%) and Colletotrichum

gloeosporioides (26.7%) following pathogenicity test. The results of the pathogenicity tests

carried out showed that all the organisms were pathogenic and were the actual causal agents of

spoilage of the fruits used. The soft rot symptoms obtained were similar to those observed

previously on the fruits when subjected to identification procedures. The moulds seen were the

same as those of the isolated fungi of fresh fruits which were subject to spoilage. The fruits

changed colour slightly after infection, becoming soft, easily punctured with a finger at the point

of inoculation and each infected fruit gave the initial organism that caused the spoilage of the

fruit. The test also established the fact that fungi caused deterioration of the fruits when they

gained entrance into them through mechanical injuries such as bruises and wounds as noted by

Zitter (1985). Mucor spp and Aspergillus niger grew at a faster rate than the remaining fungal

isolates which was evident in their cause of spoilage in the fruits at a faster rate leading to rapid

disintegration of treated fruits in 3-5 days when compared to the other fungi and Aspergillus

flavus was least pathogenic causing the least amount of rot on fruits. The isolation of these

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(1999), Baiyewu et al., (1994, 2007) and Chukwuka et al., (2010) that Rhizopus spp and A. niger

found associated with rotten papaya are highly pathogenic causing appreciable losses in papaya

fruits at post-harvest. These organisms have been implicated in food spoilage both in the field

and when stored (Baiyewu et al., 1994). Microorganisms are generally ubiquitos in nature which

shows why the same microbes isolated from the fruits were also isolated from the stems and

leaves be it on the surface or internal regions of these samples.

Numerous microbial defects (signs and symptoms) of agricultural crops are characterized by the

types of microorganism responsible for the deterioration; the process of infection in the case of

fungal invasion follows the development of fungal penetrating structure (appresorium).

Colonization of fungi is a critical phase in the microbial spoilage of fruit and involves the ability

of the microorganism to establish itself within the produce, initiated when fungi depolymerises

certain specific cell wall polymers (such as protopectin, the cementing substance) of the produce

(Snowdown, 1988). Susceptibility of fruits and vegetables is largely due to differential chemical

composition such as pH and moisture content. The occurrence of fungal spoilage of fruits and

vegetables is also recognized as a source of potential health hazard to man and animals due to

their production of mycotoxin compounds which are capable of causing mycotoxicoses in man

following ingestion or inhalation (Eaton et al., 1994; Baiyewu, 2007). The prevalence of fungi

as the spoilage organism of fruits and vegetables is due to a wide range of factors which are

encountered at each stage of handling from pre-harvest to consumption and is related to the

physiological and physical conditions of the produce as well as the extrinsic parameters to which

they are subjected. Damage inflicted on produce at the time of harvest is a major cause of

infection since most of the spoilage microorganisms invade the produce through such damaged

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Furthermore, the incidence of infection is worsened by poor sanitary practices such as crosscontamination, contact infection during the transportation of produce (Effiuvwevwere, 2000).

The magnitude of the symptoms of the induced disease is a reflection of the extent of

colonization (Chuku et al., 2008). Efiuvwevwere, (2000) reported that high moisture and relative

humidity led to greater fungal growth in agricultural produce and thus low storability of fruits

and vegetables. The main causes of post-harvest losses for tropical crops include: mechanical

damage due to mishandling along the supply chain; loss of moisture through evaporation and

transpiration which causes shriveling; early senescence and death of tissue due to interruption of

metabolic rate when stored in higher than optimum or extremely low temperature; low shelf life

due to ethylene biosynthesis; and spoilage and rot due to the invasion of pathogens on injured

fruits. The high prevalence of some fungi demand that appropriate control measures against

infection, should be employed if farmers expect good performance of their produce. Adequate

microbiological knowledge and handling practices of these produce would therefore help

minimize wastes due to deterioration and unacceptability. The high moisture content of fruits and

vegetables will be a serious limiting factor in their preservation.

