INVITRO EFFECT OF AQUEOUS AND METHANOL EXTRACTS OF DIFFERENT PARTS OF *MURRAYA KOENIGII* (L.) SPRENG ON FUNGI ISOLATED FROM ROTTEN TOMATOES (*SOLANUM LYCOPERSICUM* L.)

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLOUNIVERSITY, ZARIAIN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE, MASTERS IN BOTANY

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ZARIA, NIGERIA

FEBRUARY, 2014

DECLARATION

I declare that the work in this thesis entitled '*invitro* Effect of aqueous and methanol extracts of different parts of *Murraya koenigii* (L.) Spreng on Fungi isolated from rotten tomatoes (*Solanum lycopersicum* (L.)' has been carried out by me in the Department of Biological Sciences under the supervision of Prof. A. U. Khan and Prof. S. O. Alonge. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Grace Abu POLYCARP _____ _____

Signature Date

CERTIFICATION

This thesis entitled "*IN VITRO* EFFECT OF AQUEOUS AND METHANOL EXTRACTS OF DIFFERENT PARTS OF *MURRAYA KOENIGII (*L.) SPRENG ON FUNGI ISOLATED FROM ROTTEN TOMATOES (*SOLANUM LYCOPERSICUM*L.)" by Grace Abu POLYCARP meets the regulations governing the award of the degree, Master of science degree in Botany of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to my Beloved husband Engr. Evarastics Polycarp who stood by me through thick and thin during the course of the research. My beautiful angels and Daddy's pride Jemima (Toks) and Genievieve (Tumuku) for their patience, My Parents Mr and Mrs Stephen Abu who took care of my kids when I was always on the road to Zaria. Most importantly late Dr. Tiseer Aweever, My mentor and Daddy who showed me love when I needed it may your soul rest in peace Amen.

ABSTRACT

The *invitro* effects of extract of different parts (leaves, stem bark and root) of *Murraya koenigii* were determined on fungi associated with Tomato fruits rot. In the present study, the fungi isolated from infected tomato fruit were identified based on Macroscopic and Microscopic characteristics. These included *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Mucor* spp *Rhizoctonia solani* and *Rhizopus stolonifer*. Two different extractive solvents (Water and Methanol) were used. Different concentrations (5%, 10%, 15%, 20% and 25%) of both extracts were used in this study. All the plant extracts, both aqueous and methanol showed significant ($p<0.05$) reduction on mycelia growthof the isolated pathogens when compared with the control. Maximum percentage inhibition was observed with the leaves extract, followed by the stem bark and the Root. Methanol extracts was more effective. Higher concentration of both aqueous and methanol (25%) favoured higher mycelia growth reduction. These Plants are available and the extracts, particularly, *M. koenigii* offers potentially safe alternative for use as botanical fungicides and could be exploited for the effective management of post-harvestharvest fruits disease of tropical fruits.

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ABBREVIATIONS

C_1- Initial concentration

C_2- Final concentration

Dc- Diameter in control Plate

Dt- Diameter in Treated plate

DMRT- Duncan Multiple Range Test

E.g- for example

E- East

‘E- Minute East

e.t.c- and so forth

g- Gramme

hrs- Hours

m- Metre

mg- Milligramme

ml- Millilitre

µm- Micrometre

‘N- Minute North

^{0}N- Degree North

NaOCL- Sodium hypo chloride

PDA- Potatoes Dextrose agar

P^H- Power of Hydrogen

Syn- Synonymous

Sp- Species

SDA- Sabouraud dextrose agar

S.E.M- Standard Error Means

U.S- United States

U.S.A- United States of America

U.T.C- variety of Tomatoes

UV-C- Ultraviolet radiation

V_1- Initial volume

V_2- Final volume

±- Plus or Minus

%- Percentage

‹- Less than

›- Greater than

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Tomato (*Solanumlycopersicum* L), members of Solanaceae, is a native of Peru and Ecuador (Ijato *et, al.,* 2011). Tomato had spread in pre-columbianera far north and Mexico. Tomato was introduced into Europe from Mexico by the Spanish in 1523. At first, it was little used for food because of its relationship with the deadly night shade family (Suleiman, 2011). Tomato was referred to as an object of affection and therefore known as love apple; 'pommels d'amour' in French (Ijato *et, al.,* 2011).

In earlier times, tomatoes were consumed only in Italy but with the beginning of the second half of the 19^{th} century the crop has been widely appreciated for the fine food that it provides. From Europe, it was carried across the Pacific into south eastern Asia. Today it is widely cultivated throughout the warm temperate and tropical regions of the world. Worldwide about 36million tones of tomatoes are produced annually mostly in Europe and North America. The major tomatoes producing countries in order of importance are: United States, followed by Italy, the Soviet Union, Spain, Turkey, Egypt, Greece, Romania and the Arabian countries. Mexico, Japan, Brazil, India and Argentina are other important producers (Wani, 2011).

The tomato fruits are largely composed of water (about 94%) but have moderate quantities of soluble sugars several organic acids (especially citric and Malic acid) mineral salt and relatively large quantities of the vitamin C. Effective and efficient management of

phyto diseases was generally achieved by the use of vitamin C and chemicals (Kiran *et, al.*, 2006, Stinson *et, al.,* 1981, Philips, 1984). Fungi are the most important and prevalent pathogens of a wide range of host plant and causing destructive and economically important disease of most fresh fruits and vegetables during storage and transportation (Sommer, 1985*).*

The use of synthetic fungicides in control approach has proved effective in the control of phyto diseases. Most synthetic fungicides used in controlling some of these phyto pathogens are costly and therefore, not economically viable. The excessive use and misuse of chemical fungicides have raised serious concern about health and environments. These have significant draw back including increase in cost of fungicides, hanging hazard, and concern about fungicides residue in food.

Due to increased awareness about the risks involved in the use of synthetic pesticides, much attention is being focused on the alternative method of pathogen control. The use of pesticides and fungicides of botanical origin has been pin-pointed by many researchers as an option to synthetic fungicides (Amadioha and Obi, 1999, Amadioha, 2000). Synthetic fungicides are not environmental friendly as they pollute the soil, water and air. And by the accumulation of obnoxious chemicals residues as a result of continuous use, leads to the development of resistant race to these chemicals (kiran*et, al.,* 2006).

1.2 Origin and Description of *Murraya koenigii*

Murraya koenigii commonly known as curry leave, the Yoruba calls it Marugbo sanyan, the Binin's calls it Ebafo. It originates from Pakistan Sri Lanka andIndia east to China and Hainan. It has been widelycultivated in South-East Asia and some parts of the

UnitedStates and Australia and the crop is usually propagated by seeds. In tropical Africa it is planted in manycountries, including Nigeria, Kenya, Tanzania and most ofthe Indian Ocean Islands, where Indian immigrants settled (Ajay *et, al.,* 2011)

M. koenigii belongs to the family Rutaceae which represent more than 150 genera and 1600 species (Satyavati *et, al.,* 1987). Rutaceae, is a deciduous to semi–evergreen aromatic tree found in many tropical and temperate region of the world. Curry leaf (*Murraya koenigii*) is an important leafy vegetable. The leaves have a slightly pungent,bitter and feebly acidic taste, and they retain their flavor and other qualities even after drying. Curry leaf is also usedin many of the Indian ayurvedic and unani prescriptions (Parul *et, al.,* 2012). *M. Koenigii* a highly valued plant for its characteristic aroma and medicinal value. The plant is a rich source of carbazole alkaloids (Kumar *et, al*., 1999). Bioactive coumarins, acridine alkaloids and carbazole alkaloids from family Rutaceae were reviewed by (Ito, 2000). Carbazole alkaloid the major constituent of the plant is known to possess cyto-toxic, anti-oxidative, anti-mutagenic and anti-inflammatory activities and anti-fungal activities (Ito *et, al*., 1993).

1.3 Traditional Uses:

Traditionally, the plant is credited with tonic and stomach ache properties (Anonymous, 1988). Its leaves are widely used in Indian and other parts of the world including Nigeria as spice due to its aromatic nature. A volatile oil, curry leaf oil, producedfrom the plant has uses in the soap industry. Green leaves are eaten raw for cure of dysentery, diarrhea and for checking vomiting. Leaves are boiled with coconut oil till they are reduced to blanked residue which is then used as an excellent hair tonic for retaining

natural hair tone and stimulating hair growth (Rao, 2011). Curry leaves are also used in calcium deficiency. It has vitamin A, Vitamin B, Vitamin C, Vitamin B2, Calciumand iron in plenty. Its nutritional value benefits both theyoung and the old alike. Women who suffer from calciumdeficiency, osteoporosis e.t.c can find an ideal naturalcalcium supplement in curry leaves.Fresh juice of curry leaves, with lime juice and sugar, is aneffective medicine in the treatment of morning sickness,nausea and vomiting due to indigestion and excessive use offats (Parul *et, al.*, 2012).

One or two teaspoons of juice of these leaves mixedwith a teaspoon of lime juice may be taken in theseconditions. The curry leaves, ground to a fine paste andmixed with buttermilk, can also be taken on an emptystomach with beneficial results in case of stomach upsets.Also used as laxative (Parul *et, al.*, 2012). Boils and similar eruptions appear onskin during summer. Most of the boils tend to subside overtime, but some may persist and remain painful. Curry leavescome handy in treating such conditions. A paste made ofcurry leaves is applied on these persistent boils for quickrelief.Along with mint leaves and coriander leaves, curry leavecan be used in treating excessive pitta conditions. Curryleaves can be used with effective result to treat burn and bruises. Cataract development can be prevented by using fresh juiceof curry leaves.Kidney pain can be cured by using juice of root of*Murrayakoenigii*. It can be used in preventing premature greying of hair (Jasim, *et, al.*,2008).

The leaves are rich in mono-terpenoids and sequenterpenoids which exhibit antifungal activities (Goutam and Purohit, 1974). Minor furano-coumarines are also reported from the seed (Adebajo and Reisch, 2002).Bark and roots are used as stimulant and externally to cure eruptions and bites of poisonous animals. Leaves and roots are also

used traditionally as bitter, analgesic, curing piles, inflammation, itching and blood disorders (Nadkarni, 1976, Kirtikar and Basu, 1993). It is used as an analgesic, stomach ache, carminative and for the treatment of dysentery and skin eruptions (Chakra borty *et, al*., 1965).Fresh leaves, dried leaf powder, and essential oil are widely used for flavorings soups, curries, fish and meat dishes, eggs dishes, traditional curry powder blends, seasoning and ready to use other food preparations. The essential oil is also utilized for soap and cosmetic aromatherapy industry (Gupta and Nigam, 1970).

The roots extracts also attribute many medicinal properties like antibiotic and anti-inflammatory e.t.c which have been use to relieve pains associated with kidney.

1.4 Phytochemistry of *Murraya koenigii*

Mature leaves contains 63.2 % moisture, 1.15 % total nitrogen, 6.15 % fat, 18.92 % total sugar, 6.8 % crude fiber, ash 13.06 %, acid insoluble ash 1.35 %, alcohol soluble extractive 1.82%, cold water (20˚C) extractive 27.33% and a maximum of hot water soluble extractive 33.45%.30 Constituents that have been stimulated the most interest includes a wide range of carbazole alkaloids, essential oil and carotenoids. (Rao, 2011).

1.5 Statement of Research Problems

Fungi are the most important and prevalent pathogen infecting a wide range of host plants and causing destructive and economically important diseases of most fresh fruits and vegetables during storage and transportation (Jerry *et, al.,* 2005). Some workers reported that Pathogens however, invade this fruit through wound without protective surface. Injuries could occur during harvesting and handling which are good sites for decay development on the fruit surfaces. Tomato fruits are being affected by a large numbers of

fungi which has become detrimental leading to substantial yield loss worldwide. This has become a source of serious concern (Ijato *et, al.*, 2010).

In Nigeria, for instance, about 98% of the food consumed is produced by the local farmers. Most of these farmers are least educated and therefore in most cases cannot practice the various control measures recommended for disease control (Chiejina and Ukeh, 2013, Amadioha, 2000 and Okigbo, 2009). At present the use of synthetic fungicides in controlling these diseases has posed a serious threat to human health and non-target organisms (Ebele *et, al.*, 2011). These chemicals are not available and in addition to they are, very expensive and are not environment friendly and at times not available to peasant farmers in sustainable agriculture. Therefore searching for alternative sources in the need of time (Sallam *et, al.*, 2012)

1.6 Justification

The demand for tomato is very high but the yield is too low to meet the demand due to substantial yield loss by these pathogens (fungi) worldwide. Synthetic fungicides have been used in controlling these fungal diseases and millions of naira is spent by tropical and sub-tropical countries in importing them. A greater number of farmers have no access to synthetic fungicides or cannot afford them.

There is equally a reported complex of health and ecological problems caused by in proper use and over-use of fungicides. This has prompted the search for an alternative plant control because this disease has posed a serious health problem to both humans and non–target organisms (Costa *et, al.* 2000, Duru *et, al.* 2003).) Therefore this calls for a serious

attention for scientists to device other means (alternative methods) through which the growth and development of the pathogens will be prevented.

It is obvious that there has been a considerable interest in the extract and essential oil from aromatic plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganism in food (Soliman and Badeaa, 2002). These extracts have been reported to exhibit anti-fungal properties under laboratory trials and are environmental friendly (Mohana and Raveesha, 2006 and Okigbo and Ogbonnaya, 2006).

This study therefore will enhance understanding the importance and advantages for therapeutic uses of medicinal plants in various diseases of plant and their safety, besides being economically effective and their easy availability. Natural plants products are now used in disease management and this is gaining importance (kiran *et, al*., 2006 and Okigbo, 2009. In this research therefore, aqueous and methanol extracts of the leaves, root and stem of *Murraya koenigii* plants was used as it has been reported to posses anti-oxidative activities due to the presence of carbozole alkaloids one of the potential bioactive component isolated from the leaves as reported by many workers (Vandana *et, al.,* 2012). Ramsewak *et, al.,* (1999) reported anti-oxidative activities due to the presence of Mehanine from the leaves of *M.koenigii.* Carbazole alkaloids the major constituents of the plants are known to posses cytotoxic, anti-mutagenic and anti-inflammatory activities (Manvi and Renu, 2010).

1.7 Aim of the Study

The aim of the research is to isolate and identify fungi associated with post–harvest rot disease of tomato fruits and to assess the efficacy of aqueous and methanol extractsof leaves, stem bark and root of *Murraya koenigii* (L.)(Curry Tree) on the growth isolated fungi.

1.8 Objectives of the Study

The objectives of the study include:

1. To isolate and identify fungi associated with post-harvest rot of tomato fruits.
2. To determine the effects of various concentrations of Aqueous and Methanol extract of *Murraya koenigii* leaves, stem bark and rooton the growth of fungi associated with tomato rot*invitro*.
3. To compare the efficacy of Aqueous and Methanol extracts of *Murraya koenigii*on the growth of fungi associated with tomato rot.

1.9 Research Hypotheses

1. Fungi are not associated with post-harvest rot disease of tomato fruits
2. Aqueous and Methanol Extracts of *Murraya koenigii*leaves stem bark and root have no effect on the growth of fungi associated with tomato rots.
3. There are no significant differences in the efficacy of Aqueous and Methanol extracts of *Murraya koenigii*on the growth of fungi associated with tomato rot.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin,Descriptions, Distribution and Cultivation of Tomato Fruits.

Tomato*Solanum lycopersicum* (L) formerly *Lycopersicon esculentum* belongs to the family Solanaceae and very important vegetable worldwide (Faith *et, al.*, 2005).It'soriginated from the Central America where it was then cultivated as an ornamental plant (Smith, 1994).Tomato was referred to as an object of affection and therefore known as love apple; 'pommels d'amour' in French (Suleiman, 2011). Formerly Tomatoes was believedto be a member of the deadly nightshade family, and as such were considered toxic causing many conditions like appendicitis, "brain fever" and cancer. At first, it was little used for food because of its relationship with the deadly night shade family. In fact they may have just the opposite effect. Tomatoes were not even eaten in the US until the early 1800s, when an eccentric New Jersey gentleman Colonel Robert Gibbon Johnson brought them back from a trip overseas. Always want to take advantage of a dramatic opportunity he announced an amazing display of courage that would take place on September 26, 1820 (Debjit *et, al*., 2012).

He shocked his hometown of Salem by consuming an entire basket of tomatoes in front of a crowd of spectators, expecting it to kill him any second. Obviously, he didn't and since then tomatoes have been a staple food in the American diet and with good reason.As a common fruits vegetable, it rated second in significance to potato in many countries and an important commercial crop in the world (Ijato*et, al.*, 2010). Tomato was introduced into Europe from Mexico by the Spanish in 1523.

It is a relatively short duration crop and gives a high yield. Tomato is an annual herbaceous plant, which can reach a height of over two meters (Sahu *et, al.,* 2012). Tomato is a weak stem, trailing, extensively branched, short leaved perennial, but treated as an annual under cultivation, the leaves are spirally arranged, pinnate and divided simple. The flowers are bond in clusters on both the main axis and lateral branches. The fruits are berries and are hairy when young but becoming smooth, juicy and shiny when ripe (Ijato*et, al.,* 2011).The shape of the fruit differs from cultivar, temperature lowers within the crops and the fruits grow in the shade of the leaves. Because they are covered, the sun does not damage the fruits and they ripen more slowly. Slower ripening and high leaf/fruit ratio improves the taste of the fruits and in particular the sweetness. Vigorous tap roots system that grows to a depth of 50cm or more.

