THE EFFECT OF AQUEOUS AND METHANOL EXTRACTS OF *MORINGA OLEIFERA* (LAM) AERIAL PARTS ON FUNGI ISOLATED FROM SELECTED VARIETIES OF *SORGHUM BICOLOR L.*

BY

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BY

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DEPARTMENT OF BIOLOGICAL SCIENCES,
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NIGERIA.

FEBRUARY, 2015.

DEDICATION

This work is dedicated to the Almighty God, and my late Brother Mohammed Sani, and the entire members of my family. I love you all.

DECLARATION

I declare that the work in this thesis entitled 'The effect of aqueous and methanol extracts of *Moringa oleifera* (Lam) aerial parts on fungi isolated from selected varieties of *Sorghum bicolor* L.has been carried out by me in the Department of Biological Sciences under the supervisions of Prof. A.U. Khan and Dr. D.B. Dangora.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.

Idris Bala DAN'AZUMI _____ _____

Signature Date

CERTIFICATION

This thesis entitled "THE EFFECT OF AQUEOUS AND METHANOL EXTRACTS OF *MORINGA OLEIFERA* (LAM) AERIAL PARTS ON FUNGI ISOLATED FROM SELECTED VARIETIES OF*SORGHUM BICOLOR* L." by Idris Bala DAN'AZUMI, meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

This study was conducted in finding out the *invitro* effects of aqueous and methanol extracts of Moringa oleifera (Lam) aerial parts on fungi isolated from some selected varieties of Sorghum bicolor L. Different varieties of *Sorghum bicolor* L. namely Samsorg 17, Samsorg 40, Samsorg 42, Samsorg 43 were obtained from Plant Science Department IAR, and a local land race Kaura was collected from Sabon Gari Market in Zaria. Five different fungi namely *Aspergillusflavus*Link,*Aspergillus niger* VanTieghem,*Fusarium oxysporum* Schlecht, *Rhizopus stolonifer* (Ehrenb.)Lind *and Phoma herbarum* Sacc were isolated from the grains. These were isolated on both potato dextrose agar(PDA) and Saboraud's dextrose agar(SDA) using the agar plate method. The predominance of *Aspergillusspp.* indicate that they possibly act as pioneer organisms in the seed borne infection while the presence of other isolates show that there is possible succession of fungi in the seed borne infection. On *invitro* culture of these organisms in PDA amended with varying concentrations of *Moringaoleifera*Lam extracts (Aqueous and Methanol) from the leaves, seeds and stem bark using the radial growth method, extract from the leaves were found to be more effective than the other two in the mycelia growth inhibition of the isolated fungi. Growth of these fungi inhibits more in the presence of higher concentrations as compared to lower concentrations of extract. However the cumulative effects of these extracts were found to vary with concentration, plant parts, pathogen as well as type of solvent used for the extraction.

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CHAPTER ONE

1.0 INTRODUCTION

Sorghum (*Sorghum bicolor* L.*)* is the fifth most important world cereal and an important native cereal in Africa (FAO, 1995 and ICRISAT, 1996; Murty and Renard, 2001). Total world production of sorghum in the year 2002 was estimated at about 54 million tons (FAO, 2004) and annual production of about 70 million metric tons of grains from 50 million hectares of land (National Academic of Science, 1996). Sorghum is also the dietary staple of more than 500 million people in more than 30 countries (National Academic of Science, 1996).

In Nigeria, sorghum is an important staple cereal grown in more than twenty states of the federation including Akwa Ibom and Enugu states (Wudiri and Fatoba, 1992). It accounts for about 50% of the total cereal crops grown in Nigeria.According to Alegbejo (2002), Sorghumis the second most important cereal in Nigeria. The estimated annual production is about 9 million tons produced on about 6 million hectares. Average grain yield of subsistent farmers in the West African sub-region are generally between 500 to 800 kg/ ha (Sharma *et al.*, 1992).

Sorghum is a principal source of energy, protein, vitamins and minerals to the poorest people of the semi – arid tropics. The crop is dried, stored, and later used to prepare stiff porridge, thin porridge or fried dumpling.It is also used in brewing local beer (NAS, 1996). The leaves provide fodder for farm animals and the stalk are used in fencing, roofing, weaving baskets and mats and also as fuel wood (Obilana, 1995). *Sorghum*

grains are used industrially in the manufacture of items such as wax, starch, syrup, alcohol, dextrose agar, edible oils and gluten feed. In addition, it is used to manufacturegypsum lath, paper and cloth sizing and adhesives (Onwueme and Singh, 1991; Komlaga *et al.*, 2001; Murty and Renard, 2001).

The major constraints of sorghum production are insects, birds and diseases. Fungi and bacteria are important disease causing organisms (Gwary *et al.,* 2003). Various reports have shown yield losses of up to 67% (Pande *et al.,* 1991; Marley, 1996; Gwary *et al.,* 2002; Halt, 1994; Gachomo *et al.,* 2004).

Seed borne inoculums, have severe implication for yield, seed production and distribution systems, trade, human nutrition and germplasm. The management of these pathogens during the seed – borne phase is considered to be the cheapest control strategy (Shenge, 2007).

Diseases are difficult to control in grain sorghum after they develop, therefore the most inexpensive, efficient and effective disease control procedures are preventive. Many studies have indicated that in some plants, there are many substances such as peptides, unsaturated long chain aldehydes, alkaloid constituents, some essential oils, phenols and water, ethanol, chloroform, methanol and butanol soluble compounds with potential significant therapeutic application against pathogens including bacteria, fungi and virus (Elastal *et al.,* 2005). The investigation of certain indigenous plants for their antimicrobial activity may yield useful result.

Moringa (*Moringa oleifera*) is a sole genus in the flowering family Moringaceae. The name is derived from the Tamil word Moringai or the Malayalam word muringa. English common names include Moringa, and drumstick tree.Moringagrows quickly in many types of environments. It is mainly grown in semi-arid, tropical, and subtropical areas.It grows best in dry sandy soil.It can however, tolerate poor soil including coastal areas. It is widely cultivated in Africa, Cambodia, Nepal, Indonesia, Malaysia, Mexico, Central and South America and Srilanka.Much of the plant is edible by humans or by farm animals (Ted, 2010). The leaves are rich in proteins, vitamin A, Vitamin B, Vitamin C and minerals (Sandford, 2011). Moringa also contains antioxidants, anti-inflammatory as well as essential amino acids (Ted, 2010). The bark, sap, roots, leaves, seeds, oil, and flower are used in traditional medicine in several countries (Sandford, 2011).*Moringa oleifera* is a highly valued plant, distributed in many countries of the tropics and sub-tropics. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and various phenolics (Farooq *et al*., 2007). The Moringa plant provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals. It is a very important plant for its medicinal value (Ted, 2010). Various parts of the plant such as the leaves, roots, seeds, fruits, flowers and immature pods act as cardiac and circulatory stimulants, it possess anti-tumour (Makonnen *et al*., 1997), antipyretic, antiepileptic, anti-inflammatory, and anti-ulcer (Pal *et al.*,1995) agents. Other important medicinal properties of the plant includes anti-plasmodic (Caceres *et al.,* 1992), anti-hypertensive (Dahot, 1988), cholesterol lowering (Mehta *et al*., 2003), anti-oxidant, anti-diabetic, hepatoprotective (Ruckmani *et al*., 1998), anti-bacterial and anti-fungal activities (Nickon *et al*., 2003). *Moringa oleifera*

parts are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in south Asia (Farooq *et al*., 2007). Vast fields in developing countries are blessed with abundant plants with fungicidal activities such as *Euphorbia hirta* L., *Azadirachta indica* L. and *Delonixregia* L. (Alegbejo, 2002).

Plant extracts act as contact fungicides by disrupting cell membrane integrity at different stages of fungal development, while others inactivate key enzymes and interfere with metabolic process (Murty and Renard, 2001). Crops treated with plant extracts produce and accumulate elevated levels of specialized proteins and other compounds which inhibit the development of fungal diseases (Yorinori, 1994). Plant extracts therefore, can serve as an alternative to the use of fungicide as it is effective, cheap, available and more environmentally friendly.

1.1 Statement of Research Problem

Fungi are natural enemies to farm produce both at pre and post-harvest levels, causing severe damage in both quantity and quality of the produce. They account for yield losses of up to67% (Pande *et al.,* 1991, Gwary *et al.,* 2002, Halt, 1994, Marley, 1996, Gachomo *et al.,* 2004).

Taylor and Francis (2001) reported that fungi cause about 50 – 80% damage to farmers sorghum during storage period or when conditions are favourable for their development resulting in significant loss both quantitatively and qualitatively. In addition, fungi produce mycotoxins which are hazardous to man and animal. Therefore, their prevention and control becomes obligatory. At present, many fungicides are used to control the disease, but these are not environmental friendly and are causing serious health

hazards(Obilana, 1995). At the same time, they are very costly, and are beyond the reach of peasant farmers. On many occasions, the required fungicides may not be available in the market as most of them are imported from developed countries.

1.2 Justification

Owing to the economic importance of the pathogen (fungi) in causing yield loss of Sorghum which is a threat to food security, it becomes mandatory to device means through which their growth and development will be hindered. The use of plant extracts have played significant role in the improvement of seed quality and field emergence of plant seeds. The development of non-toxic, safe and effective biodegradable alternatives to synthetic fungicides has in recent years, led to global screening of various plants for bioactivity against plant pathogenic organisms (Onifade, 2000, Yorinori, 1994). Plants generally contain a wide variety of free radicals scavenging molecules including phenols, flavonoids, vitamins and terpenoids that are rich in antifungal activity (Cai and Sun, 2003). Plant leaves, stem bark, buds, seeds and roots offer potentials for fungi control through the production and release of toxic mixtures of phytocompounds/allelochemicals in them (Barnes *et al.*, 2007). 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.

1.3 Aim

The aim of the research is to identify the fungi associated with *Sorghumbicolor* grains and to assess the antifungal properties of*Moringaoleifera* leaves, stem bark, and seed extracts.

1.4 Objectives of the Study

The objectives of the study were;

1. To isolate and identify fungi associated with sorghum grains of the selected varieties of Sorghum.
2. To assess *invitro* effect of methanol and aqueous extracts of *Moringaoleifera* aerial partsonthe growth of isolated fungi.

1.5 Hypotheses

1. Fungi are not associated with sorghum grains of the selected varieties of Sorghum.
2. Methanol and aqueous extracts of *Moringaoleifera*aerial partshave no effect on the growth of isolated fungi.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance and Production Levels of Sorghum

Sorghum is an annual plant belonging to the family Poaceae. It is an important crop in West Africa. In fresh form, the young leaves, immature pods and peas are used as vegetable, while snacks and main dishes are prepared from dried grains. All the plant parts that are used for food are nutritious, providing proteins, vitamins and minerals. FAO (2007) estimated that 3.3 million tons of sorghum dried grains were produced worldwide. Nigeria produces 2.1 million making it the world's largest producer.

Chemical composition of sorghum include: carbohydrates, proteins, water, crude fiber, ash, phosphorous, calcium and iron and these corresponds with most edible crops (Coetzee, 1993). The seeds also contain small amount of β-carotenes equivalent, thiamine, riboflavin, vitamin A, niacin, folic acid and ascorbic acid (Murty and Renard, 2001).

2.2 Pathogenic Fungi

Fungi are eukaryotic, heterotrophic, and absorptive organisms that developed tubular, rather diffuse branched body and reproduce by means of spores. This described not a single phylogenetic line, but rather a way of life shared by organisms of different evolutionary backgrounds (Kendricks, 2000). The anamorphic ascomycetous fungi are the most important terrestrial fungal group that concerns the mycologist. These fungi can asexually produce small dry spores that are readily distributed into the atmosphere by the slightest physical disturbance making them ubiquitous. This feature promotes their ability

to contaminate great varieties of field crops, stored agricultural products, and a host of other materials.

Pathogenic fungi are filamentous (mould) and many are able to produce a wide range of secondary metabolites. Some of these metabolites are pigments, some have antibiotic properties and some metabolites are toxic to plants and animals. The three genera *Aspergillus, Penicillum*and *Fusarium*are considered to be the most significant toxigenic moulds at the present time (Miller and Trenhlm, 1994). Other anamorphic ascomycetous species other than Aspergilli, Penicilliaand Fusaria include *Alternaria alternata, Pithomyces chartarium, Trichithecium rouseum, Rhizoctonia leguminicola, Stachybotry atra, Myrothecium roridum* and*Phomopsis lephtrostromiformis* (Miller and Trenhlm, 1994).

2.3 Seed-borne Fungi of Sorghum

A range of pathogens, primarily fungi damage the sorghum plant which include the Following:

2.3.1 Headsmut

This soil borne fungus attacks Sorghumappearing on the ear and tassels. Infection may befrom individual spikelets or a large mass of black spores. Usually, if the tassels are smuttedall the ears will be smutted (Coetzee, 1993)

2.3.2 Brown spot

It is generally not a serious problem. Symptoms are small, round, yellow spots on leavesand sheathes that appear in bands across the leaf lesions that turn brown and blend togetherto form large blotches (Coetzee, 1993).

2.3.3 Southern sorghum rust

Southern rust is more destructive to leaf tissue than common rust. Southern rust pustules are smaller, more round and orange in colour. The pustules that are formed cause the leaf to turn yellow and die. Yields are reduced due to heavy infection at ear filling (Coetzee, 1993).

2.4 Fungi Contaminating Foods and Feed Stuff

Cereal grains, stored grains and processed foods of both plant and animal origin harbour large number of fungi under different environmental conditions. These fungi include parasitic species that are host specific, saprophytic species which even tend to be selective in their choice of living substrate and environment. Fungi are major cause of spoilage in stored grain and seeds, and rank second only to insects causing deterioration and loss (Miller and Trenholn, 1994).