5.1

RECOMMENDATIONS AND CONCLUSION

Almost all post-harvest diseases of fruits and vegetables are caused by fungi and bacteria. Fruits

and vegetables are highly perishable products, the quality being affected by improper postharvest handling, transportation, storage and marketing thus resulting in decay and production of

microorganisms, which become activated because of the changing physiological state of the

fruits and vegetables (Wilson et al., 1991). Post-harvest diseases are often classified according to

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initiates infection of the host usually before harvest, but then enters a period of inactivity or

dormancy (walled off or inactivated by a host reaction) until the physiological status of the host

tissue changes in such a way that triggers for the reactivation of quiescent infection . Examples

of postharvest diseases arising from quiescent infections include anthracnose of various tropical

fruits including Carica papaya caused by Colletotrichum gloeosporioides (Kader, 2002). The

other major group of post-harvest diseases is those which arise from infections initiated during

and after harvest with these infections occuring through surface wounds created by mechanical

or insect injury. Common post-harvest diseases resulting from wound infections include blue and

green mould (caused by Penicillium spp.), transit rot (caused by Rhizopus stolonifer) bacteria

soft rot (Kader, 2002). Since pathogenic fungi alone caused 10-30% reduction in the yield of

major food and cash crops (Agrios, 2005), several pre and postharvest technologies have been

used to control their decay (Serrano et al., 2005). Although several synthetic chemicals in form

of pesticides and fungicides have been used to curb the menace of pre-harvest and post-harvest

diseases of papaya plants but some have been removed from the market due to possible

toxicological risks.

Therefore, there is the need to develop new and acceptable pre-harvest and post-harvest

disinfestation methods, in this way, the use of natural products, either directly as crude

preparations, or as pure compounds, can be a very attractive method for pre-harvest and postharvest disease control of papaya. These will help farmers, marketers and consumers take the

necessary precautions in preventing contamination of fruits and vegetables, thus reducing the

risk of infections with mycotoxins which are harmful to humans. Pre-harvest controls basically

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including:

Sanitation: This involves any measures that can be taken to reduce the spore load and is

regarded as most desirable such as removal or destruction of diseased plant parts is an obvious

first step. Removal or destruction of infected parts reduces sources of primary and secondary

infection (Wills et al., 1998).

Chemical application: Fungicide sprays during the flowering period may provide excellent

control. Latent infections with certain organisms can also be controlled after they become

established by the use of the newer “systemic” fungicides such as benomyl.

Plant nutrition: Higher or excess nitrate fertilization increases susceptibility to decay by

bacteria soft rot organisms.

While Post-harvest control methods which focus on reducing the growth of pathogenic

microorganisms in the wounded areas, can be achieved through the following measures which

include:

Sanitation: The inoculum load on field-bins, picking bags, brushes, rollers, and graders can be

very high. Attention to hygiene in these areas, filtering and chemical treatment of water are

primary means of reducing the risk of severe infection of harvested produce. One of the most

effective chemicals for sterilization of water and surfaces is hypochlorous acid, prepared by

adding calcium or sodium hypochlorite to water.

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atmosphere through various manipulations can be reduced to 00C which will inhibit the growth

of fungi.

Chemical treatments: Use of chemical treatments for post-harvest disease control requires a

knowledge of the fungus and of the mode of action of the chemical. In general, chemicals are

most effective during the lag phase before infections become firmly established in the tissues.

Pre-harvest treatment of Captan 5000ppm is effective against watery rot of papaya (Pathak et al.,

1976). Field spray of mancozeb reducing Rhizopus soft rot incidence by reducing field initiated

fruit diseases (Nishijima et al., 1990). Use of Bordeaux mixture for Colletotrichum

gloeosporioides at pre-harvest period can control pre-harvest as well as post-harvest infections.

Radiation: Used for elimination of fungal and bacterial diseases of fruits and vegetables using

some radiation treatments like gamma radiation. The use of gamma radiation has also been

developed to slow down the ripening process and to disinfect and pasteurize the fruit surface.

Techniques like waxing and gene manipulation have also been used to reduce the ripening rate.

This field of biotechnology is now the focus of much research to reduce the problem of losses

due to poor shelf life of tropical fruits. In storage under cold conditions, use of modified

atmosphere packaging (MAP) has been employed for temperate fruits (Morris et al., 2003).

Hot water treatments: Effect of hot water treatment between 50 - 60°C for a few minutes can

kill fungi but can be tolerated by the plant hence care must be taken to ensure good temperature

control and duration as injury to the product could result. Use of hot water treatment combined

with the vapour heat treatment provides adequate control of rot diseases (Yaguchi et al., 1993).

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to produce toxins that inhibit the pathogen and also out compete pathogen for nutritional

resources. Two products registered in 1995 namely; Pseudomonas syringae (Bio-Save) and

Candidia oleophila (Aspire) can be applied as dips, drenches or sprays on the plant. Planting of

disease-resistant cultivars is also a biocontrol measure of reducing infection rates.