The main root produces dense lateral and adventitious roots. Growth habit ranges between erects and prostrate. The stem is coarse, hairy and glandular. Spirally arranged, leaflets are ovate to oblong covered with glandular hairs, small pinnate appears between larger leaflets. Inflorescence is clustered, flower are bisexual regular and grow opposite or between leaves. (Sahu*et, al.,* 2012), Calyx tube is short and hairy; sepals are persistent usually 6 petals up to 1cm in length yellow and when mature. 6 stamen, anther are bright yellow in colour surrounding the style with an elongated style with an elongated sterile tip.

Ovary is superior and with 2-9 compartments, mostly self but partly also cross-pollinated. Bees and bumble bee are the most important pollinators. Fruits are fleshy berry; globular to oblate in shape the immature fruit is green and hairy. Ripe fruits range from yellow, orange to red. It is usually round, smooth or furrowed. Seed are numerous, kidney or pear shaped. (Sahu*et, al.,* 2012)

In Nigeria, tomato is regarded as the most important vegetable after onions and pepper and most widely grown crop after Potato and sweet Potato (Fawusi, 1978). It is also the world's largest vegetable crop after potato and sweet potato but it tops the list of canned vegetables (Wojciech and Korsten, 2002).Tomatoes is economically attractive and the area under cultivation is increasing daily (Smith, 1994).

In Nigeria, tomato is mainly cultivated in the region north of 10^{o}N latitude. This covers the savannah belts where its cultivation is supported with irrigation (Suleiman, 2011). In the south, the excessive precipitation and the associated high relative humidity tend to limit tomato production during the raining season as it favors the multiplication of the pathogen responsible for the tomato rot with hot and humid weather being conducive for growth; the fungus is more devastating in tropical and sub-tropical regions of the world (Agrios, 2005).

It is economically attractive and the area under cultivation is increasing daily (Smith, 1994). Tomato was referred to as an object of affection and therefore known as love apple; 'pommels d'amour' in French (Suleiman, 2011). Tomatoes are planted by an estimated 85% of the gardens each year (Lynn *et, al*., 2000). If well managed, tomato is highly productive (Denton and Swarup, 1983). The first harvest is possible 45-55 days after flowering or 90-120 days after sowing (Sahu *et, al.,* 2012). Worldwide, about 36million tones of tomatoes are produced annually mostly in Europe and North America. A total of one million hectares per annum is reportedly used for its cultivation in Nigeria (Ebimieowei *et, al.,* 2013).Genetic evidence shows that the tomatoes fruit have a Centre of diversity in the highland of Peru where one species, *Solanum lycopersicum* was transported to Mexico where it was grown and consumed by pre-historic humans(Smith, 1994). However, the

fruit, grown by the Aztec of central Mexico, who called it 'xitomati' meaning plump thing with a naval and later called tomato by other Mesoamerican people (Peralta and Spooner, 2001). In earlier times, tomatoes were consumed only in Italy but with the beginning of the second half of the 19th century the crop was widely appreciated for the fine food that it provides. From Europe, it was carried across the pacific into south eastern Asia (Ijato*et, al.*, 2011).

2.2 Production of Tomato Fruit

The major tomatoes producing countries in order of importance are: the United State, followed by Italy, the Soviet Union, Spain, Turkey, Egypt, Greece, Romania and the Arabian countries. Mexico, Japan, Brazil, India and Argentina are other important producers (Ijato*et, al.*, 2011). Nigeria is second largest producer of tomato in Africa second only to Egypt and 13th in the world, and produces 6 million tonnes of tomato annually (Erinle, 1989).

2.3 Nutritional Quality of Tomato

Nutritional value of tomato makes it a widely accepted vegetable by consumers and tomato fruit production can be adopted as a strategy for improving livelihood and alleviating the nutritional status of the people. It is the answer to the perpetual problems of hunger and malnutrition in the country. Since in Nigeria, a lot of children suffer from vitamin "A "deficiency, a nutritional problem that can be reduced by regularly eating fruit and green leafy vegetables (South Pacific Foods, 1995).

Tomato plant, also with its fruit is of great nutritional importance because it is an excellent source of thiamine, niacin, riboflavin and of minerals like iron and calcium (Suleiman, 2011).

The tomato fruits are largely composed of water (about 94%) but have moderate quantities of 90% soluble sugars, several organic acids (especially citric and malic acid) mineral salt and relatively large quantities of the vitamin C (Faith *et, al.,* 2005) compared with oranges, tomatoes contain nearly 20 times as much vitamin A, the same amount of vitamin B; slightly more vitamin B2. It also contains a large quantity of water (%), calcium (%) and Niacin all of which are of great importance in the metabolic activities of man. Tomato is a good source of vitamins A, C and E and minerals that are very good for body and protect the body against diseases (Taylor, 1987)

Tomato is an important condiment in most diet and a very cheap source of vitamins. It is a familiar ingredient of salad, being valued for its colour, sweet flavour and pleasing acidic taste of the flesh in a caned or preserved state. Fresh ripe fruit is refreshing and appetizing. Its fibers serve as roughage for the promotion of digestion (Chiejina, 2005).

In view of its varied uses which includes salad vegetable, puree, paste, ketchup, juice and for cooking soups and stews, it can also be used as garnish, in canned or pickled the seed contains about 24% of oil which is used as salad oil and also in the production of margarine and soup (Chiejina, 2005). The residual mass or 'press cake' is used as a stock feed and fertilizer (Ijato *et, al.,* 2011). Unfortunately tomato are attacked by large numbers of diseases causing pathogens when green and small and may not show any symptoms until ripen which result in the great reduction in the quality and quantity of fruit yield (Jude*et,al*.,

2012). The pathogens can however, infect tissue that are wounded and expose without a protective surface.

Mechanical injuries (e.g. cut and punctures) that occur during harvest and handling are good sites for decay development on the fruit surface (Jerry *et, al.*, 2005). In contrast, internalized pathogen, causes lesion that begin inside the fruit, high nutrient composition makes the fruits susceptible to attacks by many fungi, which in addition to cause rot, may also make them unfit for human consumption due to myco-toxins production (Stinson *et, al.,* 1981 and Philip *et, al.,* 1984).

2.4 Plants Diseases Caused by Fungi

Plant disease can be divided conveniently into those caused by parasitic organisms or Pathogen and non-parasitic disease or disorders, the major groups of plants pathogens are fungi, bacteria viruses, and nematodes but Fungi are the most important common cause of plant disease (Persley, 1993) since they are the most widespread and destructive parasites of plants (Ingold and Hudson, 1993). More than 100,000 species of fungi are known to cause diseases in humans and about as many cause disease in animals, most of them superficial disease of the skin or appendages (Agrios, 1997). The effects of fungi on plants can be devastating cellular structure can be destroyed, physiological function of the plant impaired, and the rate of metabolism and metabolic path ways can be altered.

These biological processes have widespread consequences for infected plants because the impairment of any one function of organ has effects on others (Moore, 1996). Estimate on crops losses that can be directly attributed to fungi vary worldwide. Weeds cause up to 32% losses, animal pest 18% and pathogen about 15%. For example individual

crop losses can be 100% if a susceptible cultivar is planted or the climate is favorable all year and down to 0% if resistance varieties are planted, and fungicides used and good husbandry employed losses tend to be higher in the humid tropics than in cooler temperate zones for examples, losses in rice and maize were higher than losses for wheat and barley. Losses in fleshly staples, for examples potatoes, sweet potatoes, yams were higher than for cereals crops. Losses in barley due to pathogens were 9% worldwide, 5% in North West Europe and 15% in south Asia. For Potatoes, losses in Europe were about 25% in North West Europe, and 50% in Africa (Oecke and Dehne, 2004).

Myco-toxin production is always a food safety, as opposed to a food spoilage issue, although they will still contribute to losses as the crop is downgraded reprocessed or destroyed. Direct crop losses in field are caused by plants pathogens by reducing crop yields. Some plants pathogens may produce mycotoxins either as incidental products or as chemical associated with the infection process for example, some species of *Fusarium*. Plant pathogens may also rendered the plant a lower grade by causing blemishes, blight or other quality problems. Some species may not be able to attack crop in the field but cause problems once crop is harvested, if conditions allows (Peter, 1978).

Plant disease influence on human welfare ranges from major catastrophes to minor annoyances (Yulia, 2005). The potato late leaf blight Epidemic caused *Phytopthora infestan* (Mont) de bary (responsible for the Irish potato famine of the 1840's), rice brown spot caused by *Helminthosporium oryzea* Breda de Haan (responsible for the Bengel nee famine of the 1940's) Economic loss caused by southern corn leaf blight reduced by *Helminthosporium maydis* in U.S.A in 1970 and aesthetic problem caused by Dutch elm disease from 1930's through the 1970's in the U.S.A illustrate that disease have been

devastating on a large (Fry, 1982). In a short time plants diseases cause economic losses to growers, increase price of produce to consumers, have direct and severe pathological effects on humans and animals and destroys the building of the landscape (Yulia, 2005).

2.5 Synthetic Fungicides and Natural Fungicides

At presents quick and effective management of plant diseases and microbial contamination in several agricultural products is generally achieved by the use of synthetic fungicides. Synthetic fungicides are being used on tomato, pepper and many other plants products for effective control of diseases in several countries (Anonymous, 2000). Though the use of synthetic fungicides has proved effective in the control of phyto diseases but most synthetic fungicides that are used in controlling some of these phyto pathogens have caused serious hazard in animals, humans and environment as they pollute the soil, water and air, due to their residual toxicity (Satish *et, al.*, 1999 and Strange, 1993).

These chemicals are also expensive and inaccessible to purchase especially by peasants farmers in the rural area and indigenous farmers who are the bulk producers of Tomato in Nigeria (Amadioha, 1998 and Onuegbu *et, al.,* 2001) and therefore, not economically viable. In recent years, a large number of pesticides and fungicide has been banned in the western world because of their undesirable attribute such as high and acute toxicity, long degradation periods accumulate in the food chain, and extension of the power to destroy both useful organism and harmful pest (Strange, 1993). In developing countries, they are still being use despites harmful effects (Satish *et, al.,* 1999 and Strange 1993).

The natural plant products, known as botanical pesticides or herbal medicines, have long been used in the control of microorganisms causing plant and human diseases.

However, with the employment of synthetic pesticides in agriculture, the use of botanical pesticides has significantly diminished. Although highly effective, synthetic pesticides often have undesirable side effects such as toxicity to mammals and causing environmental pollution (Sahu *et, al.,* 2012). Of the herbal medicine and botanical pesticides, much attention has been given to the use of phenolic-rich plant extracts. Tannins are important water soluble plant phenolics. Tannin-rich plant extracts have traditionally been used as medicines to treat infectious human diseases (Djipa*et, al.,* 2000 and Tegegne*et, al.,* 2008)and they exhibit antimicrobial activity against phytopathogenic fungi and bacteria (Alstrom, 1992, Fowcett and Spenser, 1970).

Many pathogenic microorganism and insect pest have developed resistance against chemical Fungicides and Pesticides this seriously hinder the managements of disease of crop and agricultural products. (Satish *et, al.,* 1999). Considering this undesirable attribute of synthetic fungicides, there is urgent need to develop alternative treatments that are less hazardous to animals, humans and environments. Within this context the use of pesticides and fungicides of botanical origin has been pin-pointed by many researches as an option to synthetic fungicides (Amadioha and Obi, 1999; Amadioha, 2000). A sizeable portion of the world population living below poverty line in the developing and underdeveloped countries of Africa are suffering from health problems associated with consuming mycotoxin contaminated grains, cereals, fruits and vegetables (Majumder *et, al.,* 1997).

Even though effective and efficient control of seed borne fungi can be achieved by the use of synthetic fungicides, the same cannot be applied to fruits and vegetables for reasons of fungi-toxic effects, and thus, there is a need to search for alternative measures of protecting fruits and vegetables for human consumption from fungal infection. As a result

scientists have been looking for alternative methods of pathogens control which are environmental friendly, less destructive and cheap (Kiran *et ,al.,* 2006 and Okigbo, 2009). These have significant draw back including increase in cost of synthetic fungicides, hanging hazard, and concern about fungicides residue in food (Chiejina, and Ukeh, 2012).

The continued use of natural plant products is particularly important in countries like Nigeria where synthetic fungicides are not readily available farmers are poorly equipped to handle them and their use is uneconomical. Fungi are more devastating in tropical and subtropical regions in the world. In Nigeria, the pathogen is of major concern to vegetable growers in the northern Savannah belts, which potentially rank among the world's best vegetable production zone (Yorinori, 1994).

2.6 Use of Plants Extracts as Fungicides

Plant extracts of many higher plants have been reported to exhibit antifungal antimicrobial, anti-inflammatory, antioxidants and antifungal properties.Keeping in view the drawback of chemical management of plant, the use of plant extract in disease management is therefore gaining importance (Kiran *et, al.,* 2006 and Okigbo, 2009).

Other research workers (Amadioha and Obi, 1999, Amadioha, 2000 and Okigbo, 2009) see the significance of fungicides of plant origin as possible means of fungal disease control in fruits and vegetables as they are easily biodegradable and non- toxic to humans. Extracts obtained from many plants have recently gained popularity and scientific interest for their antifungal activities (Sownumi and Akinusi, 1983). Therefore, the expression of fungi-toxic activities by plant extracts is an indication that such plants could be used as fungicides by peasant farmers who cannot afford the costly synthetic agrochemicals to

control fungi. In addition to environmental concerns, there are high chances of development of multi-resistant strains of the fungal phyto pathogens.

Herbal and natural products of medicine have been used for centuries in every culture throughout the world. Scientists and medical professionals have shown increased interest in this field as they recognize the true health benefits of these remedies. “Let food be your medicine and let medicine be your food.” Plants are one of the most important sources of medicines. (Mahesh and Satish, 2008) The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance.

The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability.Medicinal plants represent a rich source of antimicrobial agents (Mann *et, al.,* 2008). Plants generally produce many secondary metabolites which constitute an important source of fungicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997 and Ogundipe *et, al.,* 1998).

Laboratory studies of extracts of plants species have revealed powerful fungitoxicities in relation to many fungal pathogens (Hay and Waterman 1993). Such inorganic and organic chemicals in the plants either inhibit germination, growth and multiplication of pathogens, or are lethal to the pathogens. For example, *Murraya koenigii* were tested for its antifungal activity against *Penicillium, Aspergillus niger, Aspergillus flavus* and *Fusarium* sppthey were found to be effective against the fungal pathogens

(Bertha *et, al.*, 2012). Studies also showed the antifungal property of aqueous, ethanol and hexane extracts of *Murraya paniculata* on fungi pathogen and they were found to showed positive inhibitory effect (Mohana *et, al.*, 2011).

Some constituents of *Murraya koenigii* which were very active were reported to have anti-fungal activity (Das *et, al*., 1965). (Ramsewak *et, al*., 1999 and Rahman and Gray, 2005) also investigated the antimicrobial activity of *Murraya koenigii* which gave an excellent result on inhibition. (Sowjanya and Chary, 2012) studied aqueous extracts at two different concentrations (5% and 10%) of *Azadirachta indica, Lawsonia inermis, Allium sativum, Murraya koenigii, Ocimumsanctum* to test their antifungal properties against the keratinophilic fungus *Microsporum gypseum* result revealed that the extracts were fungitoxic on the fungiwith *Allium sativum* and *Ocimum sanctum* at 10% conc. were more pronounced compared to all the other extracts followed by *Azadirachta indica, Lawsonia inermis* and *Murraya koenigii*. (Muthumani *et, al.*, 2010) investigate the antibacterial and antifungal activity of *Murraya Koenigii* stem bark extracts. The compound isolated was tested for their antibacterial efficacy against *Staphylococuus aureus* and antifungal efficacy against *Candida albicans*. The antimicrobial effect produced by the isolated compounds was comparable to that of Amikacin and Griseofulvin. The isolated compounds showed high enzyme inhibitory activity.

Several researchers have shown that extracts of many plants may control different fungal plants pathogens (Agrios, 1997) plant extracts and oils have been shown to be effective against other Ascomycota and Basidiomycota leaf pathogens (Kamalakannan *et, al.,* 2001). Studies have also showed that extracts from plant materials have been

successfully used for the treatment of fungal and bacterial infections of post-harvest rots of tomato (Lattanzio, 2003).

Previous reports by (Akpomedaye and Ejechi, 1998, Ejechi and Ilondu, 1999 and Ejechi *et, al.*, 1999) showed that spices, herbs and other plant materials possess antifungal activity. Antifungal efficacy of stem and leaf-extracts of *Mirabilis jalapa*in reducing mycelial growth of four different strains of fungi were reported by Akinyosoye and Oladunmoye (2000). According to Ebele (2011), aqueous plant extracts from the leaves of *Carica papaya,Chomolaena odorata,* and *Acalypha ciliata* was tested on the growth of the pathogenic fungi rot on pawpaw fruits. The different concentrations (10, 20 and 30%) significantly reduced the mycelia growth of *Aspergillus niger i*n-*vitro* by (26.83%), *Botryodiplodia theobromae* (31.71%) and *Fusarium solani* (39.02%) respectively.