In 1974, Christensen, classified the fungi identified from cereal into three groups namely; Field, storage and advanced decay fungi. Field Fungi invade developing and mature seeds before harvesting and include species of *Altenaria, Fusarium, Helminthosporium, Cladosporium, Chaetomium* and*Curvularia*in order of predominance (Javis, 1997). All field fungi require moisture content of between 20-25% to grow and so are referred to as hydrophilic fungi (Brull *et al*., 1999).

The storage fungi that invade grains after harvesting in storage consist of the few yeast (Christensen, 1974, Javis 1997). This group is also known as mesophytic storage fungi that has the following as their representative species: *Aspergillus flavus, A. fumigatus,*

A.niger, A. terreus, Paecilomyces variotti, Penicilium guarantio griseum, P. citrinum and*P. viridicatum* (Brull*et al*., 1999).

The major factors influencing the development of this group of fungi are moisture content of the stored grain, temperature, storage period, degree of earlier invasion before arrival to storage site, amount of foreign material and the activities of insects and mites (Bhikane *et al*., 1981).

Advanced decay fungi require the same general moisture range as field fungi but rarely develop on seed in the field and consist of the genera, *Fusarium*and *Chaetomium*. This fungus grows after considerable damage from other micro-organism has occurred (Javis, 1997).

2.5 Factors Influencing Fungal Development and Mycotoxin Production

The occurrence of mycotoxins in foods and feeds depends on factors such as geographical locations, season and the conditions under which a particular crop is grown, harvested, processed and stored. Production of mycotoxins can occur in the field before harvest, or post-harvest during storage, processing, or feeding. Several factors that influence the degree of fungal growth include:

2.5.1 Moisture

The singularly most important environmental condition controlling mould growth is moisture. Generally, hot humid conditions enhance fungal growth and mycotoxins production in tropics (Bhikane *et al*., 1981).

2.5.2 Water activity

All micro organisms including toxigenic fungi have minimum, optimum, and maximum water activity for growth. The minimum water activity of most species colonizing stored cereal is about 0.70 (70% aw) (Bhikane *et al*., 1981).

2.5.3 Temperature effects

Like fungal growth rates, mycotoxins production is influenced by ambient temperature. Fungi are known to grow slightly below 0 to as high as 60^0C with each fungal species having its characteristic minimum, optimum, and maximum temperature requirement for growth. Generally, fungi grow readily and produce mycotoxins between 20-30^0C, although certain fungi e.g. *Fusarium* spp do not grow at low temperature and produce toxin (Bhikane *et al*., 1981).

2.5.4 Substrate material

Many studies on species such as S*tachybotrys chartarum* performed from cultures grown on white rice clearly indicated that under this condition, the species could produce a wide range of mycotoxins than when grown on different substrates. Their presence in a substrate of different materials that support fungal amplification may be one of the key factors influencing mycotoxins production (Fog, 2001).

2.5.5 The presence of other species

Several species of *Penicillum* have been shown to increase mycotoxins production, and in some instances to change mycotoxins in response to adjacent colonies of other species. The influence of other species appears to some extent to be dependent on the type and quality of mycotoxins being produced (Bhikane *et al*., 1981).

2.6 Fungi Metabolites

Fungi have been reported to produce many metabolites known as toxins. These toxins have been reported to cause many adverse effects on human beings and livestock (Fog, 2001). They include Aflatoxin, Ochratoxin, Fumonism, Trichothecenes and Zearalenone (Bhat and Vasanthi, 2003).

2.7 Use of Plant Botanicals as Fungicides

Plant extracts have played significant role in the inhibition of seed borne pathogens and in the improvement of seed quality and field emergence of plant seeds. The development of non toxic, safe and effective biodegradable alternative to synthetic fungicides has in recent years, led to global screening of various plants for bioactivity against plant pathogenic organisms (Onifade, 2000 and Yorinori, 1994). However, it was estimated that only about 10% of the over 280,000 different plant species in the world today have been examined chemically for antimicrobial activity (Udoh and Cardwel, 2000).

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 of 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).

Fungi toxic activity of vernolepin and vernodalin isolated from *Vernonia amygdalina* showed that *Aspergillusniger*and *Candidaalbicans* were sensitive to both pigments. Two new flavones 4,6,7-trihdroxy 3,5 – dimethyl flavones and 5,5 – dihydroxy 3,4,8 –

reimethoxy flavones were isolated from *Artemisia giraldii*. These two new flavones also showed antitoxic activity against *Aspergillusflavus* and*Trichoderma viride* (Zheng *et al*., 1996). Essential oils from *Azadirachta indica* and *Morinda lucida* were found to inhibit the growth of toxigenic *A.flavus* and significantly reduced aflatoxin synthesis in inoculated maize grains (Soliman and Badea, 2002).

The efficacy of plant materials in controlling mycotoxigenic moulds has not been well documented.There has not been any concerted effort of a large scale trial of these plants on the farmer's field. Udoh and Cardwel, (2000) were of the view that caution must be exercised in using plant materials to control mycotoxins, because some of these materials are natural media for *Aspergillus flavus*. *Khayasenegalensis* bark used to protect maize against insect increased the risk of aflatoxin development and that even the farmers were aware of low efficiency of the indigenous products. Most of the plants being screened for ability to control storage Fungi are traditional medicines (Hell *et al*, 2000).

2.8 *Moringa oleifera*(Lam)

It is a fact that natural products from plants remain vastly underutilized resources for the discovery of novel antimicrobial compounds, yet we live in a world where most pathogens can be controlled by these natural products. The majority of higher plant species are yet to be explored as potential sources of antimicrobial agents. The use of botanicals in disease management has been going on for a very long time in traditional practices (Ted, 2010).

Moringa oleifera Lam. is a member of the family Moringaceae.It is the most widely cultivated species which is a multipurpose tree native to the foothills of the Himalayas in

north western India and cultivated throughout the tropics. *Moringa stenopetala*, an African species, is also known but to a much lesser extent than *Moringa oleifera*. The plants market potential is wide spread given its easy growth and high nutrient value of its leaves and high protein seeds. It can also be made into defatted meal for consumption by hypertensive patients (Sandford, 2011).

Moringa oleifera siviculture is being promoted as a means to combat poverty and malnutrition. The leaves are rich in protein, vitamin A, B, C and minerals. They also contains antioxidants, anti-inflammatory and essential aminoacids (Ted, 2010). Feeding the high protein leaves to cattle has been shown to increase weight gain by up to 32% and milk production by 43 to 65% (Sanford,2011).

The seeds contain 30 to 40% oil that is high in oleic acid, while degreased meal is 61% protein. The defatted meal is a flocculant and can be used in water purification to settle out sediments and micro organisms (Schwarz, 2000). Most recently, the *Moringa oleifera* is being cultivated in poverty-stricken nations such as Niger, as a primary source of food and nutrients and a rich source of anti oxidants (Ted, 2010). A portion of the leaves and stem bark are drunk by hypertensive and diabetic patients while in some cases, the stem bark is used to treat typhoid fever by inhalation or hydrotheraphy (Ruckmani *et al*., 1998).

Chromatographically, the plant contains several phytocompounds such as phenol, terpenoids, cardiac glycosides, steroid, alkaloid, saponins, anthraquinones, tannins, flavanoids as well as reducing sugars (Elastal *et al*., 2005). Due to its phytochemical

nature, it has been used as fungicide, antibacterial, and antiviral agent, for dermatological infections, dental treatment, pain relief, and fever reduction (Nickon *et al*., 2003).

Researchers have found that the leaves stem bark, seeds, oil, fruits, and flowers of *Moringa oleifera* have fungicidal activity and could also inhibit mycotoxin formation (Farooq *et al.*, 2007). Over the years, *Moringa oleifera* has been shown to possess active principles in leaves, bark, seeds and then can be exploited for their medicinal, biological and biocidal properties. Makonnen *et al*. (1997) found out that the various parts of Moringa also have antitumour properties.

2.9 Mycotoxins in Sorghum

Although humans face health risks stemming from contamination of grains with other naturally occurring substances, mycotoxins are unique in that they are produced naturally on grains. Their presence is usually associated with uncontrollable factor such as climatic conditions. Mycotoxins are produced by certain fungi (e.g. *Aspergillus* spp, *Penicilliumspp*, and *Fusarium* spp) that grow on grain and feed ingredients such as sorghum, maize, wheat, barley, and peanuts.

Research results support the contention that generally soft endosperm sorghum is more susceptible to seed pest than harder endosperm in types and that the biggest problem with the dense genotypes is endosperm softness (Gropler, 1994). Despite insecticide application to reduce core earworm, these insects cause major cob damage in predisposition of plants to seeds mould damage by providing entry point and vectoring moulds if a substrate is spoiled by the growth of mould, that is caused above all by the production of mycotoxins (Abbas and Micrha, 1989).

Mycotoxins occurrence is not restricted to underdeveloped countries. Aflatoxin in sorghum in the south eastern United States is the known mycotoxins that is more likely to occur at higher concentration in the Tropical or subtropical developing countries of the world, the bulk of mycotoxin research in Latin America has been conducted on sorghum and specified on aflatoxin although other toxin such as zearalenone, T-2 toxin, deoxynivalenol, penicillin acid, Kojic acid and ochratoxins have been detected in sorghum.

Aflatoxin is the major food borne carcinogenic hepatoxins of great public health importance in Africa and indeed in the world. They are produced by *Aspergillus niger, Aspergillus flavus, Aspergillus parsiticus and Aspergillius nominus* on cereal, nuts, dried fruits and even water (Candlish and Smith, 2001) which when consumed by man and animal caused damage to liver and kidney, intestinal hemorrhages and death of affected organisms (Sahin and Korukluoglu, 2000).

Ochratoxins *A.* (OTA) is a mycotoxin produced by different species of *Aspergillus* and *Penicillium*, though it was first isolated from cultures of *Aspergillusochreaceus* (Kawashima and Valente, 2006). It is found as natural contaminants in many food stuffs such as dried fruits, corn etc.

Zearalenone is produced mainly by *Fusariumgraminearum* and related species. It is found principally in maize and wheat. Zearalenone and its derivatives produce estrogenic effects in various species (infertility vulvae, oedema, prolapsed and mammary hypotrophy in females and fertilization of males in Puerto Rico). Zearalenon was found in the blood of children with precocious sexual development and contaminated food

(Kawashima and Valante, 2006). It was also found together with other fusarium mycotoxin in “scabby grain toxicosis” (Roddricks, 1978).

Trichothecenes are mycotoxins produced mostly by members of the fusarium genus. The most frequent contaminants are deoxynivalenol (DON) known as vomitoxin, nivalenol (NIV), diacetoxy scripenol (DAS), while T-2 toxin is rare (Bhat and Vasanthi, 2003). Common manifestions of trichothecenes toxicity are depression of immune responses, nausea, and sometimes vomiting. The first recognized trichothecenes mycotoxicoses was alimentary toxin leukemia in the USSR in 1932 with mortality rate of 60% (Roddricks, 1978).

Fumonisms was discovered in South Africa in 1978 and is produced by *Fusarium verticilloides* and *Fusarium proliferatum*whichare recently receiving increasing attention in scientific literature because they have been implicated in a number of animal diseases such as leukoencephalomalacia in equines, which involves massive liquefactions of the cerebral hemisphere of the brain (Richard *et al*., 1998). Fumonism have also been identified as a carcinogen in humans, in addition to adversely affecting brain, liver and lung function, it can cause hepatotoxicity and nephrotoxicity in many animals (Kawashima and Valente, 2006).

Due to the presence of Fungi and mycotoxin in sorghum grains, the different methods of prevention that could be used inciude the use of pesticides on grains, evidence of physical damage to the grain, inhibition of biosynthetic pathway all seen or observed before harvest (pre-harvest prevention). Post harvest detoxification methods include the use of

physical methods like temperature, moisture content, time, irradiation and chemical method such as application of acids, oxidizing agent etc. (Miller and Trenholm, 1994).

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 located in Kaduna state between latitude $11^0$09′N, Long 7^0 42′E at an altitude of 686 meters above sea level.

3.2 Source of Materials

Five different varieties of *Sorghum bicolor* L. were used in this study. Four different sorghum varieties namely Samsorg 17, Samsorg 40, Samorg 42, Samsorg 43 were obtained from Plant Science Department, IAR and a local variety "Kaura" was purchased from Sabon Gari market, Zaria.Moringa seeds, leaves and stem bark were collected from trees growing in the Botanical garden of Biological Sciences, ABU main campus. The extracts were obtained from all these parts and were used in this study.

3.3 Preparation of Media

Two kinds of media Potato Dextrose Agar (PDA) and Saboraud's Dextrose Agar(SDA) were prepared and used as described by manufacturer's recommendations.

3.4 Dispensing of Media

15ml of both PDA and SDA were dispensed into individual Petri-dishes with the aid of a syringe.

3.5 Isolation ofFungi from Sorghum Grains

This wasdone by inoculating the two prepared growth media in separate petridishes. Before inoculation, the grains were randomly selected and surface sterilized with 1% sodium hypochlorite solution (NaOCl) for 2 minutes to remove surface contaminants and then rinsed with sterilized distilled water three times to remove remains of sodium hypochlorite. Five grains of each of the selected varieties were splitted with sterilized forceps and then inoculated in each of the prepared media and incubated at room temperature for seven days. Every plate with two different media was in triplicates. After the incubation period, the numbers of visible colonies that appear werecounted. All the discrete colonies of fungi were sub-cultured to obtain pure cultures.

3.6 Identification ofFungi

Isolated fungi were identified by using both microscopic and macroscopic characters. The identification was aided by reference to the description of fungi using identification keys ofBarnett and Hunter (1972),Larone (2002), Klich (2002) and Samson*et al.* (2004).