Growth regulator treatments: The use of growth regulators to maintain the harvested organ’s

own defenses and prevent decay.

5.2

CONTRIBUTION TO KNOWLEDGE

From this study, the following were noted as the contributions to knowledge and they include:

•

The identification and characterization of the pathogenic microorganisms associated with

the soft rot of papaya fruits present on Covenant University Papaya Plantation to be

Mucor spp and Aspergillus niger.

•

The experimental preparation and use of papaya fruit agar.

•

The use of the papaya fruit agar in supporting and confirming the growth of

microorganisms that are pathogenic as well as non-pathogenic species.

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APPENDIX

A: PREPARATION OF SOLID MEDIA AND REAGENTS

Nutrient Agar (Oxoid)

Composition

Peptone

5.0

Beef

3.0g

Agar

15.0g

Preparation method

The nutrient agar was prepared according to manufacturer’s direction as written on the media

container, 28g of the media was diluted in 1000ml(1L) of distilled water. The agar was then

mixed with a stirrer and allowed for a while to sit in the water bath until when completely

dissolved. It was then sterilized in an autoclave for 15 minutes at 1210C.The sterilized medium

was cooled and then dispensed into Petridishes already containing the innoculum or the

innoculum streaked directly on the agar surface.

Sabouraud dextrose agar (Oxoid)

Composition

Potato Extract

40g/l

Dextrose

20g/l

Agar No 1

15g/l

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The media was prepared according to manufacturer’s direction as written on the media container,

62g of the media was diluted in 1000ml(1L) of distilled water. The agar was then mixed with a

stirrer and allowed a for a while to sit a water bath until dissolved. It was then sterilized in an

autoclave for 15 minutes at 1210C. The sterilized medium was cooled and then dispensed into

Petridishes already containing the innoculum or the innoculum streaked directly on the agar

surface.

McConkey agar(Oxoid)

Composition

Lactose

10g

Bile Salts

1.5g

Sodium Chloride

5g

Neutral Red

0.03g

Crystal violet

0.001g

Agar

13.5g

Preparation method

The media was prepared according to manufacturer’s direction as written on the media container,

50g of the media was diluted in 1000ml(1L) of distilled water. The agar was then mixed with a

stirrer and allowed a for a while to sit a water bath until dissolved. It was then sterilized in an

autoclave for 15 minutes at 1210C. The sterilized medium was cooled and then dispensed into

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surface.

Urease

Composition

Peptone

10.0g

Potassium chloride

5.0ml

Agar

9.0g

Preparation method

The media was prepared according to manufacturer’s direction, 13g of the media was diluted in

1000ml(1L) of distilled water. The agar was then mixed with a stirrer and allowed a for a while

to sit a water bath until dissolved. It was then sterilized in an autoclave for 15 minutes at 1210C.

The sterilized medium was cooled for another 10minutes and 20ml of 4% urea solutionwas

added and mixed thoroughly with the agar. The medium was then aseptically dispensed into

Bijou bottles and allowed to set in a slant position.

Sugars’s phenol red broths( glucose,sucrose, lactose, fructose, maltose, arabinose and galactose

phenol red broths)

Composition

Crypticase

10g

Sugar

5g

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0.018g

Preparation method

The medium was prepared according to manufacturer’s direction, 5g of each sugar was dissolved

in 100ml(0.1L) of peptone water(preparation is described below). The medium was dispensed

into Bijou bottles and Durham tubes were inverted in the bottles, the tubes were completely filled

with the medium.The agar was then gently mixed with a stirrer and allowed a while to sit in a

water bath until completely dissolved and sterilized by autoclaving at 1210C for 15 minutes.

Peptone water/broth

Composition

Peptone

4g

Preparation

The medium was prepared according to manufacturer’s direction,1 5g of

the media was

dissolved in 1000ml(1L) of distilled water. The medium was then mixed with a stirrer and

allowed a while to sit in a water bath until it dissolved for 10 minutes. The medium is not

sterilized yet because it is used in the preparation of the sugars’ broth and so finally sterilized

after that preparation.

NOTE: About 20ml of the agar medium is dispensed per petri dish/ plate while 10ml of the agar/

broth medium is dispensed per Bijou bottles.