Antimicrobial effects of extract of *Venonia amygdalina* and *Tridax procumbens* on the pathogenic fungi isolated from infected tomato fruit parts by two different extractive solvents (water and ethanol) aqueous concentrations of 80 and 60% as well as ethanol concentration of 30 and 20% were used in the study where all the plant extracts, all extracts significantly reduces the mycelia growth of isolated pathogens. Higher concentration of both aqueous and ethanol favoured higher mycelia growth reduction (Ijato *et, al.*, 2011).

Nashwa and Abo-Elyousr (2012) reportedthe antimicrobial activity of six plant extracts from *Ocimum basilicum* (Sweat Basil), *Azadirachta indica* (Neem), *Eucalyptus chamadulonsis* (Eucalyptus), *Datura stramonium* (Jimsonweed), *Nerium oleander* (Oleander), and*Allium sativum* (Garlic) was tested for controlling *Alternaria solani in vitro* and *in vivo*. All treatments with plant extract both *invivo* and *invitro* significantly reduced

the early blight disease as well as increased the yield of tomato compared to the infected control under field conditions.

` Pandey *et, al.,* (1981) studied the fungi toxicity of some higher plant with special reference to the synergistic activity amongst some volatile fungi toxicants. leaves of 25 plants spp were screened using *Fusarium lateritium* f. sp *cajani* (*Giberella baccata*) as test pathogen the oil of *Aegle marmelos* was fungistatic and those of lime (citrus) and *Mentha arvensis* were fungicidal combinations of oils was more toxic than individual oils, the citrus menthe mixture having a broad fungitoxic spectrum against a range of fungi. Allelopathic effect of leaf extract of *Azardirachta indica* and *Chromolaena odorata* were reported by Ijato *et, al*., (2010) against post- harvest and transit rot of tomato (*Lycopersicum esculentum* L).

According to Suleiman, (2011), Plant extracts was employed to control the vegetative myceliagrowth from neem *(Azadirachta indica*) and tobacco (*Nicotiana tabacum*) at 20%, 30%,40%, 50% and 60% concentrations. *In vitro* application of extracts for the control showed thattobacco and neem (*Nicotiana tabacum* and *Azadirachta indica*) had fungi toxic effect that controlled the mycelia growth and there wascomplete inhibition on all the fungi. Sahu *et al*., (2012) used ten medicinal plants for the control of diseases, and its antibacterial and antifungal effect was tested against*Clavibacter michigenesis, Alternaria solani,* and *Septoria lycopersici, Pythium debaryanumand* and *Phytopthoracapsici* that cause *Alternaria canker,* early blight, leaf spot, fruit spot, blossom end rot and sunscald disease in*Lycopersicum esculentum.* Extract of *Ageratum conyzoides* was efficient in inhibiting the growth of *Clavibacter michigenesis* while mycelial growth of *Alternaria solani, Septoria lycopersici, Pythium debaryanum* &*Phytopthora capsici* was

inhibited by *Piper nigrum, Aegle marmelos*& *Ageratum conyzoides*. Among the plant extract tested in field, *Ageratum.conyzoides* was found most effective in reducing the *Alternaria* canker disease by78.20% and *Azadirachta indica* reduced the early blight & leaf spot disease by 53.84% &40.78% respectively, *Aegle marmelos* reduced the fruit spot disease by 61.29%. *Pongamia piñata*& *Brassica campestris* reduced the Blossom end rot disease by 86.95% and 82.17% and *Ageratum.conyzoides* &*Pongmia piñata* reduced the sunscald disease by 90.08% & 76.85% respectively in *Lycopersicum esculentum*.

The inhibitory properties of the methanolic extracts of *Aframomum melegueta* and *Zingiber officinale* were also reported by Chiejina and Ukeh(2012) on *Helminthosporium solani, Mucor piriformis, Penicillium digitatum* and *Aspergillus niger* pathogens isolated from tomato. Various concentrations of the extracts ranging from 0-30% were used. Antifungal effects of these extracts on the mycelial growth of the pathogens were significant at $P< 0.05$ for all treatments. The *in vitro* inhibitory effects of these extracts indicated that they could be used for the control of tomato fruit rot.

The *in vitro* fungitoxic activity of crude extracts of ginger (*Zingiber officinale)*, bitter-kola (*Garcinia cola*), aloe (Aloe vera) and neem (*Azadirachta indica*) was tested on *Pythium aphanidermatum* isolated from root rot of tomato (*Lycopersicon esculentum)* at different concentration of (methanol) extracts of leaves of neem and aloe, seeds of bitter kola, and rhizomes of ginger at 40%, 60%, 80% and 100%. The results showed that all the extracts did well in the mean percentage inhibition of mycelia growth (Suleiman, 2011). (Nashwa and Abo-Elyousr 2012), reported the effects of various plant extracts *Ocimum basilicum* (Sweat Basil), *Azadirachta indica* (Neem),*Eucalyptus chamadulonsis* (Eucalyptus), *Datura stramonium* (Jimsonweed), *Nerium oleander* (Oleander), and*Allium*

sativum (Garlic) *Alternaria solani in vitro* and *in vivo* against the early blight disease of tomato plants under greenhouse and field conditions. In *invitro* study theleaf extracts of *D. stramonium*, *A. indica*, and *A. sativum* at 5% concentration caused the highest reduction ofmycelia growth of *A. solani* (44.4, 43.3 and 42.2%, respectively), while *O. basilicum* at 1% and 5% concentrationand *N. oleander* at 5% concentration caused the lowest inhibition of mycelia growth of the pathogen.

Ingreen house experiments, the highest reduction of disease severity was achieved by the extracts of *A. sativum*at 5% concentration and *D. stramonium* at 1% and 5% concentration. The greatest reduction of disease severitywas achieved by *A. sativum* at 5% concentration and the smallest reduction was obtained when tomato plantswere treated with *O. basilicum* at 1% and 5% concentration (46.1 and 45.2 %, respectively). *D. stramonium* and*A.sativum*at 5% concentration increased the fruit yield by 76.2% and 66.7% compared to the infected control.All treatments with plant extracts significantly reduced the early blight disease as well as increased the yield of tomato compared to the infected control under field conditions.

Taiga *et, al.*, (2008) reported the inhibitory effects of cold extracts of five fungicidal plants (*Aloe barbadensis, Azdrichta indica, Nicotiana tabacum, Tridaxprecubens* and *Carica papaya*) were screened for *in vitro* inhibitory effects on *Fusarium oxysporium*, using 25, 50, 75 and 100% concentrations of each extract. The four different concentrations of *N. tabacum* completely inhibited radial growth of *F. oxysporium* mycelium *in vitro*, 75 and 100% concentrations of *A. barbadensis* had a similar effect, while only the 100% concentration of *A. indica* completely prevented the fungal growth. Generally *C. papaya* and *T. precumbens* showed fungicidal properties, but they could not completely inhibit

radial mycelia growth of the fungal pathogen at any of the concentration levels tested. *N. tabacum* was found to be most fungi toxic as exhibited by all the concentration levels used.

Evaluations of the methanol extract of *Ficus exasperate* stem bark, leaf and root for phytochemical analysis andantimicrobial activities against some human pathogenic organisms was reported by in the work of Banso (2008) *Acacia nilotica* was assessed for active principles. The results showed that the stem bark extract of theplant possessed the active principles e.g. terpenoids, tannins, alkaloids, saponins and glycosides.

Theantimicrobial activity of the extracts was assayed against *Streptococcus viridans, Staphylococcus aureus, Escherichia coli*, *Bacillus subtilis* and *Shigella sonnei* using the agar diffusion method. Theplant extract exhibited antimicrobial activity against all the test microorganisms. *B. subtilis* was themost susceptible to the plant extract while *Candida albicans* was the most resistant. The minimuminhibitory concentration of the stem bark extract of the plant ranged between 35 and 50 mg/ml while theminimum bactericidal concentration ranged between 35 and 60 mg/ml. *A. nilotica* could be a potentialsource of antimicrobial agents.Charles *et, al.,* (2012) reported *in-vitro* the control of fungal species found associated with the storage of four staple food crops at Ibadan in the humid forest of southern Nigeria using some indigenous plant extracts. *Aspergillus flavus, Aspergillus niger, Penicillium expansum and Rhizopus stolonifer* were found associated with the spoilage of horticultural crops in storage. The results of the investigation revealed that, *Acalypha ciliata,* Aloe vera, *Azadirachta indica and Vernonia amygdalina* were effective in the inhibition of *Aspergillus flavus and Penicillium expansum. Annona squamosa* effectively inhibited the mycelial growth and sporulation of *Aspergillus niger*and*Rhizopus stolonifer*. Aloe vera extract was equally effective in the reduction of mycelial growth and

sporulation of *Aspergillus niger*. In the overall, a significant reduction in mycelial growth and sporulation of the pathogens was found associated with treatment with most of the plant extract tested.

Enyiukwu and Awurum (2011) reported the Phytochemicals in the form of aqueous extracts of *Piper guineense* seeds and *Carica papaya* roots and seeds were evaluated *invitro* for efficacy against Colletotrichumdestructivum O'Gara, the incitant of cowpeaanthracnose, at five concentrations of 0, 25, 50, 75 and 100%. All the extracts inhibitedthe germination of spores of the fungus. The fungitoxicity of the extracts increased asextract concentration increased. *P. guineense* was the best in inhibition effects against theorganism, this was followed by C. papaya roots and then *C. papaya* seeds. Benomyl had superior inhibition effects over the extracts, at ($P \geq 0.05$)with 100% concentration of *P. gunieense* seeds extracts infungitoxicity against the pathogen

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in the Department of Biological sciences, Ahmadu Bello University, Zaria which is located between latitude 11^{o}9’5.46”N and longitude 7^{o} 39’6.72 E, at altitude of 686m above sea level (figure 1).

3.2 Source of Materials

Three varieties of Tomatoes Fruits U.T.C, Derica and Panchy were used in the study. Tomatoes showing symptoms of rot infection were purchased from Samaru markets stalls in Zaria, Kaduna State, Nigeria. These were collected by interviewing the sellers on the different names of the tomatoes fruits and how they can be differentiated. They were collected in both rainy and dry season. These were packed in a sterile polyethane bag and brought to laboratory where they were used immediately. Fresh leaves stem bark and root of *Murraya koenigii* were collected from different residential areas of Ahmadu Bello University, Zaria. These were placed in a clean polyethene bags and brought to the laboratory for preparation of extracts.

3.3 Preparation of Plant Extracts

Freshleaves, stem bark and root were washed separately 3 times in tap water to remove dirt and rinse in sterile distilled water before being surface sterilized with 90% alcohol (Kumar *et, al.,* 2013) after which they were shade dried on laboratory bench at room temperature. Thereafter the individual part was ground separately into fine powder using pestle and

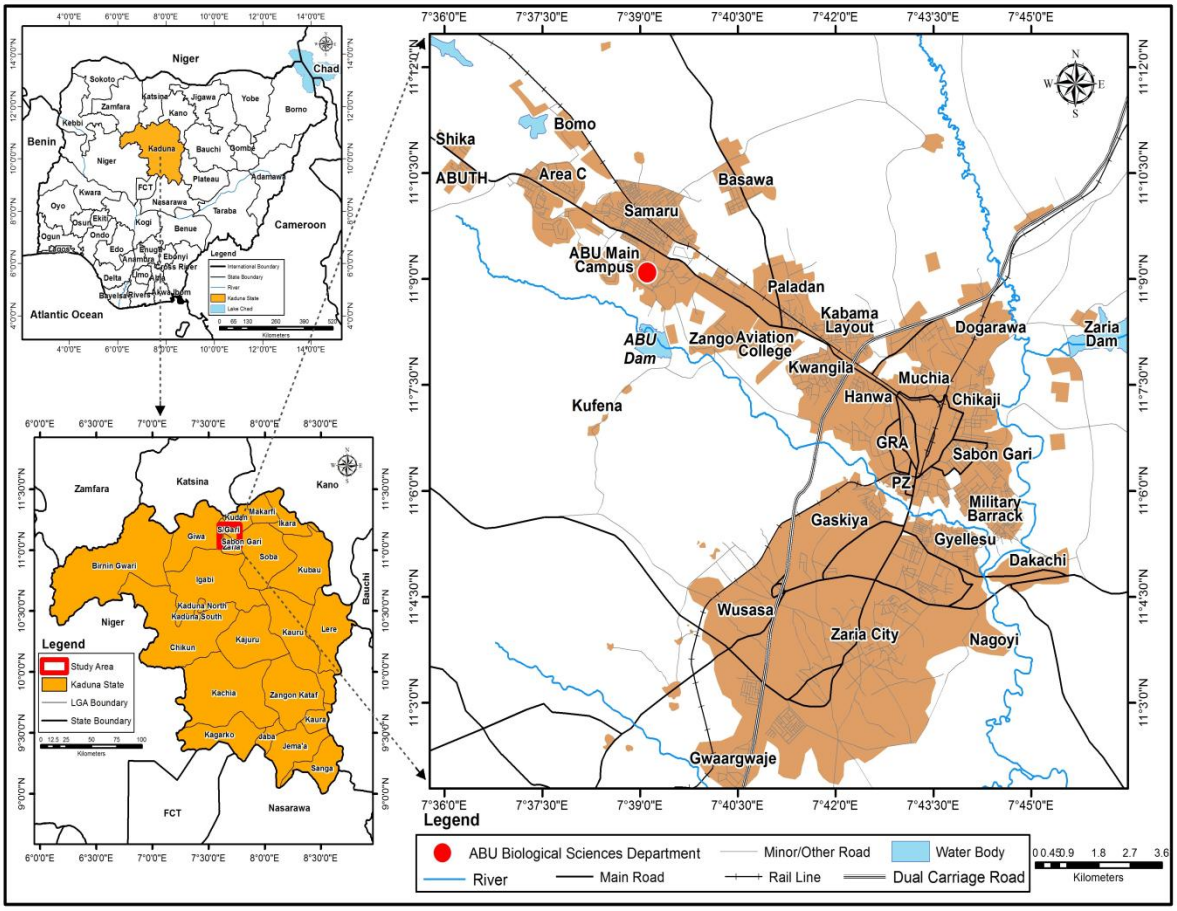


Figure 1: Map of Zaria showing ABU Biological Sciences Department
Source; Modified from the Administrative Map of Sabon Gari L.G.A., 2014

morter. 30g of each powder specimen of different part of *M. koenigii* was then placed in a porous cellulose thimble. The thimble was placed in an extraction chamber which was suspended above the flask containing the solvents and below a condenser. The flask was heated and the solvents (aqueous and methanol separately) evaporated and moved up into the condenser where it was converted into a liquids and trickled into the extraction chamber containing the sample. The liquid continued to accumulate in the extractor containing the sample until it reached a point and overflowed and trickled back into the boiling flask.

At the end of the extraction process which lasted for a minimum of six hours the flask containing the extracted ingredients was removed and the solvents removed using a rotary evaporator, to obtain the active ingredients in a paste form and different concentration viz: 5, 10, 15, 20, and 25% of both aqueous and methanol extracts were prepared by dilution method from stock solution.

3.4 Types of Media used

Potato dextrose Agar (PDA) and Sabouraud dextrose agar (SDA) were prepared and used throughout the experiment. 200g of peeled Irish potato was cut into small pieces, washed thoroughly, placed in 1000ml of sterile water and boiled in a metal pot for one hour. The liquid was then filtered using muslin cloth and the volume was made up to 1000ml with distilled water to compensate for loses due to evaporation and 20g each of agar and dextrose were added. The mixture wastransferred into a conical flask capped with cotton wool and aluminum foil.This was then autoclaved at 121°C for 15 minutes. 1g of Streptomycin was dissolved in 2000 ml of distilled water and 10 ml of the solution was

added to the medium to avoid bacteria growth and then poured into sterilized Petri dishes and allowed to solidify.

The SDA was prepared by dissolving 38g of the Sabouraud dextrose agar in distilled water and then heated gently to dissolve the agar completely these was prepared as described by the manufacturer instruction. The mixture was transferred into a conical flask capped with foil paper and autoclaved at 121°C for 15min. Streptomycin was also added into the media. After sterilization 15ml of the medium were dispensed into sterilized Petri dishes and allowed to cool for 24hrs in the laboratory.

3.5 Isolation of Fungi from Rotten Tomato Fruits

Tomatoes were washed in running tap water and then surface sterilized with 1% sodium hypo chlorite solution (NaOCl)) for 3 minutes (Suleiman, 2011). These were then rinsed with three changes of sterile distilled water to remove residue of NaOCl and was dried on sterile tissue paper. Pieces of rotten tomato fruits including unrotten part were cut from the periphery and placed on a sterile soft paper and were plated out on a15ml potato dextrose agar (PDA) and Sabouraud dextrose Agar (SDA) in the Petri-dishes using plating methodsbased on Suleiman, (2011).