3.7 Preparation of Extracts of *Moringaoleifera*

3.7.1 Methanol extract

This was done using the soxhlet extraction method. The leaves, seeds and stembark of *Moringa oleifera*collected were rinsed thoroughly with tap water followed by distilled water to remove all forms of dirt. The samples were then shade dried under room temperature for three weeks after which the samples were ground using clean pestle and mortar.Thirty grams of different parts of the ground powders were placed in a porous cellulose thimble. The thimble was placed in an extraction chamber containing 50ml of

methanol, which was suspended above a flask containing 50ml of methanol and below a condenser. The flask was heated and the methanol evaporated up into the condenser where it got converted into liquid and trickled back into the extraction chamber containing the different ground parts. The liquid continued to accumulate in the extractor containing the samples until it reached a point where it overflowed and trickled back into the boiling flask. At the end of the extraction process which took a minimum of six hours, the flasks containing the extracted ingredients were removed and the solvent was recovered using a rotary evaporator leaving behind the active compounds which were removed and stored in the form of paste for further use.

3.7.2 Aqueous extract

This was obtained using the soxhlet extraction method. The leaves, seeds and stembark of *Moringa oleifera* collected were rinsed thoroughly with tap water followed by distilled water to remove all forms of dirt. The samples were then shade dried under room temperature for three weeks after which the samples were ground using clean pestle and mortar. Thirty grams of different parts of the ground powders were placed in a porous cellulose thimble. The thimble was placed in an extraction chamber containing 50ml of distilled water, which was suspended above a flask containing 50ml of distilled water and below a condenser. The flask was heated and the distilled water evaporated up into the condenser where it got converted into liquid and trickled back into the extraction chamber containing the different ground parts. The liquid continued to accumulate in the extractor containing the samples until it reaches a point where it overflowed and trickled back into the boiling flask. At the end of the extraction process which took a minimum of six hours, the flasks containing the extracted ingredients were removed and the solvent

was recovered using a rotary evaporator leaving behind the active compounds which were removed and stored in the form of paste for further use.

3.8 EffectofDifferent ConcentrationsofMethanol andAqueous Extracts of*Moringa oleifera*ontheGrowth ofFungi Species

5ml each of varying concentrationsviz:5, 10, 15, 20 and 25% of both aqueous and methanol extract were dispensed separately onto the 15ml moltenmedium in petri dishes with the help of a syringe. These were thoroughly mixed and allowed to solidify. A portion of fungal growth from the periphery was cut with the help of a sterilized cork borer of 5mm in diameter and placed onto the middle on each petri dish and incubated at room temperature. A week after incubation, measurement of the colony was taken directly with the help of a scale. Control experiment was set up without adding any plant extract. Difference in the measurement between the mycelial growth of the fungi and that of the cork borer gives the growth of colony and the effect of different concentration of*Moringaoleifera* on the growth of the species. Fungi toxicity was recorded in terms of percentage mycelial growth inhibition which was calculated using the formular as described by Nene *et al*.(1979).

$$I = \frac{DC - DT}{DC} \times 100$$

Where, I = Percentage inhibition

DC = Average diameter of control (8cm)

DT = Average diameter of growth with treatment

3.9 Statistical Analysis

Data collected was subjected to one way analysis of variance and where significant difference was observed between the treatments, the means were separated using Duncan Multiple Range Test (DMRT). Student t-test was used to compare the efficacy of the two extracts on the growth of isolated fungi. All statistical analyses were performed using SPSS version 20.

CHAPTER FOUR

RESULT

4.1 Isolation of Fungi from Grains of *Sorghum bicolor* Varieties

Fungi were isolated from the grains of all the selected varieties of *Sorghum bicolor* namely Samsorg 17, Samsorg 40, Samsorg 42, Samsorg 43 which were obtained from Plant Science Department IAR, and a local land race(Kaura) was collected from Sabon Gari market, Zari*a.*

4.2 FungiIsolatedfrom *Sorghum bicolor*L. Grains

Five different fungi namely *Aspergillus flavus*Link, *Aspergillus niger*Van Teighem,*Fusarium oxysporum* schlecht, *Rhizopus stolonifer*(Ehrenb.)Lind, and *Phoma herbarum*Sacc.were isolated from the grains. These were isolated on both potato dextrose agar (PDA)and Sabouraud dextrose agar (SDA). It is clear that *Aspergillus flavus* and *Aspergillus niger* were the most prevalent than others (Table 4.1).

4.3 Systematic Description ofFungi Isolates from Sorghum Grains

Isolated fungi were identified using both macroscopic and microscopic characteristics and compared with already described species using the identification key by Barnett and Hunter (1972), Larone (2002), Klich (2002) and Samson*et al*. (2004).

4.3.1 *Aspergillus flavus* Link

4.3.1.1 Macroscopic characteristics

The colonies in both PDA and SDA were regularly shaped measuring 8cm in one week at room temperature. The color was greenishin the beginning becoming dark green at the

Table 4.1 Fungi Isolated from *Sorghum bicolor* Varieties on PDA and SDA

	Samsorg 17		Samsorg 40		Samsorg 42		Samsorg 43		Kaura*	
Fungi species	PDA	SDA	PDA	SDA	PDA	SDA	PDA	SDA	PDA	SDA
Aspergillus flavus	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Aspergillus niger	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Fusarium oxysporum	-	-	-	-	-	-	-	-	✓	✓
Phoma herbarum	-	-	✓	✓	-	-	✓	✓	-	-
Rhizopus stolonifer	✓	✓	✓	✓	✓	✓	✓	✓	-	-

✓ = Present, - = Absent, * = local land race of Sorghum

upper surface, and green with yellow tint on the reverse side of the plate which were consistent on both media(Plate I).

4.3.1.2 Microscopic characteristics

The mycelium produced long, erect, hyaline, and aseptate conidiophore which narrow slightly towards the base. The conidiophores terminated on top with globose vesicle. Conidia were globose (plate I).

The conidiophores were measured. They ranged between 130-500µm in length with an average length of 383.404µmand a width of 20-40µm near the vesicle, 10-40µm at the middle and also 10-40 µm at the base with an average width of 28.6µm near the vesicle, 28.2µm at the middle and 25.4µm at the base. Vesicle measured 130-500µm in length with an average length of 383.404µm. Conidia were measured and ranged from 4-6µm in diameter with an average diameter of 4.64µm.

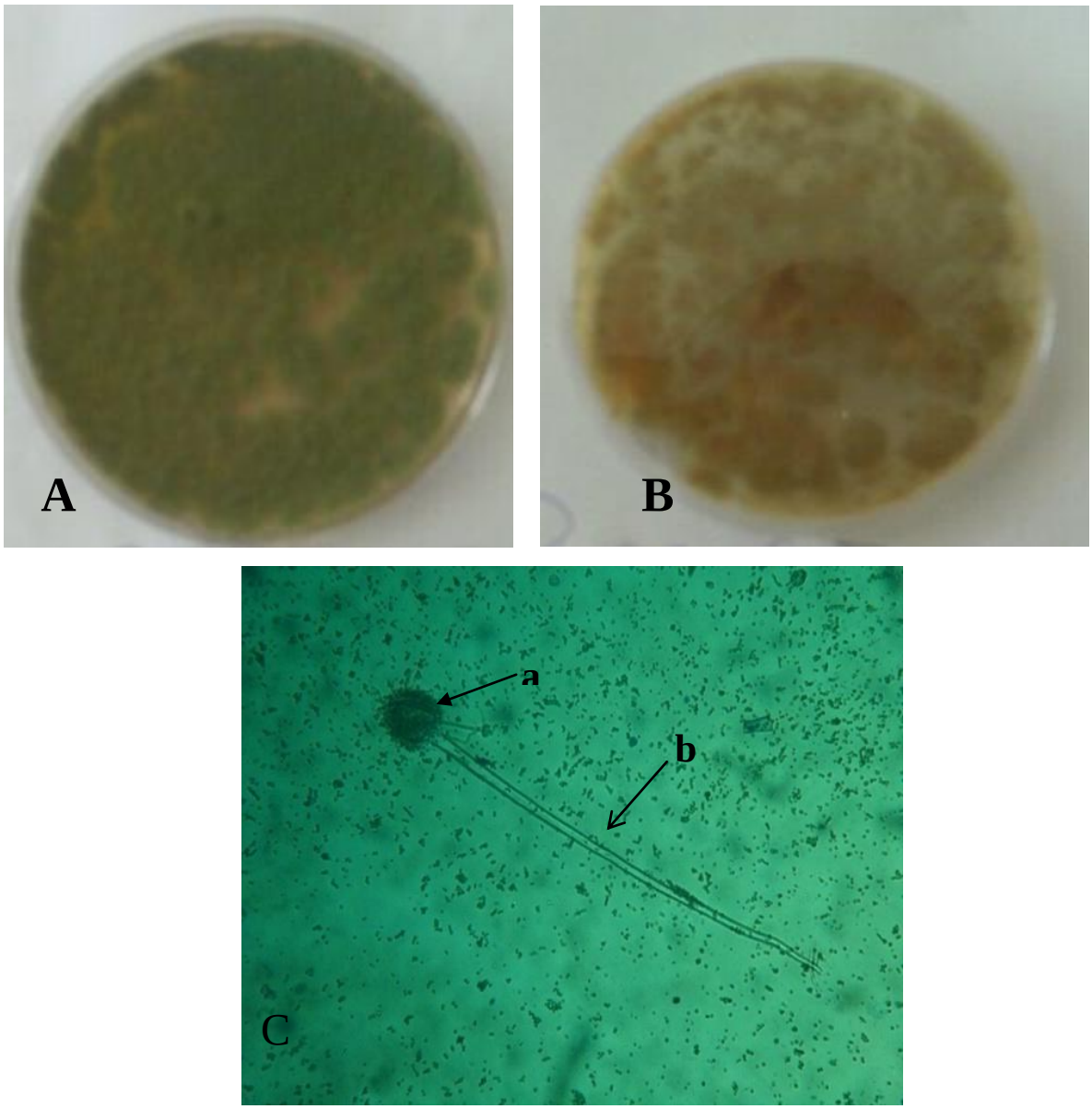
4.3.2 *Aspergillusniger* van Tieghem

4.3.2.1 Macroscopic characteristics

Cultures in both media produced roundish black colonies at the upper surface and green with yellow tint on the reverse side of the petri plate. Colonies expanded to 8cm in one week at room temperature (Plate II).

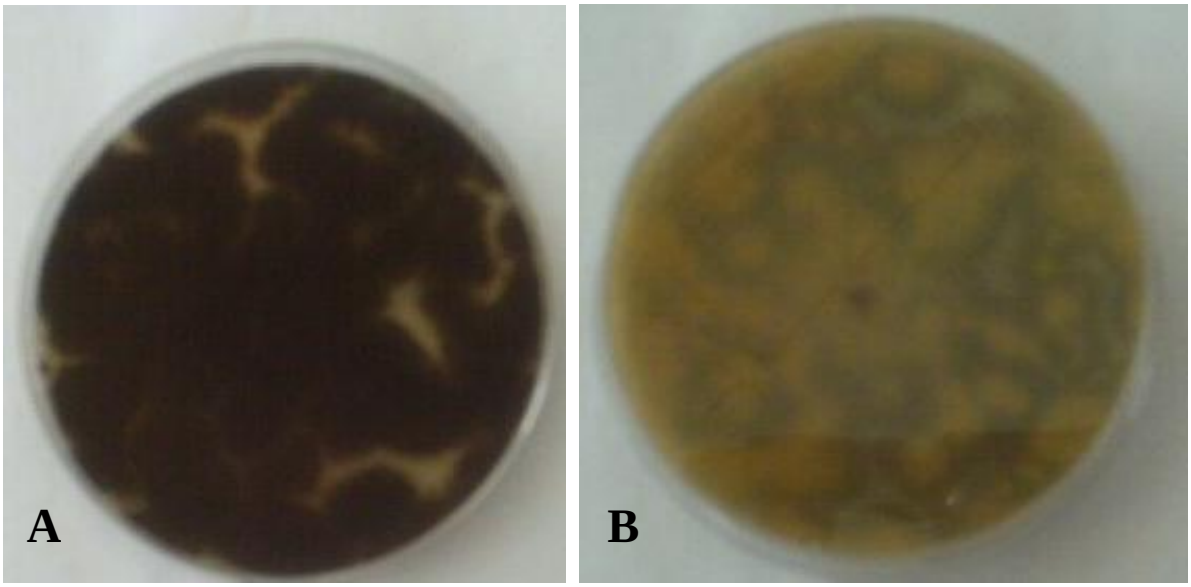
4.3.2.2 Microscopic characteristics

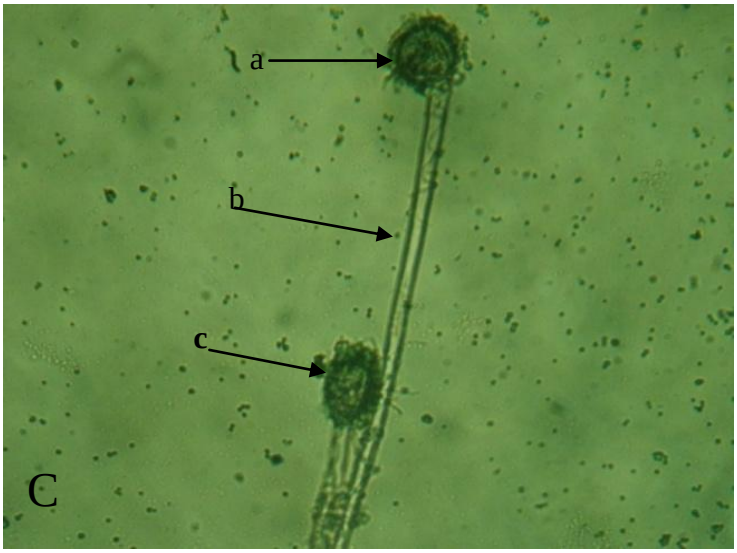
The mycelium consist of profusely branched hyaline hyphae which produced long, upright, erect, hyaline and smooth conidiophores which narrow slightly towards the base.



