POTENTIALS OF ALKALOIDS FROM *PANICUM MAXIMUM* FLORETS INFECTED WITH THE FUNGUS *TILLETIA AYRESII* IN CONTROLLING UTERINE CONTRACTION IN *SPRAGUE- DAWLEY RATS*

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DEDICATION

This work is dedicated to Almighty God, the only true and wise God, to who belong the secret of success and knowledge of witty inventions, my husband and children for their endurance, patience and support and my brothers, for their encouragement and support.

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ABSTRACT

The potential of alkaloids from *Panicum maximum* floret infected with the fungus *Tilletia ayresii* in the control of uterine contraction in *Sprague-Dawley* rats was investigated using bioassay guided fractionation technique. Disease incidence study revealed that smut disease was significantly higher in Ikorodu site (95.00%) when compared with Akoka site (69.00%) and Isolo site (72.00%). The height of disease incidence was between the months of August and November. Thirteen species of fungi were identified as fungi associated with smutted florets and out of these, *Aspergillus niger, A. fumigatus* and *Tilletia ayresii* occurred most frequently (100%), *A. flavus* (80%) while others such as *A. wentii, Trichoderma* sp.., *Mucor* sp., *Penicillium pinophyllum, Fusarium* sp., *F. fujikuroii* recorded 40%. *Syncepalastrum racemosa, Curvularia lunata and Paeciliomyces* sp. were least frequent (20%). Only *T. ayresii* was pathogenic and five spore types were isolated from *T. ayresii* (Basidiospores, 'Y- shaped conidia, blastic conidia, ballistospores, and teliospores). The genus and species of *Fusarium* sp. and *F. fujikuroii* were further confirmed by DNA sequencing. Growth studies of the fungal isolates on six solid and three broth media showed that the best media for their cultivation are potato dextrose agar, potato dextrose broth and malt extract agar. Ethnobotanical studies of crude aqueous, ethanol and chloroform extract of infected *Panicum maximum* florets, produced dose-dependent contraction of uterine smooth muscle of non-pregnant rat with ethanol extract being more potent than others. However, the healthy *P. maximum* floret extracts (aqueous, ethanol, and chloroform) produced relaxation effects. Infected ethanol fraction produced a similar pattern of contraction with ergometrine (standard) stimulated uterine muscle. The ethanol fractions spotted on Thin Layer Chromatography plates revealed three compounds under UV light (366nm) with R_f values of 0.20 (zone A), 0.30 (zone B) and 0.90 (zone C) respectively. Further fractionation and bioassay of the three compounds showed that the compounds in zone A with R_f value of 0.20 was active while the others were not. Effect of ethanol and chloroform extracts of infected *Panicum maximum* florets on six fungal isolates showed that they inhibited the growth of all the fungal isolates except *Tilletia ayresii* at varying concentrations. However Benlate (plant orthodox fungicide) inhibited the growth of *T. ayresii* while ketaconazole (animal

orthodox fungicide) did not. A preliminary phytochemical screening of the healthy and infected *P. maximum* floret extracts showed the presence of alkaloids, tannins, saponins and flavonoids in varying proportions. The percentage of alkaloids, tannins and sapoinins was higher in the crude ethanol extracts of infected floret than in healthy florets. The acute toxicity value (LD_{50}) of infected ethanol and chloroform extract were determined to be 9.0g/kg body weight. Significant reduction in body weight was observed in groups treated with highest dose of the extracts at the third week of administration ($p<0.05$). There was a non-significant reduction in plasma protein, creatinine and total bilirubin while aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed appreciable reduction at low to moderate doses ($p>0.05$) but increased significantly only at the highest dose of the extract ($p<0.05$). The levels of high and low density cholesterol were not affected significantly . The haemoglobin, red blood cells, white blood cells and packed cell volume increased in treated animals compared to the control. In this study alkaloids were extracted from *T. ayresii* infected *Panicum maximum* florets and purified. They demonstrated potency in inducing contraction and controlling bleeding after childbirth. The high LD_{50} value (9.0g/kg) obtained was a clear indication that the infected extracts is safe to use, since it reduced level of cholesterol significantly and did not induce toxic effects on the liver, kidney and heart.

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background of study

Panicum maximum Jacq. most commonly known as guinea grass is indigenous to Africa, widely distributed throughout the tropics and subtropics but most commonly in moist soils of southern Nigeria and some parts of the North (Stanfield, 1970; Arohkesi, 1997). The economic value of *P. maximum* cannot be overemphasized. It is the best forage grass in the tropics, often classified among the important pasture grasses of the tropics (Lenne and Sonoda, 1990; Aganga and Tshwenyane, 2004). *Panicum maximum* grass is drought resistant and it has been reported to be highly nutritive due to the presence of the following components per 100g fresh weight- 26g dry matter, 9.37g crude protein, 60.0g natural detergent fibre, 38.0g acid detergent fibre, 7g lignin, 31g cellulose, 22g hemicelluloses and 12g ash. It is thus highly palatable, digestible and acceptable to livestock (Sodeinde *et al*., 2006; Oderinde and Babayemi, 2008). Its role in phytoremediation and control of soil erosion, and other domestic uses such as compost manuring and nesting has been reported by several workers (Mellory, 1972; Lago-Palva, 1996; Merki *et al.*, 2004; Olabode *et al.*, 2007; Carmo, 2008; Ogbo *et al.*, 2009).

Fungal attack of parts of *P.maximum* (stem, leaves and florets) has been reported. *Cercospora fusimaculans* and *Fusarium spp* attack the stem causing stem blight, *Phyllachora spp* cause black spots (tar spots) while *Curvularia spp* and *Colletotrichum graminicola* cause leaf spot disease. Smut disease of the floret is caused by *Tilletia ayresii* (Calpouzos *et al.*, 1962; Mabadeje, 1978; Arohkesi, 1997). During floral infection by *T. ayresii*, it colonizes the ovary and replaces the contents with fungal mycelia and

spores (Vanky and Bauer, 1992; Arohkesi, 1997; Piepenbring, 2000).Other tissue replacement diseases such as grain smut of *Sorghum vulgare* incited by *Claviceps africana*, ergot of rye caused by *C. purpurea*, and corn smut caused by *Ustilago maydis* have been reported (Patience *et al* 1999; Langenhein, 2003; Curran *et al.*, 2009).

Panicum maximum being a forage grass is often grazed upon by camels, horses, sheep, rabbits and guinea pigs and since these animals do not discriminate between infected and healthy plants during grazing, naturally infected grass forms part of their diet. Studies have shown that ingestion of infected grasses often results in poisoning with changes in biological activities (circulation and neurotransmission), physiological and anatomical damage of some organs or death commonly referred to as ergotism. These defects are probably due to the presence of toxic substances in the infected grasses (Hudler, 1998; Woolf, 2000; Tudzynski *et al* ., 2001). Several workers have shown that pathogenic fungi produce metabolites (toxic substances) in the course of their infection which play several roles in disease development (Scheffer, 1983; Komolong *et al.*, 2003; Eziashi, 2008).

1.2. Statement of Problem

Bleeding after childbirth (postpartum haemorrhage) is a leading cause of maternal mortality in developed and developing countries. Statistics from World Health Organization suggests that 25% of maternal death are due to postpartum haemorrhage (PPH) accounting for more than 100,000 maternal death per year as cited by Abouzahr, (1998). The causes of PPH are many but the most common by a wide margin are uterine atony (failure of the uterus to contract following delivery of baby) and incomplete expulsion of placenta (Jackson *et al.*, 2002; Sheiner et *al.*, 2005).

The drug, ergometrine has long been used for strong uterine contraction and control of postpartum haemorrhage due to its pronounced effect of direct stimulation of the rate and force of rhythmical contractions (Daniel and Maria, 2000; Sele-Ojeme, 2002). This drug is produced from ergot alkaloids stored in sclerotium during infection of the rye plant by the fungus *Claviceps purpurea.* During the infection process, this fungus grows inside the ovary of rye flower, colonizes it, and replaces the ovarian content. The sphacelia converts into sclerotium with accumulation of alkaloids which is useful in pharmaceutical industries for the production of drugs (Hudler, 1998; Tudzynski *et al.*, 2001). Methods for artificial infection of the temperate rye plant with *Claviceps purpurea* as well as induction of sclerotia formation have not been discovered. In addition, the rye is a temperate plant and may not be able to grow in tropical climate; consequently making the commercial production of these ergot alkaloids in Nigeria difficult. Therefore, huge amount of money is spent annually in importing this drug from western countries.

Tilletia ayresii (smut fungus) grows inside the ovary of a tropical grass (*Panicum maximum*) just like *Claviceps purpurea* in rye plant, colonizing and replacing ovarian contents. Reports however have shown that this fungus produces toxic substances in the florets of *P. maximum* which could be the cause of physiological and anatomical changes in animals that feed on the infected grass (Arohkesi, 1997). Investigation of the nature and neurotropic property of these toxic substances produced during the infection of *P. maximum* florets by the smut fungus is neccesary. Moreover there is lack of information in this area of research. Although Arohkesi (1997) reported the effects of infected florets on some organs of guinea pig, there has been no report on the effect of infected *P. maximum* floret on contractility of uterine muscle of animal. Furthermore, there is no

report so far on the bioactive ingredient responsible for the contractility of infected *P. maximum* florets.

1.3. Aims and Objectives

In view of the highlighted problems above, the aim of this work is to produce easily accessible alkaloids of tropical origin which can be used in the formulation of drugs for controlling uterine contraction and postpartum haemorrhage.

The objectives of this research are to:

I. study the etiology of smut disease incidence in selected sites in Lagos state .

II. isolate and identify the causal organism of smut infection in *Panicum maximum* floret and other fungi associated with it and determine the best media for their cultivation.

III. determine the pathogenicity of the fungal isolates from infected *P. maximum* florets.

IV. extract, purify, identify and characterize phytochemical compounds from *Tilletia ayresii*-infected *P. maximum* florets.

V. compare the effects of the crude and purified extracts from healthy and infected *P. maximum* florets and the orthodox drug (ergometrine) on uterine contractility on primed Sprague-Dawley rat.

VI. determine the toxicological and histopathological effects of crude extract of the infected *P. maximum* florets in Sprague-Dawley rats.

1.4. Significance of Study

1. Possible production of easily accessible and alkaloids in Nigeria (tropics) from a local plant for production of bioactive component for controlling uterine contraction and bleeding after childbirth (PPH).

2. In other to reduce foreign exchange spent in the importation of ergometrine drug and possibly make the drug available for use during child birth , thus contribute to reduction in maternal mortality in child birth

1.5. Research Questions

1. Are there smut infection disease incidence in selected sites in Lagos state?

2a. Are there fungi associated with infected *P. maximum* florets?

b. What are the best media for cultivating *Tilletia ayresii* and other fungi associated with infected *P. maximum* florets?

3. Which of the fungal isolates is pathogenic on healthy *P .maximum* florets?

4. Are there phytochemical compounds (crude/purified) in *Tilletia ayresii*-infected *P. maximum* florets extracts that affects uterine contractility?

5. Are these crude/purified extracts active for control of uterine contraction compared with the orthodox drug (ergometrine) on rats?

6. Are the extracts toxic or not to animals?

1.6. Operational definition of terms

Biotechnology: Use of microorganisms (bacteria, fungi and yeast) or biological substances (enzymes) to perform specific industrial or manufacturing process / use of biologic substances to solve problems and make useful products.

Anamorph: Asexual state of a fungus.

Anisocytosis: Unequal red blood cells/excessive inequality in size of red blood cells.

Agglutination: Clumping of red blood cells.

Biocoenosis: Group of organisms that live closely together in a specific habitat.

Blastoconidia: An asexual conidium that forms by budding process.

***Claviceps*:** Fungus parasitic on the ovaries of various grasses.

Chromatography: Any technique that separates substances in a mixture on the basis of their physical and chemical properties.

Dikaryotic mycelium: Mycelium with two different and distinct nuclei per cell.

Dikaryotic Cell: A cell that contains two separate haploid nuclei (n+ n) which is different from being haploid (n) or diploid (2n).

Enclampsia: Coma and convulsion before, during or shortly after childbirth.

Etiology: Origin and cause of disease or abnormal condition.

Ergot: Dried sclerotium of the fungus – C*laviceps purpurea* which is developed on rye plant.

Echinulate: Covering with delicate spine.

Fungi: Organism with distinct cellular structures but lack the ability to use energy from sun directly due to absence of chlorophyll.

Floret: Little flower in grasses or capitulum of composite flowers.

Glume: Bract found at the base of spikelet.

Hypha: Basic filamentous element of fungi.

Hyaline hyphae: Clear, transparent hyphae which are non-septate with thin wall.

Haematological analysis: Analysis of blood forming tissues.

Hepatocytes: Parenchymal liver cells that perform all the functions ascribed to liver.

Haemoconcentration: Decrease in plasma volume resulting in increase in the concentration of red blood cells.

Hepatotoxic: Capacity of a substance to have damaging effect on the liver.

Inflorescence: A reproductive shoot bearing commonly a number of flowers.

Immunosuppressive: Purposely thwarting of immune system due to presence of toxic chemical.

Lanceolate: **S**haped like a spear.

Lethal dose: Amount of drug that would prove fatal to the majority of persons/dose of chemical that is likely to cause death.

Myometrium: Muscular wall of uterus.

Myocardium: Muscular tissue of the heart.

Nephrotoxicity: Poisonous effect of toxic substances on kidney.

Neurotransmitter: Chemicals located and released in the brain and allows impulse from one nerve cell to pass to another nerve cell.

Ovary (Plant): Enlarged basal portion of the pistil, which becomes the fruit.

Panicle: An inflorescence whose main axis is branched and bear loose racemose clusters.

Poikilocytosis: Presence of abnormally shaped red blood cells in the blood.

Phytotoxins: Poisonous substances derived from plant.

Postpartum Haemorrhage: Excessive bleeding (a loss of more than 500ml of blood) during the first 24 hours after childbirth.

Pathogenicity: Ability of a pathogen to produce an infectious disease in an organism.

Pathogenesis: Mechanism by which a disease is caused/chain of events that leads to disease development.

Pre-Eclampsia: This is a condition in pregnancy characterized by sharp rise in blood pressure.

Ruminant: Animals that regurgitates food from their stomach and chews their cords (cow and sheep).

Rhizome: This is modified underground stem usually growing horizontally.

Sclerotium: A dense mass of branched hyphae, as in certain fungi, that contain stored food and are capable of remaining dormant for long periods.

Spores: A general name for reproductive structure in fungi, bacteria and yeast.

Species (spp): Population of similar organism that interbreed in nature.

Spikelet: Small spikes with one or few flowers (florets).

Spike: An inflorescence in which the main axis is elongated and lower flowers are sessile.

Teleomorph: Sexual state of a fungus.

Tiller: Shoot that arises from the base of a stem

Uterine Atony: Failure of uterus to contract after delivery.

Uterotonic: An agent that is used to induce contraction

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. *Panicum maximum* (guinea grass)

The plant *Panicum* belongs to the family poaceae, subfamily panicoideae, tribe paniceae which is highly diverse in morphological and physiological characters. In Nigeria, these genus has 25 species, the commonest being *Panicum maximum* Jacq. (Guissani *et al.,* 2001; Zuloaga *et al.,* 2009).This grass is indigenous to Africa and widely distributed throughout the tropics and subtropics, commonly occurring in moist soils and wetter parts of Southern and Northern Nigeria (Stanfield,1970; Arohkesi,1997).

Pancium maximum is a perennial, tufted grass with short creeping rhizome. The stem can reach a height of about 70cm. The lanceolate leaf blades are 1.0cm to 5.0cm long and 1.3cm to 9.1cm wide, often tapering at each end. There is a sheathing leaf-base which is slit open on the side facing the leaf blade. When young, the leaf sheath completely encircles the stem but slits open to expose the stem when matured (Bogdan, 1977; Stanfield, 1970; Dutta, 2000). The inflorescence of this plant is a panicle of spikelet up to 70.0cm long with numerous horizontal branches. The lowest ones are usually whorled and up to 35.5cm long. The upper branches are irregularly spaced and progressively shorter. The secondary branches averages 5.0cm long while the spikelet are 3-4cm long. Each spikelet consists of 1-5 florets with bracts (glumes, lemma and palea). The flower is bisexual with 3-4 stamens and brown anthers making up the androecium (Purseglove, 1972; Ivens *et al.,* 1978; Gibbs *et al.,* 1991; Dutta, 2000; Reinheimer *et al.,* 2005) (Plate 1).

Plate1*:* ***Panicum maximum*** **Jacq. (Poaceae), (i), (Portrait view) Mag. x0.06**

2.2 .0 Cultivation of *Panicum maximum* Plant

Panicum maximum is propagated by seed or rhizome. It grows in tropical and subtropical areas with adequate rainfall on wide range of soils. The dense and fibrous root system allows guinea grass to survive quite long drought periods, but it performes best on well drained soils in high rainfall regions. Guinea grass is tolerant of shade and fire, but not water logging or severe drought. It produces high yield of palatable fodder and responds well to manuring, but rapidly declines in nutritive value with age (Pieterse *et al*., 1997; Aganga and Tshwenyane, 2004).

2.3.0 Fungal diseases of *Panicum maximum*

Many fungi have been found to attack parts of *Panicum maximum*. Leaf spots are the most important and familiar disease symptoms produced by fungi (Mundkur, 1953). Fungi belonging to the family Dothideales example *Phyllachora spp* cause black spots which are slightly raised and are known as tar spots. Dickson (1956) reported that tar spots are localized and rarely affect all plants in nursery, field or pasture. In Nigeria, Arohkesi (1997) isolated *Phyllachora bonaniesis* from *Panicum maximum* leaves. Mabadeje (1978) isolated and confirmed the pathogenicity of *Fusarium semitectum* from *Panicum maximum* leaves.

Srivastara and Rao (1963) reported that spots resemble blight in the expression of symptoms as well as mechanisms of pathogenesis, but the difference lies in the extent of host tissue breakdown. Blight organisms destroy host tissue extensively and many systematically disperse toxic substances far beyond the area of infection but the spot organisms are more united in their area of action (Wheeler, 1969). Diseases that kill leaf tissues reduces forage quality by increasing the lignin content and decreasing host

protein content, acceptability and digestibility (Burton and Wells, 1981). Stem diseases also result in field and quality reduction of the plants. In Brazil, *Cercospora fusimaculans* causes stem blight of *P. maximum* while in Nigeria, *Fusarium pallidoroseum* was isolated from stem of *Panicum maximum (*Mabadeje, 1978*;* Porto *et al.*, 1988*).*

Fungi also affect flowers and their ability to infect host flowers offers important ecological benefits to plant parasitic fungi. This flower – infecting fungi can be opportunistics, unspecialized pathogen and specialist pathogens which infect inflorescence either through the gyneocium or systemically through the apical meristem (Ngugi and Scherm, 2006). Chandra and Hieff (2008) reported that *Salmacisia buchoeana* infected the ovary of *Buchoe dactyloides* (Buffalo grass) displacing the contents of the ovary with dirty brown agglutinated teliospores. These often results in sterility of the flowers (parasitic castration). The ovary becomes sterile and thus unable to set seed.

Several moulds (*Aspergillus* spp., *A. fumigatus, and Rhizopus stolonifer)* were isolated during preliminary survey of cotton flower mycoflora from Sudan. Curran *et al., (*2009) also reported infection of oxalis flowers by an anther smut fungus known as *Thecaphora capensis* in Cape floristic region of South Africa. In recent years, ergot of sorghum (*Sorghum bicolor*) caused by the fungus *Claviceps africana* has emerged as a serious threat to sorghum production world wide (Bandyopadhyay *et al.*, 1996). The presence of alkaloids in sclerotia of *C. africana* has caused major concern in the commercial sorghum industry as small amounts in feed ration can adversely affect livestock (Ryley *et al.*, 2000; Blaney *et al.*, 2001). *Claviceps africana* caused ergot disease resulting in famine in 1903 – 1906 in Northern Cameroon, West Africa and in Eastern and Southern Africa,

especially Zimbabwe and South Africa. Infection by these pathogen is associated with cold temperatures that are below 12^{o}C occurring 2-3 weeks before flowering (Komolong *et al.,* 2003). Bacon *et al.* (1979) reported that species of *Babanisia* such as *B. epichloe, B. hennigsiana, B. strangulans* parasitized grasses such as *Sporobugus poiretii, Panicum anceps* and *Panicum hians* respectively. Infection of flowers of some cereals and grasses such as *Paspalum spp*., *Pennisetum typhoides, Zea mays, Sorghum vulgare* by *Claviceps paspali, C. fusiformis, C. gigantes* and *Sphacelia sorghi* have also been reported (Mantle, 1975; Patience *et al.,* 1999; Langenheim, 2003).

Infection of the flower of rye plant *Secale cereale* (a temperate grass) by spores of *Claviceps purpurea* to produce an ergot (Sclerotium) was reported by Hudler, (1998). The infection process mimics a pollen grain growing into an ovary during fertilization. The first stage of ergot infection manifests itself as white soft tissue (known as sphacelia) producing sugary honey dew, which often drops out of the grass florets. This honey dew contains millions of asexual spores (conidia) which are dispersed to other florets by insects. Later the sphacelia convert into a hard sclerotium inside the husk of the floret. At this stage, alkaloids and lipids accumulate in the sclerotium. *Claviceps purpurea* is a biotrophic flower pathogen of rye and other grasses and deleterious toxic effects of infected rye seeds on humans and grazing animals have been known since the middle ages (Eadie, 2003).