Three pieces per Petri dish were plated out and replicated thrice and incubated at room temperature for 3-7 days. These were observed regularly for any fungal growth. The total numbers of colonies observed were counted. Pure culture of the Pathogen were obtain by sub culturing each discrete colony into a freshly prepared potato dextrose Agar and Sabouraud dextrose agar in sterilized petri dishes. Each fungus was plated at the Centre of the Petri dish. They were incubated at Room temperature for seven (7). The pure isolates

were stored in slant for further use. All these process were carried out aseptically. Isolation was done both in rainy season and dry season (Akintobi *et, al.*,2011).

3.6 Identification of Isolated Fungi

3.6.1 MacroscopicCharacteristics

Macroscopic Characteristics such as colour of the colony, texture, topography shape and diameter of the colony were used for fungal identification.

3.6.2 MicroscopicCharacteristics

Microscopic characteristics such as the shape, size nature and the colour of the conidiophore, conidia, Mycelium, Sporangiophore, Sporangiospore and vesicles were all studied. To achieve that, a small fungal growth from the petri dish was mounted in drop of lacto phenol cotton blue on a clean glass slide and studied under the microscope. The characters were then compared with already identified species with the help of identification key by Barnett and Hunter (1972), Klich (2002), Samson *et, al.*, (2004) and Larone (2002). Digital microphotography of each species was taken.

3.7 Determination of the Percentage Frequency of Occurrence of the Isolated Fungi:

The frequency of occurrence of the different types of fungi associated with tomato fruit rot diseases was determined. The percentage frequency of occurrence was calculated using the following formula:

$$\% \text{ frequency of occurrence} = \frac{\text{Number of times a fungus is encountered}}{\text{Total Number of Fungi isolated}} \times 100$$

3.8 *Invitro* Assay of Plants Extracts

The method of Amadioha and Obi (1999) was used for the in vitro assay of the plants extracts on fungal growth by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The point of intersection was indicated as the centre of the plate this was done before dispensing PDA into each of the plates.

2mls of the different plants extracts were incorporated into the Petri-dishes containing 15ml of PDA medium with the help of a sterilized syringe. The control was PDA on which no extracts was added. 5mm diameter disc was cut from the periphery of colony from the pure culture with a sterilized cork borer and placed at the Centre of the Petri dishes containing the amended PDA. These were replicated thrice and incubated at room temperature. The Petri-dishes were laid out in a completely randomized design and were terminated after 7 days.

The toxicity of the extracts to fungi was determined as the percentage inhibition ofgrowth thus: % inhibition $=\frac{dc-dt}{dc} \times 100$

Where, dc = average diameter of fungi colony in control plate and dt = average diameter of fungal colony in treated plate.

The growth of the fungi was measured using meter rule by turning the reverse side of each Petri-dishes just at the point of intersection of the two lines drawn at the bottom of the Petri-dish plates 7 days after inoculation

3.9 Statistical Analysis

Data collected were subjected to one way analysis of variance where significant differences were observed between treatments. The means were separated using the Duncan Multiple Range Test (DMRT) and student t-test was used to compare the efficacy of the two extracts.

CHAPTER FOUR

4.0 RESULTS

4.1 Fungi Isolated from Three Varieties of Tomato Collected from Samaru Market

Result showed that the same fungi were isolated on PDA and SDA from the three Tomatoes varieties. 273 colonies belonging to five genera and six species of different fungi were recorded these included *Aspergillus niger* Vanteighem, *Aspergillus flavus* Link, *Fusarium oxysporum* Schlecht, *Mucor sp* Mich, *Rhizoctonia solani* Khunand *Rhizopus stolonifer* Ehrenb.However, *A. niger, A. flavus, F. oxysporum,* and *R. stolonifer* was isolated from all the variety in both seasons and both media. *Mucor sp.* was isolated only during rainy season from all the three varieties both from PDA and SDA on the other hand *R. solani* was not recorded from Panchy variety in both season in both season and on both nutrient media. (Table 4.1 and 4.2).

4.2 Identification of Isolated Fungi

Allthe isolated fungi were identified by studying both macroscopic and microscopic characters. They were then compared with already described species using identification keys by Barnett and Hunter(1972), Klich(2002), Samson *et al.*, (2004) and Larone (2002).

4.2.1 *Aspergillusniger*Van Tieghem

4.2.1.1 *MacroscopicCharacteristics*

The surface was black in colour while the reverse was white to yellow. The shape of the colonies was roundish. Topography of the colony was downy to powdery. The colonies expanded from 5-8cm in diameter at room temperature (Plate I: A and B).

Table 4.1 Fungi Isolated from Three Varieties on PDA and their Distribution.

Fungi isolated	Tomato Variety					
	U.T.C		Derica		Panchy	
	Season					
	Rainy	Dry	Rainy	Dry	Rainy	Dry
Aspergillus niger	+	+	+	+	+	+
Aspergillus flavus	+	+	+	+	+	+
Fusarium oxysporum	+	+	+	+	+	+
Mucor sp	+	_	+	_	+	_
Rhizoctonia solani	+	+	+	+	_	_
Rhizopus stolonifer	+	+	+	+	+	+

+ = present, - = absent.

Table 4.2 Fungi isolated from three varieties on SDA and their distribution.

Fungi isolated	Tomato Variety					
	U.T.C		Derica		Panchy	
	Season					
	Rainy	Dry	Rainy	Dry	Rainy	Dry
Aspergillus niger	+	+	+	+	+	+
Aspergillus flavus	+	+	+	+	+	+
Fusarium oxysporum	+	+	+	+	+	+
Mucor sp	+	_	+	_	+	_
Rhizoctonia solani	+	+	+	+	_	_
Rhizopus stolonifer	+	+	+	+	+	+

+ = present, - = absent.

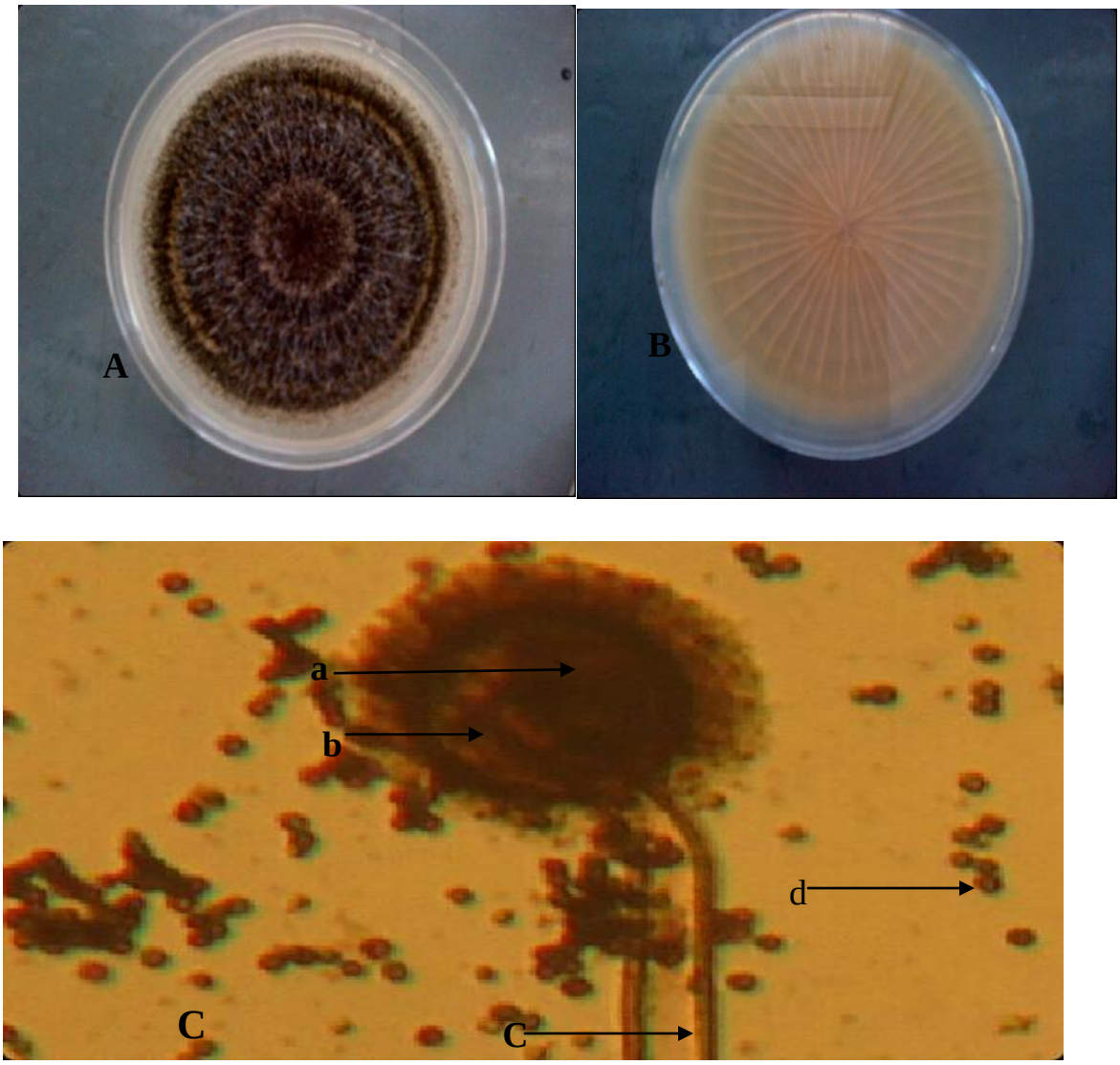


Plate I: Pure culture of *Aspergillus niger*Vantieghem.A. Upper Surface, B. Reverse side,C. Microscopic features: a. vesicle, b. Phialides, c. Conidiophore and d. Conidia

4.2.1.2 *MicroscopicCharacteristics*

Mycelium consists of profusely branched hyphae which produced long erect and smooth conidiophore which measured between 100-700µm long with an average length of 490.4µm, a width of 20-30µm near the vesicle, 20-30µm at the middle and also 20-30µm at the base with an average width of 26.4µm near the vesicle 25.6µm at the middle and 25.2µm at the base respectively. Vesicle measures 20-110µm in diameter with an average diameter of 66.8µm. Conidiophores are smooth-walled, upright, simple and terminating in globose swelling bearing phialides at the apex or radiating from the entire surface. The conidiophore is colourless or brown, sometimes hyaline or turning dark towards the vesicle. Conidia 1-celled, globose, measured 2-5µm in diameter with an average diameter of 2.65µm(Plate IC: a, b, c, and d).

4.2.2 *Aspergillusflavus*Link

4.2.2.1 *MacroscopicCharacteristics*:The surface was green to lime green while the reverse was cream. The shape of the colony was irregular and topography of the colony was downy to powdery. The colony expanded 3-4cm in diameter in one week at room temperature (plate II: A and B).

4.2.2.2 *MicroscopicCharacteristics*

Mycelium profusely branched whitish in the beginning becomes darker with time. Conidiophore long, erect, hyaline measured between 190-600µm long with an average length of 406.2µm and a width of 20-40µm near the vesicle, 20-30µm at the middle and 20-40µm at the base with an average width of 26.00µm near the vesicle, 24.20µm at the middle and 25.40µm at base respectively.

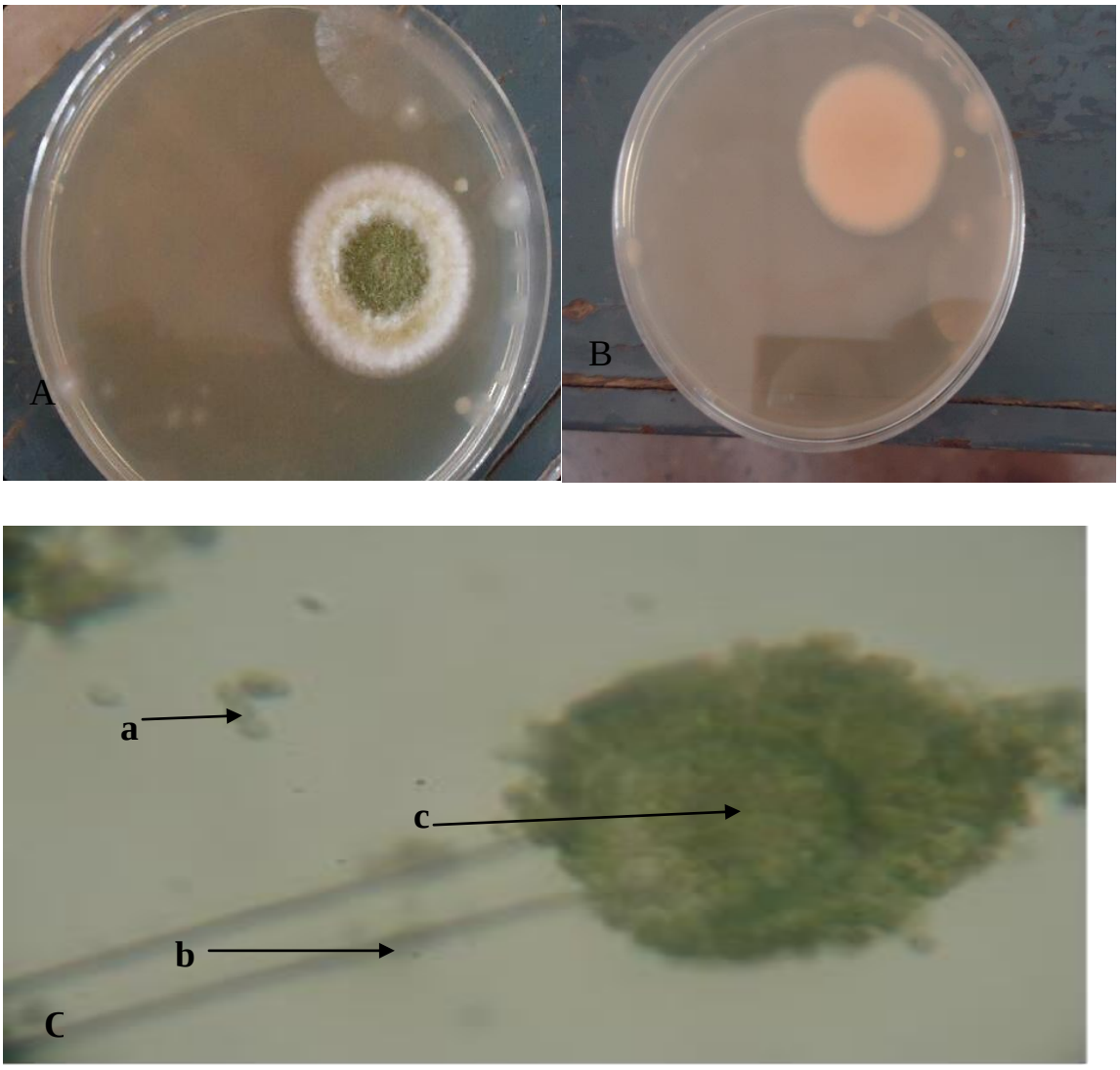


Plate II: Pure culture of *Aspergillus flavus* Link. A. Upper Surface, B. Reverse side, C. Microscopic features: a. conidia, b. conidiophore, c. vesicle

Each conidiophore tapering at the base, Vesicle measured 30-100µm in diameter with an average of 70µm. The vesicle radiate to loosely columnar with age. Conidia are smooth to very finely rough globose to subglobose. It measures between 2-5 µm in diameter with an average diameter of 3.92µm(Plate IIC: a, b and c).

4.2.3 *Fusariumoxysporum*Schlecht

4.2.3.1 *MacroscopicCharacteristics*

The surface of the colony was white but later became purple. The colony extended from 3-4cm in diameter in one week at room temperature.The topographywas cottony in nature as shown in Plate (III: A and B).

4.2.3.2 *MicroscopicChracteristics*

Conidia variable, principally of two kinds Macroconidia and Microconidia and often held in small moist heads.

Macroconidia: Hyaline Fusiform, slightly curved pointed at the tips. They are septate with three to five septations (3-5) celled, typically canoe in shape. The conidia measures 50-130µm in length with an average length of 70.96µm while the width measures 20-30µm at the top, 20-30µm at the middle and 20-30µm toward the end with an average width of 20.00µm at the top, 20.80µm at the middle and 20-20µm towards the end respectively (Plate IIIC).