Mag x10

Plate I: Pure culture of *Aspergillus flavus* link.'A' upper surface. 'B' reverse side. 'C' microscopic features *a.* vesicle b.conidiophore.





Mag x10

Plate II: Pure culture of *Aspergillus niger*Vantieghem. 'A' upper surface. 'B' reverse side 'C'.Microscopic features a vesicle. b conidiophores. c conidium

Each conidiophore terminated in a globose vesicle. Conidia were globose with rough orechinulated surfaces (Plate II)**.** The conidiophores were measured and ranged between 160-600µm in length with an average length of 410.4µm and a width of 20-30µm near the vesicle, 20-30µm at the middle and 10-30µm at the base with an average width of 28.0µm near the vesicle, 25.0µm at the middle and 20.0µm at the base. Vesicles measured 20-100µm in diameter with an average diameter of 58.0µm. Conidiawere 4-6µmin diameter with an average diameter of 4.96µm.

4.3.3 *Fusarium oxysporum* Schlecht

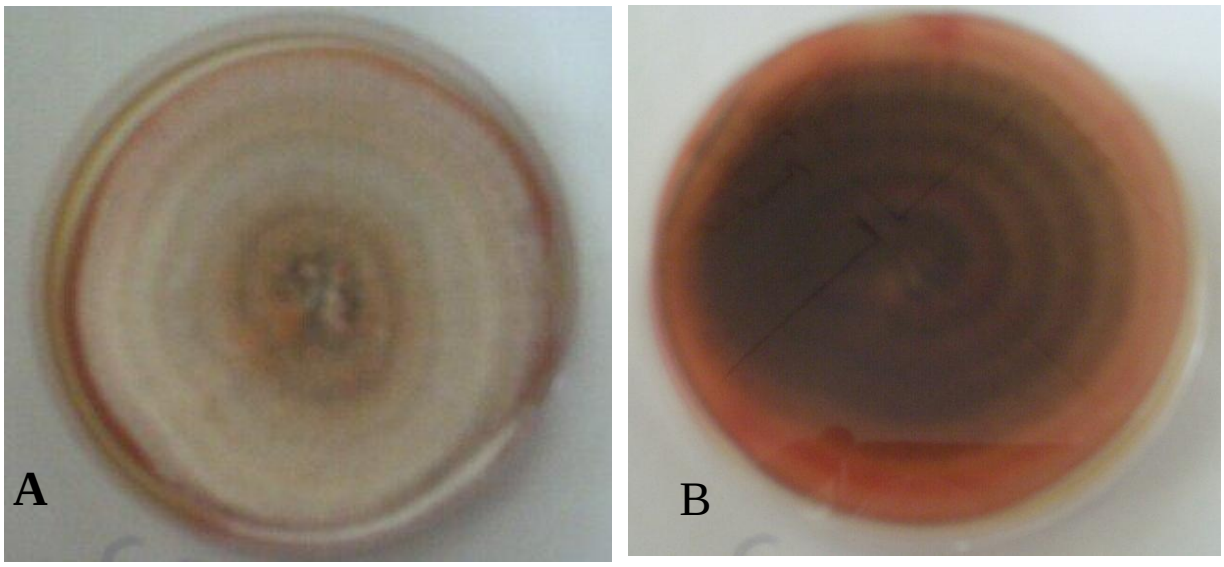
4.3.3.1 Macroscopic characteristics

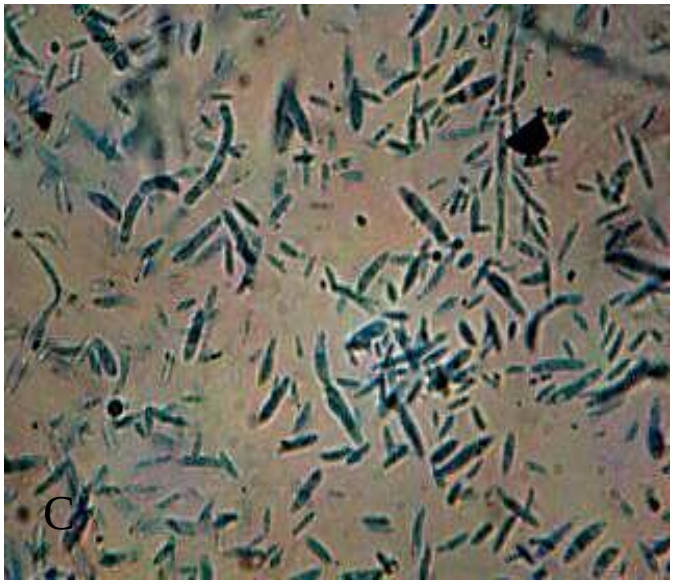
Mycelium was extensive, initially white and cottony in both media with tinge of pink and orange colour at the upper surface,with orange and dark brown on the reverse side of the plate. The mycelium expanded to 8cm in one week at room temperature (Plate III). The growth was fluffy and showed concentric ring.

4.3.3.2 Microscopic characteristics

Mycelium produced conidia (phialospores) which are hyaline and principally of two kinds.

1. **The macro conidia:** They are slightly curved and tapering at both ends typically canoe shaped(Plate III). They are septate with three to six septations. The conidia were measured and ranged between 30-100µm in length with an average length of 62.80µm. In the middle they ranged between 20-30 µm with an average width of 7.60µm.





Mag x40

Plate III: **Pure culture of *Fusarium oxysporum* schlecht. 'A' upper surface. 'B' reverse side. 'C' mixture of micro and macro conidia**

2. **The micro conidia:** They are single celled, ovoid or oblong borne singly or in chains. Some conidia are intermediate, 2 or 3 celled, oblong or slightly curved (plate III). The conidia were measured and ranged between 20-40µm in length with an average length of 32.40µm. The width was alsomeasured which measured between 10-20µm at the middle with an average width of 4.60µm.

4.3.4 *Phomaherbarum*Sacc

4.3.4.1 Macroscopic characteristics

Colonies of *Phoma herbarum*grows rapidly in both media and expanded to 6cm in one week at room temperature. The mycelium was flat, spreading powdery to velvety and often largely submerged in the media with dark brown and dark colouration at the upper and reverse side of the plate respectively (Plate IV).

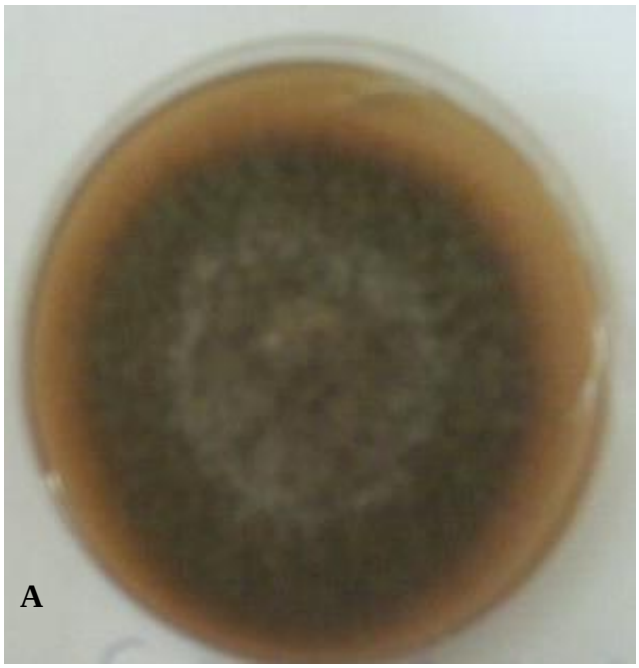
4.3.4.2 Microscopic characteristics

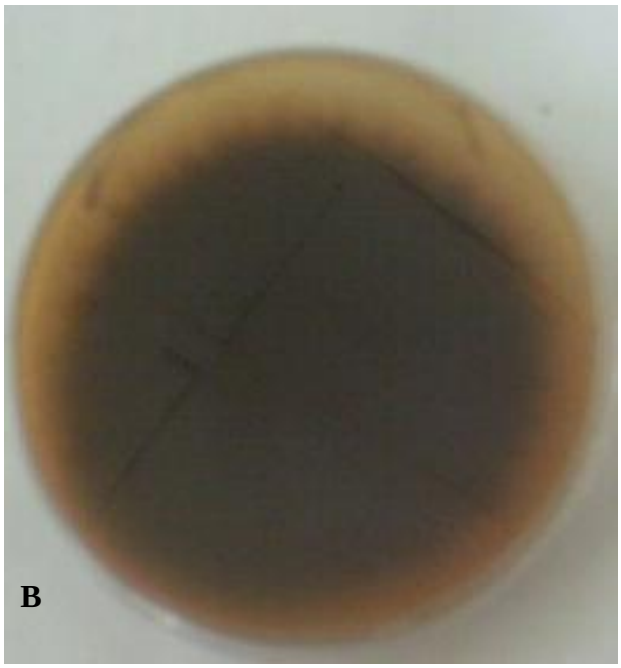
The mycelium produced septate hyaline phialides producing pycnidia. Pycnidia are large, round to pyriform asexual fruiting bodies. They are dark in colour bearing phialides at their inner linings. Pycnidia may be singlyarranged or in chains (Plate IV). The pycnidia were measured and ranged between 30-60µm in diameter with an average diameter of 41.40µm.

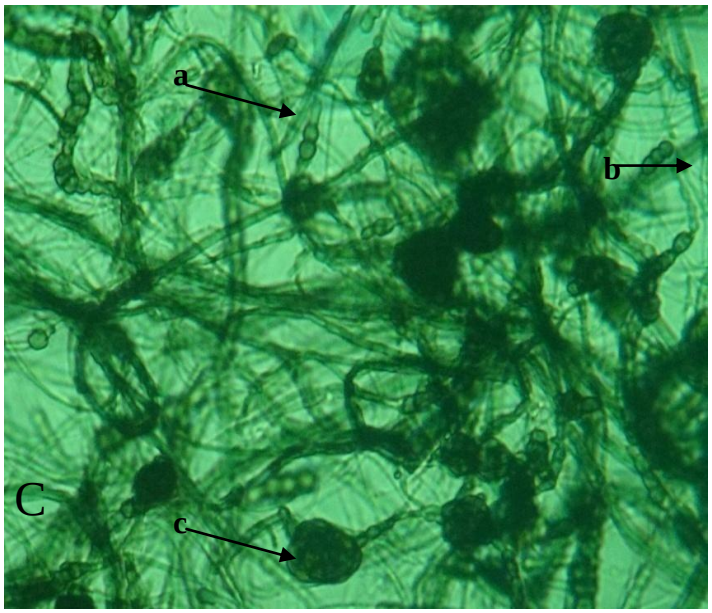
4.3.5 *Rhizopus stolonifer*(Ehrenb.) Lind.

4.3.5.1 Macroscopic characteristics

Colonies in both media were very fast growing with some tendency to coallase, white cottony initially becoming brownish grey to blackish grey with white and light brown colouration on the reverse side of the plate. Colonies expanded rapidly to 8cm in one







Mag x40

Plate IV: Pure culture of *Phoma herbarum* Sacc. 'A' upper surface. 'B' reverse side. 'C' microscopic features.a chlamydospores. b hyaline phialides. c pycnidium

week at room temperature covering the entire surface of the media in a petri-plate (Plate V).

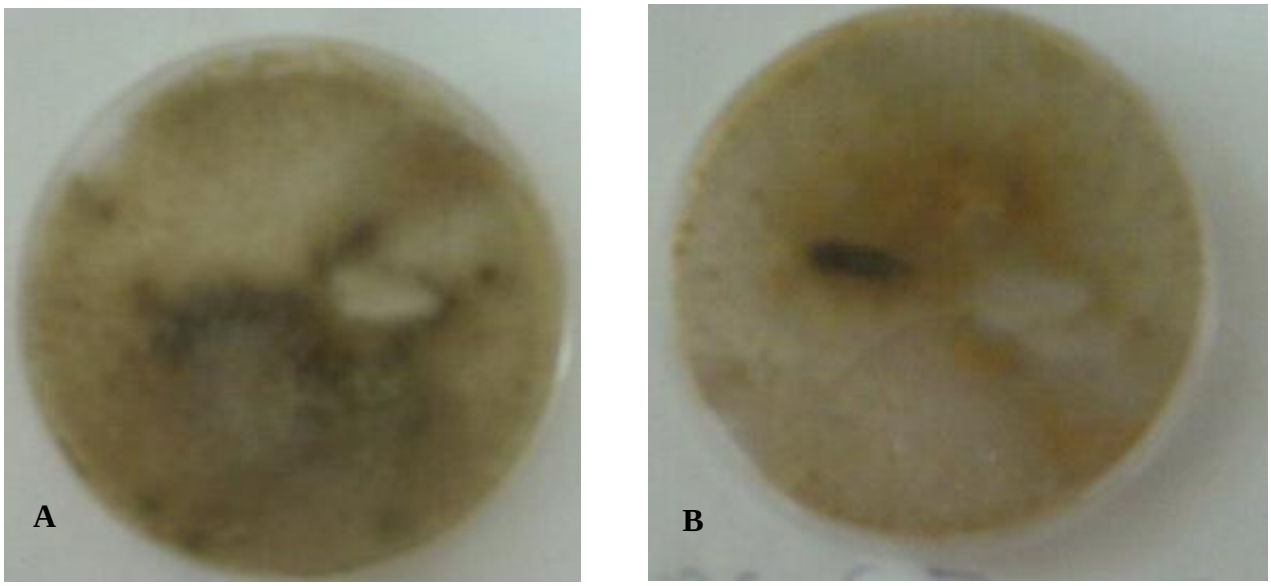
4.3.5.2 Microscopic characteristics

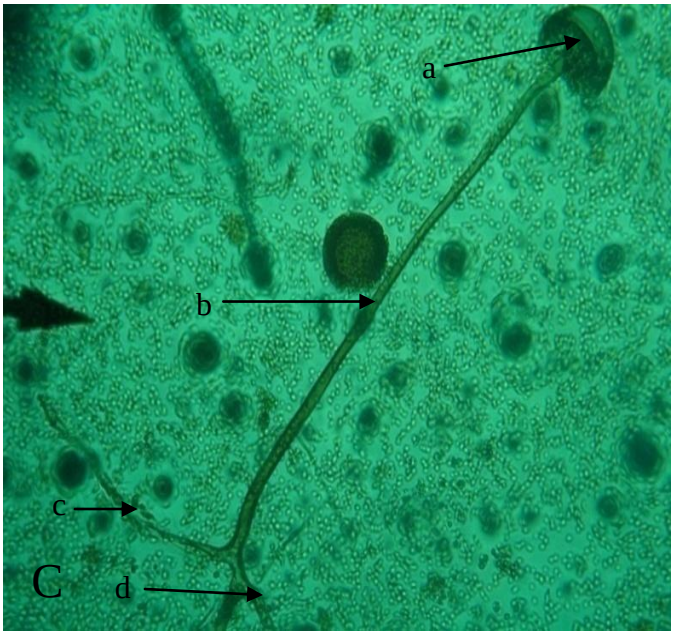
Mycelium produced sporangiophore which were smooth walled, non-septate, simple, occurring singly or in cluster arising from the stolon usually one or two in number with rhizoids from the same point usually in group of two or three and 30µm towards the base with an average width of 23.0µm near the sporangium, 25.60µm at the middleand 30µm towards the base. The sporangium ranged between 30-100µm in diameter with an average diameter of 63.80µm.