Gram’s stain

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Composition

Solution A

Crystal violet(90% dye content)

2g

Ethanol(95%)

20ml

Solution B

Ammonium oxalate

0.8g

Distilled water

80ml

Preparation

Solution A and B were mixed together and stored in a glass-stopped bottle.

Gram’s iodine solution

Composition

Iodine

1g

Potassium chloride

2g

Distilled water

300ml

Preparation

The above constituents were mixed thoroughly and stored in an amber glass bottle.

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Composition

Ethanol(100%)

95ml

Distilled water

5ml

Preparation

The above constituents were mixed thoroughly and stored in a glass-stopped bottle.

Counter stain(Stock Safranin)

Composition

Safranin O

0.25g

Ethanol(95%)

10ml

Distilled water

100ml

Preparation

The above constituents were mixed thoroughly and stored in a glass-stopped bottle.

Catalase

Reagents: Hydrogen peroxide(2H 2 O 2 (aq))

Principle: The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and

water . 2H 2 O 2 (aq)→2H 2 O(l) + O 2(g)

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inoculums is emulsified on a glass slide already containing 3% hydrogen peroxide and the rapid

elaboration of oxygen bubbles occurs.

Catalase positive- copious bubbles produced

Catalase negative- no or few bubble produced

Citrate Test

Medium: Simmon’s Citrate

Composition

Sodium ammonium hydrogen phosphate

0.15g/l

Potassium dihydrogen phosphate

0.10g/l

Magnesium sulphate

0.20g/l

Sodium citrate

0.20g/l

Bromotymol blue

0.016g/l

Distilled water

1000ml

Preparation

Simmon’s citrate agar was weighed as stipulated by manufacturer and dissolved in 1000ml(1L)

of distilled water. The medium was dispenced into test tubes which were plugged with cotton

wool and sterilized at 1210C for 10 minutes. A pure culture was inoculated into the medium

using a sterile inoculating loop and the citrate agar culture was incubated at 370C for 3 days.

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Medium: Tryptone Soy Broth

Composition of the Medium

Peptone

5g

Sodium chloride

5g

Tryptone

5g

Distilled

100ml

Reagent: Kovac’s Reagent

Composition

Para-dimethyl aminobenzaldehyde

10g

Para amyl alcohol

150ml

Conc.Pure hydrochloric acid

50ml

Preparation

Tryptone broth was prepared according to the manufacturer’s instructions and dispensed in

McCartney bottles. Each bottle was inoculated with a pure culture of the isolate with the aid of a

sterile inoculating loop and incubated at 370C for 6 days. After 6 days of incubation three drops

of Kovac’s reagent were added to each bottle, swirled gently and allowed to stand.

Urease Test

Medium: Urea agar

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Peptone

0.1g

Sodium chloride

0.5g

KHPO 4

0.2g

D(+) glucose

0.1g

Phenol red(0.2% in 50% ethanol)

0.6ml

Agar

2.0g

Distilled water

1000ml

Preparatipon

Urea agar was weighed according to the manufacturer’s instructions and dissolved in distilled

water.It was dispensed in McCartney bottles which were then autoclaved at 1210C for 15

minutes. The bottles were then allowed to cool and set in a slanting position. The prepared agar

slants were inoculated with a pure cultures with the aid of a sterile inoculating loop and

incubated at 370C for 7 days.

Methyl Red Test

Medium: Methyl red-Voges Proskauer medium

Composition

Dextrose

0.5g

K 2 HPO 4

0.5g

Peptone

0.5g

115

1000ml

Preparatipon

A total of 17g of MRVP medium was weighed into 1 litre of distilled water dispensed into

McCartney bottles which were then autoclaved at 1210C for 15 minutes. The bottles were then

allowed to cool and inoculated with the isolates with incubation carried out at 370C for 24 hours.

After the incubation period, five drops of methyl red were added to the cultures.

Sugar Fermentation Test

Composition

Peptone

5g

Sodium chloride

5g

Tryptone

5g

Distilled water

1000ml

Indicator(bromocresol)

0.0025

Fermentable sugar

1.0%

The fermentable sugars used were glucose, fructose, maltose, galactose, lactose, sucrose and

arabinose.

Preparation

20g of peptone water was weighed into a conical flask to which 1 litre of distilled water was

added.1% of the sugar was then added to the peptone water. The peptone water containing the

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15 minutes. The bottles were then allowed to cool and inoculated with the isolates with

incubation carried out at 370C for 48 hours hours. After the incubation period, five drops of

methyl red were added to the cultures.

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