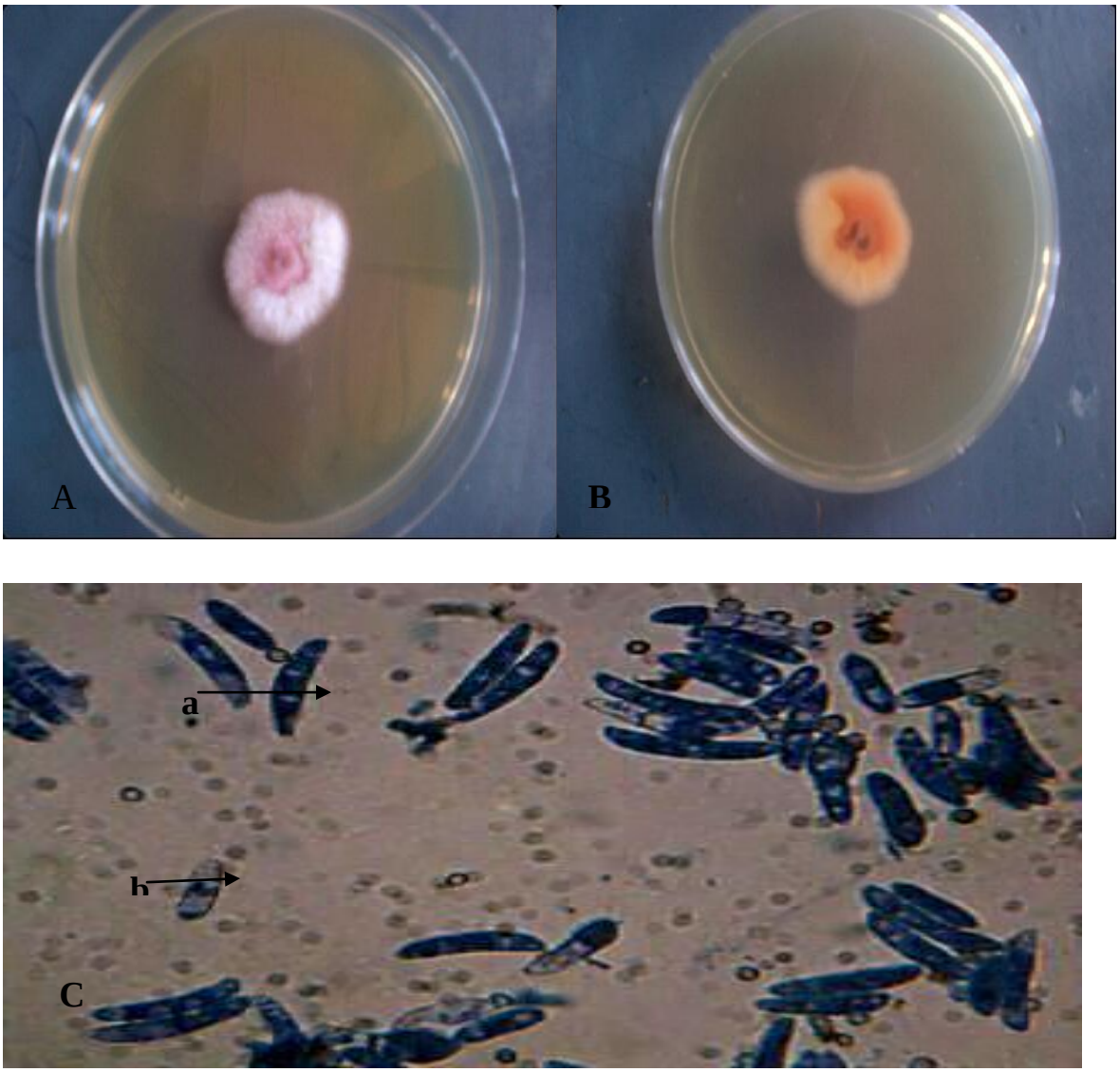


Plate III: Pure culture of *Fusarium oxysporum* Schlecht.A. Upper surface, B. Reverse side, C. Microscopic features of: a. Macroconidia, b. Microconidia

Microconidia: Microconidia are abundants never in chains, usually unicellular but 1-2 celled also occurred. Hyaline, fusiform to ovoid. The conidia measures and ranged between 20-30 µm in length with an average length of 30.20µm and the width measures 10-20µm with an average of 4.62µm(Plate IIIC).

4.2.4*Mucor*spMich

4.2.4.1 *MacroscopicCharacterestics*

Colonies are very fast growing expanded rapidly and cover the entire surface of the agar media 6cm in diameter in one week at room temperature. Cottony to fluffy, the colony was white in the begining becoming dark grey with time. The reverse was white in colour (Plate IV: A and B).

4.2.4.2 *MicroscopicCharacteristics*

Mycellium is non-septate, sporangiophore are errect and simple. Sporangiophore, measured 70-360µm in length with an average length of 237.80µm and width 20-30µm at the top, 20-30µm at the middle and 20-30µm at the base with an average diameter of 21.60µm. Sporangia are round 30-150µm in diameter with an average diameter of 51.20µm grey or black in colour and filled with sporangiospore. Sporangiospore hyaline, globose and smooth 3-4µm with an average diameter of 2.92µm (Plate IVc: a and b).

4.2.5*Rhizopusstolonifer*Ehrenb

4.2.5.1 *MacroscopicCharacterestics*

The colonies of *Rhizopus stolonifer* grew very rapidly from 5-8cm in diameter, fill the petridish and mature in 4 day. Texture typically cotton cottony, from the surface. The

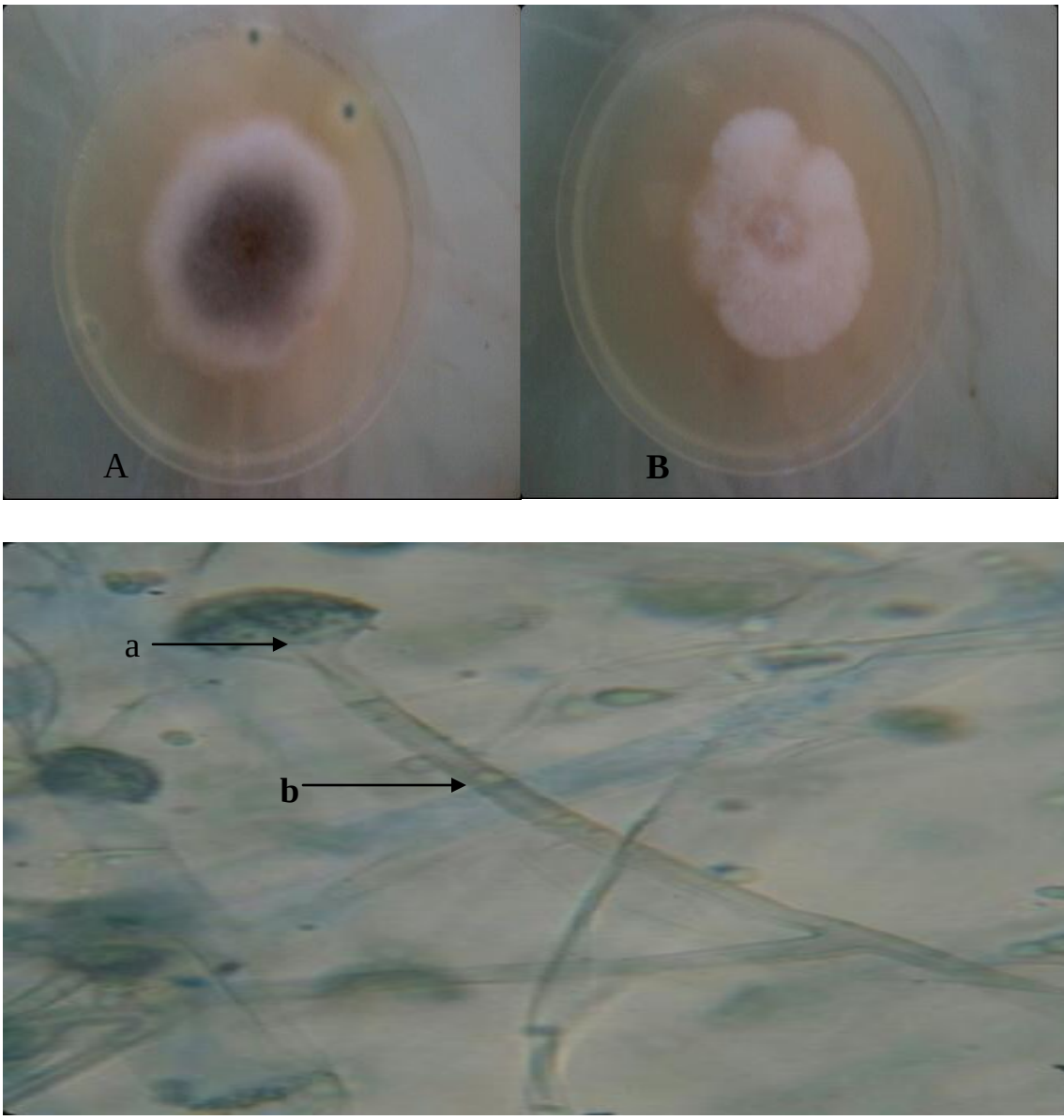


Plate IV: Pure culture of *Mucor sp* MichA. Upper surface, B. Reverse side, C. Micoscopic features, a. Sporangium, b. Sporangiophore

colour of the colonies were white initially and turns greyish to brown in time.The reverse is white to pale, colony expanded rapidly to about 5-8cm in diameter in one week at room temperature (Plate V: A and B).

4.2.5.2 *MicroscopicCharacterestics*

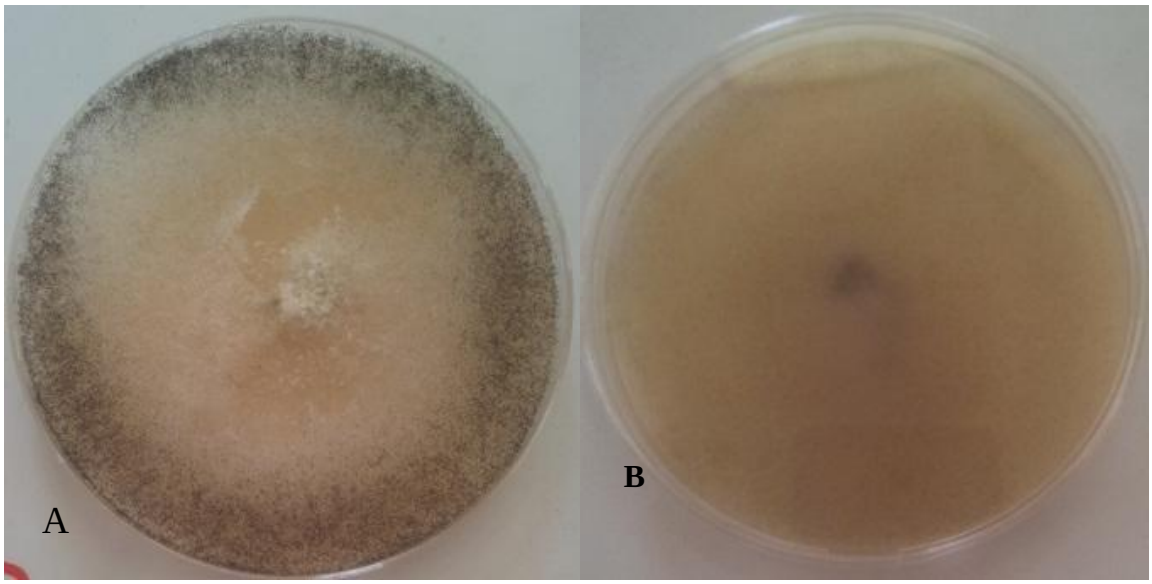
Mycelium was non–septate hyaline and white. Sporangiophore and are brown in colour and usually unbranched.Rhizoids are located at the points where the stolon and Sporangiophore errect brown in colour and usually unbranched Rhizoid are located at the points where the stolon and sporangiophore meet. Each Sporangiophore measured 100-540µm in length with an average length of 200.20µm and width 20-30µm at the top, 20-40µm at the middle and 20-30µm at the base with an average width 26.00 µm at the top, 26.20µm at the middle and an average width of 24.80µm near the base.

Sporangia are globose often with a flattened base greyish, black powdery in appearance up to 30-100µm in diameter with an average of 60.40µm. sporangia are located at the tip of the sporangiophores. sporangiospore are unicellular,smooth and brown in colour. Sporangiospore measures 2-4µm with an average of 2.64µm (Plate VC: a, b, c,d and e).

4.2.6*Rhizoctoniasolani*Khun

4.2.6.1 *MacroscopicCharacterestic*

The surface of the colony was light tan to black and the reverse was black in colour. The topography was cottony and concentric in nature and colony expanded from 5-7cm in diameter in one week at room temperature (Plate VI: A and B).



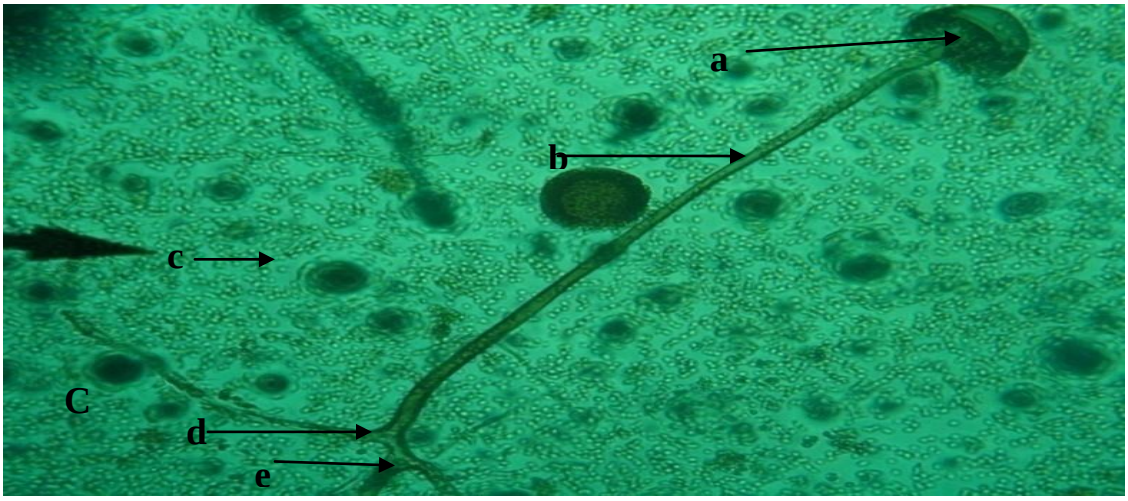


Plate V: Pure culture of *Rhizopus stolonifer*Ehrenb A. Upper surface, B. Reverse side,C. Microscopic features: a. Sporangiophore,b. sporangia,c. rhizoid, d. stolons

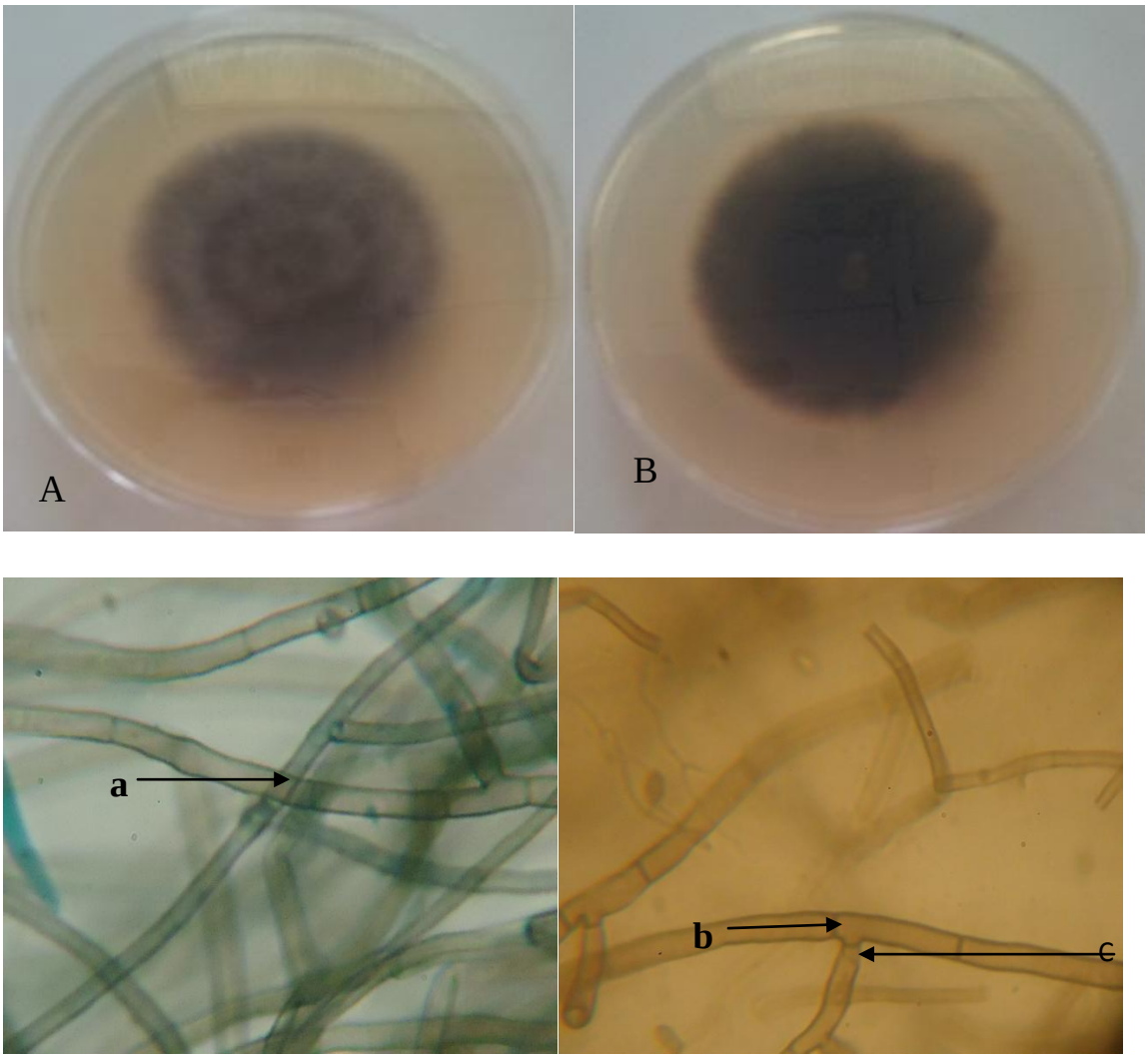


Plate VI: Pure culture of *Rhizoctonia solani* Khun.A.Upper surface,B. Reverse side,C. a. Mycelium, b. Septum, c. Branched mycelium

4.2.6.2 *MicroscopicCharacterestics*

Mycellium was hyline in some cases dark, usually long and septate, width measured 50-83µm with an average diameter of 54.20µm. Branches usually set off from main hyphae. Sexual fruit bodies and spores or conidia are absent or lacking. The hyphae (mycelium) often lightly pigmented with right angled branched septa (cross wall) occures near branching points (Plate VI: a, b andc).