The first report of smut disease of *Panicum maximum* inflorescence in Nigeria was by Arohkesi, (1997). Smuts are diseases caused by fungi in the order Ustilaginales and are characterized by production of dusty brown or black spore masses on their hosts. They spend part of their life cycle saprophytically (sporidia) and a part (mycelium and spores)

parasitically within the plant tissues (facultative parasites). Generally the life cycle of smut is complicated in that it initiates its infection with a dikaryotic mycelium. The dikaryotic cells differentiate into teliospores which have dark, thick warty walls. The teliospore on germination produces haploid spores and other additional spores which in culture forms yeast – like colony of spores. In this yeast-like phase many smuts are capable of prolonged saprophytic growth (Mehrotra and Aggarwa, 2003). In smut diseases, the host tissues are replaced by the pathogen hyphae and spores. Smut sori could be formed on the roots, stems, leaves or flowers. For example *Urocystis brassicae* induced gall formation on the roots of *Brassica campestris*, *Entyloma oryzae* caused the leaf smut of *Oryza sativa* and *Urocystis tritici* formed smut sori on the leaves of *Triticum vulgare* . Smut may completely destroy individual flowers as seen in *Triticum vulgare* attacked by *Ustilago tritici* (Pers) or the whole floral axis may be smutted as in *Saccharum officinarum* L. infected by *Ustilago scitaminea*. At times only a part of a flower may be invaded. Dianthus smut caused by *Ustilago violacea* affects the anthers of the host. Smut sori are also formed in the ovaries as those of *Avena sativa* L. attacked by *Ustilago kollneri*. Ovary infections may result in complete replacement of the ovary thus turning the entire ovary into a sori (Mehrotra and Aggarwal, 2003).

Examples of smut fungi are *Ustilago tritici, U. violacea, Claviceps spp, Tilletia baclayana, T. indica, T. secalis and T. ayresii. Tilletia ayresii* was first described by British naturalist known as Miles Joseph Berkeley in 1899 as cited by Vanky and Bauer (1992). It grows in ovaries of various grass species such as *Hypaorhemia* spp., *Panicum* spp. and *Setaria* spp. all in the family poaceae. The *Tilletia ayresii* Berk found in the tropics shows several characters such as presence of balls of conidia between the

ornamented sterile cells and spores uncommon for other species such as *T. baclayana, T. secalis, and T. indica*. The presence of these spores and their structures as well as their germination is unique and peculiar. The sori are swollen masses of spores with apical opening surrounded by sac-like membrane comprising tissue of both host and fungal origin (Vanky and Bauer, 1992). The sori is often closed when young and ruptures only most times with stellate lobes. The teliospores are single, spherical; with dimension of 13-16 or 12-13μm; brown. Wall which is about 2.5 – 3.5μm thick is covered by warts. The sterile (non spore – producing) single cells is spherical to globose, and colourless. Transitional forms between single sterile cells and teliospore are present. The conidia are globose, loosely formed; mostly "y" – shaped; up to 20μm long hyaline; with smooth wall. Germination of teliospore occurs after several days; with holobasidia apically carrying numerous filiform basidiospores (Lisa *et al.,* 2005). Conidia germinates within few days producing hyphae that can form both blastic conidia and ballistospore. All these spore types have not been reported to be isolated in Nigeria.

Tar (1955), isolated *Tilletia ayresii* from *P. maximum* florets in Sudan. Arohkesi (1997) identified *Tilletia ayresii* as the causal agent of smut disease in *Panicum maximum* florets. *T. ayresii* is a soil borne fungus and is spread mainly through rain splash and wind. The smutted florets are usually pinkish and bigger in size (about 2 times the size of healthy floret) as a result of spores within the ovary. Studies on infection process revealed that during this process, the teliospores germinate in water on the stigma branch to produce germ tubes. These penetrate the stoma branches and grow downward through the style into the ovary. The invading hyphae parasitize the ovary; disintegrate the tissues whose cells become obliterated and establish internal mycelium. Further development

result in conversion of the ovarian tissues into fungal hyphae. After several days of inoculation the spores mature and together with the pathogens hyphae form the sorus enclosed in a membrane which is actually the remains of the ovary wall. With the maturing of the membrane and the parting of the palea and glume the spores are exposed to the air to be blown about

2.4.0 Microbial Toxins and Disease Development

Fungi produce secondary metabolites through antagonistic activity which is attributed to one or more complex mechanisms, including nutrient competition, antibiosis, activity for cell wall-lytic enzymes induction of systemic resistance, and increased plant nutrient availability (Jeffries, 1995; Lonto *et al.*, 1996; Naseby *et al.*, 2000). The fungal toxins produced during infection process may have adverse or stimulatory effects on plants such as suppression of seed germination , malformation and retardation of seedling growth (Lynch and Clerk,1984). Some cause reduction in yield of crops such as *Sorghum bicolor* (Komolong *et al.*, 2003). Gold *et al.*, (2001) reported that Cercosporin produced by *Cercospora* spp play important role in pathogenesis of host plant by producing substances which destroy membrane of host plants. Identified metabolites from other pathogens include fusaric acid from *Fusarium moniliforme* which alters brain and pineal neurotransmitters and contribute to toxic effects of *Fusarium* contaminated feeds (Porter *et al.*, 1995).

Deoxynivalenol (DON) commonly produced by *Fusarium gramineae* (telemorph = *Gibberella zeae*) have been reported to cause emesis, feed refusal and growth depression in animals, especially dogs and swine (Malone, 2000). Studies on fungi and some mycotoxins (Aflatoxin B_1 – AFB_1, Ochratoxin A and *Zearalenone*) contaminating rice

(*Oryza sativa*) in Niger State of Nigeria revealed that the following major fungal genera were isolated from one hundred and ninety six mouldy rice samples- *Aspergillus spp, Penicillium spp, Fusarium spp, Alternaria spp, Mucor spp, Rhizopus spp, Trichoderma spp, Curvularia spp, Helminthosporum spp and Clasdosporum spp*. Aflatoxin was detected in 97 of the samples analyzed at concentrations between 20-1642 µg/kg, Ochratoxin contaminated toxin between 24-1164 µg/kg while zearalenone was found at concentrations of between 24 – 1169µg/kg (Malone, 2000).

Aspergillus species produces many types of mycotoxins including aflatoxins, Ochratoxins, tremagens and more than a dozen other toxins (Scott, 1994). These toxins are involved in many human and animal (Gbodi and Nwude, 1998) maladies. Of major concern is the presence of aflatoxin $B_{1,}$in our foods, one of the most potent naturally occurring carcinogens. Ochratoxin A causes kidney and liver impairment in animals and man especially pigs. *Penicillium* species also produce wide variety of mycotoxins including petulin and citrunin which are neurotoxic and nephrotoxic respectively ((Scott, 1994; Thurvander *et al.,* 2001; Carios *et al.,* 2004)

Zearalenone, Fumonisms and Trichothecenes are metabolites of *Fusarium* spp and the adverse impact of these Fusariotoxins are well documented by Bottalico (1998). Zearalenone causes infertility in animals and is associated with outbreaks of precocious pubertal changes in children in Puerto Rico and has been suggested to have a possible involvement in human cervical cancer. Furthermore, Rhizorin A produced by *Rhizopus* and M*ucor* spp. elicit degenerative necrosis of heptocytes of liver when grasses infected with these organisms are fed to experimental animals (Willson *et al.,* 1984).

The genus Alternaria is known to secrete a host of mycotoxins namely alternariol, alternariol monomerthyleste, L-tenuazonic acid, Altertoxins I, II, III, Altenuene, Brefeidin A, Cytochalasins A and B, Destruxin (Visconti and Sibilia, 1994). According to their review alternariol and altenuene have no significant biological activities in man and animals while altertoxins induced mutagenesis and transformation of mammalian cells. L-tenuazonic acid inhibits protein synthesis causing salivation, gastro intestinal haemorrhage and convulsion in pigs, mice, rabbits, dogs and monkeys. In the same review, it was reported that cytochalasins inhibit cytokinesis and protein synthesis and cause pulmonary haemorrhage and brain oedema in mice (Visconti and Sibilia, 1994).

Species of *Geotrichum candidum* have also been shown to be a common contaminant of grains and produce gliotoxin which can cause secondary infection in association with tuberculosis such as lesions of the skin, mouth, lungs and intestine. Beauvericin is a cyctohexadepsipeptide mycotoxin which has insecticidal properties and which can induce apoptosis in mammalian cells. Beauvericin is produced by some phytopathogenic *Fusarium* species, (F*usarium proliferatum*, *F. semitectum*, and *F. subglutinans*. Beauvericin induces programmed cell death and causes cytolysis accompanied by internucleosomal DNA fragmentation into multiples (Beerdall and Miller, 1994).

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They are the first natural product isolated from medicinal plants in the 19th century. Being nitrogen – containing bases, they form salts with acids. Alkaloids are produced by large variety of organisms including bacteria, fungi, plants and animals. Many of them are toxic to organisms and often have pharmacological effects. Many have neuroactive properties and interact with the receptors at nerve endings due to the presence of

fragments buried within overall structure. These structures resemble the natural substances (the neurotransmitters) that bind to these receptors and are thus used in medicine (Tridzynski *et al.,* 2001; Simon, 2003).

Ergot alkaloids produced by the fungus *Claviceps purpurea* on *Secale cereal(* rye) was first mentioned around 600BC by the Assyrians when it was probably used in medicine as an oxytocic . In 1582, Adam Loncier in Germany made the first note of ergot stimulating uterine contractions of labour by administering sclerotia to pregnant women (Boicheuko *et al.,* 2001). It was the most effective drug for this purpose at the time resulting in a rapid and sudden termination of labour, with a delivery time lasting less than three hours. But ergot was eventually deemed unsuitable for this purpose as the dosage could not be given accurately due to large variations in the active ingredients.

The ergot alkaloids (peptide alkaloids) have high biological activity and a broad spectrum of pharmacological effects; hence they are of considerable importance to medicine. They have therapeutic effect on some forms of migraine, postpartum haemorrhages, and a sedative effect on the central nervous system (Boicheuko *et al.,* 2001). Ergometrine alkaloids (lysergic acid) is linked to amino alcohol and was isolated in 1932 by Dudley and Moir and synthesized by Stoll and Hoffmann in 1943 (Evans, 1996). This drug among the other natural alkaloids of ergot significantly increased the motor activity of the uterus. After small dose administration, contractions are increased in force or frequency, or both. As the dose is increased, contraction becomes more forceful and prolonged, resting tonus is markedly increased and sustained contraction often results. Due to the oxytocic property of ergometrine its medical use in obstetrics is in facilitating delivery of placenta and prevention of bleeding after childbirth by causing smooth muscle tissue in

the blood vessel walls to narrow, thereby reducing blood flow. The gravid uterus is very sensitive and small doses of ergot alkaloid can be given immediately after delivery to obtain a marked uterine response, usually without a significant side effect. This drug acts at alpha-adrenergic receptors to exert a powerful stimulant effect on the uterus. It can also induce spasm of the coronary arteries. It is most frequently delivered intramuscularly or rarely by mouth. Nevertheless, women with pre-eclampsia, eclampsia, high blood pressure or heart disease should use these drug with caution (Schuurmans *et al.*, 2000).

Postpartum haemorrhage (bleeding after childbirth) is one of the major cause of maternal death in developed and developing countries. It is estimated that it causes 125,000 deaths per year, affecting 5-15% of women after young birth and increases morbidity in about 20 million women undergoing delivery (Schuurmans *et al.*, 2000; Sele-ojeme, 2002). It can also be described as blood loss of more than 500ml during or immediately after the 3rd stage of labor in a vaginal delivery or more than 1000ml in a cesarean delivery. Causes of postpartum haemorrhage include uterine atomy (most common), lacerations of the genital tract, extension of an episiotomy, uterine rapture, bleeding disorder, uterine invation and incomplete expulsion of placenta(Abouzahr,1998).

Other drugs commonly used for control of uterine contraction are **oxytocin, prostaglandins** and **misoprostol.** Oxytocin, a small peptide of amino acids, is well known from clinicians for its potent uterotonic effect (Mitchell *et al.*, 1998). Indeed, administration of oxytocin in late pregnancy stimulates powerfully the contraction of uterine smooth muscle cells. It is therefore widely used clinically for the induction and argumentation of labour in women. It is also used for control of bleeding after childbirth especially in severe cases in combination with ergometrine. The uterine myometrium

contains receptors specific to oxytocin. Oxytocin stimulates contraction of uterine smooth muscle by increasing intracellular calcium concentrations, thus mimicking contractions of normal spontaneous labour and transient impeding uterine blood flow. Amplitude and duration of uterine contractions are increased leading to dilation and effacement of cervix. The number of oxytocin receptors and therefore uterine response to oxytocin increases gradually throughout pregnancy, reaching the peak at term (Blakemore and Petrie, 1988; Godwin and Zograbyan, 1998).

Prostaglandins are naturally occurring fatty acid produced by many tissues in the body. Prostaglandin E1 causes myometrial contractions by interacting with specific receptors on myometrial cells. This interaction results in a cascade of events, including a change in calcium concentration thereby initiating muscle contraction (Blanchard *et al.*, 2002). Prostaglandin E2 (PGE2) tablets have been used since 1971 to induce labour at term. The active ingredient of PGE2, dinoprostone, is obtained from the coral *Flexaura homomala* (or sea whiff), which is found in the Caribbean (Karim and Sharma, 1971).

Misoprostol is an analog of prostaglandin E1. By interacting with prostaglandin receptors, misoprostol causes the cervix to soften and uterus to contract, resulting in the expulsion of the uterine contents. Misoprostol is relatively metabolically resistant and thus has prolonged action (Baird, 2000).

2.5. Natural Products

A natural product is a chemical compound or substance produced by a living organism - found in nature that usually has a pharmacological or biological activity and its use in pharmaceutical drug discovery and drug design. Not all natural products can be fully synthesized and many natural products have very complex structures that are too difficult and expensive to synthesize on an industrial scale. Such compounds can only be harvested from their natural source (Newman and Cragg, 2007).

The searches for new biologically active compounds are most often based on hints coming from ethno botany but there are still a huge number of unstudied plants. Thus structure elucidation has still much to offer especially when combined with biological tests (Hanson, 2003).

2.5.1 Alkaloids

An alkaloid is a plant-derived compound that is physiologically active, contains nitrogen in a heterocyclic ring, basic with a complex structure and is of limited distribution in the plant kingdom. Many of the earliest isolated pure compounds with biological activity are alkaloids. This is due to the ease of isolation. The nitrogen generally makes the compound basic and the compound exists in the plant as a salt. Thus, alkaloids are often extracted with water or mild acid and then recovered as crystalline material by treatment with base. Alkaloids may be grouped based on structural similarities with known compounds, including non-nitrogenous compounds or by the organism(s) from which the alkaloid is isolated. They can also be grouped based on structure of the ring system

containing nitrogen atom. Examples are pyridine group: piperine and coniine, pyrroiidine group: nicotine and hygrine, tropane group: atropine and cocaine, indole group: ergolines (the ergot alkaloids): ergine, ergotamine, lysergic acid, purine group: xanthines: caffeine, theobromine and Terpenoid group: steroid alkaloids containing a steroid skeleton in a nitrogen containing structure. Among the most famous of the alkaloids are the Solanaceae or tropane alkaloids. Plants containing these alkaloids have been used throughout recorded history as poisons, but many of the alkaloids do have valuable pharmaceutical properties (Simon, 2003)

2.5.2 Tannins

These are oxygen-substituted derivatives of phenols. Tannins have been defined as astringent bitter-tasting plant polyphenols of high molecular weight (ranging from 500 to 20,000) which are water soluble. Sometimes the high molecular weight structures bind and precipitate proteins forming soluble tannin-protein complexes.Tannins have been previously subdivided into two groups: hydrolysable tannins and proanthocyanidines. Hydrolyzing tannins are hydrolysed by weak acid or weak bases to yield carbohydrate and phenolic acids. Proanthocyanidines are more widely distributed in plants than hydrolysable tannins which are usually present in low amounts. Anthocyanin pigment is responsible for the wide array of colors in flowers, fruits and wines. They are also responsible for the astringent taste of fruits and wines (Bryant et al., 1992). Antinutritional effects of tannins were outlined by Fahey and Jung (1989). They depress food intake and form complexes with digestive enzymes thus interfering with normal digestion. They cause internal malaise inhibiting digestive enzymes such as proteases, pectinases, amylases and lipases. Furthermore, tannins work by stimulation of

phagocytotic cells, host-mediated tumor activity, and arrangement of anti-infective actions. Their mode of antimicrobial actions may be related to their ability to inactivate microbial adhesions enzymes and cells envelop transport proteins (Provenza *et al.*, 1991).

2.5.3 Saponins

Saponins are natural detergents found in a variety of plants. They contain a polycyclic, lipophylic nucleus and one or more water solube sugar side chains, thus their surfactant activity is a result of both fat-soluble and water –soluble moieties in the same molecules (Chandler, 1985). Saponins can be found in vegetables, beans and herbs and have many health benefits though some are toxic. Apart from the expectorant, diuretic and spasmolytic properties of saponins, studies have illustrated the beneficial effects on blood cholesterol levels, cancer and stimulation of immune system (Rao and Sung, 1995; Plock *et al.*, 2001)

2.5.4 Flavonoids

Flavonoids (or bioflavonoids) are a class of plant secondary metabolites. They are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants. They have health-modulating effects in animals which eat them and are most commonly known for their antioxidant activity (Kaufman, 2007).

2.5.5 Screening of Natural Products

Pharmacognosy provides the tools to identify, select and process natural products destined for medicinal use. Usually, the natural product compound has some form of biological activity and the compound known as the active component can act as a lead

compound (not to be confused with compounds containing the element lead). Many of today's medicines are obtained directly from a natural source. On the other hand, some medicines are developed from a lead compound originally obtained from a natural source. This means that the lead compound: can be produced by total synthesis, or can be a starting point (precursor) for a semi synthetic compound, or can act as a template for a structurally different total synthetic compound. This is because most biologically active natural product compounds are secondary metabolites with very complex structures. This has an advantage in that they are extremely novel compounds but this complexity also makes many lead compounds synthesis difficult and the compound usually has to be extracted from its natural source. The lead compound (or active principle) present in a mixture of other compounds from a natural source, is often isolated and purified. However, the ease with which the active principle can be isolated and purified depends much on the structure, stability and quantity of the compound (Hanson, 2003; Newman and Cragg, 2007).

2.6 Toxicity

Toxicity is defined as "the potential of a substance to exert a harmful effect on humans or animals, and a description of the effect and conditions or concentration under which the effect takes place" (Akhila *et al.,* 2007). In order to support an application for a chemical trial or for the registration of a new drug, it is necessary to satisfy legislation that requires that certain data should be produced from a variety of toxicological investigations that show the safety profile of the compound to which human may be exposed (Prascoe, 1983). In toxicity study, the animal species selected will depend partly on the type of toxicity test, existing data available and also on ethical and financial considerations. The

most common species used are rats and mice for reasons of size, accumulated knowledge on these species and cost, besides the similarity of their metabolism to that of humans (Timbrell, 2002). Therefore, in the majority of cases of evaluation of toxicity of most substances rodents and non-human primates are first used in preclinal animal safety studies before further studies are done in humans. These animals are mainly used because of their biological similarity to humans that allows them to be regarded as the suitable metabolic models for humans in a broad range of investigations (Loomis and Hayes, 1996).

In general, according to Lorke (1983), toxicity testing methods can be divided into two categories: the first category comprises tests that are designed to evaluate the overall effects of compounds on experimental animals. Individual tests in this category differ from each other basically in regard to the test and the extent to which the animals are evaluated for general toxicity. Toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of new drugs involve acute, subchronic and chronic toxicity. The second category of tests consists of those that are designed to evaluate specific types of toxicity in detail. The sub-chronic and chronic tests do not detect all forms of toxicity, but they may reveal some of the specific toxicities and indicate the need for more detailed studies. Acute and chronic toxic effects differ principally from each other with respect to the amount of chemical compound involved and the time intervening before the effect is seen (Timbrell, 2002). Acute effects are normally observed soon after exposure and result from the uptake of large amounts of poison, generally as a single dose. On the other hand, chronic effects are often detected over an extended period of time during which exposure may be continuous or

intermittent, though obviously at levels which are lower than to produce an acute effect (Ghosh, 1984; Loomis and Hayes, 1996).

2.6.1 Acute Toxicity

Acute toxicity tests are those designed to determine the effects, which occur within a short period after dose. They serve to establish the lethal .dose range of the test substance and provide prompt warning if a highly toxic compound is being dealt with ((Loomis and Hayes, 1996). They also provide information on the limiting toxicity arising from the pharmacological effects of the compound on target organs and often, on the maximum dose to be used in subsequent chronic studies. This latter information is particularly important for predicting the amount of chemical required for future toxicological studies. The initial procedure, in an acute toxicity test programme, is to test a series of range-finding single doses of the compound in a single animal species. This necessitates selection of a route of administration, preparation of the compound in a form suitable for administration via the selected route and selection of an appropriate experimental animal species (Timbrell, 2002). Additionally, such testing may also indicate the viability of the oral route for use in subsequent, more extensive and prolonged toxicity studies. Normally, the significance and use of the data that are obtained are limited to those routes of administration that were used in the actual experiment. As usual, all initial acute toxicity tests are performed on either rats or mice because of the low cost, the availability of the animals, and the fact that abundant reference toxicologic data for many compounds in these species are available (Loomis and Hayes, 1996).

Before the experiment is performed, a total number of animals of similar body weight and same sex, or equal numbers of both sexes, are selected and randomly assigned to test (treatment) and control groups. After exposure to single doses of the test compound (or treatment), the animals are monitored for a minimum of 24hrs for any clearly recognized effect (such as changes in locomotive activity; bizarre reactions; sensitivity to pain, sound and touch; changes in social interaction; aggressive behavior; convulsions; paralysis, etc.) seen, as an index of toxicity, shortly or/and consistently after the administration of the chemical (Lorke, 1983). However, the most easily recognized and certainly the most significant of effects is that of death and this outcome is usually used as a primary measure of acute toxicity. If the animals appear to be healthy at the end of 24hrs, they are monitored at daily intervals for at least a further one to two weeks for the appearance of delayed toxicity (Prascoe, 1983). In rats and mice, three types of acute toxicity studies may be performed. For the first type of study, it is usual to establish the maximum tolerated dose (i.e. the highest dose after which the animals recover completely from all effects of the chemical) and the minimum lethal dose for the compound (or treatment). The second type of study is the single dose study to establish the target organ(s) for toxicity while the third type of study is for the determination of the precise LD_{50} or median lethal dose. The results of the latter type of study may be required, in most countries, for a clinical trial's certificate or even for a product license (Prascoe, 1983; Timbrell, 2002).