4.4 Percentage occurrence of fungi isolates from SorghumGrains

A total of 48 colonies of different fungi developed on PDA.*Aspergillus niger* had the highest occurrence of 21 with percentage occurrence of 47.91%. *Aspergillus flavus*occurred 14 times with percentage occurrence of 29.16%, *Rhizopus stolonifer* occurred five times with percentage occurrence of 10.41%, *Fusarium oxysporum*had 8.33% and occurred four times. *Phoma herbarum* had occurrence of two with percentage occurrence of 4.16% (Table 4.2).

44 colonies of different fungi developed on SDA.*Aspergillus flavus* had the highest occurrence of 21 with percentage occurrence of 47.72%. *Aspergillus niger* occurred 12 times with percentage occurrence of 27.27%.*Rhizopus stolonifer* occurred five times with percentage occurrence of 11.36%. *Fusarium oxysporum* and *Phoma herbarum* both occurred three times with percentage occurrence of 6.81% (Table 4.3).





Mag x10

Plate V: Pure culture of *Rhizopusstolonfer*(Ehrenb) Lind. 'A' **upper surface. 'B' reverse side. 'C' microscopic features.a sporangium. b sporangiophore. c stolon. d rhizoid**

Table 4.2: Percentage occurrence of different fungi species in Sorghum varieties on PDA

Fungi species	No. of discrete colonies	Percentage occurrence (%)
*Aspergillus niger*VanTieghem	23	47.91
Aspergillus flavus Link	14	29.16
Rhizopus stolonifer(Ehrenb.)Lind.	05	10.41
Fusarium oxysporum Schlecht	04	08.33
Phoma herbarum sacc	02	04.16

Table 4.3: Percentage occurrence of different fungi species in Sorghum varieties on SDA

Fungi species	No. of discrete colonies	Percentage occurrence(%)
Aspergillus flavus Link	21	47.72
Aspergillus niger VanTieghem	12	27.27
Rhizopus stolonifer(Ehrenb.) Lind	05	11.36
*Fusarium oxysporum*schlecht	03	06.81
*Phoma herbarium*sacc	03	06.81

4.5 Effect of Different Concentrations of Seed Aqueous Extractof *Moringa oleifera* on Percentage Mycelia Growth Inhibition of Isolated Fungi from Sorghum Grains.

It is evident from Table 4.4 that seed aqueous extract possess toxic effect on the isolated pathogens. The table further revealed that the inhibitory effect vary with concentration and the test organisms. The higher the concentration of the extract, the higher the inhibitory effect and vice-versa. Thus, at 5%, 10%, 15%, 20% and 25% seed aqueous extract concentration, *Aspergillus niger* was inhibited by 22.5%, 27.5%, 31.4%, 50.0%, and 51.3% respectively in comparison to the control.

Similarly, *Aspergillus flavus* was inhibited by 20.0%, 22.5%, 26.3%, 38.8%, and 62.5% at the same concentrations of the seed aqueous extract respectively. *Fusarium oxysporum* was inhibited by 21.3%, 22.9%, 28.8%, 38.8% and 46.3%, respectively. Inhibitory effect of 37.3%, 38.8%, 40.0%, 50.0% and 51.3% were also recorded on *Phoma herbarum* at 5%, 10%, 15%, 20%, and 25% respectively of the extract. Like all other pathogens, *Rhizopus stolonifer* was also inhibited by 18.8%, 22.5%, 25.0%, 27.5%, and 30.0%% respectively in seed aqueous extract concentrations of 5%, 10%, 15%, 20% and 25% as compared with control. The effects of the different concentrations of the extract on the test organisms were significantly different.

Table 4.4: Effect of different concentrations of seed aqueous extract of *Moringa oleifera* on percentage mycelia growth inhibition of isolated fungi from Sorghum Grains

Concentrations (%)	Plant part	Test organisms				
		A. niger	***A. flavus***	***F.oxysporum***	***P.herbarum***	***R.stolonifer***
0.0		0.0±0.00^{e}	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{b}	0.0±0.00^{c}
5.0		22.5±1.44^{d}	20.0±2.89^{c}	21.3±3.31^{c}	37.3±1.45^{a}	18.8±0.00^{b}
10.0		27.5±0.72^{c}	22.5±1.44^{c}	22.9±1.50^{c}	38.8±6.88^{a}	22.5±2.60^{ab}
15.0	Seeds	31.4±1.18^{b}	26.3±9.01^{bc}	28.8±1.91^{bc}	40.0±6.25**a**	25.0±2.89^{ab}
20.0		50.0±0.00^{a}	38.8±6.96^{b}	38.8±10.03^{ab}	50.0±6.41^{a}	27.5±2.89**a**
25.0		51.3±0.00^{a}	62.5±1.91^{a}	46.3±0.00^{a}	51.3±0.00^{a}	30.0±2.89^{a}

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

4.6: Effect of Different Concentrations of Stem bark Aqueous Extract of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungifrom Sorghum Grains.

Table 4.5shows that the stembark aqueous extract of *Moringa oleifera* possess inhibitory effect on the test organisms. It is also shown from the table that inhibitory effect increased with increase in concentration of the extract and vice – versa. Thus in 5%, 10%, 15%, 20% and 25% stembark aqueous extract, *Apergillus niger* was inhibited by 18.8%, 21,3%, 21.3%, 32.1% and 37.5% respectively. Similarly, *Aspergillus flavus* was inhibited by 20.0%, 22.5%, 25.0%, 28.8% and 35.0% respectively of the stem bark aqueous extract concentrations. The results further indicates that at 5%, 10%, 15%, 20% and 25% extract concentrations, *Fusarium oxysporum* was inhibited by 20%, 22.1%, 27.5%, 28.8% and 32.5% respectively. *Phoma herbarum* shows growth inhibition of 32.5%, 33.8%, 41.3%, 43.8%, and 45.8% at same concentrations of the extract respectively. The results also reveal the efficacy of seed aqueous extract on *Rhizopus stolonifer* with growth inhibition of 18.8%, 21.3%, 23.8%, 25.0% and 26.3% at 5%, 10%, 15%, 20% and 25% of the extract respectively in comparison to control. There were significant differences on the effects of different concentration of the extract on the percentage mycelia growth inhibition of isolated fungi.

Table 4.5: Effect of Different Concentrations of Stem bark Aqueous Extract of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungifrom Sorghum Grains

Concentrations (%) (Aqueous extracts)	Plant part	Test organisms				
		A. niger	***A. flavus***	***F.oxysporum***	***P.herbarum***	***R. stolonifer***
0.0		0.0±0.00^{c}	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{c}	0.0±0.00^{b}
5.0		18.8±3.31^{b}	20.0±3.31c	20.0±4.33^{c}	32.5±2.17^{b}	18.8±2.89^{a}
10.0		21.3±3.61^{b}	22.5±1.44^{bc}	22.1±1.10^{bc}	33.8±2.17^{b}	21.3±3.31^{a}
15.0	Stem bark	21.3±3.31^{b}	25.0±0.00^{bc}	27.5±1.91^{ab}	41.3±3.31^{a}	23.8±3.61^{a}
20.0		32.1±8.30^{ab}	28.8±1.91^{b}	28.8±2.17^{ab}	43.8±3.31^{a}	25.0±4.73^{a}
25.0		37.5±0.00^{a}	35.0±2.50^{a}	32.5±1.25^{a}	45.8±1.10^{a}	26.3±4.73^{a}

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

4.7: Effect of Different Concentrations of Leaves Aqueous Extract of *Moringa oleifera*on Percentage Mycelial Growth Inhibition of Isolated Fungifrom Sorghum Grains

Table 4.6 indicates that leave aqueous extract possess fungistatic activity on the isolated fungi. However, inhibitory activity varies with pathogen and concentration. The higher the concentration of the extract, the higher the mycelial growth inhibition and vice – versa. Hence, at 5%, 10%, 15%, 20% and 25% extract concentrations, *Aspergillus niger* was inhibited by 38.8%, 40.4%, 42.5%, 63.8% and 72.5% respectively. Inhibitory activity of 22.5%, 23.8%, 33.3%, 60% and 75.0% were recorded at the same concentration on *Aspergillus flavus.* The results also reveals that mycelia growth of *Fusarium oxysporum* was inhibited at 37.5%, 37.5%, 62.5%, 68.8% and 70.4% at 5%, 19%, 15%, 20% and 25% of the leaf aqueous extracts respectively. Toxic activity of 37.5%, 50.8%, 54.2%, 56.3% and 58.8% were also shown on *Phoma harbarum* at same concentration. Like all other pathogens, *Rhizopus stolonifer* was inhibited at 26.3%, 30.0%, 31.3%, 32.5% and 35.0% of the leaf aqueous extract concentrations respectively. However, the effects of different concentrations of the extract on mycelia growth inhibition of the test organism were significantly different.

4.8: Effect of Different Concentrations of Seed methanol Extract of *Moringa oleifera* on Percentage Mycelia Growth Inhibition of Isolated Fungi from Sorghum Grains

It is evident from Table 4.7 that the efficacy of seed methanol extract varies with concentration and individual test organism. Higher concentration of the extract possesshigher inhibitory effect and vice – versa.

Table 4.6: Effect of Different Concentrations of LeavesAqueous Extract of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungifrom Sorghum Grains

Concentrations (%) (Aqueous extracts)	Plant parts	Test organisms				
		A. niger	***A. flavus***	***F.oxysporum***	***P. herbarum***	***R. stolonifer***
0.0		0.0±0.00^{c}	0.0±0.00^{c}	0.0±0.00^{d}	0.0±0.00^{c}	0.0±0.00^{d}
5.0		38.8±6.96^{b}	22.5±1.25^{b}	37.5±0.72^{c}	37.5±3.31^{b}	26.3±0.00^{c}
10.0		40.4±3.33^{b}	23.8±3.61^{b}	37.5±4.33^{c}	50.8±5.42^{a}	30.0±1.25^{bc}
15.0	Leaves	42.5±8.75^{b}	33.3±11.58^{b}	62.5±0.72^{b}	54.2±5.07^{a}	31.3±1.25^{ab}
20.0		63.8±0.00^{a}	60.0±0.00^{a}	68.8±0.00^{a}	56.3±3.82^{a}	32.5±1.91^{ab}
25.0		72.5±2.89^{a}	75.0±5.77^{a}	70.4±1.10^{a}	58.8±4.39^{a}	35.0±1.91^{a}

NOTES: 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 4.7: Effect of Different Concentrations of Seed Methanol Extract of *Moringa oleifera* on PercentageMycelial Growth Inhibition of Isolated Fungifrom Sorghum Grains

Concentrations (%) (Methanol extracts)	Plant part	Test organisms				
		A. niger	***A. flavus***	***F.oxysporum***	***P. herbarum***	***R. stolonifer***
0.0		0.0±0.00^{d}	0.0±0.00^{c}	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{c}
5.0		25.0±0.00^{c}	46.3±6.61^{b}	30.4±1.82^{c}	37.5±0.00^{c}	25.0±0.00^{b}
10.0		28.8±0.1.91^{bc}	58.8±0.00^{a}	32.5±1.91^{c}	52.5±6.41^{b}	50.1±5.51^{a}
15.0	Seeds	38.8±0.00^{b}	63.8±2.60^{a}	46.3±6.88^{b}	58.8±2.17^{ab}	50.8±3.97^{a}
20.0		52.9±7.23^{a}	68.8±6.25^{a}	56.3±0.00^{ab}	66.3±3.81^{a}	50.8±3.97^{a}
25.0		62.1 ±6.31^{a}	70.4±1.10^{a}	60.8±3.00^{a}	67.5±331^{a}	53.3±4.58^{a}

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

Hence, at 5%, 10%, 15%, 20% and 25% seed methanol extract concentration, *Aspergillus niger* was inhibited by 25.0%, 28.8%, 38.8%, 52.9% and 62.1% respectively in comparison with the control. In 5%, 10%, 15%, 20% and 25% of the extract, *Aspergillus flavus* shows growth inhibition of 46.3%, 58.8%, and 63.8%, 68.8% and 70.4% respectively. The results also indicate that *Fusarium oxysporum*was also inhibited by 30.4, 32.5%, 46.3%, 56.3% and 60.8% of the same extract concentrations. Similarly, *Phoma herbarum* was inhibited by 37.5%, 52.5%, 58.8%, 66.3% and 67.5%. Like all the isolated fungi, *Rhizopus stolonifer* shows growth inhibition of 25.0%, 50.1%, 50.8%, 50.8%, and 53.3% of the seed methanol extract concentrations respectively. However the effects of different concentrations of the extract on mycelia growth inhibition of the test organisms were significantly different

4.9: Effect of Different Concentrations of Stem bark Methanol extract of *Moringa oleifera* on Percentage Mycelial growth Inhibition of Isolated Fungi from Sorghum Grains

Table 4.8 shows the effects of different concentrations of stem bark methanol extract on the inhibitory activity of isolated fungi. The results revealed that mycelia growth inhibition differs with concentration and pathogen. Thus, the higher the concentration, the higher the toxicity and vice – versa. Therefore, at 5%, 10%, 15%, 20% and 25% stem bark extract concentration, *Aspergillus niger*was inhibited at 25.0%, 25.0%, 30.0%, 35.8%, and 50.0% respectively. Furthermore, growth of *Aspergillus flavus* was also inhibited at 32.9%, 37.5%, 40.8%, 50.4% and 66.3% of the stembark methanol concentration.