4.3 Percentage Frequency of Occurrence of Isolated Fungi from UTC, Derica and Panchy on PDA

A total of 237 colonies belonging to five genera and six species were isolated from the rotten tomatoes fruits collected from Samaru Markets, Zaria,Kaduna State. Of all the fungi isolated *Aspergillus species (A. niger*and *A. flavus*) were the most frequently isolatefungi in the two season from the three Tomatoes varieties. UTC in the rainy season recorded (29.03 and 19.35%) for *Aspergillus niger* and *A. flavus* (Table 4.3). This was followed by *Rhizoctonia solani* with (16.13%), *Rhizopus stolonifer* and *Fusarium oxysporum* (12.91%) while *Mucor sp* was the least encountered (9.67%). In dry season *Aspergillus niger*recorded the highest with (41.67%) and the least was *Rhizoctonia solani* with (8.33%). Derica in rainy season recorded (50.00% and 18.18%) for *Aspergillus sp* (*A.niger and A.flavus*) and the least was *Mucor sp* and *Rhizoctonia solani* with the same percentage frequency of occurrence of (4.5%) respectively (Table 4.3). In the dry season, *Aspergillus sp* (*niger*and *flavus)* recorded the highest with (31.58% and 36.84) while *Rhizoctonia solani* recorded the least with (5.26%). Panchy variety In rainy season recorded (35.00% and 25.00%) for *Aspergillus niger*and *Aspergillus flavus.* while the least wasrecorded for *Mucor sp* and *Rhizopus stolonifer* with (10.00%). In dry season,*Aspergillus niger*(52.94%)

Table 4.3: **Percentage Frequency of Occurrence of Fungi Isolated on PDA from Three Varieties of Tomatoes**

Isolated fungi	Tomato variety					
	UTC		Derica		Panchy	
	(%) occurrence		(%) occurrence		(%) occurrence	
	Season					
	Rainy	Dry	Rainy	Dry	Rainy	Dry
Aspergillus niger	9(29.03)	10(41.67)	11(50.00)	6(31.58)	7(35.00)	9(52.94)
Aspergillus flavus	6(19.35)	4(16.67)	4(18.18)	7(36.84)	5(25.00)	3(17.65)
F. oxysporum	4(12.91)	3(12.50)	3(13.64)	2(10.53)	4(20.00)	3(17.65)
Mucor sp	3(9.67)	0(0.00)	1(4.54)	0(0.00)	2(10.00)	0(0.00)
Rhizoctonia solani	5(16.13)	2(8.33)	1(4.54)	1(5.26)	0(0.00)	0(0.00)
Rhizopus stolonifer	4(12.91)	5(20.83)	2(9.09)	3(15.79)	2(10.00)	2(11.76)
Total	31(100)	24(100)	22(99.99)	19(100)	20(100)	17(100)

Value outside the parenthesis is number of time a fungus is encountered. Value inside the parenthesis is the percentage occurrence

Followed by *Aspergillus flavus* and *F.oxysporum* with the same percentage of occurrence of (17.65%) and *Rhizopus stolonifer* recorded the least (11.76%) (Table 4.3).

4.4: Percentage Frequency of Occurrence of Isolated Fungi from UTC, Derica and Panchy on SDA

Aspergillus sp also recorded the highest percentage frequency of occurrence. UTC in the rainy season *Aspergillus niger*and *Aspergillus flavus* recorded (28.00% and 24%) while *F.oxysporum* recorded the least with (0.8%) and in the dry season *Aspergillus niger*recorded (40.00%) while the least was *F.oxysporum* with (0.4%). Derica in the rainy season recorded *Aspergillus niger*recorded (30.77%) and the least was *Rhizoctonia solani* (3.85%). While in the dry season *Aspergillus sp (niger and flavus*) recorded (25% and

29.17%) and the least was recorded in *Rhizoctonia solani* and *F. oxysporum* recorded (12.50%). Panchy variety, in the rainy season *Aspergillus niger*and *Aspergillus flavus* recorded (22.72% and 36.37%) and the least was recorded in *Mucor sp* (09.09%). While in the dry season,*Aspergillus niger*and *A. flavus* recorded (44.44% and 33.33%) and the least was recorded in *F.oxysporum and Rhizopus stolonifer* with (11.11%) percentage frequency of occurrence (Table 4.4).

4.5 *Invitro* Assay

4.5.1 Effects of Aqueous Extracts of leaf on percentage mycelial growth inhibition of isolated Fungi.

M. koenigii leaves aqueous extracts had significant effect on the percentage mycelial growth inhibition of the isolated fungi. All the concentrations of *M. koenigii*

Table 4.4: **Percentage Occurrence of Fungi Isolated on SDA from Three Varieties of Tomatoes**

Isolated fungi	Tomato variety					
	UTC		Derica		Panchy	
	(%) occurrence		(%) occurrence		(%) occurrence	
	Season					
	Rainy	Dry	Rainy	Dry	Rainy	Dry
Aspergillus niger	7(28.00)	10(40.00)	8(30.77)	6(25.00)	5(22.72)	8(44.44)
Aspergillus flavus	6(24.00)	4(16.00)	5(19.23)	7(29.17)	8(36.37)	6(33.33)
F. oxysporum	2(08.00)	1(04.00)	4(15.38)	3(12.50)	4(18.18)	2(11.11)
Mucor sp	3(12.00)	0(00.00)	2(7.69)	0(00.00)	2(09.09)	0(00.00)
Rhizoctonia solani	3(12.00)	5(20.00)	1(3.85)	3(12.50)	0(00.00)	0(00.00)
Rhizopus stolonifer	4(16.00)	5(20.00)	6(23.08)	5(20.80)	3(13.64)	2(11.11)
	25(100)	25(100)	26(100)	24(100)	22(100)	18(100)

Value outside the parenthesis is number of time a fungus is encountered. Value inside the parenthesis is the percentage occurrence

leaves aqueous extract resulted in significant mycelial growth inhibition of each of fungus than the control where there was no inhibition. The percentage mycelial growth inhibition for all the fungi were however, not the same even at the same concentration. The highest percentage mycelial growth inhibition for all the fungi occurred at 25% concentration (Table 4.5).

The growth inhibition of only *A.flavus* and *F.oxysporum* were comparable at 15% and 20% concentration with that at 25% while the the rest of them the percentage mycelial growth inhibition was not comparable. In addition, the degree of growth inhibition are not the same for all the isolated fungi in different concentration of extracts (Table 4.5).

4.5.2 Effects of Aqueous Extracts of the Stem of *Murraya koenigii* on Percentage Mycelial Growth Inhibition of Isolated Fungi.

M. koenigii stem aqueous extracts also had significant effect on the percentage mycelial growth inhibition of the isolated fungi. At all, concentrations *M. koenigii* Stem aqueous extract resulted in significant percentage mycelial growth inhibition of all fungi than the control where there was no inhibition. The percentage mycelial growth for all the fungi varies even at the same concentration. The highest percentage mycelial growth inhibition of each fungus occured at 25% concentration. This however, was comparable with *Mucor* sp at 10%, 15% and 20% concentration and *R. solani* at 20% concentration. For mojority of the fungi, the percentage mycelial growth inhibition were not comparable (Table 4.6).

Table 4.5: Percentage Mycelia Growth Inhibition of Isolated Fungi with Different Concentrations of Aqueous Extracts of Leaves of *Murraya koenigii*

Concentrations (%)	Fungal species					
	A. niger	***A. flavus***	***F.oxysprum***	***Mucor sp***	***R.solani***	***R.stolonifer***
0.0	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
5.0	37.50 ± 0.72^{c}	59.33 ± 2.38^{ab}	53.81 ± 7.66^{b}	36.11 ± 7.35^{b}	40.48 ± 3.12^{c}	18.75 ± 3.15^{c}
10.0	50.00 ± 7.22^{bc}	67.62 ± 5.79^{a}	64.29 ± 7.14^{ab}	$46.67.\pm5.30^{b}$	53.34 ± 0.48^{bc}	18.75 ± 2.89^{c}
15.0	52.92 ± 7.23^{b}	71.43 ± 7.87^{a}	68.57 ± 2.18^{a}	50.56 ± 2.94^{ab}	57.14 ± 7.56^{b}	25.00 ± 1.44^{bc}
20.0	65.00 ± 5.05^{ab}	72.86 ± 2.18^{a}	71.43 ± 2.47^{a}	51.67 ± 3.47^{ab}	67.14 ± 6.23^{ab}	31.25 ± 4.02^{ab}
25.0	76.67 ± 1.50^{a}	77.14 ± 4.36^{a}	77.91 ± 0.48^{a}	55.00 ± 11.10^{a}	71.43 ± 2.97^{a}	36.25 ± 3.15^{a}

Values are expressed as means ± SEM (Standard error of means).Means having different superscripted alphabet(s) along columns are significantly different at $P < 0.05$.

Table 4.6: Percentage Mycelia Growth Inhibition of Isolated Fungi with Different Concentrations of Aqueous Extracts of Stem Bark of *Murraya koenigii*

Concentration (%)	Fungal species					
	A. niger	***A. flavus***	***F.oxysporum***	***Mucor sp***	***R.solani***	***R.stolonifer***
0.0	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}
5.0	32.50 ± 1.91^{b}	53.67 ± 3.72^{c}	50.95 ± 2.65^{c}	32.22 ± 4.01^{b}	32.38 ± 1.91^{c}	12.50 ± 1.91^{bc}
10.0	30.00 ± 2.17^{b}	60.38 ± 3.90^{b}	56.19 ± 4.69^{bc}	46.67 ± 2.54^{a}	43.81 ± 3.33^{b}	12.50 ± 3.61^{bc}
15.0	37.50 ± 5.77^{ab}	62.10 ± 4.83^{ab}	65.71 ± 3.78^{ab}	47.22 ± 0.55^{a}	50.00 ± 4.36^{b}	15.00 ± 1.91^{b}
20.0	37.50 ± 0.00^{ab}	70.00 ± 1.65^{ab}	69.05 ± 4.83^{ab}	49.45 ± 1.47^{a}	62.86 ± 3.78^{a}	20.00 ± 3.61^{ab}
25.0	47.50 ± 5.45^{a}	73.81 ± 0.48^{a}	71.43 ± 6.60^{a}	50.00 ± 7.88^{a}	68.57 ± 3.30^{a}	23.75 ± 4.73^{a}

Values are expressed as means ± SEM (Standard error of means).Means having different superscripted alphabet(s) along columns are significantly different at $P < 0.05$.

4.5.3 Effects of Aqueous Extracts of the Root of *Murraya koenigii* on Percentage Mycelial Growth Inhibition of Isolated Fungi.

M. koenigii root aqueous extracts possess significant toxic effects on the mycelial growth of the isolated fungi. It also revealed that all the concentrations of extract resulted in significant percentage mycelial growth inhibition of each of the fungi than the control. Thehighest percentage mycelial growth inhibition of each fungi occured at 25% concentration. This was comparable with*A. flavus* at 20% concentration, *F. oxysporum* and *R. solani* at 15% and 20% concentration. For the the rest of them the percentage mycelial growth inhibition was not comparable at 25% concentration. It was also observed that inhibition increased with concentration(Table 4.7).

4.6 Comparison of the Efficacy of Different Concentration of Aqueous Extracts of Leaf, Stem bark and Root of *Murraya koenigii*.

Efficacy of different concentrations of aqueous extracts of leave, stem and root when compared. It was observed that leaves extracts was more effective in all concentration as compared to stem and root. Stem bark followed the leaves extracts at 5% and 10%.While the root extract proved to be less effective. However, at 15%, 20% and 25% concentration, the stem bark and root were almost comparable with one another in most cases. It was also observed that different organism had differential response to different concentration of different part. *F. oxysporum* did not show any significant difference in all the concentration except 25% concentration. *A. niger,* showed significant difference in all the concentration except 15%. *A. flavus* showed significant response in all concentration except 25%. *Mucor sp.* showed significant response in all the concentration except at 15%. *R. solani* shows significant difference only at 5% and 10% and *R. stolonifer* shows significant response in

Table 4.7: Percentage Mycelia Growth Inhibition of Isolated Fungi with Different Concentrations of Aqueous Extracts of Root of *Murraya koenigii*

Concentration (%)	Fungal species					
	A. niger	***A. flavus***	***F.oxysporum***	***Mucor sp***	***R.solani***	***R.stolonifer***
0.0	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
5.0	25.00 ± 0.72^{b}	44.76 ± 5.49^{c}	42.86 ± 2.97^{c}	16.67 ± 5.77^{b}	30.00 ± 7.95^{b}	6.25 ± 0.00^{cd}
10.0	25.00 ± 6.88^{b}	51.43 ± 3.59^{b}	51.90 ± 3.33^{b}	25.55 ± 11.88^{b}	30.00 ± 8.37^{b}	8.75 ± 1.91^{c}
15.0	37.50 ± 3.82^{ab}	61.91 ± 3.90^{b}	62.86 ± 2.18^{a}	33.33 ± 8.55^{b}	49.05 ± 3.81^{a}	12.50 ± 1.91^{bc}
20.0	37.92 ± 4.10^{ab}	63.81 ± 2.52^{a}	66.19 ± 1.26^{a}	36.67 ± 8.55^{b}	57.14 ± 4.13^{a}	17.50 ± 3.82^{ab}
25.0	43.75 ± 5.05^{a}	71.90 ± 2.90^{a}	67.14 ± 4.29^{a}	45.00 ± 5.85^{a}	61.43 ± 4.36^{a}	21.25 ± 3.31^{a}

Values are expressed as means ± SEM (Standard error of means).Means having different superscripted alphabet(s) along columns are significantly different at $P < 0.05$

all the concentration except at 10% concentration (Table 4.8a and b.). It is also evident that higher concentrations of all the extracts were more effective than the lower one.In addition,the degree of growth inhibition were not the same for all the isolated fungi at different concentration of extracts and for different part.

4.7 Effects of Methanol Extracts of the Three Parts.

4.7.1 Effects of Methanol Extracts of Leaf on Percentage Mycelial Growth Inhibition of Isolated Fungi.

M. koenigii leaves methanol extracts had significant effect on the percentage mycelial growth inhibition of the isolated fungi. All the concentrations of *Murraya koenigii* leaves methanol extract resulted in significant mycelial growth inhibition though the rate for each fungus in the same concentration varied. (Table 4.9) It is also evident that for each fungus, the highest growth inhibition was accomplished at higher concentration (25%) while in all the fungi, lower growth inhibition was obtained at lower concentration (5%). The growth inhibition of *A. flavus* and *F.oxysporum* were comparable at 20% concentrationwith that at 25% concentration. For those of *A.niger, Mucor* sp,*R. solani R.stolonifer* the growth was not comparable.It is also evident from the table that, growth inhibition increases with increase concentration.

4.7.2 Effects of Methanol Extracts of the Stem of *Murraya koenigii* on Percentage Mycelial Growth Inhibition of Isolated Fungi.

M. koenigii stem methanol extracts had significant effect on the percentage mycelial growth inhibition of the isolated fungi. All the concentrations of *Murraya koenigii* stem methanol extract resulted in significant mycelial growth inhibition of each of the fungus isolated

Table 4.8a: Comparison of the Efficacy of Different Concentration of Aqueous Extracts of Leaf, Stem and Root of *Murraya koenigii*

Concentration (%)	Plant parts	Fungal species					
		A. niger	***A. flavus***	***F.oxysporum***	***Mucor sp***	***R.solani***	***R.stolonifer***
0.0	Leaves	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]
	Stem barks	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]
	Roots	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]
5.0	Leaves	37.50±0.72[a]	59.33±2.38[a]	53.81±7.66[a]	36.11±7.35[a]	40.48±3.12[a]	18.75±3.15[a]
	Stem barks	32.50±1.91[b]	53.67±3.72[ab]	50.95±2.65[a]	32.22±4.01[a]	32.38±1.91[b]	12.50±1.91[b]
	Roots	25.00±0.72[c]	44.76±5.49[b]	42.86±2.97[a]	16.67±5.77[ab]	30.00±7.95[b]	6.25±0.00[c]
10.0	Leaves	50.00±7.22[a]	67.62±5.79[a]	64.29±7.14[a]	46.67±5.30[a]	53.34±0.48[a]	18.75±2.89[a]
	Stem barks	30.00±2.17[ab]	60.38±3.90[a]	56.19±4.69[a]	46.67±2.54[a]	43.81±3.33[b]	12.50±3.61[a]
	Roots	25.00±6.88[b]	51.43±3.59[ab]	51.90±3.33[a]	25.55±11.88[b]	30.00±8.37[bc]	8.75±1.91[a]

Values are expressed as means ± SEM (Standard error of means). Means having different superscripted alphabet(s) in each column, under each concentration are significantly different at P < 0.05.