2.6.2 Median Lethal Dose (LD_{50})

This is defined as the concentration of a given substance that will kill 50% of the test population in a given time, typically a short time (LD_{50}). The LD_{50} values provide many indices of potential types of drug activity. The lower the LD_{50}, the more toxic the chemical (Prascoe, 1983). The LD_{50} value depends on the route of administration. Usually, the values are found to increase with the following sequences of routes: intravenous, intraperitoneal, subcutaneous and oral administration (Turner, 1965). The intravenous route is preferable to the intraperitoneal route (because many drugs get detoxified if the intraperitoneal route is employed; Ghosh, 1984). Since a great range of concentrations or doses of various chemicals may be involved in the production of harmful effects, the LD_{50} has been used by some authors to devise categories of toxicity on the basis of the amounts of the chemicals necessary to produce harm.

2.6.3 Chronic Toxicity

Chronic toxicity is defined as "the capacity of a substance to cause poisonous health effects in humans, animals, fish and other organisms after multiple exposures occurring over an extended period of time or over a significant fraction of an animal's or human's lifetime ". The study determines the systemic effect of repeated doses of materials or their extracts for no less than 24 hours and no greater than 10% of the total life span of the test animal. The purpose of the chronic toxicity test is to investigate the harmful effects that foreign compounds that are introduced to animals in repeated doses or in continuous exposure over an extended period of time may produce. The dose levels of compounds used usually range from a very low fraction of the therapeutically effective dose to doses that approach the maximum non-lethal dose (as established in rodent acute toxicity

studies; Loomis and Hayes, 1996). Different approaches to dose ranging studies are applied depending on the species being used. The procedures used can vary, but usually involve exposing the experimental animals (in typical group sizes of two to five animals/sex/group) to various doses of the test compound, i.e. from the maximal non-lethal dose (determined in the acute studies) down to doses in the pharmacological dose range. Clinical chemistry and haematological parameters are then measured at the start of the study (i.e. within 48 hours after the first dose) and at the end of the study, along with full histopathology analysis of all abnormal tissues plus the major organs (such as the heart, liver, kidneys, lungs and brain of the animals), at least at the end of the study (Timbrell, 2002). In a subchronic (prolonged) toxicity study, the test substance or extract is administered to the animals for about four to eight weeks, while the chronic toxicity study lasts for three to six months or more. In all cases of chronic toxicity studies, the animals are observed daily for signs of toxicity: weight changes, appetite and signs of disease or abnormal behavior, throughout the study period (Timbrell, 2002).

2.6.4 Toxicity and Routes of Administration

Routes of administration refer to the way in which drugs or compounds are introduced into animals or humans. To evaluate the toxicity of a compound in animals, various routes may be used, but the two most commonly used modes of administration for animal studies are via intraperitoneal injection or the oral route (Waynforth, 1980). The route of administration is important in demonstrating toxicity of plants. A plant that is toxic in a particular route may not necessarily be toxic in another route of administration (Ajibesin e*t al.*, 2002). In an acute toxicity study of some selected plants using rats and estimating the LD_{50}, results showed variations in mode to toxicity. Only the extract of

Stachytarpheta indica was toxic by oral and intraperitoneal route. *Anchomanis difformis* and *Caladian bicolor* tubers were not toxic by either oral (PO) or intraperitoneal (IP) administration, *Telfaria occidentalis* root, *Coula edulis* roots, *Euphorbia kamerounica* leaves, *Pterocarpus milbreadii* leaves and *Blighia sapinda* leaves were toxic to various extents through the intraperitoneal route (Ajibesin *et al.*, 2002).

2.6.5 Oral Route of Administration

The oral route is probably one of the most common means by which a chemical enters the body. In short, the oral administration is the form of administration involving the gastrointestinal tract, which may be viewed as a tube going through the body, starting at the mouth and ending at the anus. Although it is within the body, its contents are essentially exterior to the body fluids. Most orally administered chemicals can otherwise have a systemic effect on the organism only after absorption has occurred from the mouth or the gastrointestinal tract. Oral administration of chemicals that are rapidly absorbed from the gastrointestinal tract would theoretically expose the liver to concentrations of the agent that would not be obtained if other routes of administration were used (Hayes, 1989; Loomis and Hayes, 1996). Furthermore, if a compound entered the enterohepatic cycle, at least a portion of the compound would be localized in the organs involved in the cycle. Compounds that are known to be toxic to the liver would be expected to be more toxic following oral administration on repeated occasions, whereas their administration by other routes may be less hazardous (Waynforth, 1980).

2.6.6 Evaluation of 'Herbal' Toxicity

Diligent evaluation of the potential for harmful effects, usually evaluated through toxicity studies, is an important consideration when investigating the medicinal properties of an unknown or known plant (Verkman, 2004). Herbal toxicity can be evaluated by: (1) Observing human or animal populations exposed to the plant material, (2) Administering the 'herbal' medicine to animals under controlled conditions and observing the effects (in vivo) and (3) Exposing cells, sub-cellular fractions or single-celled organisms to the plant material (in vitro) (Timbrell, 2002).

Ethically, toxicity of a compound cannot randomly be evaluated in humans. Nevertheless, the exposure of humans to toxicity with herbal products previously may occur accidentally when these are part of their therapeutic activities or intentionally as with drugs and food additives (Chan, 2003). In such cases, the accidents resulting from this type of exposure may, if well monitored and recorded (i.e. by measuring substances and their metabolites in body fluids and using biological indices of pathological change), provide important information about the toxicity of a plant material in humans. However, acquiring such data is often difficult and rarely complete, and the latter is the main reason why procedures for animal toxicity testing have been maintained as a successful alternative for evaluating the harmfulness of compounds for humans .The majority of data on the toxicity of chemicals, including drugs and herbal medicines, is gained from experimental studies done in animals (in vivo). The data so acquired are used for the risk assessment and safety evaluation of drugs (or herbal medicines) prior to human exposure. Because animal tests can be carefully controlled with the exact known doses being used, the quality of the data obtained is generally reliable (Timbrell, 2002). The number of

animals used should be enough to allow statistical significance to be demonstrated and the application of human conditions and proper treatment of the animals are essential, for scientific as well as ethical reasons, to help ensure that the data are reliable and robust (Chan, 2003).

All chemicals may be considered toxic under certain conditions, but some chemicals present a greater hazard than others (Prascoe, 1983). Particularly, there is a concern that many herbal practitioners continue to use plants for the treatment of diseases without any knowledge of the toxicity profiles or safety of their plant materials (Chan, 2003). The traditional prolonged use of herbal remedies has enabled those producing acute and obvious signs of toxicity to be well recognized and their use avoided. However, a history of traditional usage is not always a reliable guarantee of safety since it is difficult for traditional practitioners to detect or monitor delayed effects, rare adverse effects, and adverse effects arising from long-term use (Ernst, 1998). These more subtle and delayed forms of toxicity, such as carcinogenicity, mutagenicity, and hepatotoxicity, may well have been overlooked by previous generations and it is these types of toxicity that are of most concern when assessing the safety of herbal remedies.

The problem of toxicity is more relevant when one realizes that even now mistakes are made with the collection of food plants and this usually leads to death of families. A common example is pounded yam. It is known that some species are poisonous e.g. *Dioscorea dumentorum* with the poison being dioscorine and dihydrodioscorine (Williaman *et al.,* 1953). Another example is *Stachytarpheta indica* (L.) Vahl which is used in Akwa Ibom state (Nigeria) for the treatment of malaria. It has been reported to be toxic by oral and intraperitoneal route at high doses while its co-generic specie,

Stachytarpheta jamaicensis (L.) Vahl is a sedative and is non-toxic (Ajibesin *et al.*, 2002). Today, still over 75% of all marketed orthodox pharmaceutical medicines is either derived from plant sources or from derivatives of secondary plant metabolites (Chan, 2003). Biological screening is therefore important not only for establishing the therapeutic efficacy of the medicinal plants also to validate their historical utilization by traditional healers and herbalists. This is especially important since the plants may have evolved over a period of time leading to changes in their chemical composition and thus the biological activity. This method outlines the determining of the quantitative relationship between the dose and concentration of a drug and the magnitude of biological response it evokes. It provides the means of standardizing and evaluating potency, safety and efficacy of pharmaceutical drugs (Ernst, 1998).

2.7 Chemotaxonomy of Poaceae

Among the constituents found in the family are different classes of alkaloids, saponins, cyanogenetic substances, phenolic acids, flavonoids, terpenoids (Trease and Evans, 1985). Literature review revealed that no investigation has been done on therapeutic property of secondary metabolites in infected *Panicum maximum* floret for the control of uterine contraction and postpartum haemorrhage. Also toxicity studies on the crude extract of the plant to ascertain its safety, has not been done in Nigeria.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Incidence of Smut Disease in the Field in Lagos

Disease incidence was studied at three sites.Samples were taken from fifty locations in each site. Each sampled plot was divided into 1.0m x 1.0m units for easy sampling. The sites chosen were at Akoka, Isolo and Ikorodu in Lagos state. At each site, the total number of tillers were noted. Those tillers bearing only healthy inflorescences were counted separately from those bearing smutted panicles. The percentage of smutted panicles out of the total number of inflorescences on each site and the percentage smutted panicles out of the number of flowering tillers were calculated. The average number of florets per inflorescence and the average number of smutted florets per panicle were counted and from these, the percentage number of smutted florets per inflorescence was calculated (Arohkesi, 1997; Mehrotra and Aggarwal, 2003).

The disease symptoms were made by observation of intact *P. maximum* plants in the field and on detached inflorescences transported to the laboratory. Two hundred each of randomly selected healthy and diseased *P. maximum* panicles were used to assess the colours, shapes and sizes of healthy and diseased florets using a magnifying glass. The protective floral parts such as bracts, glumes and palea were separately taken apart to expose the stamens and gynoeciums of both healthy and diseased florets. Each of the floral parts was placed on a clean microscope slide and stained with cotton blue in lacto phenol for observation. With sterile inoculating needle, the ovaries were teased on microscope slides, stained and observed under the light microscope.

3.2 Isolation of Fungi from Naturally Infected *Panicum maximum* Florets

Fifty randomly selected diseased *Panicum maximum* florets were obtained from the field (Akoka, Enugu, Isolo, Ikorodu and Ifo) each time. They were surface sterilized for two minutes in 0.26% NaOCl (5% v/v commercial bleach), rinsed in four changes of sterile distilled water and blotted dry using sterile filter paper (Whatman No. 1). Using a sterile pair of forceps, the floral parts of diseased florets were taken apart and each part placed in separate sterile Petri dish. The palea, lemma and glumes were plated (three pieces per Petri dish) separately on sterile Potato dextrose agar (PDA). Ovaries were opened to expose their contents. With sterile water agar at the tip of a sterile inoculating needle, the contents of the ovaries were picked out into sterile PDA plates and incubated at room temperature (28 – 32^{o}C) and were inspected daily for ten days. Fungal colonies growing from the inocula were successively subcultured until pure. They were used for further studies.

The ovary contents left over from above were also used to make single spore isolation (Booth, 1971). They were transferred to a sterilized 10.0ml test tube containing two millimeters of sterile distilled water. The test tube was shaken and the contents were serially diluted from which 1ml of 10^{-4}, 10^{-5}, and 10^{-6} dilutions were plated on sterile solid water agar. Using a sterilized inoculating needle, single spores were then transferred to sterilized Potato dextrose agar plates and incubated at room temperature with daily inspections for the growth of fungal colonies.

3.2.1 Identification of Fungal Isolates from Naturally Infected *Panicum maximum* Florets

Fungal isolates that emerged from the serial dilutions and plated single spores were stained in cotton blue in lacto phenol and examined under the light microscope. The identities of these fungi were certified using cultural, morphological characteristics, as well as comparing them with confirmed representatives of different species in relevant texts such as Clements and Shear (1931); Bassey (1964); Webster (1973); Alexopolous *et al.,* (2007). Photomicrographs of fungal isolates were taken with motic camera 2.0. Pure cultures of some of the fungi that had several strains and could not be identified were grown on PDA and Malt yeast peptone agar (MYPA) slants in 5.0ml McCartney bottles and transported to Centre for Agriculture and Bioscience International (CABI) identification services, Kew, England along with smutted florets obtained in the field after proper sealing with copper sulphate and sterilized ciggerate foil. Two of the fungal isolates were sequenced according to CABI standard protocols.

3.3 Determination of Pathogenicity of Fungal Isolates on Healthy *Panicum maximum* Flowering Plot

All the fungal isolates obtained from the infected florets were subjected to pathogenicity test according to the method described by Cashion and Luttrell (1988), which was specific for the grass inflorescence. Ten healthy 12 week old *P. maximum* plants were raised as follows: A parcel of land measuring 6.25m x 6.0m was tilled in the Botanic and Zoological Garden of the University of Lagos. Four beds each of 2.5m x 1.0m were constructed 25cm apart and the soil enriched with animal manure. Ten rhizomes of *P. maximum* each bearing three buds were planted, 20cm apart, in each of the four rows.

They were watered daily. Six weeks later, old and senescing leaves were removed. The beds were weeded when necessary and plants used as the need arose. The inocula were prepared from the ovary contents of infected florets and from culture plates. To prepare the inoculum from the infected florets, intact diseased florets were picked off the inflorescences, placed into sterilized envelopes and transported to the laboratory. The smutted florets were each opened after surface sterilization, with a sterile pair of forceps. The teliospores were transferred from the ovaries into a sterile 50ml beaker containing sterile distilled water. The beaker was shaken and the contents filtered through sterilized muslin cloth. The spore concentration was determined at 3.2×10^3 spores /ml with haemocytometer. Another set of inocula were prepared from one-month-old cultures of each isolate and their spore concentration adjusted to 3.2×10^5 spores / ml.

The separate spore suspensions were used in 0.1% glycerin to inoculate the healthy inflorescences of the ten-healthy 12-week-old *P. maximum* plants of uniform sizes two days after the inflorescences emerged from the boots (anthesis). Inoculation was done with a sterilized hypodermic needle over a period of 3 days. After each day's inoculation, panicles were covered with clear sterile polythene bags to create microclimate. Inoculation was done in the evenings. The controls consisting of ten healthy 12-week-old *P. maximum* plants inflorescence were inoculated with sterile distilled water. Four days after inoculation, the sterile plastic bags were removed from the panicles and the plants allowed to mature normally. The time of the appearance of symptoms on the inoculated florets was noted. Fifty inoculated florets having symptoms of the disease were surface sterilized, teased and plated on potato dextrose agar (PDA). Fungal colonies growing from these floret parts were compared in every respect with the fungi originally isolated.

The ovaries of 50 artificially smutted florets were teased and their contents compared in every respect with ovary contents of naturally smutted florets. The isolate that produced disease symptoms was studied further.

3.4 Growth Studies of Fungal isolates

Growth studies of all the isolates were carried out according to modified methods of Khattab, (2006) to determine the best media for their cultivation. The following media were used for this study – Potato dextrose agar (PDA), Carrot agar (CA), Malt extract agar (MEA), Water agar (WA), *Panicum maximum* leaf extract agar (PLEA), *Panicum maximum* floret extract agar (PFEA), Potato dextrose broth (PDB), Carrot dextrose broth (CDB), Malt yeast peptone broth (MYPB).

3.4.1 Preparation and the components of solid and liquid media used in the studies

Potato Dextrose Agar (Factory Prepared)

PDA	39g
Distilled water	1 Litre

Carrot Agar

Carrot tubers	100g
Plain agar	12g
Distilled water	500ml

Preparation: Carrot tubers sliced into 250ml of water were cooked for one hour in a steamer or 40 minutes in an autoclave**.** At the same time the agar was melted in 250ml distilled water. The carrot juice was strained into the melted agar and the volume adjusted

with water to 250ml. The medium was autoclaved at a temperature of 121^{o}C at 1.0kg/cm^{3} for 20min.

Malt Extract Agar (MEA)

Difco bacto malt extract	15g
Difco bacto agar	20g
Distilled water	1 Litre

Plain Agar

Difco bacto agar	20g
Distilled water	1 Litre

***Panicum Maximum* Leaf Extract Agar**

Healthy *Panicum maximum* leaf	150g
Difco bacto Agar	12g
Distilled water	500ml

***Panicum Maximum* Floret Extract Agar**

Healthy *Panicum maximum* floret	150g
Difco bacto Agar	12g
Distilled water	500ml

Potato Dextrose Broth

Potato tubers	50g
Dextrose	10g
Distilled water	250ml

Malt Yeast Peptone Broth

Malt extract agar	3g
Peptone	2g
Yeast extract	4g
Distilled water	250ml

Carrot Dextrose Broth

Carrot tubers	50g
Dextrose	10g
Distilled water	250ml

Healthy *Panicum maximum* leaf and floret were homogenized separately with electric blender into 250ml beakers of water. The agar for the two beakers were also melted into 250ml-distilled water. The juices of the leaf and floret were strained into melted agar and the volumes adjusted with water. The media were autoclaved at temperature of 121^{o}C at 1.0kg/cm^{3} for 20min. The components of each media were dissolved in boiled water and autoclaved at the temperature of 121^{o}C at 1.0kg/cm^{3} for 20min.

Each medium was poured in triplicates into plate and a cross was drawn with permanent marker at the bottom of the plate to indicate the centre as the origin. Seven days old cultures of the fungus were used. Each fungus was transferred by cutting with a sterilized cork borer of 6mm in diameter and placed at the centre of each plate. The plates were incubated at room temperature. They were observed daily at 24 hours interval. The growth diameter were measured and recorded for 7 days. The isolates used for the growth

studies were *Fusarium* sp., *Fusarium fujikuroii, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus wentii, Penicillium pinophyllum, Mucor* sp., *Syncephastrum racemosa* and *Trichorderma* sp.

3.4.2 Preparation of potato dextrose broth, malt yeast peptone broth and carrot dextrose broth for growth studies of *Tilletia ayresii.*

Fifty grams of potato tuber was cut into small pieces and boiled to soften for 30-45 minutes in 100ml of distilled water in a water bath. The filtrate was strained into another 250ml conical flask with cheese cloth. Ten grams of dextrose sugar was added into the conical flask and made up to 250ml. The medium was autoclaved at a temperature of 121^{o}C at 1.0kg/cm^3 for 20minutes.

The stated quantity of malt extract, yeast extract and peptone were measured into conical flask with 250 ml of distilled water, dissolved and autoclaved at temperature of 121^{o}C at 1.0kg/cm^3 for 20 minutes. The carrot dextrose broth was prepared like carrot agar and autoclaved though without plain agar. The three broth media, potato dextrose broth (PDB), malt yeast peptone broth (MYPB), and carrot dextrose broth (CDB) were poured into test tube (10ml each) in triplicate. The yeast stage of *Tilletia ayresii* was inoculated into two test tubes leaving the third one as control (without organism). They were incubated at 18^{oC} with 12hours light and 12hours dark, and observed daily at 24hours interval. The turbidity of the content and optical density were measured and recorded at 530nm wavelength for seven days.

3.5.0 Ethnobotanical Studies of Crude Extracts of Healthy and Infected *Panicum maximum* Florets

3.5.1 Extraction Procedures

Two kilogrammes of healthy and infected *Panicum maximum* floret were subjected separately to sequential extraction procedure according to the method of Sofowora (1982) using the following solvents: four litres of distilled water, six litres of chloroform (analytical grade) and five litres of 100% ethanol (absolute). The extracts were concentrated in the rotatory evaporator under reduced pressure and controlled temperature (40^{o}C). The dried solid products were weighed to determine yield and transferred to airtight containers. They were stored in the refrigerator until used.

The % yield was calculated using the expression:

$$\% \text{ Yield} = \frac{\text{Weight of dry extract}}{\text{Weight before extractions}} \times \frac{100}{1}$$

3.5.2 Preparation of Experimental Animals

Thirty adult female *Sprague-dawley* rats identified by Dr. I.A. Taiwo at the Department of Cell Biology and Genetics, University of Lagos weighing 140-200g were obtained from animal house of the College of Medicine Idi-Araba, University of Lagos. They were placed in standard metal cages (90cm x 40cm x 35cm) in the metabolic laboratory of Department of Biochemistry at temperature of 28 ± 31^{o}C and 12 hours light and 12 hours dark periodicity. The cages and the surroundings were cleaned out every two days and disinfected. The animals were fed with standard rat chow (Nimeth Livestock Feeds, Ikeja) and water *ad libitum* and allowed to acclimatize for 7 days before bioassays. The

estrous cycle monitoring of the animals was routinely done in the morning between the hours of 7.00am and 8.00am in the metabolic laboratory where the animals were kept. This laboratory has no illumination other than the natural light from the day. It is known that the amount of light rats are exposed to affects the frequency of estrous cycle (Olatunji-Bello *et al.,* 2000). Vaginal smear was collected with a plastic pipette filled with 5ml of normal saline (NaCl 0.9%) by inserting the tip of a blunt edge dropping pipette into the vagina, but not deeply. In preparing vaginal smears normal saline is used due to its ability to prevent lyses of cell because of its isotonicity. This allows for the smear to last long for examination under the microscope without drying. On examination of smears collected from the rats under low and high power magnification, four types of cells (nucleated cells, leucocytes, epithelial cells and cornified cells) were recognized. The proportion of the cells in the smears was used to determine the estrous cycle phases. Only the cycling animals were selected and kept in different cages for the assay.