Table 4.8: Effect of Different Concentrations of Stem bark Methanol Extract of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungi from SorghumGrains

Concentrations (%) (Methanol extracts)	Plant part	Test organisms				
		A. niger	***A. flavus***	***F.oxysporum***	***P. herbarum***	***R.stolonifer***
0.0		0.0 ± 0.00^{c}	0.0 ± 0.00^{d}	0.0 ± 0.00^{c}	0.0 ± 0.00^{b}	0.0 ± 0.00^{c}
5.0		25.0 ± 0.72^{b}	32.9 ± 2.20^{c}	21.3 ± 3.31^{b}	52.5 ± 6.41^{a}	25.0 ± 6.88^{b}
10.0		25.0 ± 0.72^{b}	37.5 ± 4.33^{bc}	22.5 ± 1.25^{b}	53.0 ± 0.00^{a}	43.8 ± 3.31^{a}
15.0	Stem bark	30.0 ± 0.00^{b}	40.8 ± 1.67^{bc}	50.4 ± 5.79^{a}	55.8 ± 3.00^{a}	46.3 ± 6.61^{a}
20.0		35.8 ± 6.01^{b}	50.4 ± 5.79^{b}	52.5 ± 6.41^{a}	60.0 ± 1.44^{a}	46.3 ± 6.61^{a}
25.0		50.0 ± 8.51^{a}	66.3 ± 8.32^{a}	55.8 ± 3.00^{a}	62.5 ± 6.61^{a}	48.8 ± 6.88^{a}

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

The results also shows that *Fusarium oxysporum* shows mycelia growth inhibition of 21.3%, 22.5%, 50.4%, 52.5% and 55.8% at same concentrations of the extract. Thus, at 5%, 10%, 15%, 20% and 25% concentrations of the extract, *Phoma herbarum* was inhibited by 52.5%, 53.0%, 55.8%, 60.0% and 62.5% respectively with regards to control. The growth of *Rhizopus stolonifer* was also inhibited by the stem bark extract. Thus, at 5%, 10%, 15%, 20% and 25%, there was growth inhibition of 25.0%, 43.8%, 46.3%, 46.3% and 48.8% respectively. Statistically, there were significant differences in the effects of different concentrations on mycelia growth inhibition of the test organisms.

4.10 Effect of Different Concentrations of Leaves Methanol Extract of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungi from Sorghum Grains

Like seed, and stembark methanol extracts, the leaves methanol extract also showed antifungal effects on the test organisms (Table 4.9). However, it is shown that the inhibitory effect of the extract varies with concentration and pathogen. Hence, at 5%, 10%, 15%, 20% and 25% leaves methanol concentrations, growth of *Aspergillus niger*was inhibited at 38.8%, 40.4%, 52.5%, 65.0% and 76.3% respectively. Similarly, *Aspergillus flavus* was inhibited by 50.0%, 61.7%, 66.3%, 68.8% and 80.0% at the same concentrations of the extract. The results further show that the leaf methanol extract also showed inhibitory effect on *Fusarium oxysporum.* Thus at 5%, 10%, 15%, 20% and 25%, there was inhibitory activity of 40.4%, 62.4%, 68.8%, 70.4% and 75.0% of the leaf methanol extract concentrations respectively. Growth inhibition of 40.4%, 53.8

Table 4.9: Effect of Different Concentrations of Leaves Methanol Extract of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungifrom Sorghum Grains

Concentrations (%) (Methanol extracts)	Plant part	Test organisms				
		A. niger	***A. flavus***	***F.oxysporum***	***P. herbarum***	***R. stolonifer***
0.0		0.0±0.00^{d}	0.0±0.00^{e}	0.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{c}
5.0		38.8±6.96^{c}	50.0±0.00^{d}	40.4±3.33^{c}	40.0±6.25^{c}	26.3±0.00^{b}
10.0		40.4±3.33^{c}	61.7±4.10^{c}	62.4±0.08^{b}	53.8±0.00^{b}	50.8±6.96^{a}
15.0	leaves	52.5±6.41^{bc}	66.3±0.00^{bc}	68.8±6.25a^{b}	67.5±1.91^{a}	51.3±0.00^{a}
20.0		65.0±5.05^{ab}	68.8±0.00^{b}	70.4±1.10^{ab}	70.0±0.00^{a}	56.3±3.81^{a}
25.0		76.3±1.91^{a}	80.0±0.00^{a}	75.0±0.00^{a}	71.3±5.44^{a}	60.4±4.40^{a}

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

67.5%, 70.0% and 71.3% were recorded on *Phoma herbarum* at the same concentrations of the extract. At 5%, 10%, 15%, 20% and 25%, *Rhizopus stolonifer* was inhibited at 26.3%, 50.8%, 51.3%, 56.3% and 60.4% respectively. The effects of the different concentrations of the extract were significantly different on mycelia growth inhibition of the test organism.

4.11 Efficacy of Different Concentrations of Aqueous Extracts of Different Plant Parts of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungi from SorghumGrains

Table 4.10 reveals the comparison of efficacy between aqueous extracts of *Moringa oleifera* on the test organism. It is shown that at all concentrations, the leafaqueous extract was more effective on growth inhibition of the pathogens, followed by seed extracts and lastly, the stem bark extract. Thus at 5% aqueous extract concentration, antifungal activity of 22.5%, 18.8% and 38.8% of seed, stem bark and leaf respectively were recorded on *Aspergillus niger.*At the same concentration, *Aspergillus flavus* was inhibited by 20.0%, 20.0% and 22.5% of seed, stem bark and leaf extracts respectively. *Fusarium oxysporum* was also inhibited at 21.3%, 20.0% and 37.5% of the seed, stembark and leaves extracts respectively at 5% concentration. Similarly, *P. herbarum*was inhibited at 37.3%, 32.5% 37.5% of seed, stembark and leaf extracts respectively. Like all other pathogens, *R. stolonifer* recorded 18.8%, 18.8%, and 26.3% of the seed, stembark and leave extracts respectively. At 10% aqueous extract concentrations, mycelia growth inhibition of 27.5%, 21.3%, and 40.4% of seed, stembark and leaves extracts respectively were recorded on *A.niger.*Inhibitory activity of 22.5%, 22.5%, and 23.8% of seed, stem bark and leave extracts respectively were shown on *A.*

*flavus.*At 10% aqueousextract concentrations, *F.oxysporum* was inhibited at 22.9%, 33.8% and 50.8%

Table 4.10a Efficacy of different concentrations of Aqueous Extracts of Different Plant Parts of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungi from Sorghum Grains.

Plant parts	Conc. (%) (AE)	Test organisms				
		A. niger	***A. flavus***	***F.oxysporum***	***P.herbarum***	***R. stolonifer***
Seeds		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 bark	**0.0**	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]
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]
Seeds		22.5±1.44[b]	20.0±2.89[a]	21.3±3.31[b]	37.3±1.45[a]	18.8±0.00[b]
Stem bark	**5.0**	18.8±3.31[b]	20.0±3.31[a]	20.0±4.33[b]	32.5±2.17[a]	18.8±2.89[b]
Leaves		38.8±6.96[a]	22.5±1.25	37.5±0.72[a]	37.5±3.31[a]	26.3±0.00[a]

NOTES: 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 4.10b Efficacy of different concentrations ofAqueous Extracts of Different Plant Parts of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated fungi from Sorghum Grains

Plant parts	Conc. (%) (AE)	Test organisms				
		A. niger	***A. flavus***	***F.oxysporum***	***P. herbarum***	***R. stolonifer***
Leaves		40.4±3.33[a]	23.8±3.61[a]	37.5±4.33[a]	50.8±5.42[a]	30.0±1.25[a]
Seeds		27.5±0.72[b]	22.5±1.44[a]	22.9±1.50[b]	38.8±6.88[a]	22.5±2.60[a]
Stem bark	**10.0**	21.3±3.61[b]	22.5±1.44[a]	22.1±1.10[b]	33.8±2.17[a]	21.3±3.31[a]
Seeds		31.4±1.18[a]	26.3±9.01[a]	28.8±1.91[b]	40.0±6.25[a]	25.0±2.89[a]
Stem bark	**15.0**	21.3±3.31[a]	25.0±0.00[a]	27.5±1.91[b]	41.3±3.31[a]	23.8±3.61[a]
Leaves		42.5±8.75[a]	33.3±11.58[a]	62.5±0.72[a]	54.2±5.07[a]	31.3±1.25[a]
P-values		**0.09**	**0.76**	**0.00**	**0.17**	**0.20**

NOTES: 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 4.10c Efficacy of different concentrations ofAqueous Extracts of Different Plant Parts of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungifrom Sorghum Grains

Plant parts	Conc. (%) (AE)	Test organisms				
		A. niger	***A. flavus***	***F.oxysporum***	***P. herbarum***	***R. stolonifer***
Seeds		50.0±0.00[a]	38.8±6.96[b]	38.8±10.03[b]	50.0±6.41[a]	27.5±2.89[a]
Stem bark	**20.0**	32.1±8.30[b]	28.8±1.91[b]	28.8±2.17[b]	43.8±3.31[a]	25.0±4.73[a]
Leaves		63.8±0.00[a]	60.0±0.00[a]	68.8±0.00[a]	56.3±3.82[a]	32.5±1.91[a]
P-values		**0.01**	**0.01**	**0.01**	**0.25**	**0.35**
Seeds		51.3±0.00[b]	62.5±1.91[a]	46.3±0.00[b]	51.3±0.00[ab]	30.0±2.89[a]
Stem bark	**25.0**	37.5±0.00[c]	35.0±2.50[b]	32.5±1.25[c]	45.8±1.10[b]	26.3±4.73[a]
Leaves		72.5±2.89[a]	75.0±5.77[a]	70.4±1.10[a]	58.8±4.39[a]	35.0±1.91[a]
P-values		**0.00**	**0.00**	**0.00**	**0.04**	**0.26**

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

of the various parts respectively *P. herbarum* showed growth inhibition of 38.8%, 33.8 and 50.8% of the seeds, stembark and leave extracts respectively. Toxic activity of 22.5%, 21.3% and 30.0% were recorded on *R. stolonifer* by the extracts. However, at 15% of seed, stembark and leaf aqueous extracts, growth inhibition of 31.4%, 21.3% and 42.5% respectively were shown on *A.niger.* Furthermore, inhibitory activity of 26.3%, 25.0% and 33.3% were revealed on *A. flavus* of the various plant parts respectively. At same concentrations of 15% seed, stembark and leaf extract, antifungal activity of *F.oxysporum*was 28.8%, 27.5%, and 62.5%. Mycelia growth inhibition of 40%, 41.3% and 54.2% were indicated on *P. herbarum* by the individual plant parts respectively. However 25.0%, 23.8%, 31.3%, Mycelial growth inhibition were shown on *R. stolonifer* by seed, stembark and leaf extracts respectively. At 20% aqueousextract concentrations of seed, stembark and leave, *A.niger* was inhibited by 50.0%, 32.1%, and 63.8% of the extracts respectively. Percentage growth inhibitionof 38.8%, 28.8% and 60.0% were shown on *A. flavus*. 38.8%, 28.8% and 68.8% were recorded on *F.oxysporum* by the various extracts. It is also indicated that *P.herbarum* was inhibited by 50.0%, 43.8% and 56.3% of the aerial parts respectively. *R. stolonifer* was also inhibited by 27.5%, 25.0% and 32.5% of the individual parts. At 25% aqueous extract concentrations of seed, stembark and leave*A.niger* was inhibited at 51.3%, 37.5% and 72.5% of the seed extract respectively. However, percentage mycelia growth inhibition of 62.5%, 35.0% and 75.0% was also shown by the extracts from the various parts on *A. flavus*. At 25% extract concentrations of the aerial parts, fungitoxic activity of 46.3%, 32.5% and 70.4% were revealed on *F.oxysporum*. 51.3%, 45.8% and 58.8% were recorded on *P.herbarum*. It is also observed that 30.0%, 26.3% and 35.0% mycelia growth inhibition were recorded on

*R. stolonifer*by the seed, stembark and leafaqueous extracts respectively. However, the comparison of efficacy between aqueous extracts of different plant parts on mycelia growth inhibition of the test organisms was significantly different. It is also revealed that in rare occasions the stembark and seed extracts possess similar antifungal property on the test organism.