Table 4.8b: Comparison of the Efficacy of Different Concentration of Aqueous Extracts of Leaf, Stem and Root of *Murraya koenigii*

Concentration (%)	Plant parts	Fungal species					
		A. niger	***A. flavus***	***F.oxysporum***	***Mucor sp***	***R.solani***	***R.stolonifer***
15.0	Leaves	52.92±7.23^{a}	71.43±7.87^{a}	68.57±2.18^{a}	50.56±2.94^{a}	57.14±7.56^{a}	25.00±1.44^{a}
	Stem barks	37.50±5.77^{a}	62.10±4.83^{ab}	65.71±3.78^{a}	47.22±0.55^{a}	50.00±4.36^{a}	15.00±1.91^{b}
	Roots	37.50±3.82^{a}	51.91±3.90^{b}	62.86±2.18^{a}	33.33±8.55^{a}	49.05±3.81^{a}	12.50±1.91^{b}
20.0	Leaves	65.00±5.05^{a}	72.86±2.18^{a}	71.43±2.47^{a}	51.67±3.47^{a}	67.14±6.23^{a}	31.25±4.02^{a}
	Stem barks	37.50±0.00^{b}	70.00±1.65^{ab}	69.05±4.83^{a}	49.45±1.47^{ab}	62.86±3.78^{a}	20.00±4.73^{ab}
	Roots	37.92±4.10^{b}	63.81±2.52^{b}	66.19±1.26^{a}	36.67±8.55^{b}	57.14±4.13^{a}	17.50±3.82^{ab}
25.0	Leaves	76.67±1.50^{a}	77.14±4.36^{a}	77.91±0.48^{a}	55.00±11.10^{a}	71.43±2.97^{a}	36.25±3.15^{a}
	Stem barks	47.50±5.45^{b}	73.81±0.48^{a}	71.43±6.60^{ab}	50.00±7.88^{a}	68.57±3.30^{a}	23.75±3.61^{b}
	Roots	43.75±5.05^{b}	71.90±2.90^{a}	67.14±4.29^{b}	45.00±5.85^{b}	61.43±4.36^{a}	21.25±3.31^{b}

Values are expressed as means ± SEM (Standard error of means). Means having different superscripted alphabet(s) in each column, under each concentration are significantly different at P < 0.05

Table 4.9: Percentage Mycelia Growth Inhibition of Isolated Fungi with Different Concentrations of Methanol Extracts of Leaf of *Murraya koenigii*

Concentration (%)	Fungal species					
	A. niger	***A. flavus***	***F.oxysporum***	***Mucor sp***	***R.solani***	***R.stolonifer***
0.0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}
5.0	44.17 ± 5.22^{c}	62.38 ± 1.72^{c}	65.24 ± 6.19^{b}	60.00 ± 2.89^{b}	45.71 ± 2.47^{c}	21.25 ± 4.39^{b}
10.0	52.92 ± 6.05^{bc}	72.38 ± 1.72^{b}	68.10 ± 5.49^{b}	61.67 ± 2.89^{b}	60.00 ± 3.60^{b}	23.75 ± 6.88^{b}
15.0	59.17 ± 8.46^{bc}	76.19 ± 3.33^{b}	75.24 ± 6.19^{ab}	66.67 ± 2.89^{ab}	64.29 ± 5.15^{ab}	18.75 ± 8.78^{b}
20.0	66.25 ± 1.91^{ab}	86.19 ± 3.72^{a}	85.24 ± 0.47^{a}	71.11 ± 3.89^{ab}	70.00 ± 7.87^{ab}	35.00 ± 6.61^{ab}
25.0	76.67 ± 1.67^{a}	91.43 ± 0.83^{a}	86.19 ± 0.48^{a}	76.67 ± 5.85^{a}	78.09 ± 4.54^{a}	40.00 ± 4.73^{a}

Values are expressed as means ± SEM (Standard error of means). Means having different superscripted alphabets along columns are significantly different at $P < 0.05$.

when compared with the control. The rate of mycelial growth inhibition for each fungus in the same concentration varies. It is also evident that for each fungus, the highest growth inhibition was accomplished at higher concentration (25%) while lower growth inhibition was obtained at lower concentration (5%).

The growth inhibition of *A.flavus, F.oxysporum, R. solani* and *R. stolonifer* at 20% concentrationwere comparable with that at 25% concentration while mojarity of the cases the inhibition of the fungi were not comparable. It also evident that, growth inhibition increases with increase concentration (Table 4.10).

4.7.3 Effects of Methanol Extracts of the Root of *Murraya koenigii* on Percentage Mycelial Growth Inhibition of Isolated Fungi.

M. koenigii root methanol extract showed significant toxic inhibition on the mycelial growth of the isolated fungi. It was revealed that all the concentrations of extract resulted in significant percentage mycelial growth inhibition of each of the fungi than the control. The highest percentage mycelial growth inhibition of each fungi occured at 25% concentration. These were comparable with only *F. oxysporum* at 20% concentrtionand *R. solani* at 10%, 15% and 20% concentration with that at 25% concentration. For the the rest of them the percentage mycelial growth inhibition was not comparable (Table 4.11).

4.8 Comparison of the Efficacy of Different Concentration of Methanol Extracts of Leaf, Stembark and Root of *Murraya koenigii*.

Efficacy of different concentration of aqueous leave, stem and root when compared, It was observed that leaves extracts were more effective in all the concentrations as compared to

Table 4.10: Percentage Mycelia Growth Inhibition of Isolated Fungi with Different Concentrations of Methanol Extracts of Stem of *Murraya koenigii*

Concentration (%)	Fungal species					
	A. niger	***A. flavus***	***F.oxysprum***	***Mucor sp***	***R.solani***	***R.stolonifer***
0.0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}
5.0	37.50 ± 3.31^{c}	60.95 ± 3.12^{c}	59.52 ± 4.97^{c}	45.55 ± 3.64^{b}	41.43 ± 2.47^{c}	12.50 ± 0.00^{b}
10.0	43.75 ± 4.51^{bc}	63.81 ± 1.26^{bc}	64.29 ± 2.97^{bc}	49.45 ± 5.47^{b}	48.57 ± 2.18^{bc}	15.83 ± 3.33^{b}
15.0	47.50 ± 3.15^{b}	70.48 ± 4.69^{b}	70.48 ± 4.15^{b}	53.33 ± 3.47^{b}	55.24 ± 6.72^{ab}	20.00 ± 3.82^{ab}
20.0	50.83 ± 0.42^{b}	85.24 ± 0.47^{a}	82.86 ± 0.83^{a}	55.00 ± 2.55^{b}	65.24 ± 2.90^{a}	25.00 ± 1.91^{a}
25.0	61.25 ± 2.17^{a}	90.48 ± 2.52^{a}	83.33 ± 1.26^{a}	70.56 ± 5.64^{a}	66.19 ± 3.33^{a}	26.25 ± 2.60^{a}

Values are expressed as means ± SEM (Standard error of means). Means having different superscripted alphabets along columns are significantly different at $P < 0.05$.

Table 4.11: Percentage Mycelia Growth Inhibition of Isolated Fungi with Different Concentrations of Methanol Extracts of Root of *Murraya koenigii*

Concentration (%)	Fungal species					
	A. niger	***A. flavus***	***F.oxysporum***	***Mucor sp***	***R.solani***	***R.stolonifer***
0.0	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}
5.0	29.17 ± 1.10^{d}	51.90 ± 2.52^{c}	60.95 ± 8.14^{b}	45.00 ± 1.92^{c}	34.28 ± 1.43^{b}	11.25 ± 0.72^{c}
10.0	37.50 ± 0.72^{c}	55.71 ± 1.65^{c}	62.38 ± 2.38^{b}	48.33 ± 1.67^{bc}	50.48 ± 4.15^{a}	12.50 ± 0.72^{c}
15.0	46.67 ± 4.64^{b}	60.95 ± 4.83^{bc}	68.57 ± 4.95^{b}	49.44 ± 0.56^{bc}	50.47 ± 2.65^{a}	17.50 ± 3.31^{bc}
20.0	50.00 ± 0.00^{b}	69.05 ± 5.30^{b}	81.90 ± 3.81^{a}	53.89 ± 5.30^{ab}	52.38 ± 10.48^{a}	21.25 ± 2.60^{ab}
25.0	59.58 ± 2.73^{a}	88.09 ± 1.26^{a}	82.86 ± 0.83^{a}	61.67 ± 2.54^{a}	62.38 ± 2.08^{a}	25.00 ± 2.89^{a}

Values are expressed as means ± SEM (Standard error of means). Means having different superscripted alphabets along columns are significantly different at $P < 0.05$.

stem and root. Stem bark followed the leaves extracts at 5% and 10% in majority of the cases. While the root extract proved to be less effective as compared to theleaves and stem bark.However, at 15%, 20% and 25% concentration, the stem bark and root were almost comparable with one another in most cases. It was also observed thatdifferent organism had differential response to different concentration of different part. *F. oxysporum* did not show any significant difference at all concentration. *A. niger,* showed significant difference in all the concentration except 15%. *A. flavus* showed significant response in all concentration except 25%. *Mucor* sp. showed significant response in all the concentration except at 25%. *R. solani* shows significant difference only at 15% and 20% concentration and *R. stolonifer* shows significant response in all the concentration except at 15% concentration (Table 4.12a and b.).

4.9 Comparison of Efficacy between Aqueous and Methanol Extracts of *Murraya koenigii* on Percentage Mycelial Growth Inhibition of Isolated Fungi.

Efficacy between aqueous and methanol extracts of *M. koenigii* on isolated fungi showedthat, methanol extract was more effective than the aqueous *Mucor* sp. at all concentration showed significant difference when compared with aqueous extract. *A. niger*shows significant difference at allconcentration except at 5% concentration when compared with aqueous extract. *A. flavus,* showed significant response only at 20% and 25% concentration. *F. oxysporum* showedsignificant difference except at 10% and 15% concentration. *R. solani* shows significantdifference only at 10% concentration and *R.stolonifer* did not show any significant difference in all concentration (Table 4.13a and b).

Table 4.12a: Comparison of the Efficacy of Different Concentration of Methanol Extracts of Leaf, Stem and Root of *Murraya koenigii*

Plant parts	Concentration (%)	Fungal species					
		A. niger	***A. flavus***	***F. oxysporum***	***Mucor sp.***	***R. solani***	***R. stolonifer***
	Leaves	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{d}
0.0	Stem barks	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{d}
	Roots	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{d}
	Leaves	44.17±5.22^{a}	62.38±1.72^{a}	65.24±6.19^{a}	60.00±2.89^{a}	42.86±2.47^{a}	21.25±4.39^{a}
5.0	Stem barks	37.50±3.31^{ab}	60.95±3.12^{a}	59.52±4.97^{a}	45.55±3.64^{b}	41.43±2.47^{ab}	12.50±0.00^{ab}
	Roots	29.17±1.10^{b}	51.90±2.52^{b}	60.95±8.14^{a}	45.00±1.92^{b}	34.28±1.43^{b}	11.25±0.72^{b}
	Leaves	52.92±6.05^{a}	72.38±1.72^{a}	68.10±5.49^{a}	61.67±2.89^{a}	60.00±3.60^{a}	23.75±6.88^{a}
10%	Stem barks	43.75±4.51^{b}	63.81±1.26^{b}	64.29±2.97^{a}	49.45±5.47^{ab}	48.57±2.18^{b}	15.83±3.33^{a}
	Roots	37.50±0.72^{c}	55.71±1.65^{c}	62.38±2.38^{a}	48.33±1.67^{b}	50.48±4.15^{b}	12.50±0.72^{ab}

Values are expressed as means ± SEM (Standard error of means). Means having different superscripted alphabet(s) in each column, under each concentration are significantly different at $P < 0.05$.

Table 4.12b: Comparison of the Efficacy of Different Concentration of Methanol Extracts of Leaf, Stem and Root of *Murraya koenigii*

Concentration (%)	Plant parts	Fungal species					
		A. niger	***A. flavus***	***F. oxysporum***	***Mucor sp.***	***R. solani***	***R. stolonifer***
15.0	Leaves	59.17±8.46^{a}	76.19±3.33^{a}	75.24±6.19^{a}	66.67±2.89^{a}	64.29±5.15^{a}	18.75±8.78^{a}
	Stem barks	47.50±3.15^{a}	70.48±4.69^{ab}	70.48±4.15^{a}	53.33±3.47^{ab}	55.24±6.72^{a}	20.00±3.82^{a}
	Roots	46.67±4.64^{a}	60.95±4.83^{b}	68.57±4.95^{a}	49.44±0.56^{b}	50.47±2.65^{a}	17.50±3.31^{a}
20.0	Leaves	66.25±3.31^{a}	86.19±6.44^{a}	85.24±0.82^{a}	71.11±6.73^{a}	70.00±13.63^{a}	35.00±11.46^{a}
	Stem barks	50.83±0.72^{b}	85.24±0.82^{a}	82.86±1.43^{a}	55.00±4.41^{b}	65.24±5.02^{a}	25.00±3.31^{ab}
	Roots	50.00±0.00^{b}	69.05±9.18^{b}	81.90±6.59^{a}	53.89±9.18^{b}	52.38±18.15^{a}	21.25±4.51^{b}
25.0	Leaves	76.67±1.67^{a}	91.43±0.83^{a}	86.19±0.48^{a}	76.67±5.85^{a}	78.09±4.54^{a}	40.00±4.73^{a}
	Stem barks	61.25±2.17^{b}	90.48±2.52^{a}	83.33±1.26^{a}	70.56±5.64^{a}	66.19±3.33^{ab}	26.25±2.60^{b}
	Roots	59.58±2.73^{b}	88.09±1.26^{a}	82.86±0.83^{a}	61.67±2.54^{a}	62.38±2.08^{b}	25.00±2.89^{b}

Values are expressed as means ± SEM (Standard error of means). Means having different superscripted alphabet(s) in each column, under each concentration are significantly different at P < 0.05.

Table 4.13a:Comparison of Efficacy between Aqueous and Methanol Extracts of *Murraya koenigii*

Concentrations (%)	Extract	Fungal species					
		A. niger	***A. flavus***	***F.oxysporum***	***Mucor sp***	***R. solani***	***R.stolonifer***
0.0	Aqueous	0.0±0.00 ^{c}	0.0±0.00 ^{c}	0.0±0.00 ^{c}	0.0±0.00 ^{c}	0.0±0.00 ^{c}	0.0±0.00 ^{c}
	Methanol	0.0±0.00 ^{c}	0.0±0.00 ^{c}	0.0±0.00 ^{c}	0.0±0.00 ^{c}	0.0±0.00 ^{c}	0.0±0.00 ^{c}
5.0	Aqueous	31.67±1.92^{a}	51.59±2.70^{a}	49.21±2.98^{b}	28.33±4.18^{b}	34.29±2.98^{a}	12.50±2.09^{a}
	Methanol	36.94±2.83^{a}	58.41±2.07^{a}	61.91±3.39^{a}	50.18±2.85^{a}	39.52±1.72^{a}	15.00±2.03^{a}
10.0	Aqueous	35.00±4.82^{b}	60.48±3.29^{a}	57.46±3.21^{a}	35.00±4.92^{b}	42.38±4.27^{b}	13.33±2.05^{a}
	Methanol	44.72±3.13^{a}	63.97±2.53^{a}	64.92±2.10^{a}	53.15±2.83^{a}	53.01±2.46^{a}	17.36±2.78^{a}

Values are expressed as means ± SEM (Standard error of means). Means having different superscripted alphabet(s) in each column, under each concentration are significantly different at P < 0.05.

Table 4.13b: Comparism of Efficacy between Aqueous and Methanol Extracts of *Murraya koenigii*

Concentrations (%)	Extracts	Fungal species					
		A. niger	***A. flavus***	***F. oxysporum***	***Mucor sp***	***R. solani***	***R. stolonifer***
15.0	Aqueous	42.64±3.87[b]	67.14±3.21[a]	65.71±1.63[a]	43.70±3.71[b]	52.06±3.03[a]	17.50±2.10[a]
	Methanol	51.11±3.56[a]	69.21±3.11[a]	71.43±2.77[a]	56.48±2.92[a]	56.67±3.26[a]	18.75±2.95[a]
20.0	Aqueous	46.81±4.92[b]	68.89±1.71[b]	68.89±1.78[b]	45.93±3.57[b]	62.38±2.82[a]	22.92±2.98[a]
	Methanol	55.69±2.70[a]	80.16±3.35[a]	83.33±1.24[a]	60.00±3.45[a]	62.54±4.68[a]	27.08±2.95[a]
25.0	Aqueous	55.97±5.64[b]	74.29±1.70[b]	70.16±2.40[b]	50.00±4.51[b]	67.14±2.33[a]	27.08±2.86[a]
	Methanol	65.83±2.94[a]	90.00±0.98[a]	84.13±0.69[a]	69.63±3.28[a]	68.89±2.93[a]	30.42±2.98[a]

Values are expressed as means ± SEM (Standard error of means). Means having different superscripted alphabet(s) in each column, under each concentration are significantly different at P <0.05.