3.5.3 Assessment of Uterine Smooth Muscle Contractility

The uterine smooth muscle contractility testing was done in the physiology laboratory of Prof. O.A.Sofola, at Department of Physiology, College of Medicine, University of Lagos. Labeled cycling rats were pretreated with 1.5mg/kg body weight of stilboesterol orally 24hours before the experiment to ensure regular spontaneous uterine contraction. After this period each animal was sacrificed by cervical dislocation and the uterus exposed by means of dissection. The uterine horns were placed in cold De Jalon's solution in order to reduce enzymatic activity and then trimmed of excess connective tissue. The uterine horns were carefully cut into 2.5 – 3.0mm ring segments and mounted in or suspended in 20ml organ baths containing De Jalon's (NaCl 9g, $NaHCO_3$ – 0.5g,

Glucose -0.5g, 10%KCl – 4.2ml, 1M $CaCl_2$ –0.27). The organ bath was bubbled with 95% O_2 – 5% CO_2 gas mixture. The temperature and pH were maintained at 37^oC and 7.4 ± 0.2 respectively. The rings were connected to a force transducer (Grass Model TO_3), which was coupled to a 4-Channel Grass Model 7D polygraph for the recording of the isometric tension. Each tissue was allowed to equilibrate for 60 – 90 minutes under resting tension of 1g according to the methods of Calixto *et al.* (1991) and Sofola *et al*., (2008). At the end of the equilibration period cumulative aliquots of the healthy and infected aqueous, ethanol and chloroform extracts were added to the organ bath in the absence of any agonist. The contractile responses of the uterine segment to the aliquots were recorded. This experiment was repeated using graded concentration of Ergometrine maleate for equilibration (10^{-5} – 10^{-2} mol/L) and their effects noted. The final bath concentrations of the healthy and infected extracts in mg/ml were 0.0312, 0.625, 0.125, 0.25, 0.5, 1.0 and 2.0 respectively

3.6 Phytochemical Screening

In the present study the crude healthy and infected *Panicum maximum* florets extracts were subjected to preliminary qualitative and quantitative phytochemcial screening using standard tests to show the different types and quantity of chemical constituents present.

3.6.1 Qualitative phytochemical screening

3.6.1.1 Test for Alkaloids

Extracts of approximately 0.5 g was weighed and stirred it with 5ml of 1% aqueous hydrochloric acid on a steam bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated similarly with freshly prepared

Dragendoff's reagent and Wagner's reagent. Turbidity or precipitation with any of these reagents was taken as evidence for the presence of alkaloid in the extract being screened (Evans, 1989; Harborne, 1998).

3.6.1.2 Confirmatory Tests for Alkaloids

Dried extract of 0.5mg was acidified it with 2ml of dilute Hydrochloric acid and partitioned with 5mls of chloroform giving an aqueous and organic layer. The aqueous layer was then basified using 2ml of dilute Ammonia and partitioned with chloroform. The organic layer obtained after the partitioning with chloroform of the dilute Ammonia solution was then concentrated to 1ml. A drop of the concentrated solution was then taken dropped on the filter paper, allowed to dry and was then observed for colour changes (Houghton and Raman, 1998).

3.6.1.3 Test for Tannins

Dried extract of 5mg of the plant was stirred with 10ml of distilled water, filtered and 5% ferric chloride; reagent was added to the filtrate. A blue black, green, or blue – green precipitate was taken as evidence for the presence of tannins (Evans, 1989, Houghton and Raman, 1998).

3.6.1.4 Test for Flavonoids

The methods of Harborne, (1973) and Sofowora, (1993) were used to determine the presence of flavonoids in the plant extracts. Dried extract of 5mg was stirred with 10ml of distilled water.An addition of 5ml of dilute ammonia solution was made into the

filterate of the extract followed by addition of Sulphuric acid. A yellow colouration indicates the presence of flavonoids.

3.6.1.5 Test for Saponins

The ability of saponins to produce frothing in aqueous solution and to hemolyse red blood cells was used as screening test for the sample. For frothing tests the method described by Farnsworth *et al.* (1966) was used. 0.5g of the plant extracts was shaken with water in a test tube and filtered. Frothing, which persisted on warming, is a preliminary evidence for presence of saponins. In order to remove "false-positive" result, the blood haemolysis test was performed on the filtrate of the frothed extract. 2 ml of normal saline solution was added to two test tubes. To one, of these, 2ml of distilled water was added and 2ml of the extract to the other. The concentration of sodium chloride in each tube is isotonic with blood serum. 5 drops of blood was added to each test tube and inverted gently to mix the content. Complete haemolysis of red blood cells in the cells in the test tube with the extract was taken as further evidence of presence of saponin.

3.6.2 Quantitative phytochemcial screening

3.6.2.1 Alkaloid determination using Harborne (1973) Method

Dried extract of 5g was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the filterate was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate

collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed. Percentage alkaloid was calculated using the expression:

$$\%\text{Alkaloid} = \frac{\text{Weight of extracted alkaloid}}{\text{Weight of sample}} \times \frac{100}{1}$$

3.6.2.2 Tannin determination by Van-Burden and Robinson method (1981).

Extracts of approximately 500mg was weighed into 50ml and shaken for 1 hour in a mechanical shaken. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filterate was pipetted out into a test tube and mixed with 2ml of 0.1m $Fecl_3$ in 0.1m HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 720nm within 10 minutes.

Percentage tannin was calculated using the expression:

$$\%\text{Tannin} = \frac{\text{Absorbance test}}{\text{Absorbance standard}} \times \text{Conc. standard} \times \text{Dilution factor} \times \frac{\text{Total volume}}{\text{Volume used}} \times \frac{100}{1}$$

3.6.2.3 Saponin determination by Obadoni and Ochuko method (2001)

Twenty grammes of ground samples were put into a conical flask and 100ml of 20% aqueous ethanol was added. The mixture was heated over a hot water bath for 4 hours with continuous stirring at about 55^{o}C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined filterate was reduced to 40ml over water bath at about 90^{o}C. The concentrate was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously.

The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated by adding 60ml of n-butanol to the aqueous layer and washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was dried in a water bath to a constant weight and percentage saponin content determined.

$$\% \text{ Saponin} = \frac{\text{Weight of extracted saponin}}{\text{Weight of sample}} \times \frac{100}{1}$$

3.6.2.4 Flavonoid determined by the Method of Bohem and Kocipaicibyazan (1974)

Ten gammes (10g) of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filterate was later transferred into a crucible and evaporated into dryness over water bath and weighed to a constant weight.

$$\% \text{ Flavonoid} = \frac{\text{Weight of extracted flavonoid}}{\text{Weight of sample}} \times \frac{100}{1}$$

3.7 Column Chromatography of Ethanol Extract of Infected *Panicum maximum* Florets

Crude ethanol extract of infected *Panicum maximum* floret which was more active than aqueous and chloroform extracts with the least dose achieving 77% contraction, was selected for further fractionation by column chromatography (Dufresne and Salituro,1998). 40g of ethanol extract was subjected to column chromatography on silica gel 60-200 mesh. Mobile phase were n-hexane, ethyl acetate, ethanol, and methanol. The aliquots of different fractions of each of the solvents were pooled and four fractions were concentrated with rotary evaporator at very low temperature and pressure and tested for biological activity. The ethanol fraction was active and so, small aliquot of it was spotted on a single TLC plate prepared with silica gel with florescence (F_{254} –F_{366}).

3.7.1 Development of TLC Plate

The solvent system used to develop the spots of the active fraction is ethylacetate: methanol: water in the ratio (77:13:10) v/v. A vertical line was ruled 1.5cm from the base of each plate using pencil. Capillary tube was used to collect dissolved samples from concentrated ethanol fraction and spotted on the marks along the line on the coated TLC plate. This plate was developed using the solvent system. The plate was removed from the tank after solvent had moved up to 18cm of the plate. Solvent front was marked leaving about 2cm beyond the front (Dufreshne and Salituro, 1998).

The TLC plate was detected under UV light (F_{366}) and three compounds were detected. Retention factor (R_f) values of each of the three compounds were calculated as distance by sample/distance moved by the solvent (Harborne,1998). Bioassay of the compounds was further carried out to determine the active component.

3.8 Determination of effects of Crude Infected *Panicum maximum* Floret extracts on Fungal Isolates

The fungi used for this study are *Aspergillus niger, A. flavus, Penicillium pinophylum, Paeciliomyces* sp., *Fusarium fujikuroii, Mucor* sp. *and Tilletia ayresii.* These fungi were subcultured from their stock cultures in other to get working cultures. Sterilized distilled water was added to two culturePetri dishes of these organisms and sterile glass rod used to squash out the spores from the fungi plate to get the microbial suspension .About 1ml of inoculum was taken from each Petri dish and added into 10ml test tube and serially diluted from 10^{-1} to 10^{-6}. Sterile circular paper disc (6mm) was produced from Whatman no. 1 filter paper Also concentrations of infected ethanol and chloroform extract, negative control (0.5%Tween 80) and positive control (benlate and ketaconazole), were prepared as follows:50mg/ml, 100mg/ml, 150mg/ml(extracts), 5g/10ml (benlate), 100mg/ml (ketaconazole) and 10ml of Tween 80.

3.8.1 Antifungal Assay

The disc diffusion agar method by Irobi and Daramola (1994) was used for the antifungal testing. About twenty sterilized filter paper were soaked in the prepared concentrations for 24hours. Thereafter they were carefully dropped on previously prepared potato dextrose agar plate surface with the test isolates at the rate of four discs per plate with sterile forceps. The plates were carefully labeled to indicate types of fungi and extracts used. After these, they were placed in the incubator at 28^{o}C-30^{o}C temperature for 72 hours. Clear zones of inhibitions were measured and standard error of mean calculated.

3.9 Evaluation of Acute and Sub Chronic Toxicity of crude infected *P. maximum* florets

3.9.1 Acute Toxicity Test

The toxicity study was carried out using forty-five (45) male and female Swiss mice (identified by Dr.I.A.Taiwo of the Department of Cell Biology and Genetics, University of Lagos) weighing 20 – 25g obtained from Laboratory Animal Centre, College of medicine, University of Lagos. The animals were randomly distributed into one control group and eight treated groups containing five animals per group. They were housed in stainless steel wire gauzed cages and maintained on mouse cubes (Nimeth livestock Feeds, Ikeja) provided with water *ad libitum*. They were allowed to acclimatize for seven days to the laboratory conditions before the experiment. After fasting the animals over night, the control group received 0.3ml of 0.5% Tween 80 solutions orally. The doses 1.0, 5.0, 10.0, and 15.0g/kg body weight were administered orally to the groups from Tween 80 solution of the extracts which was prepared by dispersing 4g of each of the dried extracts (ethanol and chloroform) with 8ml of 0.5% Tween 80 in 50ml beaker and transferred to 20ml volumetric flask. The beaker rinsed with Tween 80 solution transferred to the volumetric flask and the volume made up to mark with the tween80 solution.. The animals were observed continuously for the first 4 hours and then hourly the next 12 hours followed by 6 hourly intervals for the next 56 hours (overall 72hours) to check for onset of any immediate toxic signs and daily for 13 days to record any delayed acute effects, mortality or changes in general behavior and other physiological activities (Shah *et al.,* 1997; Bürger *et al.,* 2005). The LD_{50} of the extracts by oral routes was estimated using the method of Lorke, (1983) and Ogbonnia *et al*. (2010).

3.9.2 Subchronic Toxicity Test

Subchronic test was carried out on infected ethanol (A) and chloroform (B) extracts in College of Medicine, Idi-Araba. Forty-five female *Sprague-Dawley* rats weighing 160 ± 20g were used. They were allowed to acclimatize to the laboratory condition for seven days and were maintained on standard animal feeds (Nimeth livestock feeds, Ikeja) and provided with water *ad libitum*. The animals were weighed and divided into 9 groups of five animals each (one control (group common to A and B) and eight treated groups). After fasting the rats overnight, the control group received a dose of 0.5ml of 0.5%Tween 80 solution orally once a day for 21 days. The eight treated groups (four groups for each) respectively received the following doses: 100, 250, 500 and 750mg/kg/body weight of the infected ethanol and chloroform extracts dispersed with 0.5%Tween 80 solutions for 21days (Pieme *et al.,* 2006; Joshi *et al.,* 2007; Mythlypriya *et al.,* 2007). The animals were then weighed every five days from the start of the treatment to note weight variation. Food and water consumption were also checked.

At the end of the experiment they were anaesthetized with warm urethane chloralose (25%: 1%v/v) at a dose of 5ml/kg body weight and blood collected via cardiac puncture in two tubes: one with EDTA for immediate analysis of haematological parameters and to separate plasma for biochemical estimations. The collected blood was centrifuged within 5minutes of collection at 4000g for 10min to obtain plasma, which was analyzed for total cholesterol, total triglyceride, and high density lipoprotein-cholesterol levels by precipitation and modified enzymatic procedures from Sigma Diagnostics (Wasan *et al.,* 2000). Low density lipoprotein-cholesterol levels were calculated using Freedwald equation (Crook, 2006). Plasma was analyzed for alanine aminotransferase (ALT),

aspartate aminotransferase (AST), and creatinine by standard enzymatic assay methods (Sushruta *et al.* 2006). Plasma protein contents were determined using enzymatic spectroscopic methods (Hussain and Eshrat, 2002).

Haematocrit was estimated using the methods of Ekaidem *et al.* (2006). Haematocrit tubes were filled by capillary action to the mark with whole blood and the bottom of the tubes sealed with plasticine and centrifuged for 4-mins. The percentage cell volume was read by studying the tube along a "critocap" chart until the meniscus of the plasma intersects the 100% line. Haemoglobin contents were determined using cyanmethaemoglobin (Drab Kin) method (Ekaidem *et al.,* 2006). Selected organs-liver, ovary, uterus, kidney, heart and brain were observed *insitu*, excised, weighed and fixed in formo-saline for histopathological/microscopic examination.

3.9.3 Histopathology

The organs removed previously were soaked in formal-saline, they were cut into pieces of about 0.3-0.5cm thickness. The tissues were processed by passing them through several reagents during the process of fixation, dehydration, clearing and impregnation. The reagents were arranged in such a way that the tissues moved from one reagent to another until the processing is completed. The processing sequence was as follows:10%formal-saline (1hr), 70% alcohol (1hr), 90% alcohol I (1hr), 90% alcohol II (1hr), 90%alcohol II (2hrs), absolute alcohol I(2hrs), absolute alcohol II (3hrs), xylene I (1hr), xylene II (2hrs), wax bath I (1hr), and wax bath II (2hrs). After the last stage of tissue processing which was embedding, a rotary microtome was used to make sections of 3-5µm in thickness from the tissues. Staining commenced about 20minutes after and followed this technique: sections were dewaxed and stained with cole's haematoxylin for

10minutes .Thereafter they were rinsed and differentiated rapidly in 1% acid alcohol and rinsed in warm water for 1-3minutes.The sections were stained with 1%eosin for 3minutes and rinsed in water. Dehydration and clearing was done with alcohol and xylene before mounting and viewing under light microscope (Dunn,1974). Photomicrographs were then taken using motic camera 2.0. The slides were read and interpreted by Dr.G.O.Mbaka of Department of Anatomy, College of Medicine,Lagos State University, Lagos.

3.9.4 Statistical analysis

All calculations and statistical analyses were done using the computer softwares:SPSS 11.0 and Graph pad prism 5. One-way analysis of variance(ANOVA) was done. A P-value of less than 0.05 was considered significant.Tables, Histogram and line graphs were also used for graphical representations.

CHAPTER FOUR

4.0 RESULTS

4.1.0 Incidence of *Panicum maximum* Smut in the Field

The results of the survey of *Panicum maximum* smut disease incidence in the selected sampled sites were shown in Figure 1. Smut disease incidence in Ikorodu(95.00%) was significantly high compared to smut incidence in Isolo(72.00%) and Akoka(69.00%) sampled sites. Observation of panicles showed that upon emergence, the inflorescence of *P. maximum* bore healthy florets which are small with closed green palea and glumes. At anthesis, the green palea and glumes parted to expose dangling purple feathery stigma and brown anthers. The protective parts closed after anthesis to protect the future seed. The stigmas and anthers fell off the florets 7-10 days post emergence while the remaining floral parts fell off the floral axis 14 to 17 days after emergence. The minimum and maximum numbers of normal florets per spikelet were one and five respectively while the mean was 3.0 florets. Healthy florets occurred all year round with minimum and maximum number of healthy florets on panicles as 200 and 3500 respectively, while 2000 and 2900 infected florets were recorded on the majority of panicles during the months of disease incidence in the field (between June and December).

Smutting of panicles occurred in the field from June to December but the height of disease season was between August and November during the four years of study (2007-2010). The smutted florets were usually pinkish in colour and bigger in size and weight than the healthy florets (due to presence of spores in the ovary).

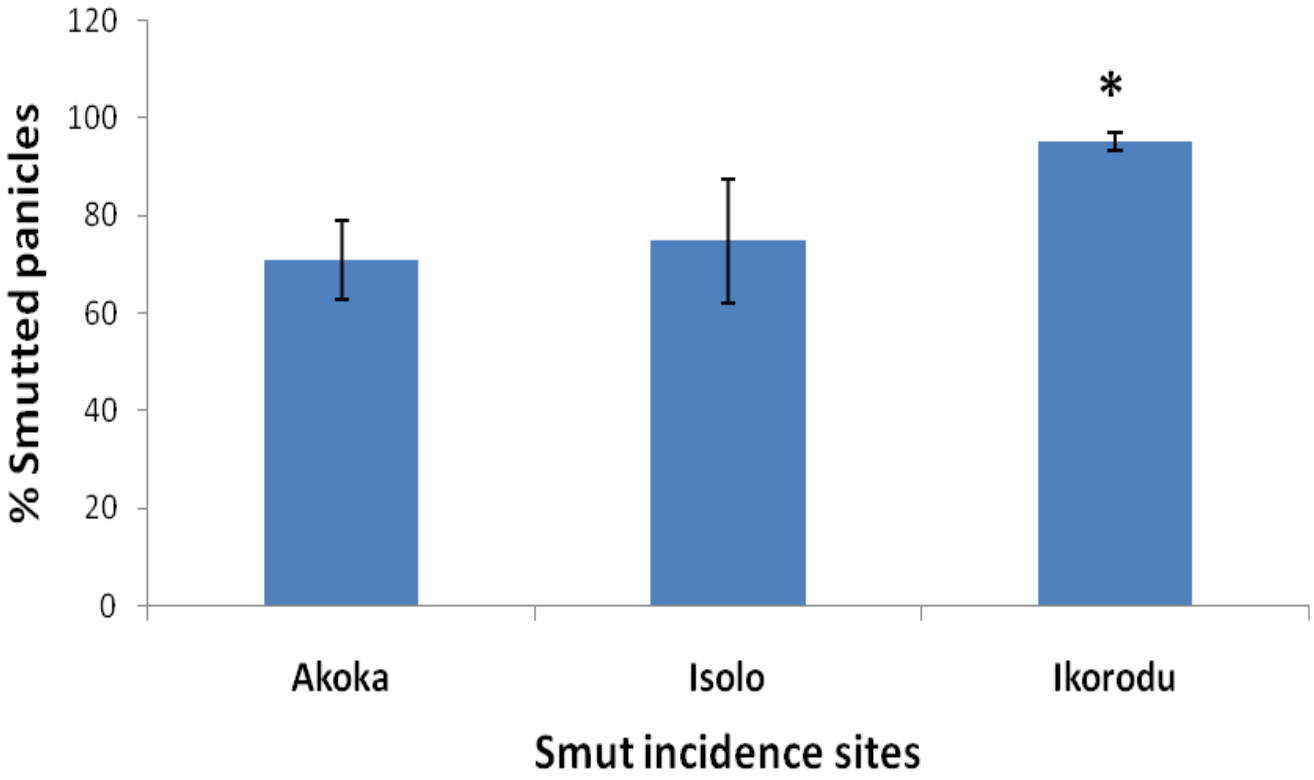
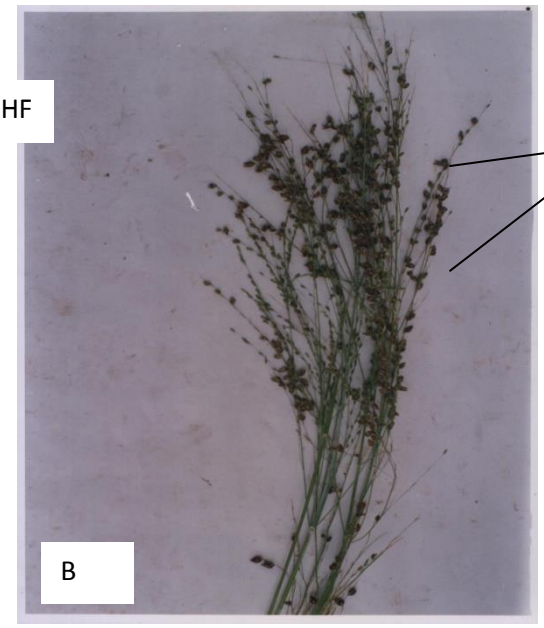


Figure1: Smut disease incidence in the three sampled sites (Akoka, Isolo, and Ikorodu). *significant at p<0.05.

Their protective part may or may not be parted. After five to seven days post emergence, infected florets lost their anthers and stigmas, and the protective parts closed again.

The protective floral parts of such enlarged florets later parted and shriveled to expose dry, greyish teliospore masses, from their ruptured ovaries which were easily blown away by wind (Plates1a-c). The minimum number of smutted florets observed per spikelet was one and the maximum number observed was five. At the height of the disease incidence the number of smutted florets was high. Also panicles that emerged when the pathogen's teliospores were abundant in the air had more smutted florets than those that emerged earlier in the season. The average minimum weight of smutted florets in all the sites studied was 0.007g while the maximum weight was 0.0109

HF



IF

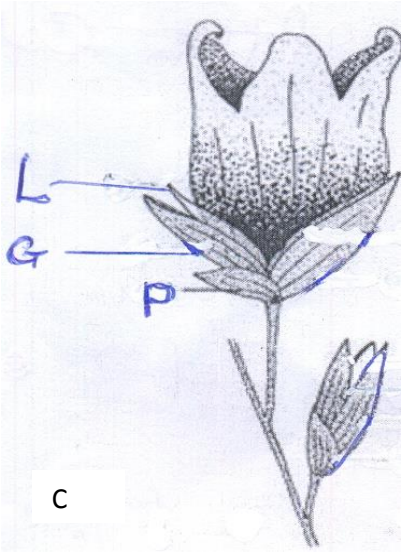


Plate2A-C: ***Panicum maximum*** **panicles and a floret**

Plate A: Panicle showing small sized healthy florets (HF) **(x0.17)**

Plate B: Panicle showing large sized infected florets (IF) **(x0.18)**

Plate C: A single infected flower with bracts-glume, lemma and palea (**x400)**

4.2 Isolation and Identification of Causal Organism and other Fungal Isolates

Culturing the palea and glumes of smutted florets yielded *Trichoderma sp*. The ovary content of intact sori was made of undeveloped and developed spherical teliospores which are grey and have echinulate ornamentation (warts), sterile cells which are spherical and colorless, globose and loosely joined, mostly 'Y'-shaped conidia. Germination of teliospores produced filiform basidiospores while the conidia produced hyphae that formed blastic conidia and ballistospores during the yeast stage of their development (Plate 3A-D).The five spores isolated are spherical sterile cells, blastic conidia, ballistospore, 'Y'-shaped conidia and teliospores.