4.12 Efficacy of Different Concentrations ofMethanol Extracts of Different Plant Parts of*Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungi from Sorghum Grains

Table 4.11 shows the comparison of efficacy between methanol extracts of *Moringa oleifera* on percentage mycelia growth of isolated fungi. It was shown that at all concentration of the extracts, the leaf methanol extract was more effective on growth inhibition of the pathogens, followed by the seed extracts with stem bark being the least. Therefore, at 5% methanol extract concentration, antifungal activity of 25.0%, 25.0% and 38.8% of seed, stem bark and leaf respectively were recorded on *A.niger.*At the same concentrations,*A.flavus* was inhibited by 46.3%, 32.9% and 50.0% of seed, stem bark and leafextracts respectively. *F. oxysporum* was also inhibited by 30.4%, 21.3% and 40.4% of the seed, stem bark and leaf extracts at 5% concentration.Similarly, *P.herbarum* was inhibited at 37.5%, 25.5% and 40.0% of seed, stem bark and leaf respectively. Like all other pathogens, *R. stolonifer* recorded 25.0%, 25.0% and 26.3% of the seed, stem bark and leaf extracts respectively. At 10% methanol extract concentrations, mycelia growth inhibition of 28.8%, 25.0% and 40.4% of seed, stem bark and leafextracts respectively were recorded*on A.niger.*Antifungal activity of 58.8%, 37.5% and 61.7% of the seed,

Table 4.11a Efficacy of different concentrations ofMethanol Extracts of Different Plant Parts of*Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungifrom SorghumGrains

Plant parts	Conc. (%) (methanol)	Test organisms				
		A. niger	***A. flavus***	***F.oxysporum***	***P. herbarum***	***R. stolonifer***
Seeds		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 bark	**0.0**	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]
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]
P-values		**Undefined**	**Undefined**	**Undefined**	**Undefined**	**Undefined**
Seeds		25.0±0.00[**a**]	46.3±6.61[ab]	30.4±1.82[**ab**]	37.5±0.00[a]	25.0±6.88[**a**]
Stem bark	**5.0**	25.0±0.72[**a**]	32.9±2.20[b]	21.3±3.31[**b**]	25.5±6.41[b]	25.0±6.88[**a**]
Leaves		38.8±6.96[**a**]	50.0±0.00[a]	40.4±3.33[**a**]	40.0±3.33[a]	26.3±0.00[**a**]
P-values		**0.04**	**0.75**	**0.01**	**0.33**	**0.03**

NOTES: 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 4.11bEfficacy of different concentrations ofMethanol Extracts of Different Plant Parts of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungifrom Sorghum Grains

Plant parts	Conc. (%) (methanol)	Test organisms				
		A. niger	***A. flavus***	***F.oxysporum***	***P. herbarum***	***R. stolonifer***
Seeds		28.81.91^{b}	58.8±0.00^{a}	32.5±1.91^{b}	52.5±6.41^{a}	50.1±5.51^{a}
Stem bark	**10.0**	25.0±0.72^{b}	37.5±4.33^{b}	22.5±1.25^{c}	53.0±0.00^{a}	43.8±3.31^{a}
Leaves		40.4±3.33^{a}	61.7±4.10^{a}	62.4±0.08^{a}	53.8±0.00^{a}	50.8±6.96^{a}
P-values		**0.00**	**0.00**	**0.00**	**0.89**	**0.29**
Seeds		38.8±0.00^{b}	63.8±2.60^{a}	46.3±6.88^{a}	58.8±2.17^{b}	50.8±3.97^{a}
Stem bark	**15.0**	30.0±0.00^{b}	40.8±1.67^{b}	50.4±5.79^{a}	55.8±3.00^{b}	46.3±6.61^{a}
Leaves		52.5±6.41^{a}	66.3±0.00^{a}	68.8±6.30^{a}	67.5±1.91^{a}	51.3±0.00^{a}
P-values		**0.01**	**0.00**	**0.09**	**0.03**	**0.69**

NOTES: 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 4.11c Efficacy of different concentrations ofMethanol Extracts of Different Plant Parts of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungifrom Sorghum Grains

Plant parts	Conc. (%) (methanol)	Test organisms				
		A. niger	***A. flavus***	***F.oxysporum***	***P. herbarum***	***R. stolonifer***
Seeds		52.9±7.23^{ab}	68.8±6.25^{a}	56.3±0.00^{b}	66.3±3.82^{ab}	50.8±3.97^{a}
Stem bark	**20.0**	35.8±6.01^{b}	50.4±5.79^{b}	52.5±6.41^{b}	60.0±1.44^{b}	46.3±6.61^{a}
Leaves		65.0±5.05^{a}	68.0±0.00^{a}	70.4±1.10^{a}	70.0±0.00^{a}	56.3±3.82^{a}
P-values		**0.04**	**0.04**	**0.03**	**0.06**	**0.42**
Seeds		62.1±6.31^{ab}	70.4±1.10^{a}	60.8±3.00^{b}	67.5±3.31^{a}	53.3±4.58^{a}
Stem bark	**25.0**	50.0±8.51^{b}	66.3±8.32^{a}	55.8±3.00^{b}	62.5±6.161^{a}	48.8±6.88^{a}
Leaves		76.3±3.31^{a}	80.0±0.00^{a}	75.0±.00^{a}	71.3±5.45^{a}	60.4±4.41^{a}
P-values		**0.03**	**0.20**	**0.00**	**0.54**	**0.37**

NOTES: 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 bark and leaves were recorded on *A.flavus*. At 10% methanol concentration also, *F.oxysporum* was inhibited at 32.5%, 22.5% and 62.4% of the various parts respectively. *P.herbarum* showed growthinhibition of 52.5%, 53.0% and 53.8% of the seed, stem bark and leaf extract respectively. Toxic activity of 50.1%, 43.8% and 50.8% were recorded on *R. stolonifer* by the respective aerial parts. However, at 15% of seed, stem bark and leaf methanol extracts, fungistatic activity of 38.8%, 30.0% and 52.5% were shown by *A.niger*. Furthermore, inhibitory activity of 63.8%, 40.8%, and 66.3% were revealed on *A.flavus*. At the same concentration of seed, stem bark and leaf methanol extracts. Antifungal activity of*F.oxysporum* was 46.3%, 50.4% and 68.8%. mycelia growth inhibitionof 58.8%, 55.8%, and 67.5% were indicated on *P.herbarum* by the individual parts respectively. However, 50.8%, 46.3% and 51.3% mycelia growth inhibitionwere shown on *R. stolonifer* by seed, stem bark and leaf extracts respectively. At 20% methanol extractconcentrations of seed, stem bark and leaf, *A.niger* was inhibited at 52.9%, of 35.8%, and 65.0% of the extracts respectively. Percentage growth inhibition of 68.8%, 50.4% and 68.0% were shown on *A. flavus*. 56.3%, 52.5% and 70.4% were revealed on *F.oxysporum* by the various extracts. It is also indicated that *P.herbarum* was inhibited at 66.3%, 60.0% and 70.0% of the aerial parts respectively. *R. stolonifer* was also inhibited at 50.8%, 46.3% and 56.3% concentrations of the individual parts. At 25% methanol extract concentration of the aerial parts, antifungal activity of 62.1%, 50.0% and 76.3% were recorded on *A.niger* of the aerial parts respectively. At the same concentrationof the respective aerial parts, *A. flavus* was inhibited at 70.4%, 66.3% and 80.0% concentrations. However, antifungal activity of 60.8%, 55.8% and 75% were revealed on *F.oxysporum*. 67.5%, 62.5% and 71.3% were shown on *P.herbarum*. It is

also shown that mycelia growth inhibition of 53.3%, 48.8% and 60.4% were exhibited on *R. stolonifer*. However, in rare cases on the test organisms, the seed and stem bark methanol extracts possess similar inhibitory activity. The comparisons of efficacy between methanol extracts of different plant parts on mycelia growth inhibition of the test organism weresignificantly different.

4.13: Efficacy of Different Concentrations ofAqueous and Methanol Extracts of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungi from Sorghum Grains.

Table 4.12 shows the comparison of efficacy between the aqueous and methanol extracts of *M.oleifera* on the test organism. It was indicated that at both concentrations on the test organisms, the methanol extract was more effective than the aqueous extract. Therefore, at 5% concentration, *A.niger* was inhibited by 26.7% and 29.6% of the aqueous and methanol extracts respectively. *A. flavus* was inhibited by 20.8% and 43.1% of the respective extracts. Similarly, *F.oxysproum* was inhibited by 26.3% and 30.7% of the aqueous and methanol extracts respectively. *P.herbarum* was inhibited at 35.8% and 43.3% of the respective extracts. Mycelial growth inhibition at 21.3% and 25.4% concentrations were revealed by the extracts on *R.stolonifer* respectively. At 10% concentration of the extracts, *A.niger* was inhibited at 29.7% and 31.4% of aqueous and methanol extracts respectively. The aqueous extract inhibited *A.flavus* at 22.9% while

Table 4.12a: Efficacy of different concentrations ofAqueous and Methanol Extracts of *Moringa oleifera* on Percentage Mycelial Growth Inhibition of Isolated Fungifrom SorghumGrains

Concentrations (%)	Extracts	Test organisms				
		A. niger	***A. flavus***	***F.oxysporum***	***P.herbarum***	***R.stolonifer***
0.0	Aqueous	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]
	Methanol	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]
	P-values	**undefined**	**Undefined**	**undefined**	**undefined**	**Undefined**
5.0	Aqueous	26.7±3.81[a]	20.8±1.38[b]	26.3±3.23[a]	35.8±1.47[a]	21.3±1.50[a]
	Methanol	29.6±3.05[a]	43.1±3.28[a]	30.7±3.13[a]	43.3±3.47[a]	25.4±2.82[a]
	P-values	**0.56**	**0.00**	**0.34**	**0.06**	**0.21**
10.0	Aqueous	29.7±3.16[a]	22.9±1.21[b]	27.5±2.85[a]	41.1±3.64[b]	24.6±1.86[b]
	Methanol	31.4±2.58[a]	52.6±4.18[a]	39.1±6.03[a]	53.8±1.89[a]	44.9±3.36[a]
	P-values	**0.69**	**0.00**	**0.10**	**0.01**	**0.00**

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

Table 4.12b Efficacy of different concentrations ofAqueous and Methanol Extracts of *Moringa oleifera*on Percentage Mycelial Growth Inhibition of Isolated Fungifrom Sorghum Grains

Concentrations (%)	Extracts	Test organisms				
		A. niger	***A.flavus***	***F.oxysporum***	***P.herbarum***	***R. stolonifer***
15.0	Aqueous	31.7±4.10[a]	28.2±4.43[b]	39.6±5.79[b]	45.1±3.38[b]	26.7±1.80[b]
	Methanol	40.4±3.76[a]	56.9±4.14[a]	55.1±4.68[a]	60.7±2.13[a]	49.4±2.37[a]
	P values	**0.14**	**0.00**	**0.05**	**0.00**	**0.00**
20.0	Aqueous	48.6±5.17[a]	42.5±5.06[b]	45.4±6.70[a]	50.0±2.97[b]	28.3±2.02[b]
	Methanol	51.3±5.23[a]	62.6±3.92[a]	59.7±3.31[a]	65.4±1.88[a]	51.1±2.88[a]
	P-values	**0.73**	**0.01**	**0.07**	**0.00**	**0.00**
25.0	Aqueous	53.8±5.16[a]	57.5±6.20[a]	49.7±5.56[b]	51.9±2.28[b]	30.4±2.11[b]
	Methanol	62.8±4.90[a]	72.2±3.16[a]	63.9±3.12[a]	67.1±2.94[a]	54.2±3.19[a]
	P-values	**0.22**	**0.05**	**0.04**	**0.00**	**0.00**

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

methanol extract inhibited the organism by 52.6%. Furthermore, inhibitory effects at 27.5% and 39.1% concentrations were indicated on *F. oxysporum* by the seed and aqueous extracts respectively. *P.herbarum* was inhibited at 41.1% and 53.8% of the respective extracts. Percentage mycelia growth inhibition at 24.6% and 44.9% were recorded on *R.stolonifer* by aqueous and methanol extracts respectively. At 15% concentration of the aqueous and methanol extract growthinhibition at 31.7% and 40.4% concentrations were revealed respectively on *A.niger.* Similarly, 28.2% and 56.9% growth inhibition were observed on *A. flavus*. At the same concentration of the respective extracts *F.oxysporum* was inhibited by 39.6% and 55.1%. *P. herbarum* was inhibited by 45.1% and 60.7% respectively.

Fungistatic activity at 26.7% and 49.4% concentrations were shown by aqueous and methanol extracts respectively on *R. stolonifer.*At 20% of the aqueous and methanol extracts concentrations, growth inhibition of 48.6% and 51.3% were revealed on *A.niger* respectively. Similar concentration of the extract inhibited *A. flavus* at 42.5% and 62.5%. The extracts show inhibitory effect at concentrations of 45.4% and 59.7% respectively on *F.oxysporum. P.herbarum* was inhibited at 50.0% and 65.4% concentrations of the aqueous and methanol extracts respectively. However, mycelia growth inhibition of 28.3% and 51.1% of the respective extracts were shown on *R.stolonifer.* At 25% aqueous and methanol extractsconcentrations,*A.niger*was inhibited by 53.8% and 62.8% respectively. Antifungal activity at 57.5% and 72.2% concentrations were shown on *A. flavus* by the respective extracts. Like all other pathogens, *F.oxysporum* showed inhibitory effects at 49.7% and 63.9%. There was growth inhibition at 51.9% and 67.1% concentrations on *P.herbarum* by the aqueous and Methanol extracts respectively.

Mycelia growth inhibition at 30.4% and 54.2% concentrations were shown on *R.stolonifer* by both extracts respectively. The comparison of efficacy between aqueous and methanol extracts on growth inhibition of the test organisms shows significant difference.

CHAPTER FIVE

5.0 DISCUSSION

Different fungal genera such as *Aspergillus flavus* link, *Aspergillus niger* VanTieghem, *Fusarium oxysporum* Schlecht, *Rhizopus stolonifer (*Ehrenb*)* lindand *Phoma herbarum* Saccwere isolated from *Sorghum bicolor* varieties on both PDA and SDA. *Aspergillusniger, Aspergillusflavus, Phoma herbarum andRhizopus stolonifer*have wide range of host (Facultative parasites) and hence, were isolated from the improved varieties. However, *Fusarium oxysporum*being host specific (Obligate parasite) was only isolated from the local land race Kaura.Microorganisms especially fungi are known to be the major cause of market and field losses of crops (Onifade, 2000). These fungi have also been shown to cause spoilage to cereals (Domijan and Sherman, 2005.