CHAPTER FIVE

5.0 DISCUSSION

The present study showed that a number of fungi areassociated with post-harvest rot diseases of Tomatofruits. These fungi includes: *Aspergillus niger* vantieghem, *Aspergillus flavus* Link, *Fusarium oxysporum* Schlecht, *Mucor* spMich, *Rhizoctonia solani* Khun and *Rhizopus stolonifer* Ehrenb. They have been previously reported as fruit rotpathogens of Tomato fruits byOgaraku*et, al.,* (2010) that isolated and identified *Aspergillus niger, Fusarium oxysporium* from deteriorated tomato.

(Ijato, 2011) isolated *Fusarium oxysporum, Fusarium solani, Aspergillus niger, Aspergillus flavus,* and *Rhizpous stolonifer* from rotten Tomato. Onyia *et, al.,* (2005) also isolated that *Fusarium monilifome*, *Aspergillus niger* and *Rhizopus stolonifer* wereisolated from rotten tomatoes fruits.These pathogens have reportedly been isolated from Pawpaw fruits in Nigeria (Baiyewu *et, al*., 2007). (Chukwuka *et, al*., 2010 and Akintobi *et, al*., 2011) isolated *Rhizopus stolonifer, Apergillus niger, A. flavus* and *Fusarium solani* from some selected fruits.

These results clearly demostrate that these pathogen are omnipresent irrespective of geographical area and involved with tomato rot. Association of these fungi with different types of fruits indicates that these fungi are not host specialized. They may be found any where food is available.

The Fungi species isolated on PDA and SDA from the three varieties of rotten tomatoes were the same. These were however, not evenly distributed. *Rhizoctonia solani*

was not even isolated from panchy variety on the two media. Possibly, Panchy variety has resistance to the growth of *Rhizoctonia solani* as reported by Isaac *et, al.,* (2014).

It is worthy to note that during isolation, *Mucor sp.* was isolated only in the rainy season in the dry season. This is an indication that *Mucor* sprequires more humidity to grow as reported by Hill and Waller, (1999). Isolation was higher inthe rainy season than in the dry season which could be an indication that moisture contents play a major role in the growth of fungi species. This also agrees with the findings of Ibrahim and Rahma (2009). It is a standing rule that fungal spore, except powdery mildew, reguires certain types of water for germination, growth and sporulation (Yarwood, 1989).*Mucor* sp. isolated where seen to share similar characteristic with those reported by Mbajiuka and Emmanuel, (2014).

However, slight differencesthat were observed in the colour of the colony and differences in microscopic measurement during identification may be attributed to variations in the environmental condition and culture media used as reported by Kalu and Khan, (2000).

The results of Percentage occurrence obtained, *Aspergillus niger* and *Aspergillus flavus* predominated. This indicates that the group possibly acts as pioneer organisms in the colonisation of wounds of tropical crops and damages stored seeds (Kalu, 2002, Kalu and Khan, 2000, Ferrante *et al.,* 2012). Ebele, (2011) reported that presence of wounds on the fruits, enabled some fungi to acts as primary invader and other as secondary invaders for disease to occur. Another reason for the high occurrence of this genus, could also be due to its ability to produce neccessary enzymes for the utilisation of the nutritional component in

the media which gave them the competitve advantage over the other fungi species as also reported by Isaac *et, al.,* (2014).

These pathogen isolated, therefore, might be responsible for post-harvest rot of tomatoes. Okigbo *et, al.,*(2009) reported that *Aspergillus niger* and *Mucor sp* were responsible to cause rot of cassava. Ijato *et, al.,* (2011) reported that *Aspergillus niger, Fusarium oxysporum, Rhizopus stolonifer Geotricum candidium* were associated with spoilage of tomato fruits.

All these fungi isolated from spoilt tomatoes have been reported toxigenic. (Kendrick 2000, Al-Hindi *et al*., 2011). These may also cause infections or allergies (Eaton and Groopman, 1994, Muhammad *et, al*., 2004, Baiyewu *et,al*., 2007 and Monso, 2004).The presence of these fungi examples *A. niger* may be a potential harzard to human health in Nigeria and most probably are the cause of increasing incidence of liver and kidney problems in west Africa and Nigeria in particular(Akintobi *et, al.,* 2011)..

In this study, both aqueous and methanol extracts of the leaves, stem bark and root were found to have significant mycelia growth inhibitions of all the isolated fungi. This may be attributed to the presence of bioactive compounds. These bioactive compounds possess antimicrobial activities (Kesari *et, al.,* 2005).

However, the efficacy of the extracts varies with the type of fungi, concentration, plant parts and the type of solvent used for extraction. The variation that occurred in the inhibition of different fungi may be due to the differences in the fungitoxic potentials between the plants extracts on the fungi species and also may be due to the sensitivity of

each of the fungus pathogen to the different concentration of the extracts. (Ahmadioah, 2000, Okigbo and Nmeka, 2005 and Nwachukwu Umechuruba 2001).

It was also evident that increase concentration caused increase growth inhibition and lower concentration resulted in low growth inhibition. This may be due to presence of higher quantity of bioactive compounds in higher concentration. These findings agree with the findings of Kumar *et, al.,* (2013). Different fungi showed different inhibition rate at the same concentration and certain fungus show comparable response at certain concentration. This is in line with the findings of Nwachukwu and Umechuruba, (2001).

When the efficacy of extracts from the three parts the leaves, stem bark and root were compared (aqueous and methanol) it was shown that in most of the cases the three part had significant differences in the percentage mycelia growth inhibition with the leaves exhibiting the highest percentage mycelia growth inhibition. This reason could be attributed to the photosynthetic nature of the leaves. As a result qualitative and quantitative compound are synthesized and stored in the leaves. Chinhiro *et, al.*,(1993) also reported that leaves extracts of *M. koenigii* possessed stronger antifungal and antibacteria activities than the root extract. Ajay *et, al*., (2011) who isolated Girinimbiol and Girinimbine from the leaves of *Murraya koenigii* and reported to be the most active carbazole alkaloids that are responsible for their antifungal activities.

Similarly, Ramsewak *et ,al.*,(1999) isolated Mahanimbine, Murrayanol and Mehane from the fresh leaves of *Murraya koenigii* and reported that these compounds have antimicrobial activities.

The stem bark follows the leaves and the root was the last in mycelial growth inhibition on each fungus isolate.This might be due to the fact that in stem bark and root, complex compound are accumulated and may not be easily dissolved in water and methanol used for the extraction. Though Das *et, al.,* (1965) isolated Murrayanine, Girinmbine and Mahaninbine from stem bark which showed antifungal activities on some pathogenic fungi.

Efficacy of different plant extracts also depend on the type of solvent used for the extraction. In this study, it was observed that, methanol extracts was more superior in the mycelial growth inhibition when compared with aqueous extracts. Bertha*et al.*,(2012) and Nwachukwu Umechuruba (2001) reported that methanol extracts were more effective in reducing mycelial growth of fungi when compared with aqueous extracts. This could be an indication that methanol could have extracted a greater number of constituents than aqueous extracts and being an organic compound, methanol must have extracted more compound.

The use of plants have been showing promising and positive response to the control of fungi and they don't have adverse effect like sythetic chemicals (Amadioha and Obi, 1999). Plant with such fungicidal properties includes *Murraya koenigii* (Bertha *et al.*, 2012).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Five (5) genera and Six (6) species were Isolated and identified macroscopically and microscopically. These include: *Aspergillus niger* Van Tieghem,*Aspergillus flavus* Link, *Fusarium oxysporum* Schlecht *Mucor sp.* Mich, *Rhizoctonia solani* Khun *and Rhizopus stolonifer* Ehrenb.

2. All the extracts leaves, stem bark and root (aqueous and methanol) significantly inhibited mycelial radial growth of fungi in all concentrations with the leave extracts (aqueous and methanol) being more effective, followed by the stem bark and lastly with the root extract.

3. Methanol extract was more effective than aqueous extract in the mycelial growth reduction of the isolated fungi and showed significant differences in most cases.

6.2 Recommendations

1. These work has shown some activities of aqueous and methanolextracts of Leaves, stem bark and root of *Murraya koenigii*. These plants have been reported to containGirinimbiol, Girinimbine, carbazole alkaloids, Mahanimbine, Murrayanol and Meha newhich are responsible for their antifungal activities.

2. The plant should also be cultivated and be used in a sustainable manner to avoid extinction.

3. I also recommend that further research be carried out on *invivo* application.

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APPENDICES I

Preparation of Different Concentrations of *Murraya koenigii* extract

Varying concentrations (%) of 5, 10, 15, 20 and 25% were prepared using serial dilution method from 30% stock solution, employing the following formula $C_I V_I = C_2 V_2$.

Where

C_1 = Initial concentration of different ground parts (30g)

V_1 = Initial volume of solvent used.

C_2 = Final concentration of extracts

V_2 = Final volume of solvent required

APPENDICES II

Mycelia growth inhibition of isolated fungi with different concentrations of aqueous extracts of leaves, stem and root of *Murraya koenigii*

Rot fungi	*A. niger*			*A. flavus*			*Fusarium oxysporium*			*Mucor sp*			*Rhizoctonia solani*			*Rhizopus stolonifer*		
Control	8cm			7cm			7cm			6cm			6cm			8cm		
treatment	L	S	R	L	S	R	L	S	R	L	S	R	L	S	R	L	S	R
Concentrations	5.0	5.2	6.0	2.6	3.6	3.1	4.0	3.2	3.6	4.0	3.6	5.6	3.9	4.6	5.3	6.3	6.9	7.5
5%	4.9	5.7	6.1	3.1	3.1	4.3	3.5	3.3	4.1	4.5	4.4	5.0	4.0	5.0	5.6	7.0	6.8	7.5
	5.1	5.3	5.9	3.1	3.1	4.2	2.2	3.8	4.3	3.0	4.2	4.4	4.6	4.6	3 8	6.2	7.3	7.5
Mean	5.0	5.4	6.0	3.3	3.0	3.9	3.2	3.4	4.0	4.0	4.1	5.0	4.2	4.7	4.9	6.8	7.0	7.5
10%	5.0	5.3	6.9	3.0	3.4	3.2	2.0	3.7	3.0	5.1	3.5	5.5	3.3	4.3	6.0	6.5	6.5	7.0
	4.0	5.9	5.0	2.2	2.7	3.1	3.5	2.9	3.3	3.0	3.1	3.1	3.2	4.0	4.7	6.1	7.0	7.5
	3.0	5.6	6.1	1.6	2.1	3.9	2.0	2.6	3.8	2.1	3.0	4.8	3.3	3.5	4.0	6.9	7.5	7.4
Mean	4.0	5.3	6.0	2.3	2.6	3.4	2.5	3.1	3.4	3.4	3.2	4.5	4.0	3.2	3.9	6.5	7.0	7.3
15%	3.4	5.0	4.6	2.9	2.9	3.2	2.5	2.0	2.9	2.7	3.2	3.0	3.8	2.9	3.3	6.0	6.7	6.7
	3.0	5.8	5.6	1.0	2.9	2.3	2.0	2.9	2.4	2.9	3.1	4.3	3.2	3.7	4.1	6.2	7.1	7.1
	4.9	4.2	4.8	2.1	2.1	2.5	2.1	2.3	2.5	3.3	3.2	4.7	2.0	3.9	3.3	5.8	6.6	7.2
Mean	3.8	5.0	5.0	2.0	2.2	2.7	2.1	2.4	2.6	3.3	3.1	4.0	3.0	3.5	3.6	6.0	6.8	7.0
20%	3.6	5.0	4.5	2.2	2.3	2.6	2.0	2.6	2.2	2.6	2.9	2.8	2.2	3.1	3.0	5.6	6.3	6.8
	2.5	5.0	5.6	1.7	2.1	2.8	1.7	2.4	2.4	2.8	3.0	4.1	1.6	2.2	2.5	6.0	5.8	6.0
	2.3	5.0	4.8	1.8	1.9	2.2	2.3	1.5	2.5	3.3	3.2	4.5	3.1	2.5	3.5	4.9	7.1	7.0
Mean	2.8	5.0	5.0	1.9	2.1	2.5	2.0	2.1	2.4	2.9	3.0	3.8	2.3	2.6	3.0	5.5	6.4	6.6
25%	2.1	4.3	5.3	2.0	1.8	2.0	2.0	2.8	2.0	3.6	3.9	3.7	2.1	2.2	3.3	5.4	6.1	6.4
	1.8	4.9	4.0	1.8	1.8	1.6	1.9	1.2	2.9	3.1	2.3	2.6	1.6	2.6	2.3	4.6	5.6	5.8
	1.7	3.4	4.2	1.0	1.9	2.3	2.0	2.0	2.0	1.4	2.8	3.6	2.3	1.8	2.5	5.3	6.6	6.7
Mean	2.6	4.2	4.5	1.6	1.8	1.9	1.9	2.0	2.3	2.7	3.0	3.3	2.0	2.2	2.7	5.1	6.1	6.3

APPENDICES III

Mycelia growth inhibition of isolated fungi with different concentrations of methanol extracts of leave, stem and root of *Murraya koenigii*

Rot fungi	*A. niger*			*A. flavus*			*Fusarium oxysporium*			*Mucor sp*			*Rhizoctonia solani*			*Rhizopus stolonifer*		
Control	8cm			7cm			7cm			6cm			7cm			8cm		
treatment	L	S	R	L	S	R	L	S	R	L	S	R	L	S	R	L	S	R
Concentrations	5.3	5.5	5.7	2.7	3.0	3.3	2.0	3.4	3.4	2.4	3.7	3.3	3.7	4.4	4.5	7.0	7.0	7.1
5%	4.0	4.9	5.5	2.8	2.9	3.1	3.3	2..9	3.2	2.7	3.0	3,5	4.0	3.8	4.8	6.0	7.0	7.0
	4.1	4.6	5.8	2.4	2.3	3.7	2.0	2.2	1.7	2.1	3.1	3.1	4.3	4.1	4.5	5.9	7.0	7.2
Mean	4.5	5.0	5.6	2.6	2.7	3.4	2.4	2.8	2.8	2.4	3.3	3.3	3.8	4.1	4.6	6.3	7.0	7.3
10%	4.2	4.7	4.9	1.7	2.5	3.3	3.0	2.6	2.8	2.0	3.2	3.0	3.0	3.7	4.0	7.2	7.0	6.9
	4.3	3.8	5.1	2.0	2.4	2.9	1.8	2.8	2.3	2.3	2.4	3.3	3.1	3.8	3.4	5.5	7.0	7.1
	2.8	5.0	5.0	2.1	2.7	3.1	1.9	2.1	2.8	2.6	3.5	3.0	2.3	3.3	3.0	5.6	6.2	7.0
Mean	3.8	4.5	5.0	1.9	2.5	3.1	2.2	2.5	2.6	2.3	3.0	3.1	2.8	3.6	3.8	6.1	6.9	7.0
15%	4.6	3.7	5.0	1.3	1.6	2.3	2.5	2.6	2.2	2.3	2.7	3.0	3.2	3.5	3.6	7.3	6.0	6.7
	2.8	4.5	4.0	2.1	1.9	3.4	1.7	2.0	2.8	1.7	3.2	3.1	2.3	3.7	3.1	7.1	6.2	6.1
	2.4	4.4	3.8	1.6	2.7	2.5	1.0	1.6	1.6	2.0	2.5	3.0	2.0	2.2	3.7	5.1	7.0	7.0
Mean	3.6	4.2	4.3	1.7	2.1	2.7	1.5	1.9	2.2	2.0	2.8	3.0	2.5	2.8	3.1	5.5	6.4	6.6
20%	2.6	3.9	4.0	1.4	1.0	1.7	1.0	1.3	1.0	1.8	2.6	3.4	2.2	2.4	2.6	5.0	5.8	6.0
	3.0	4.0	4.0	1.0	1.1	2 9	1.1	1.1	1.8	2.1	2.5	2.4	1.1	2.1	4.8	6.2	6.3	6.2
	2.5	3.9	4.0	0.5	1.0	1.9	1.0	1.2	1.0	1.3	3.0	2.5	3.0	2.8	2.6	4.4	5.9	6.7
Mean	2.7	3.9	4.0	0.9	1.0	2.2	1.0	1.2	1.3	1.7	2.7	2.7	2.1	2.5	3.0	5.2	6.0	6.3
25%	1.6	2.8	3.4	0.7	1.0	1.0	1.0	1.3	1.3	2.1	1.1	2.2	2.1	2.0	2.9	5.5	5.5	6.4
	2.0	3.1	3.5	0.5	0 6	0.7	0.9	1.2	1.2	1.0	2.2	2.1	1.0	2.3	2.6	4.2	6.0	6.0
	2.0	3.4	2.8	0.6	0.4	0.8	1.0	1.0	1.1	1.1	2.0	2.6	1.5	2.8	2.4	4.7	6.2	5.6
Mean	1.8	3.1	3.2	0.6	0.7	0.9	0.9	1.2	1.2	1.4	2.1	2.3	1.5	2.0	2.6	4.8	5.9	6.0