The fungus was identified as *Tilletia ayresii* Berk. Culturing of open smutted florets yielded twelve fungal isolates namely *Aspergillus flavus, A. fumigatus, A.niger, A.wentii ,Curvularia lunata, Fusarium fujikuroii, Fusarium* sp., *Mucor* sp., *Paeciliomyces* sp., *Penicillium pinophyllum, Trichoderma* sp. *and Syncephalastrum racemosa* (Table 1).

Out of the thirteen fungal species isolated *Aspergillus niger, A .fumigatus, Tilletia ayresii* occurred most frequently (100%), *A.flavus* (80%), *A. wentii, Trichoderma* sp., *Mucor* sp., *Penicillium pinophyllum, Fusarium* sp., *F.fujikuroii* (40%) respectively. *Syncephalastrum racemosa, Curvularia lunata and Paeciliomyces* sp. were the least frequent in all the sampling sites at 20% respectively. No organism was isolated from healthy florets (Plate 2A).Plate 4A-Y shows the culture and photomicrographs of all the fungi isolated.

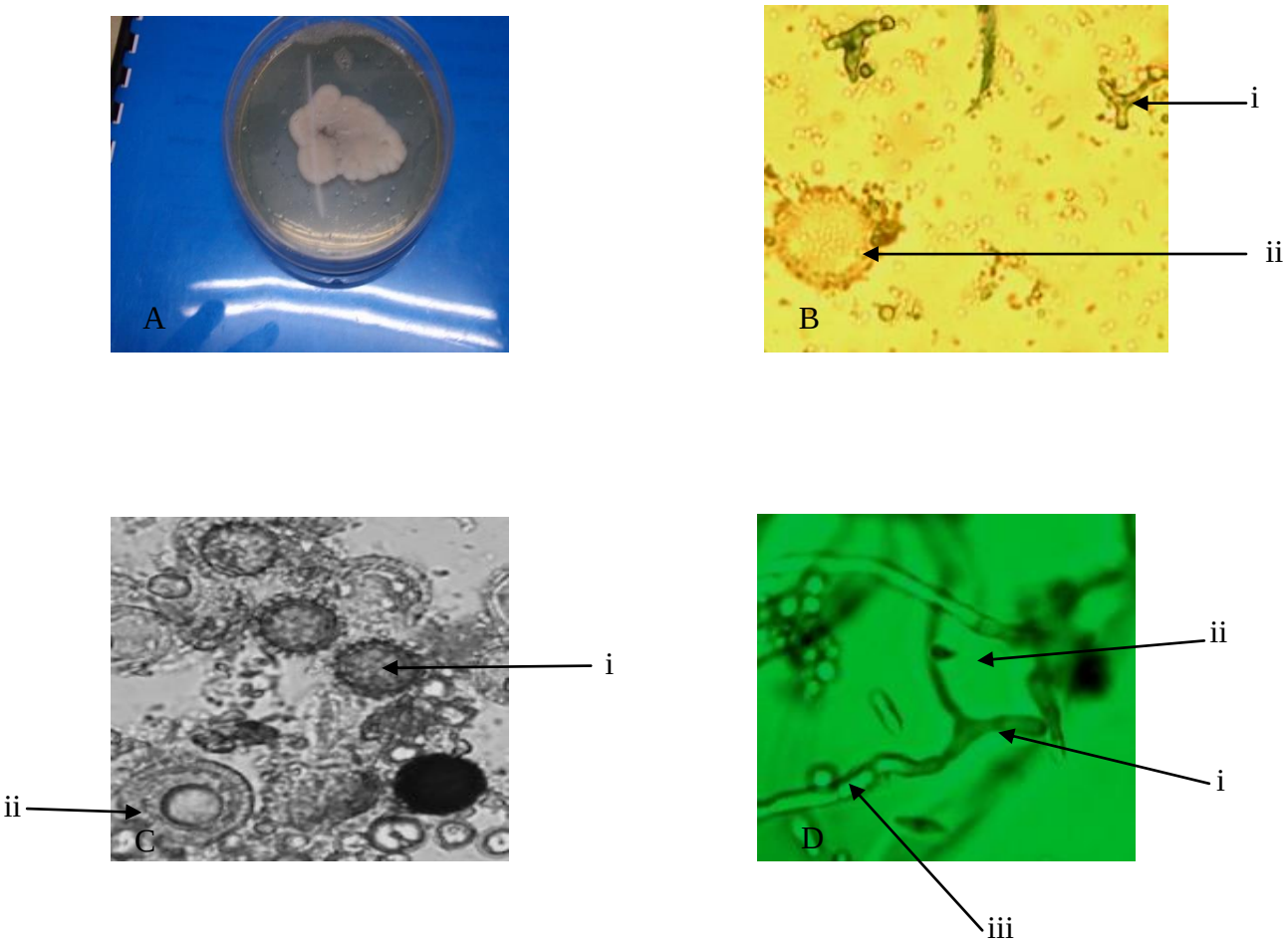


Plate 3 (A-D): Photomicrographs of spore types in *Tilletia ayresii*

Plate A Pure plate of conidial stage of *Tilletia ayresii* **(x0.53)**

Plate B Photomicrograph of germinating teliospore (ii) and Y-shaped conidia (i) **(x400)**

Plate C Photomicrograph of sori with teliospores (i) intermingled with globose sterile cells (ii) and Y-shaped conidia **(x400)**

Plate D Photomicrograph of germinating conidia (i) to form blastic conidia(ii) and ballistospores (iii) (**x400)**

Table 1: Fungal species isolated from infected florets in five sampling sites and their frequency of occurrence

Fungi isolates	IMI(KewUK) Identification number	Akoka	Enugu	Ikorodu	Isolo	Ogun	Frequency	%frequency
Aspergillus flavus	_	+	+	+	+	+	5	100
A.fumigatus	398394	_	+	+	+	+	4	80
A.niger	398396	+	+	+	+	+	5	100
A.wentii	_	+	_	_	_	+	2	40
Curvularia lunata	_	_	_	+	_	_	1	20
**Fusarium fujikuroii*	396989	+	_	+	_	_	2	40
**Fusarium*.sp.	396990	+	_	+	_	_	2	40
Mucor sp.	_	_	_	+	_	+	2	40
Paeciliomyces sp.	_	+	_	_	_	_	1	20
Penicillium pinophyllum	_	+	_	+	_	_	2	40
Syncephalastrum racemosa	_	_	_	+	_	_	1	20
Tilletia ayresii	398397	+	+	+	+	+	5	100
Trichoderma sp.	_	_	_	+	+	_	2	40

(--)= Organism absent in the site (+) = Organism present in the site

Fusarium organisms were further sequenced to confirm their identity. The sequence data of IMI396989 (*Fusarium fujikuroii*) revealed that it belongs to the *Fusarium fujikuroii* complex. It is closest to the unnamed species NRRL25615, which was isolated from rice seeds in Nigeria, but there is 98% homology between transition elongation factor (TEF) sequences of these strains. The sequence data of IMI396990 (*Fusarium* sp.) is very similar to the previous isolate and shows 99% homology with *Fusarium NRRL 25615.* (Sequence attached below).

IMI 396989 TEF sequence-- *Fusarium fujikuroii*

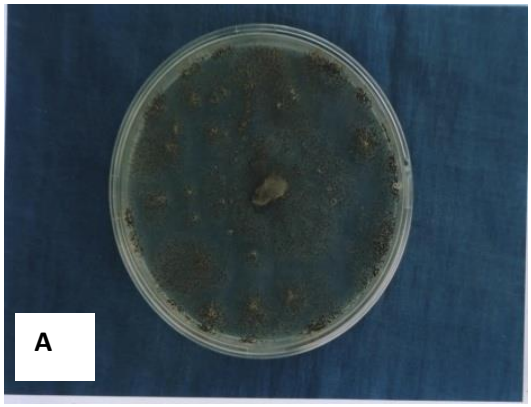
AGTGATGTGTTAATAATAGGATATATAGAACGGAGCAAGAGCGACAACATAC
CAATGACGGTGACATAGTAGCGAGGAGTCTCGAACTTCCAGAGAGCAATATC
GATGGTGATACCACGCTCACGCTCAGCCTTGAGCTTGTCAAGAACCCAGGCG
TACTTGAAGGAACCCTTACCGAGCTCAGCGGCTTCCTATTGTCGGATGGTTAG
TGACTGTTTGACACGTGACGATGCACTCAATGAGGTTGTGGAATAAAAGAGG
GCAAAAAACGCGCCGCTCGAGTGGCGGGGTAAATGCCCCACCAAAAAAATT
ACGGTCGTATCGCAAAATTTTTGGGCTCGAGCGGGGTAGCGGGCACGTTTCG
AGTCGTAGGGAGAAATCGATGGACAAAGGACGCGCGATCGAAGGGAGTGTG
ACTAACCTTCTCGAACTTCTCGATGGTTCGCTTGTCGATACCACCGCACTGGT
A

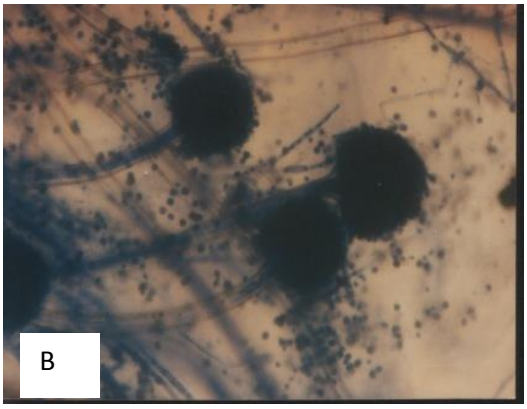
Figure 2: Sequence data of *Fusarium fujikuroii* IMI 396989

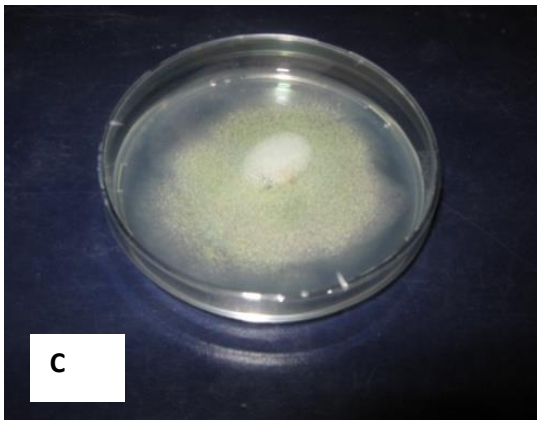
IMI 396990 TEF sequence -- *Fusarium* sp.

AGTGATGTGTTAGTAATAGGATATATAGAACGGAGCAAGAGCGACAACATAC
CAATGACGGTGACATAGTAGCGAGGAGTCTCGAACTTCCAGAGAGCAATATC
GATGGTGATACCACGCTCACGCTCGGCCTTGAGCTTGTCAAGAACCCAGGCG
TACTTGAAGGAACCCTTACCGAGCTCAGCGGCTTCCTATTGTCGGATGGTTAG
TGACTGTTTGACACGTGACGATGCACTCAATGAGGTTGTGGAATAAAAGAGG
GCAAAAAACGCGCTCGAGTGGCGGGGTAAATGCCCCACCAAAAAAAATTAC
GGTCGTATCGCAAAATTTTTGGGCTCGAGCGGGGTAGCGGGCACGTTTCGAG
TCGTAGGGAGAAATCGATGGACAAAGGACGCGCGATCGAAGGGAGTGTGAC
TAACCTTCTCGAACTTCTCGATGGTTCGCTTGTCGATACCACCGCACTGGTAG
ATCAAG

Figure 3: Sequence data of *Fusarium sp.* IMI 396990







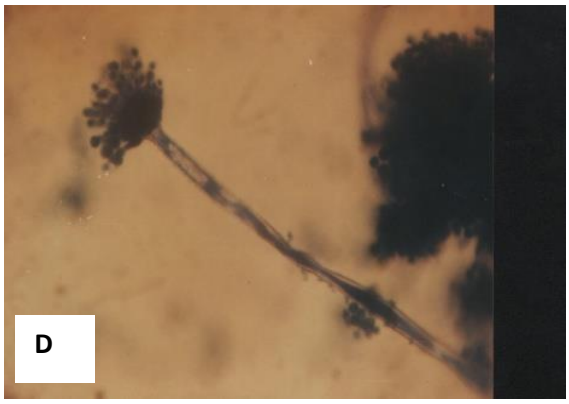


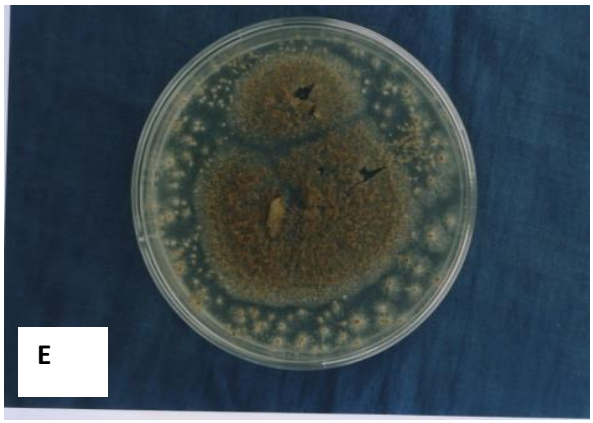
Plate 4 (A-D): Photomicrograph and Pure cultures of fungal isolates

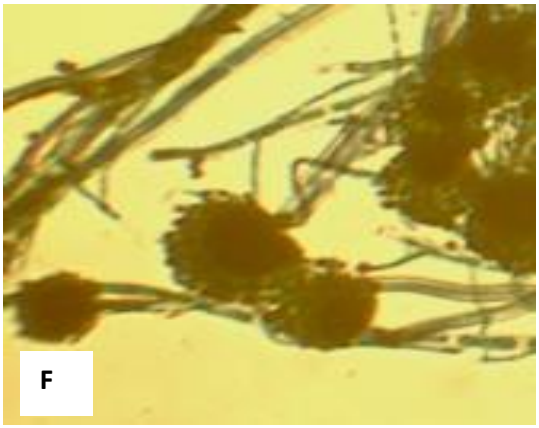
Plate A Pure culture of *Aspergillus niger* **(x0.50)**

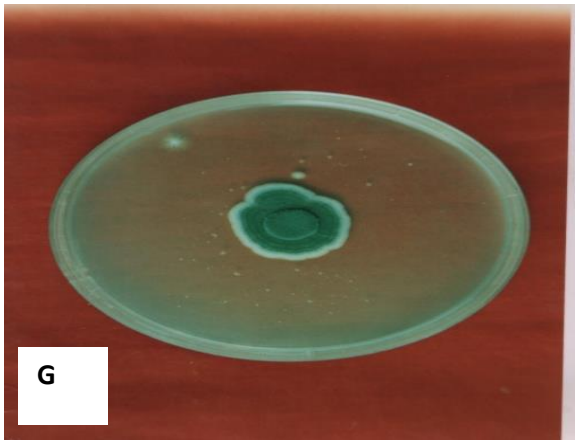
Plate B Photomicrograph of *A.niger* (**x400)**

Plate C Pure culture of *Aspergillus flavus* **(x0.41)**

Plate D Photomicrograph of *A.flavus* (**x400)**







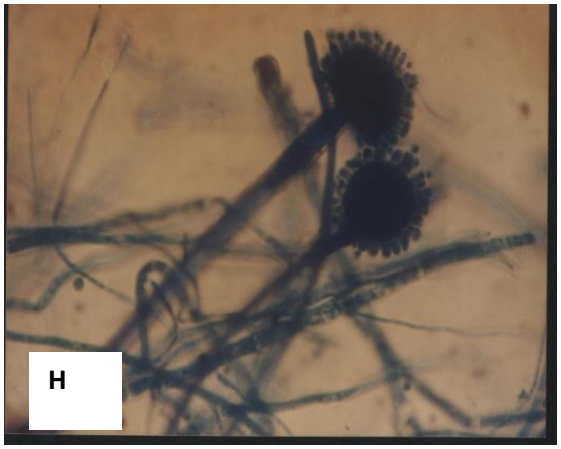


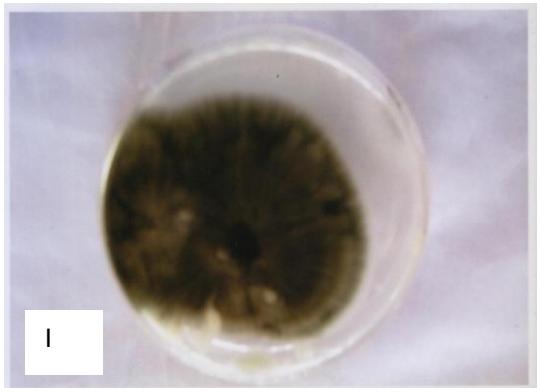
Plate4 (E-H): Photomicrograph and Pure cultures of fungal isolates

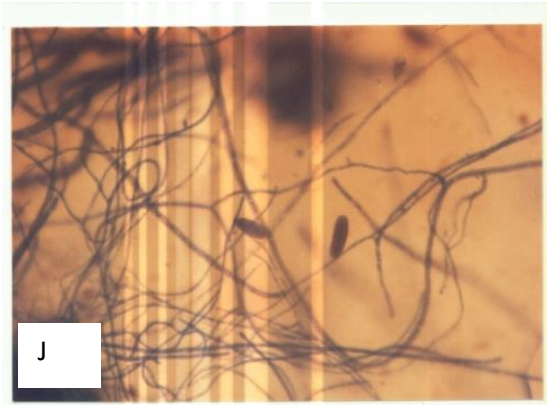
Plate E Pure culture *of Aspergillus wentii* **(x0.54)**

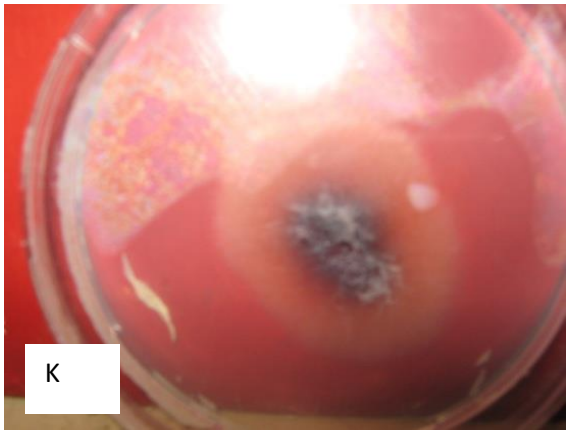
Plate F Photomicrograph *of A. wentii* (**x400)**

Plate G Pure culture of *Aspergillus fumigatus* **(x0.41)**

Plate H photomicrograph of *A.fumigatus* (**x400)**







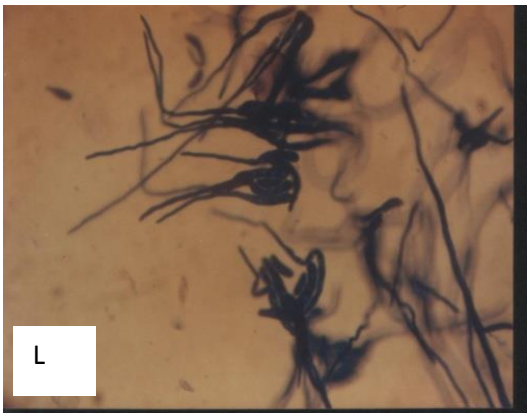


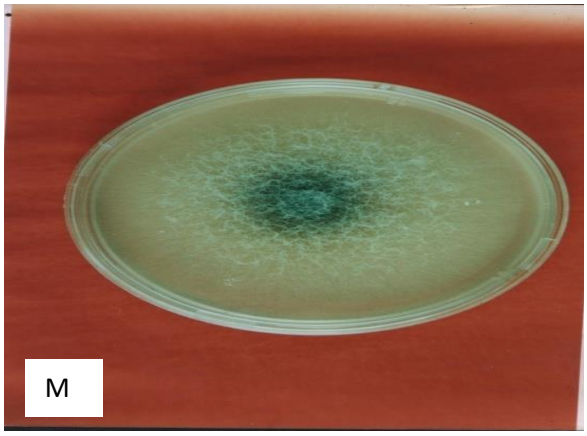
Plate4 (I-L): Photomicrograph and Pure cultures of fungal isolates

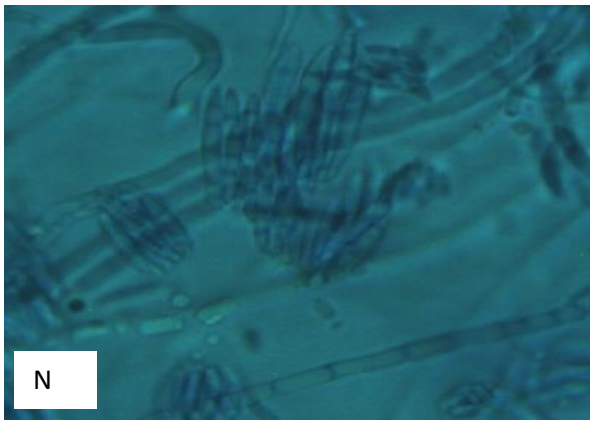
Plate I Pure cultures of *Curvularia lunata* **(x0.55)**