All the isolated fungi when compared with already described species by Barnett and Hunter (1972), Larone (2002), Klich (2002) and Samson *et al*., (2004) were found to be similar. However, there were minor variations in the measurement of the microscopic characteristics possibly due to difference in nutritional media used and environment conditions.

From the result of fungi identification, Sorghum was found to be dominated by *Aspergillus niger* (47.91%) on PDA and *Aspergillus flavus* (47.72%) on SDA. This corroborate with the work of Visconti (2001). The high occurrence of *Aspergillus* species indicates that *Aspergillus* genus possibly acts as either pioneer organisms in seed borne infection of sorghum, or the dominant species found in the tropics while the presence of

other fungi showed that there is possible succession of fungi in the seed borne infection.Hell *et al*.(2000) reported that *Rhizopus stolonifer* and *Phoma herbarum* were also found to contaminate maize (Z*ea mays*).

In this study, the radial mycelia growth of all the isolated fungi were found to be significantly inhibited by both aqueous and methanol extracts of seed stem bark and leaves of *Moringa oleifera.* Although, the efficacy of the extracts were found to vary with concentration, plant parts, type of solvent used for extraction and pathogen. This indicated that *Moringa oleifera* have fungi toxic potential though none of the extracts had 100% mycelia growth inhibition within the period of observation. This may be possibly due to the presence of alkaloids, flavonoids, tannins and saponins in the plant (Safowora, 1984). It was further revealed in this study that increase in the antifungal activities of the extracts was enhanced by increase in concentration of the extract and vice-versa. This may be due to higher quantity of antifungal compounds in higher concentration. These findings agree with the findings of Mares *et al*., 2004; Sharma and Tripath, 2007.

The leaves extract of *Moringa oleifera* upon comparison gave the best result in terms of mycelia growth inhibition, followed by the seed and then stem bark. This may be due to the fact that leaves being the site for photosynthetic activities possess more qualitative and quantitative ingredients compared to the other parts (seed and stem bark). This corroborates the findings of Nwosu and Okafor (1995) where they found leaves extract of *Moringaoleifera*to have high inhibitory effect on *Basidiobolus* and some other pathogenic fungi than stem bark, and root extracts.

From the result it is evident that extracts of stem bark have low inhibitory activity. This may be due to the fact that in stem bark, either there is low accumulation of these phytochemicals or they are more complex to be dissolved in water and methanol used for the extraction. It is possible that with some other solvent, they may yield phytochemical substances which may be more effective than leaves and seeds.

Successful prediction of botanical compounds from plant materials is largely dependent upon the type of solvent used in the extraction procedure. In this study, it was found that the plant extracts in organic solvent (methanol) provided more consistent antifungal activities compared to those extracted in water (aqueous extract). This may be due to the fact that methanol being an organic solvent have the ability to completely dissolve these phytochemicals compared to water which is an inorganic solvent resulting in more effective controlof the growth.

The antifungal activities of Moringa seed, leaves and stem bark have also been reported by Nwosu and Okafor (1995). While the seed extract was shown to be active on *Rhizopus* species (Raheela *et al*., 2008). Antifungal properties of *Moringa oleifera* against *Aspergillus flavus* and *Aspergillus niger* have been reported by Akinyemi *et al*. (2005). Foidli *et al*. (2001) reported that stem bark extract of *Moringa oleifera* possess fungicidal effect against *Fusarium* species. Extracts of *Moringa oleifera* have been reported to be active on *Phoma herbarum*, *Rhizopus stolonifer* and *Aspergillus niger* (Nickon *et al*., 2003). The antimicrobial properties of the leaf and seed of *Moringa oleifera* were also found to corroborate with the findings of Aktar *et al*. (2006). Antimicrobial activity of *Moringa oleifera* extracts has been highlighted by Madsen *et al*; 1987 and Kawo, 2007.

The antimicrobial activity of *Moringa oleifera* extracts is due to the presence of an array of phytochemicals, but most importantly due to the activity of short polypeptide benzyl-isothiocyanate (Eilert *et al*., 1981 and Guevara *et al*., 1999). The peptide may act directly on microorganisms and result in growth inhibition by disrupting cell membrane syntheses or syntheses of essential enzymes (Silvestro *et al*., 2000 and Suarez *et al*., 2003).

Biological control is generally favoured as a method of plant diseases management because it does not have demerits of chemicals (Amadioha and Obi, 1999). Kuhn and Hargreavez (1997) observed that substances found fungicidal *in vitro* in almost all cases kill the fungi *in vivo*. Plants with such fungicidal properties includes *Moringaoleifera* (Caceres *et al*., 1992).

Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (De Billeberk *et al*., 2001). They still remain the principal source of pharmaceutical agents used in orthodox medicine (De Billeberk *et al*., 2001). Several reports stated that the extracts of medicinal plants play an important role in controlling many phytopathogenic fungi (Lin *et al*., 2004, Khalil *et al*., 2005, Abd-El-Khair and Haggag, 2007, Perez*et al*., 2007). The inhibitory effect of the tested extracts might be due to natural bioactive materials present in them (Rasooli and Abyaneh, 2004).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

1. Based on the findings of this research work, it can be concluded that five fungal genera namely *Aspergillus flavus* Link, *Aspergillus niger*Van *Tieghem, Rhizopus stolonifer (*Ehrenb.*)* Lind, *Phoma herbarum sacc* and*Fusarium oxysporum* schlecht were found to contaminate *Sorghum bicolor*grains. However,*Sorghumbicolor* was found to be dominated by *Aspergillusniger* (47.91%) on PDA and *Aspergillusflavus*(47.72%) on SDA.Therefore, the null hypothesis is rejected.
2. The extracts of *Moringaoleifera* proved to be effective in the control of the isolated pathogens with leaf extract being the most effective, followed by the seed extract with stem bark being the least. However, the efficacies of the extracts were found to vary with concentration, pathogen, plant part as well as the type of solvent used for the extraction. Therefore, the null hypothesis is rejected.

6.2 Recommendations

1. It is recommended that further studies should be carried out on the fungicidal effects of the leaf extract of *Moringa oleifera*and the possibilities of producing the extracts in commercial quantities.
2. It is also recommended that the application of the extract on sorghum grains should be done via sprinkling or spraying and then dried prior to storage.

3. It is also recommended that further research on fungi isolation from *Sorghum bicolor* should be done using other media.
4. Thereisneed to identify the active ingredients responsible for inhibiting the growth of the isolated fungi

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APPENDICES I

Preparation of Different Concentrations of *Moringa oleifera*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

APPENDIX 11

Effect of Different Concentrations of Methanol Extract of Plant Parts of *Moringa oleifera* on Mycelia Growth of Fungi Isolated from Sorghum Grains

Concentrations (%)	*Aspergillus niger* (8cm)			*Aspergillus flavus* (8cm)			*Fusarium oxysporum* (8cm)			*Phoma harbarum* (8cm)			*Rhizopus stolonifer* (8cm)		
	Seed	Stem bark	Leaves	Seed	Stem bark	Leaves	Seed	Stem bark	Leaves	Seed	Stem bark	Leaves	Seed	Stem bark	Leaves
5	6.0	6.1	5.6	5.1	5.7	4.0	5.8	6.7	5.3	5.3	5.0	4.5	6.9	6.9	5.9
	6.0	6.0	5.3	4.5	5.3	4.0	5.6	6.4	4.5	5.3	5.0	4.1	6.1	6.1	5.9
	6.0	5.9	3.8	3.3	5.1	4.0	5.3	5.8	4.5	3.8	5.0	2.8	5.0	5.0	5.9
	6.0	6.0	4.9	4.3	5.3	4.0	5.6	6.3	4.8	4.8	5.0	3.8	6.0	6.0	5.9
10	5.9	6.1	5.3	3.3	5.6	3.7	5.7	6.3	3.0	3.7	4.5	3.6	4.5	4.9	5.3
	5.8	6.0	4.5	3.3	5.0	2.9	5.3	6.3	3.0	3.7	4.1	3.6	4.0	4.6	5.6
	5.4	5.9	4.5	3.3	4.4	2.6	5.2	6.0	3.0	3.7	2.8	3.6	3.0	4.0	3.8
	5.7	6.0	4.8	3.3	5.0	3.1	5.4	6.2	3.0	3.7	3.8	3.6	4.0	4.5	4.9
15	4.9	5.6	4.5	2.8	5.0	2.7	4.9	4.8	3.5	2.9	3.6	4.0	4.3	5.1	3.9
	4.9	5.6	4.1	2.6	4.6	2.7	4.8	3.9	2.0	2.5	3.3	3.4	4.2	4.5	3.9
	4.9	5.6	2.8	3.3	4.6	2.7	3.2	3.2	2.0	2.4	3.0	3.2	3.3	3.3	3.9
	4.9	5.6	3.8	2.9	4.7	2.7	4.0	5.4	2.5	2.6	3.3	3.5	4.0	4.3	3.9
20	4.9	5.8	3.6	3.5	4.8	2.5	3.5	4.5	2.5	2.4	3.3	3.4	4.3	5.1	3.9
	3.4	5.4	2.5	2.0	3.9	2.5	3.5	4.1	2.4	2.4	2.5	3.2	4.2	4.5	3.7
	3.0	4.2	2.3	2.0	3.2	2.5	3.5	28	2.2	2.4	2.3	3.0	3.3	3.3	2.9
	3.8	5.0	2.8	2.5	3.9	2.5	3.5	3.8	2.4	2.4	2.7	3.2	4.0	4.3	3.5
25	4.0	5.0	2.1	2.5	3.6	1.6	3.6	4.0	2.0	3.1	3.1	3.8	4.1	5.0	3.7
	2.8	4.3	2.0	2.4	3.1	1.6	3.0	3.4	2.0	2.2	2.5	3.2	4.1	4.2	3.3
	2.3	2.7	1.6	2.2	1.4	1.6	2.8	3.2	2.0	1.6	2.2	2.0	3.0	3.1	3.5
	3.0	4.0	1.9	2.4	2.7	1.6	3.1	3.5	2.0	2.3	2.6	3.0	3.7	4.1	3.2

APPENDIX III

Effect of Different Concentrations of Aqueous Extract of Plant Parts of *Moringa oleifera* on Mycelia Growth of Fungi Isolated from Sorghum Grains.

Concentrations (%)	*Aspergillus niger* (8cm)			*Aspergillus flavus* (8cm)			*Fusarium oxysporum* (8cm)			*Phoma harbarum* (8cm)			*Rhizopus stolonifer* (8cm)		
	Seed	Stem bark	Leaves	Seed	Stem bark	Leaves	Seed	Stem bark	Leaves	Seed	Stem bark	leaves	Seed	Stem bark	Leaves
5	6.4	6.9	5.6	6.8	6.8	6.3	6.7	7.0	5.1	5.0	5.7	4.9	6.5	6.9	5.9
	6.2	6.6	5.3	6.4	6.5	6.3	6.4	6.4	5.0	5.2	5.4	5.5	6.5	6.5	5.9
	6.0	6.0	3.8	6.0	5.9	6.0	5.8	5.8	4.9	4.8	5.1	4.6	6.5	6.1	5.9
	6.2	6.5	4.9	6.4	6.4	6.2	6.3	6.4	5.0	5.0	5.4	5.0	6.5	6.5	5.9
10	5.9	6.8	5.3	6.4	6.4	6.1	6.4	6.4	5.6	5.5	5.6	4.7	6.5	6.7	5.8
	5.8	6.3	4.5	6.2	6.2	5.6	6.1	6.2	5.0	5.4	5.3	3.9	6.3	6.4	5.5
	5.7	5.8	4.5	6.0	6.0	6.6	6.0	6.1	4.4	3.8	5.0	3.2	5.8	5.8	5.5
	5.8	6.3	4.8	6.2	6.2	6.1	6.1	6.2	5.0	4.9	5.3	3.9	6.2	6.3	5.6
15	5.6	6.7	5.3	6.9	6.0	5.7	5.9	6.0	3.0	5.3	5.1	4.4	6.4	6.6	5.7
	5.3	6.4	5.3	6.3	6.0	5.2	5.8	5.9	3.1	5.3	4.8	3.6	6.0	6.1	5.4
	5.7	5.8	3.2	4.5	6.0	3.8	5.4	5.5	2.9	3.8	4.2	3.0	5.6	5.6	54
	5.5	6.3	4.6	5.9	6.0	4.9	5.7	5.8	3.0	4.8	4.7	3.6	6.0	6.1	5.5
20	4.0	6.6	2.9	5.6	5.9	3.2	5.8	6.0	2.5	4.7	4.9	3.9	6.2	6.6	5.7
	4.0	5.4	2.9	5.3	5.8	3.2	5.6	5.7	2.5	4.3	4.6	3.7	5.8	6.1	5.3
	4.0	4.3	2.9	3.8	5.4	3.2	3.3	5.4	2.5	3.0	4.0	2.9	5.4	5.3	52
	4.0	5.4	2.9	4.9	5.7	3.2	4.9	5.7	2.5	4.0	4.5	3.5	5.8	6.9	5.4
25	3.9	5.0	2.6	3.2	5.6	2.8	4.3	5.6	2.5	3.9	4.5	3.7	6.0	6.5	5.5
	3.9	50.	2.2	3.1	5.0	2.0	4.3	5.3	2.4	3.9	4.3	3.6	5.6	6.0	5.1
	3.9	5.0	1.8	2.7	5.0	1.2	4.3	5.3	2.2	3.9	4.2	2.6	3.2	5.2	5.0
	3.9	5.0	2.2	3.0	5.2	2.0	4.3	5.4	2.4	3.9	4.3	3.3	5.6	5.9	5.2