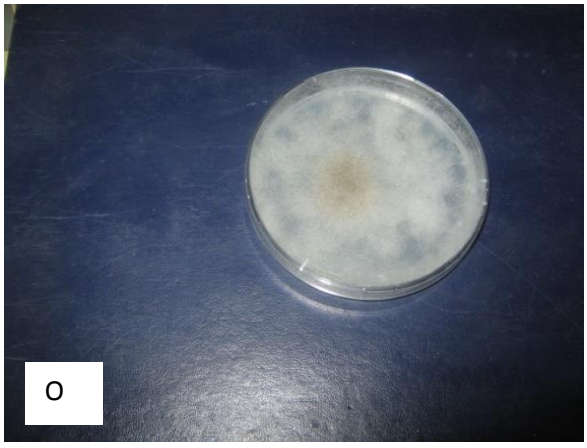
Plate J Photomicrograph of *C.lunata* (**x400)**

Plate K Pure culture of *Fusarium fujikuroii* **(x0.81)**

Plate L Photomicrograph of *F. fujikuroii* (**x400)**







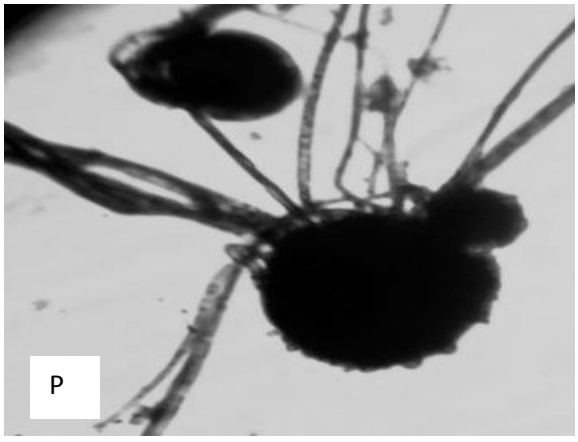


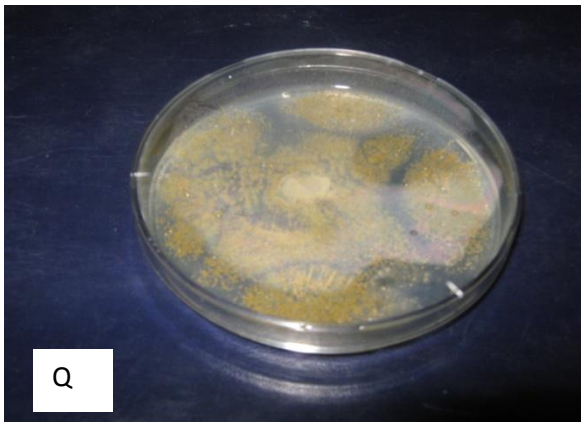
Plate 4 (M-P): Photomicrograph and Pure cultures of fungal isolates

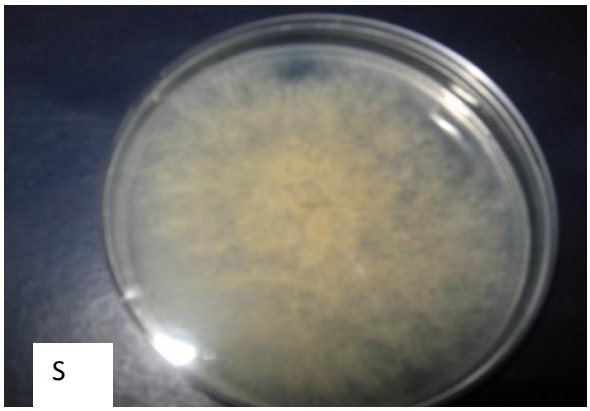
Plate M Pure culture of *Fusarium* sp. (**x0.40)**

Plate N Photomicrograph of *Fusarium* sp. (**x400)**

Plate O Pure culture of *Mucor* sp**. (x0.35)**

Plate P Photomicrograph of *Mucor sp.* (**x40)**





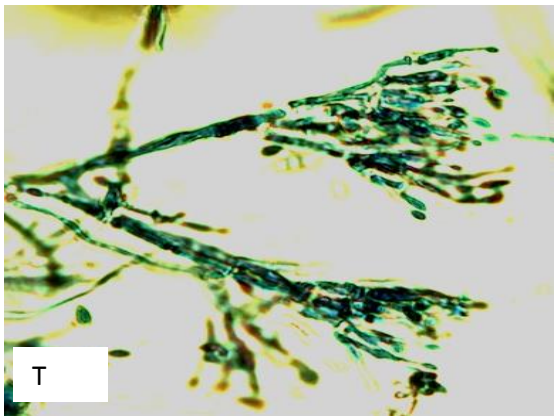


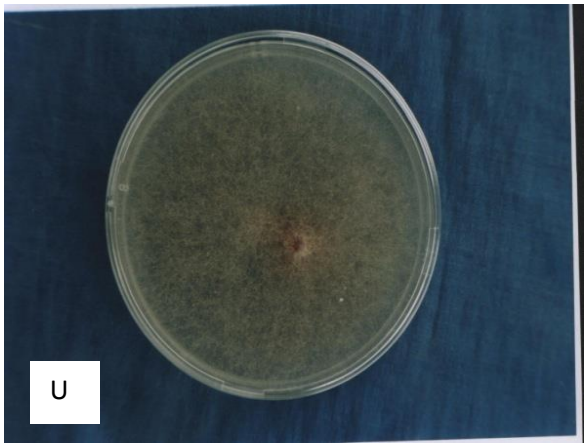
Plate 4 (Q-T): Photomicrograph and Pure cultures of fungal isolates

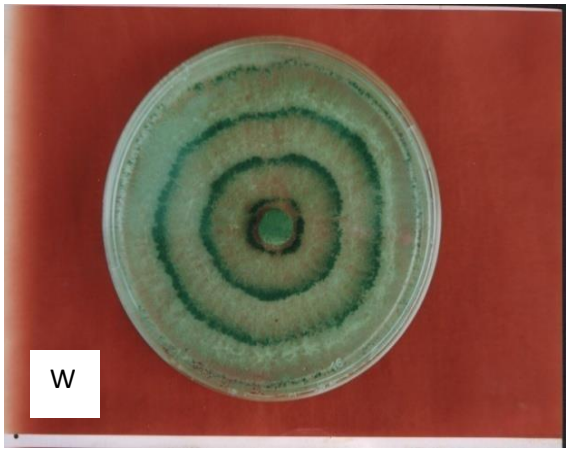
Plate Q Pure culture of *Penicillium pinophyllum* **(x0.47)**

Plate R Photomicrograph of *P.pinophyllum* **(x400)**

Plate S Pure culture of *Paeciliomyces* sp**. (x0.59)**

Plate T Photomicrograph of *Paeciliomyces* sp. (**x400)**





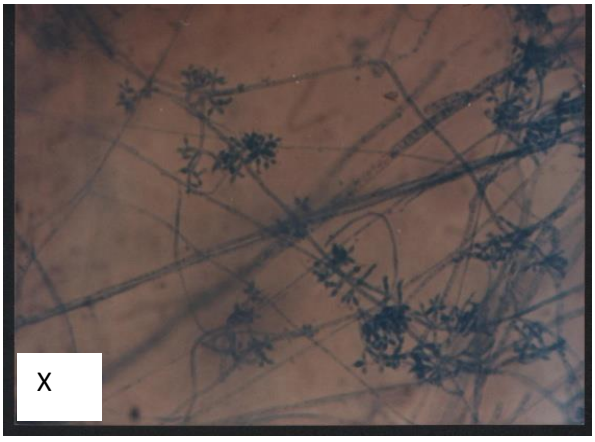


Plate 4(U-X): Photomicrograph and Pure cultures of fungal isolates

Plate U Pure culture of *Syncephalastrum racemosa* (**x0.55)**

Plate V Photomicrograph of *S. racemosa* **(x400)**

Plate W Pure culture of *Trichoderma* sp. (**x0.55)**

Plate X Photomicrograph of *Trichoderma* sp. (**x400**)

4.3 Pathogenicity Test of Fungal Isolates on Healthy *Panicum Maximum* Floret

The results of inoculation after one month showed that the plot with *Panicum maximum* plants inoculated with teliospore suspension obtained from intact smutted florets as well as conidia suspension developed symptoms similar to those observed in the field (Plate5A-B). Re-isolation of teliospores from artificially smutted florets yielded fungi similar in feature to the original isolate. Their teased ovary contents showed spores similar to those of the pathogen. The remaining twelve fungi and control plants did not show symptoms of smut on the plant (Table 2).

4.4 Growth Studies

The result of growth studies of ten fungal isolates on six growth media – Potato Dextrose Agar (PDA), Carrot Agar (CA), Panicum Floret Extract Agar (PFEA), Panicum Leaf Extract Agar (PLEA), Water Agar (WA), Malt Extract Agar (MEA) is shown in Figure 4-9 . Growth studies of fungal species on potato dextrose agar shows that there was general increase in radial growth diameter of all the organisms with increase in number of days from 2^{nd} to 7^{th} day .The growth rate of *Trichoderma* sp., *Syncephalastrum racemosa* and *Mucor* sp. increased uniformly from 3^{rd} day to 7^{th} day where they had the highest growth. However, there was no statistical difference in the growth rate of these three organisms. The radial growth of *Aspergillus niger* also increased significantly from 2^{nd} day to 7^{th} day of study. *Mucor* sp. had the best growth because by the 4^{th} day, the plate was covered with mycelium with sporangium at the tip of sporangiophore while *Aspergillus fumigatus* grew least on this medium. There was significant difference in the growth rate of *Penicillium pinophyllum, A. fumigatus, A. flavus, A. wentii, Trichoderma* sp., *Mucor* sp. *and* S. racemosa (Fig. 4). In carrot agar the radial growth of *Trichoderma sp.* increased

significantly from 2nd to 5th day where it remained constant till day 7. *Aspergillus flavus and Penicillium pinophyllum* increased in growth diameter from 2nd to 7th day.*Trichoderma* sp. grew best in this medium while *Mucor* sp. grew least in the medium as the radial growth did not exceed 30mm at 7th day of study (Fig. 5) Studies on malt extract agar showed that there was a general non-significant increase in radial growth of all the organisms as the number of days increased. *Trichoderma* sp. and *S. racemosa* were exceptions as they showed significant increase ($P<0.05$) in radial growth from day 2 to 7 of study (Fig. 6).

Plate5 (A-C): Plates of pathogenicity plots Mag. x0.10

Plate A. **Pathogenicity plot with enclosed inoculated panicles**

Plate B. **Pathogenicity plot with smutted panicles**

Plate C **Pathogenicity plot with unsmutted panicles (control)**

Table 2: Pathogenicity test of fungi on healthy *Panicum maximum* floret

Fungi	Pathogenicity
Aspergillus flavus	--
A.fumigatus	_
A.niger	_
A.wentii	_
Curvularia lunata	_
Fusarium fujikuroii	_
Fusarium sp.	_
Mucor sp.	_
Paeciliomyces sp.	_
Penicillium pinophyllum	_
Syncephalastrum racemosa	_
Trichoderma sp.	_
Tilletia ayresii	+

(-) = Non-pathogenic

(+) =Pathogenic

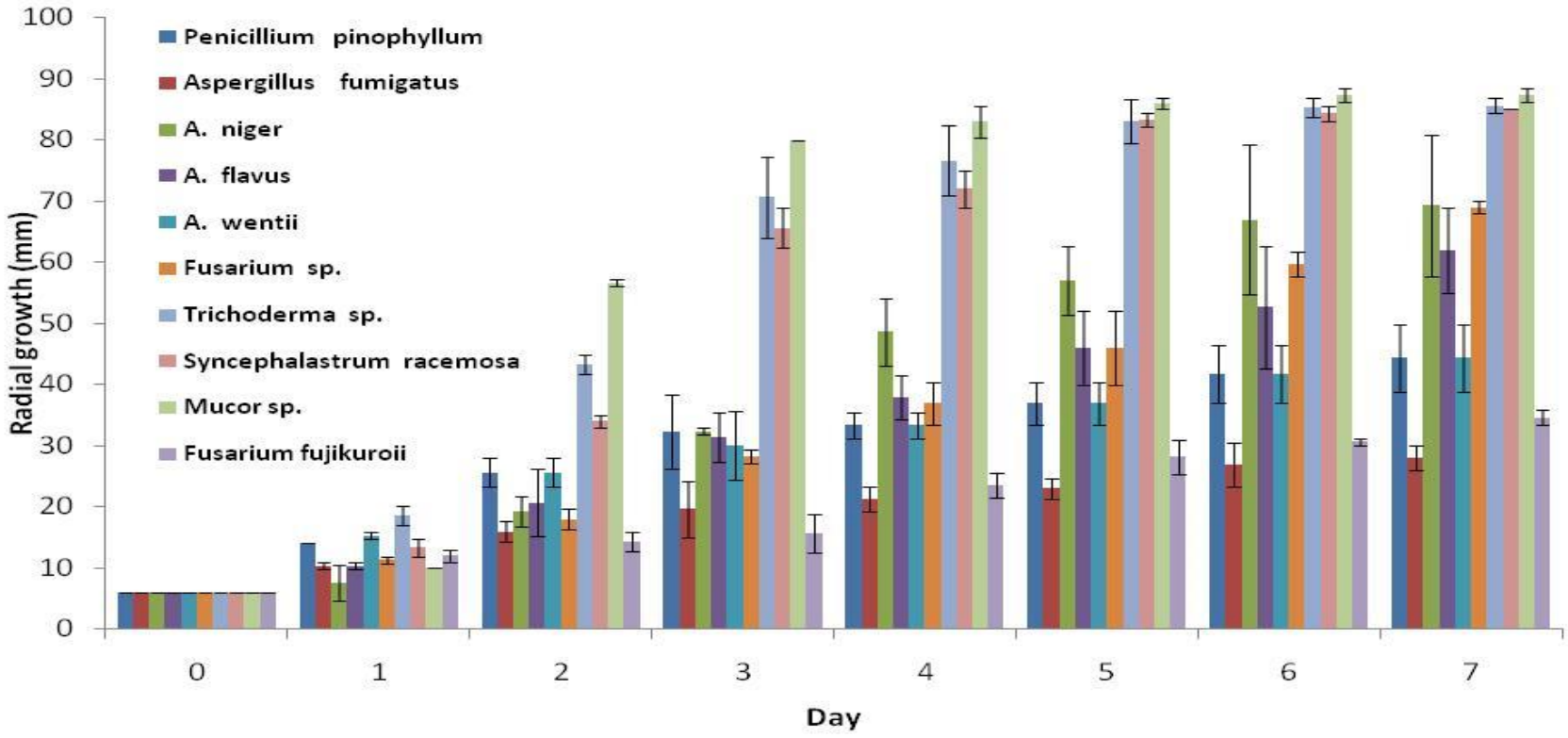


Figure 4: Radial growth of fungal species on potato dextrose agar n=3, Values are expressed as Mean±SEM

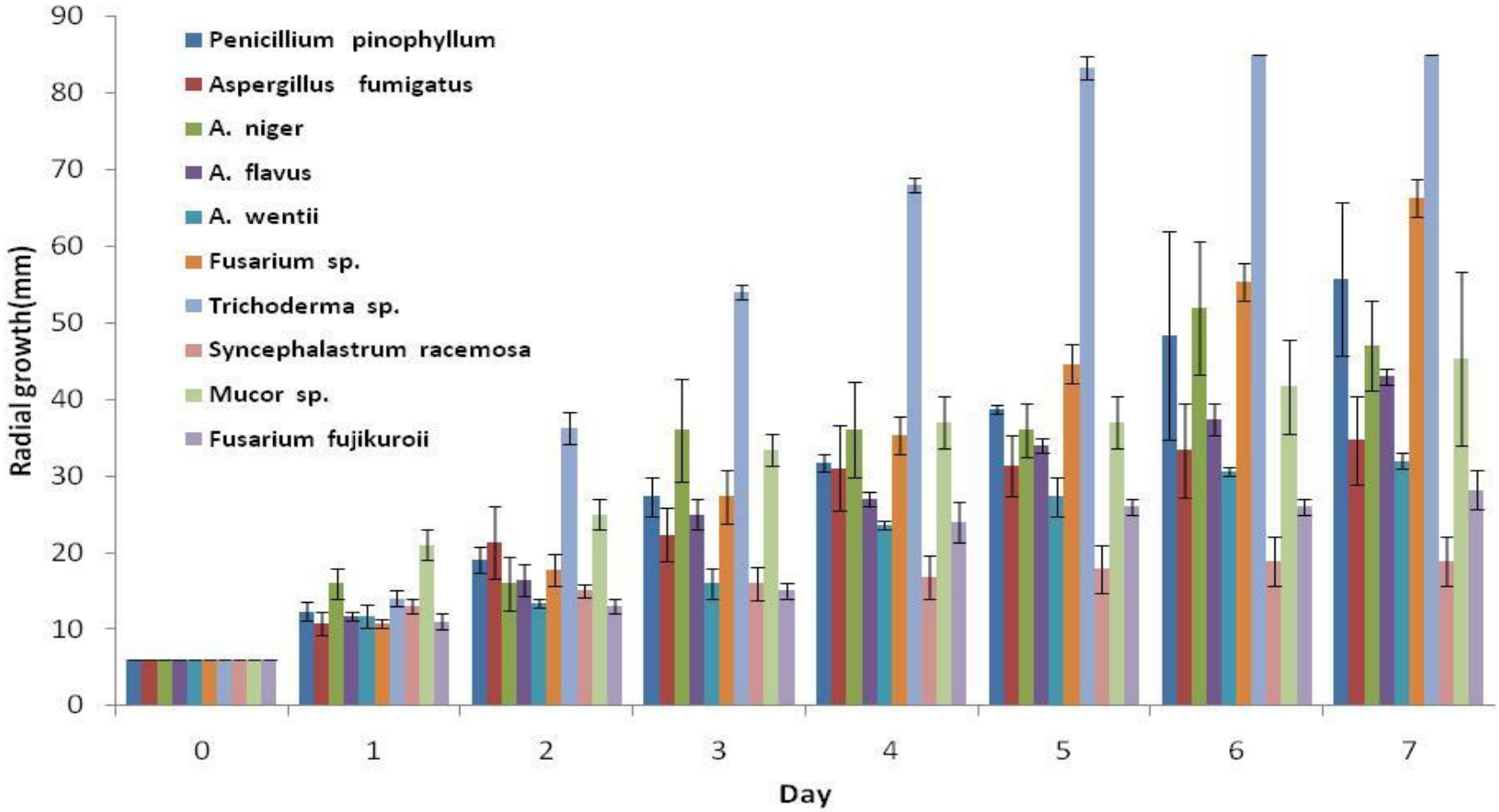


Figure 5: Radial growth of fungal species on carrot agar n=3, Values are expressed as Mean±SEM

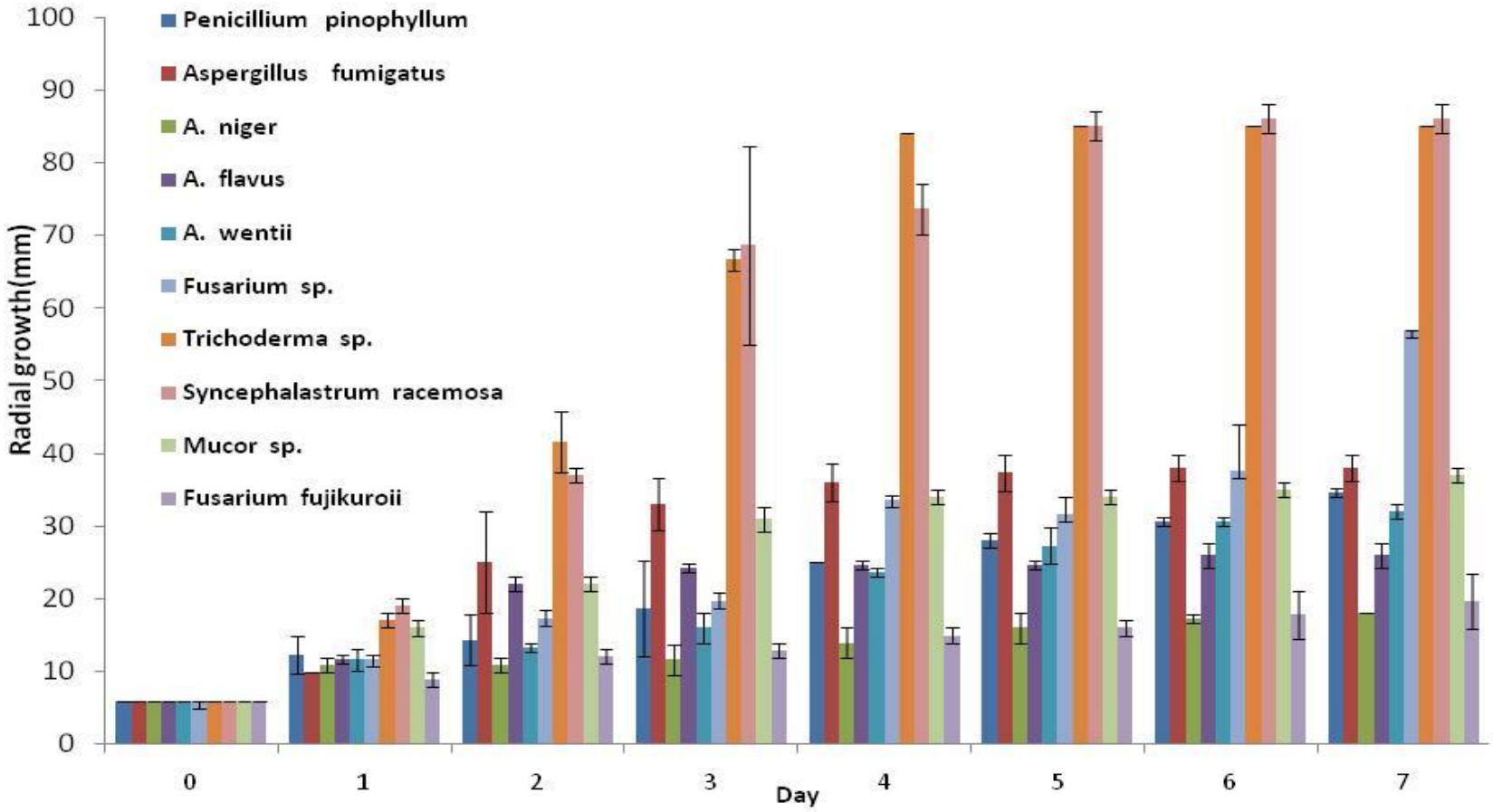


Figure 6: Radial growth of fungal species on malt extract agar n=3, Values are expressed as Mean±SEM

Growth on Panicum leaf extract agar shows that there was a non--significant increase in radial growth of all the organisms except *Trichoderma* sp. as the number of days increased. The radial length of the *Trichoderma* sp. increased significantly ($P<0.05$) from second day to fifth day and then remained constant till the 7^{th} day with a diameter of 85mm.

The least grown organism was *Fusarium fujikuroii* with growth diameter of 25mm even at the 7^{th} day of study (Fig.7). The radial growth of all the organisms in Panicum floret extract medium increased non-significantly as the number of days increased except *Trichoderma* sp. which increased significantly ($P=0.01$) from second day to fifth day and then remained constant till the 7^{th} day with a diameter of 85mm.The least grown organism however was *Fusarium fujikuroii* with growth diameter of 25mm even at the 7^{th} day of study (Fig.8). The water agar being a minimal medium supported growth of almost all the organisms minimally. The least grown organism in this medium was *A. niger* whose radial growth diameter was 17mm at 7^{th} day of the study (Fig. 9).

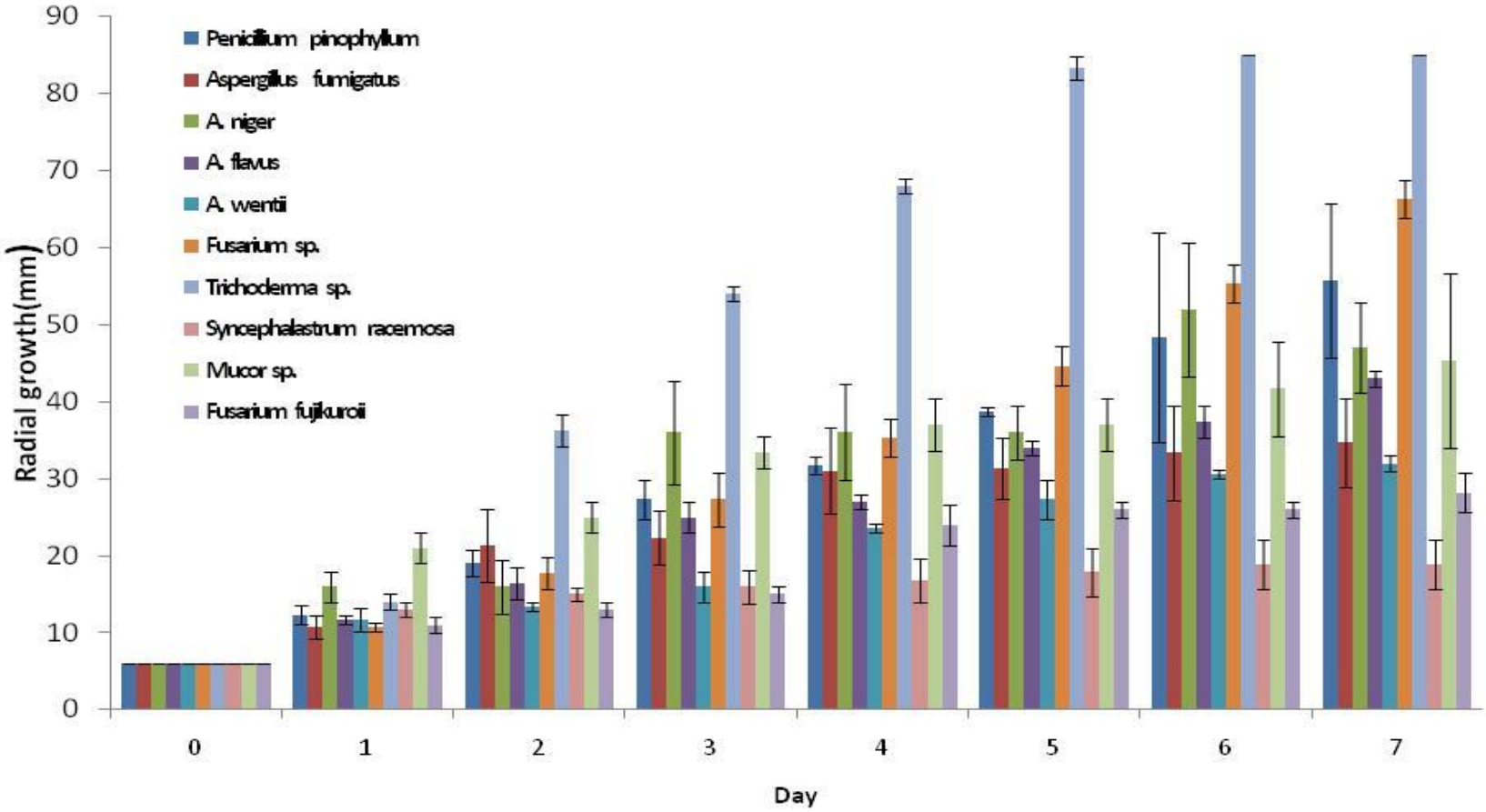


Figure 7: Radial growth of fungal species on Panicum leaf extract agar n=3, Values are expressed as Mean±SEM

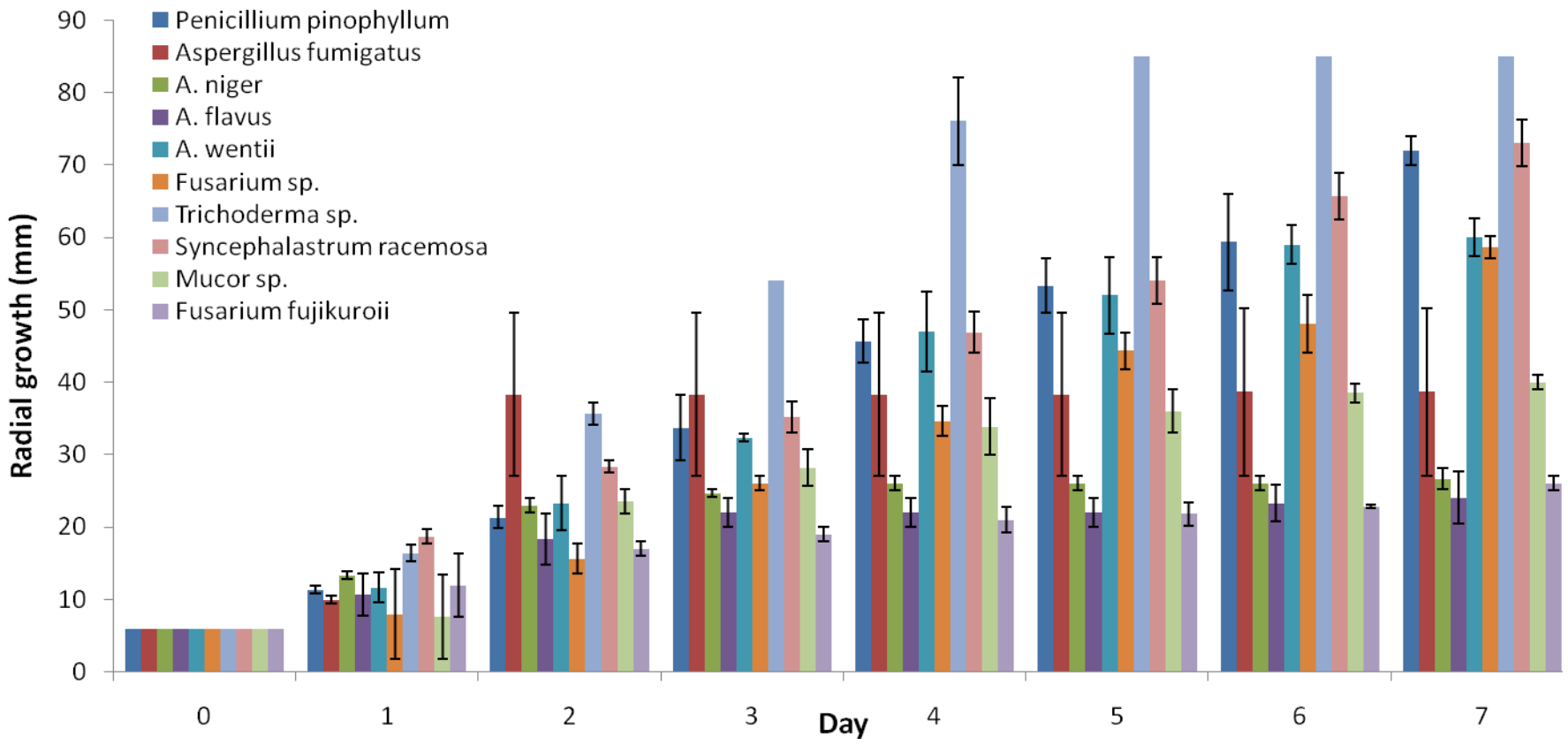


Figure 8: Radial growth of fungi species on Panicum floret extract agar n=3,Values are expressed Mean±SEM

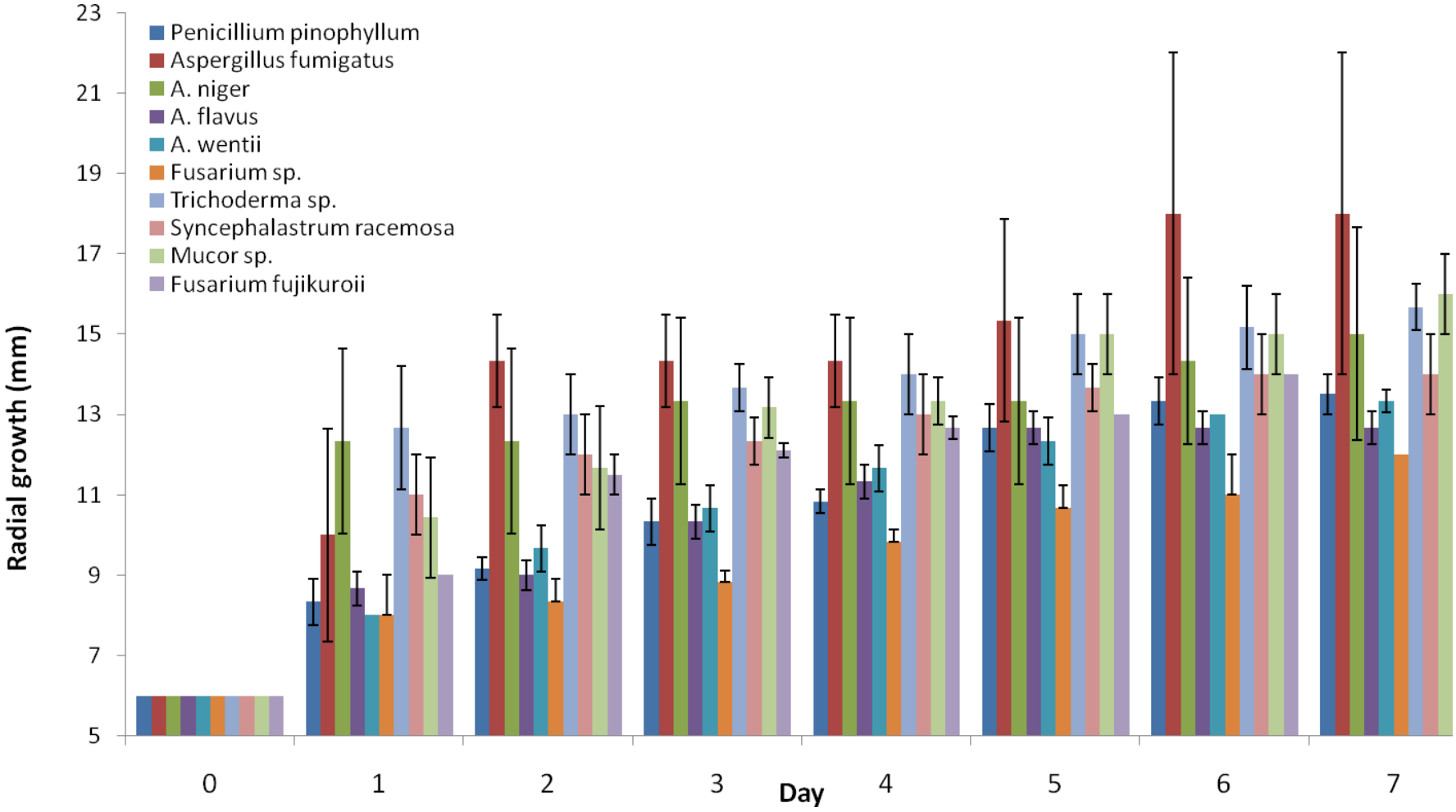


Figure 9: Radial growth of fungal species on water agarr n=3, Values are expressed as Mean±SEM

4.5 Growth Studies of *Tilletia ayresii* using Three Media

Result of growth studies of *Tilletia ayresii* using Malt yeast peptone broth (MYPB), Carrot Dextrose Broth (CDB) and Potato Dextrose Broth (PDB) with Spectrophotometer at 530nm wave length is shown in Figure9. *Tilletia ayresii* grew in the three broth media at pH6.5 and temperature of 18^{o}C.The highest optical density was attained by the organism in potato dextrose broth. In malt yeast peptone broth, a sigmoid curve growth which is the normal growth pattern of living organisms occurred. In the lag phase (day1-2) which can be regarded as adaptation phase, the fungal spores germinated even though there was no appreciable supply of nutrient availability. A sharp increase occurred from day 2 to 3 and then maintained a stationary phase during which time the birth rate of the organism equaled the death rate. A sharp decline occurred from day 4 to 7. In carrot dextrose broth there was a sharp increase in optical density (OD) up to 1.2, then sharp decline to less than 0.6 OD and an increase up to 1.1 optical density (Fig.10).

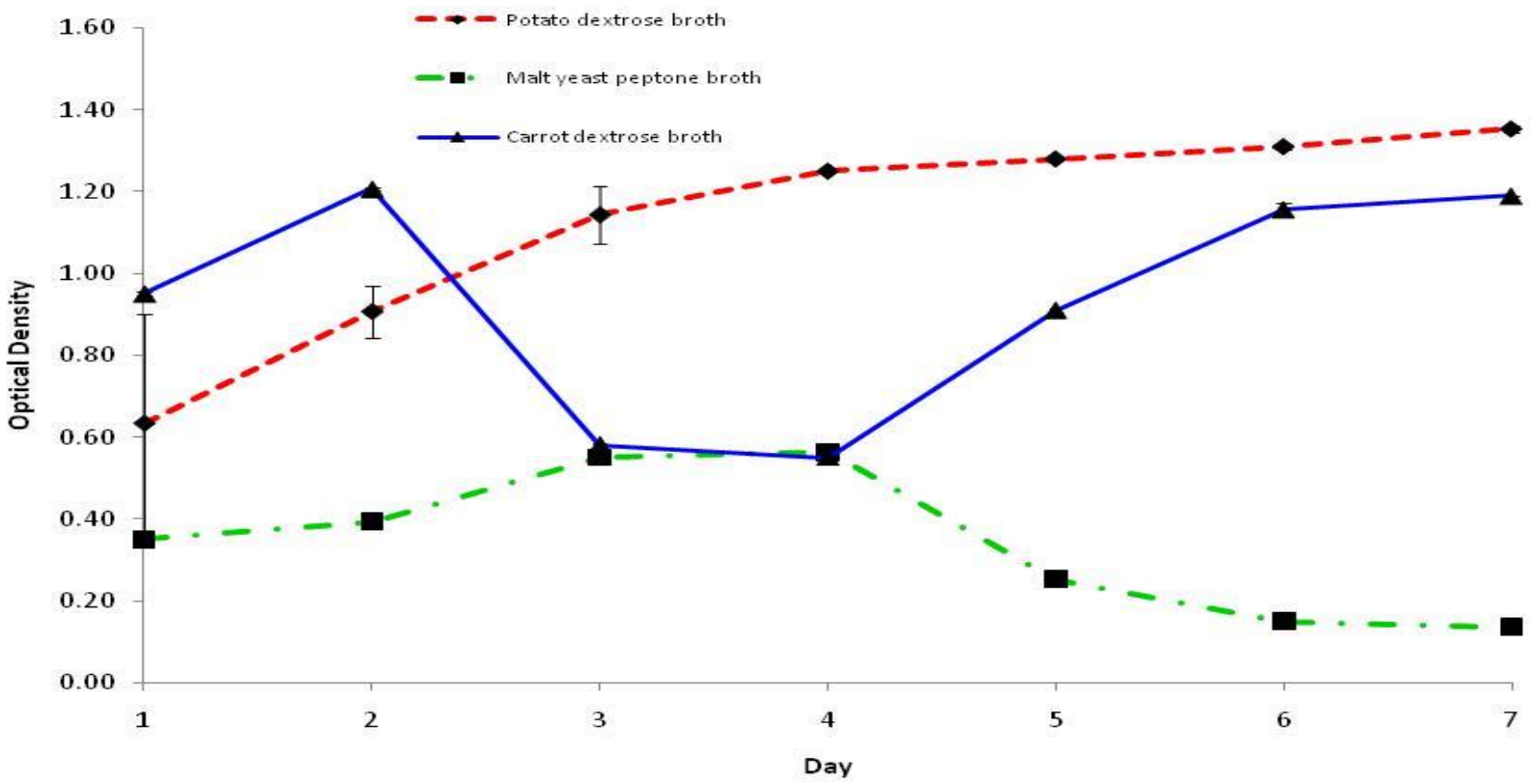


Figure 10: Growth pattern of *Tilletia ayresii* on three broth media at 530nm wavelength

4.6 Ethnobotanical Studies of Crude extracts of Healthy and Infected *Panicum maximum* Floret in Rats

Sequential extraction of infected *Panicum maximum* florets yielded varying percentage weight of crude extracts in different solvents used (aqueous-1%, (20g); chloroform-2.6%, (51g), ethanol-5%, (98g). Ergometrine (orthodox drug) is known to cause contraction of uterine muscles and in this experiment it produced a dose-dependent contraction of non-pregnant uterine muscle (Fig.11). The different extracts of healthy *Panicum maximum* florets (PMF) using sterile distilled water, ethanol and chloroform however gave a dose—dependent relaxation of non-pregnant uterine smooth muscle. The highest relaxation was produced by chloroform extract at a final bath concentration of 0.063-0.125mg/ml (Fig.12).

The contrary was the case for the infected aqueous, ethanol and chloroform PMF extracts where a dose- dependent contraction of the non-pregnant muscle was recorded (Fig.13). Ethanol extract produced the highest contraction with the least dose achieving 77% contraction at final bath concentration of 0.062mg/ml. The infected ethanol extract fraction produced a similar effect on ergometrine- stimulated uterine muscles with greater contraction compared with the unstimulated uterine muscles. However, the patterns of contraction of the two are similar (Fig.14)

Further fractionation of the infected ethanol extract (most active) and elution with four solvents yielded four fractions. Out of the four fractions produced, only ethanol fraction was active while others were inactive. Further separation of the active ethanol fraction on TLC plate revealed the presence of three compounds with the following R_f values- 0.20. 0.30, and 0.90 respectively. Contractility studies of the three compounds showed that only the compounds with R_f value-0.20 was active while the other compounds were inactive (Figures 15 and 16).

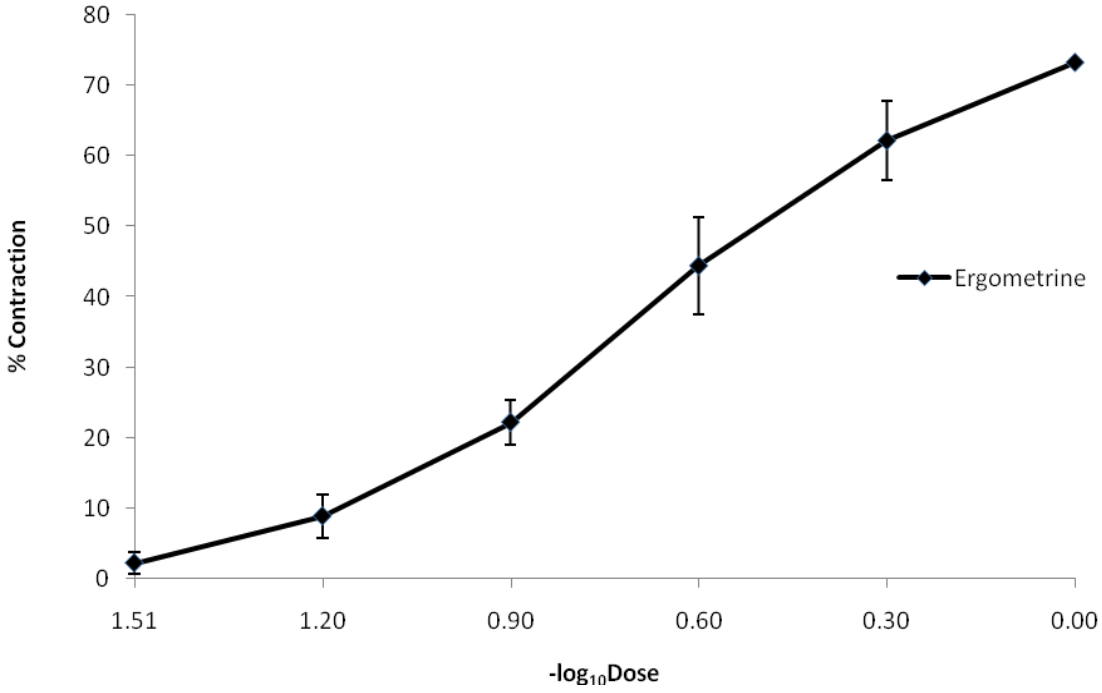


Figure 11: Effect of graded concentration of ergometrine on uterine smooth muscle of non-pregnant rat. Response is presented as percentage contraction.

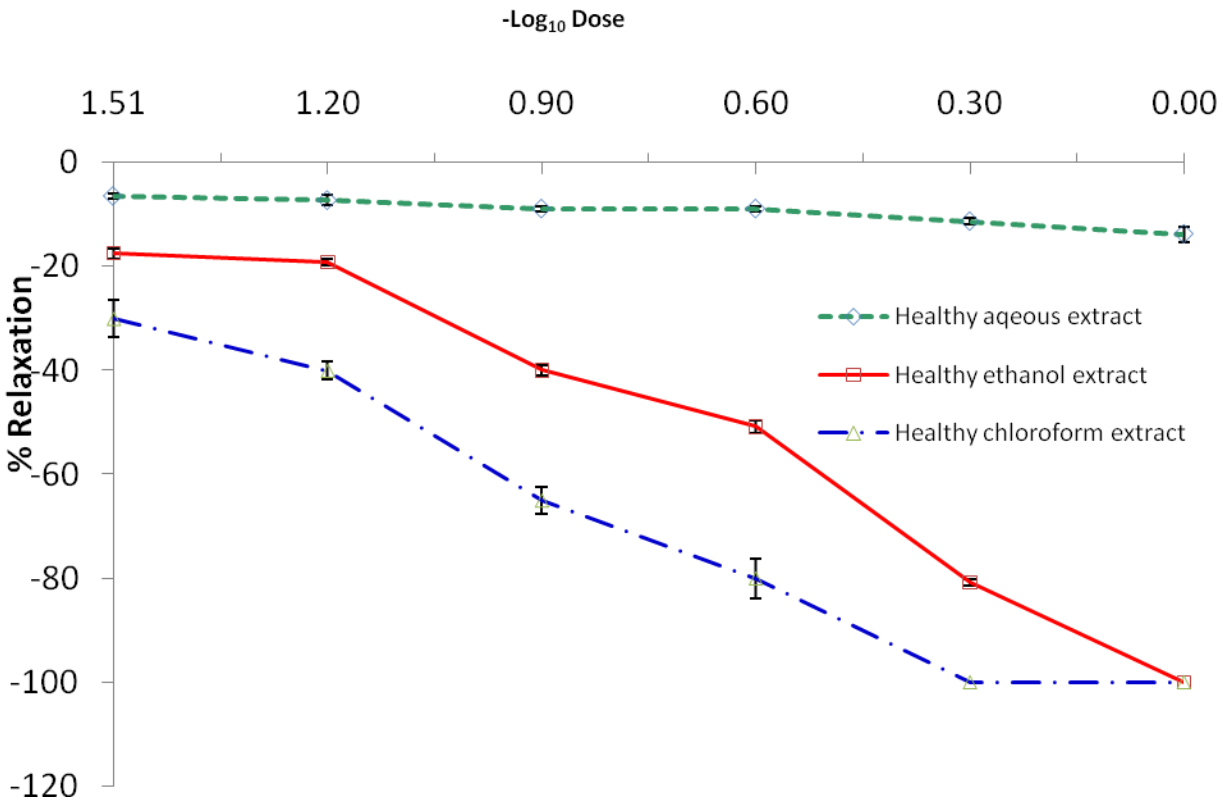


Figure 12: Effect of graded concentrations of aqueous, ethanol and chloroform extracts of healthy *P. maximum* florets on uterine smooth muscles

Response is presented as percentage relaxation.

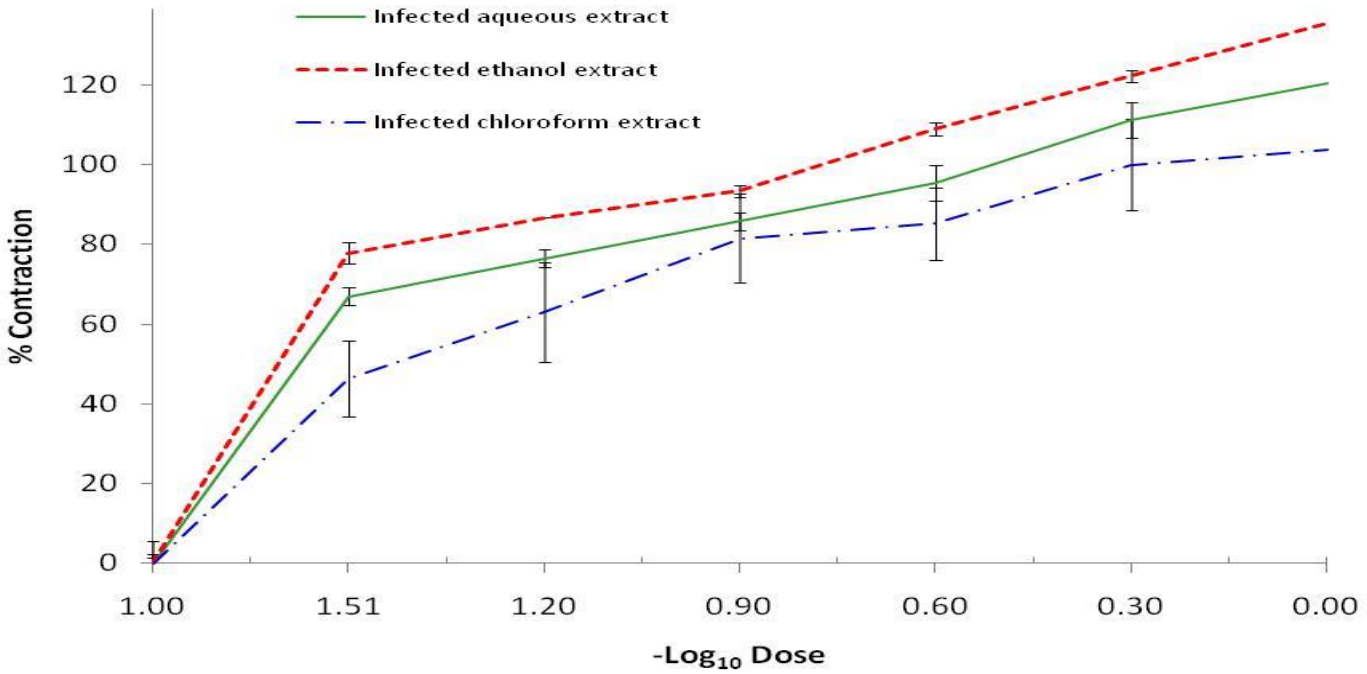


Figure 13: Effect of graded concentrations of aqueous, ethanol and chloroform extracts of infected *P. maximum* florets on uterine smooth muscle. Response is presented as percentage contraction.

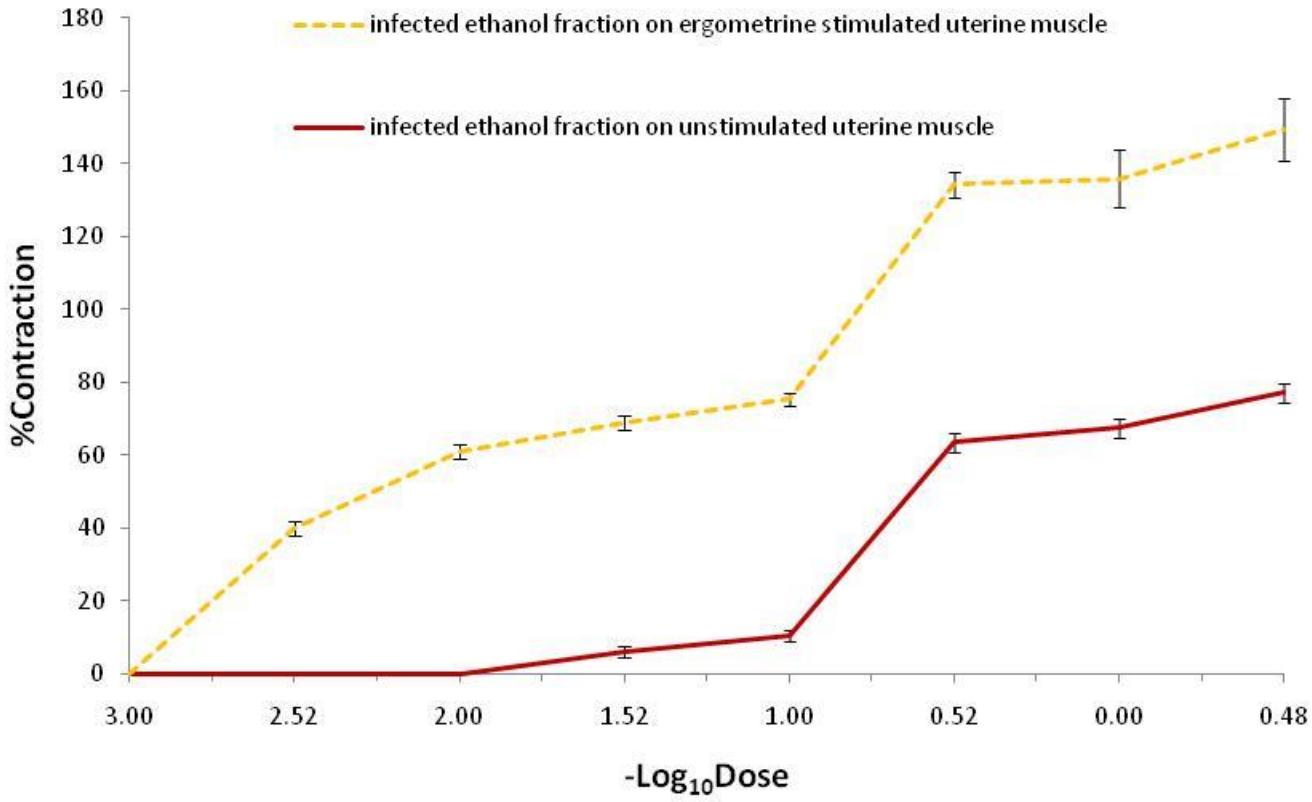


Figure 14: Effect of graded concentrations of active ethanol fraction of infected *P. maximum* florets on stimulated and unstimulated uterine smooth muscles. Response is presented as percentage contraction

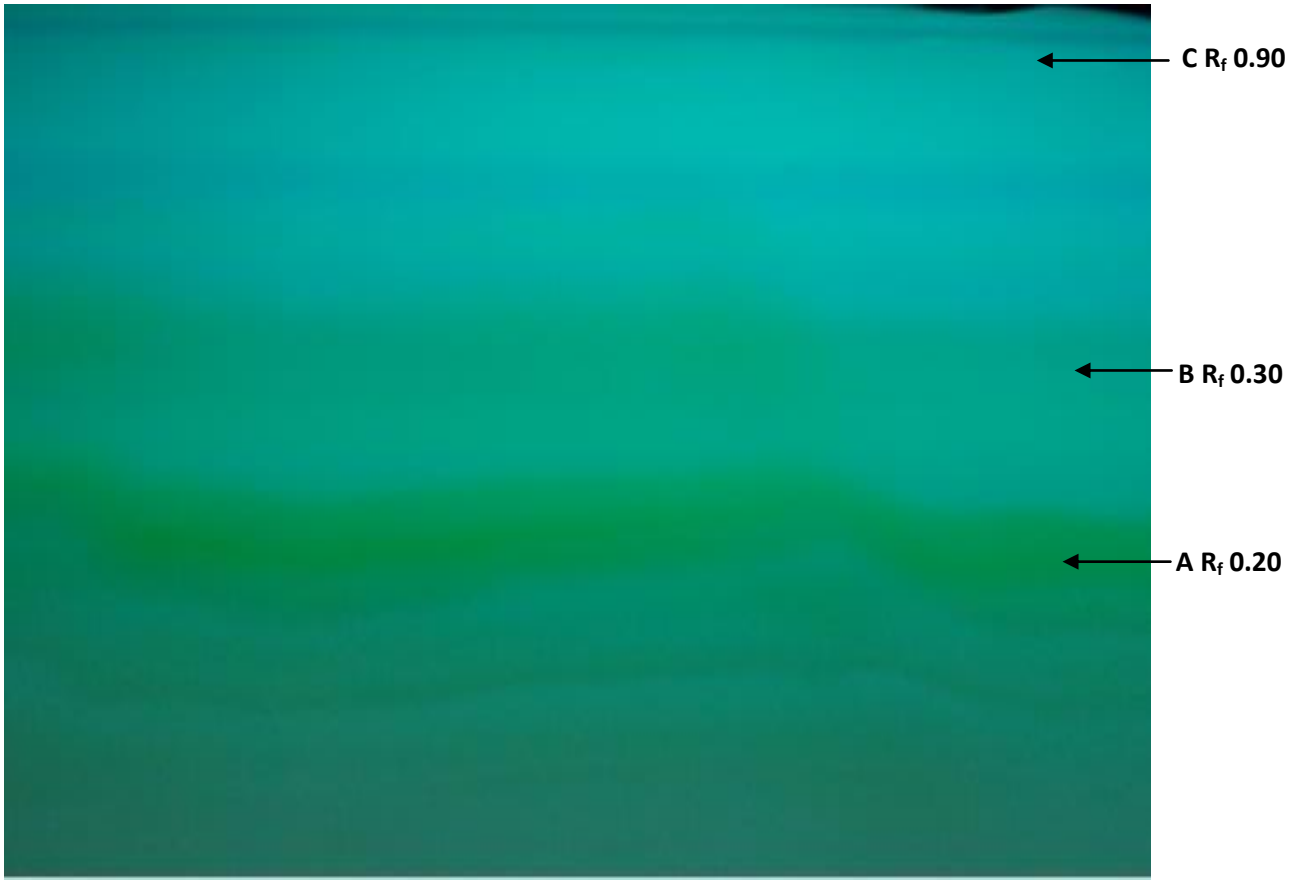


Figure15: Detection of compounds in ethanol fraction under UV F_{366} on TLC plate developed with ethyl acetate-methanol-water Mag. 0.56

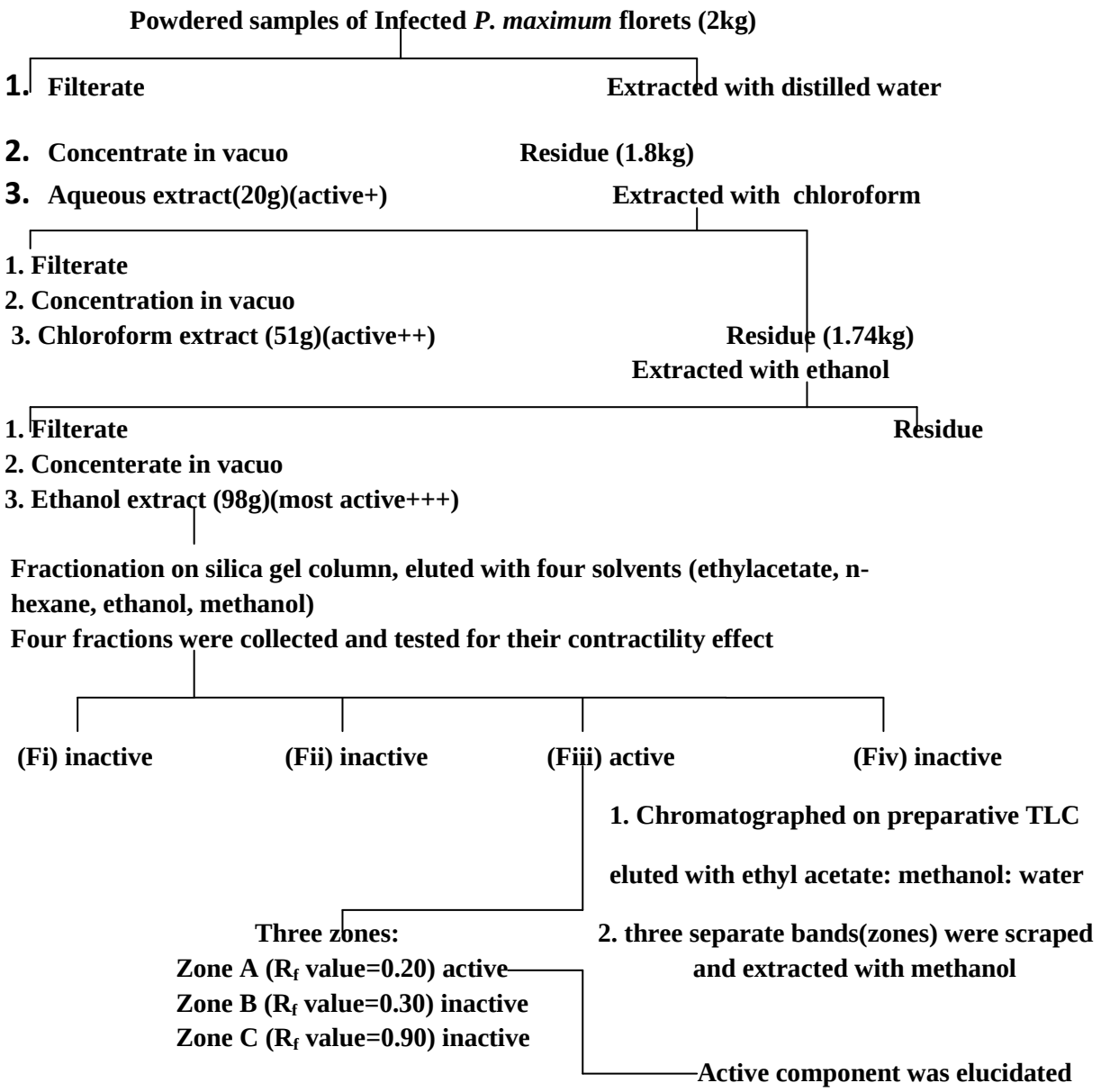


Figure 16: **Procedure for Sequential Plant Extraction and Bioassay guided fractionation/Isolation of bioactive component**

4.7 Effect of Active Crude Extracts on Fungal Isolates

The results of effect of crude extracts of infected *Panicum maximum* floret are presented in tables 3 and 4.The extracts showed significant inhibition of the fungal isolates tested. The ethanol and chloroform extract inhibited the growth of all the isolated fungi except *Fusarum fujikuroii* and *Tilletia ayresii.*. *The* ethanol extracts of the infected floret were more potent than the corresponding chloroform extract. The zones of inhibition for antifungal active extracts is above 10mm as is the case with the two extracts against all the organisms except *F. fujikuroii, Mucor* sp., *Paeciliomyces* sp. and *Tilletia ayresii.* Benlate, an orthodox plant fungicide caused a significant higher zone of inhibition than any of the plant extracts. Benlate produced inhibition against the growth of *Aspergillus flavus, A. niger, Penicillum pinophyllum, Fusarium fujikuroii, Mucor* sp., *Paeciliomyces* sp., and *Tilletia ayresii*. It caused the highest inhibition against *Fusarium fujikuroii (22.00mm).*

Ketoconazole, an orthodox animal fungicide also showed inhibition against the entire organism except *Tilletia ayresii*. The fungi that were inhibited responded to increase in concentration gradient of crude extract i.e. zones of inhibition on the fungi increased as the concentration of the crude extracts increased.

Table 3: Antifungal activities of infected chloroform *Panicum maximum* floret extract

Group/Treatment	Dose (mg/ml)	Zone of inhibition(mm) Organisms						
		Aspergillus flavus	***A.niger***	***Penicillium pinophyllum***	***Fusarium fujikuroii***	***Mucor* sp.**	***Paeciliomyces* sp.**	***Tilletia ayresii***
Control (0.05%Tween80)	10ml	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Benlate	5000	20.50±0.64*	19.75±0.85*	19.25±0.49*	22.00±1.08*	13.50±0.20*	13.50±0.20*	12.75±0.25*
Ketaconazole	100	12.63±0.63*	13.75±0.75*	14.00±0.40*	13.00±1.42*	12.00±0.20*	12.00±0.20*	0.00±0.00
Pmf $_{\text{inf.chl.}}$ I	50	10.88±0.52*	11.63±0.62*	11.25±0.59*	0.00±0.00	4.75±0.32*	4.75±0.32*	0.00±0.00
II	100	12.75±0.32*	12.25±0.32*	12.25±0.25*	0.00±0.00	5.88±0.42*	5.87±0.42*	0.00±0.00
III	150	13.50±0.20	13.63±0.55*	14.38±0.55*	6.13±0.42*	5.87±0.42	5.87±0.43*	0.00±0.00

Values are expressed as Mean±SEM

Pmf inf.chl–Infected chloroform extract of *Panicum maximum* floret

***P< 0.05-significantly different compared to control. Means without *are not significantly different compared to control.**

Table 4: Antifungal activities of infected ethanol *Panicum maximum* floret extract

Group/Treatment	Dose (mg/ml)	Zone of inhibition(mm) Organisms						
		Aspergillus flavus	***A.niger***	***Penicillium pinophyllum***	***Fusarium fujikuroii***	***Mucor* sp.**	***Paeciliomyces* sp.**	***Tilletia ayresii***
Control (0.05%Tween80)	**10ml**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Benlate	**5000**	18.63±0.24*	18.63±0.23*	18.63±0.23*	20.65±0.53*	11.50±0.50*	13.50±0.20*	12.75±0.79*
Ketaconazole	**100**	13.75±0.47*	13.38±0.62*	13.75±0.49*	13.00±1.48*	9.50±0.20*	12.00±0.70*	0.00±0.00
Pmf $_{inf.eth}$. I	**50**	11.68±0.34*	12.63±0.55*	11.68±0.34*	3.38±0.23*	0.00±0.00	4.75±0.32*	0.00±0.00
II	**100**	12.43±0.36*	13.63±0.63*	12.13±0.35*	3.88±0.12*	7.75±1.10*	5.89±0.43*	0.00±0.00
III	**150**	14.13±0.13*	14.25±0.63*	14.13±0.12*	4.50±0.29*	12.00±0.93*	5.89±0.43*	0.00±0.00

Values are expressed aS Mean±SEM

Pmf $_{inf.eth}$-Infected ethanol *Panicum maximum* floret extract

***P<0.05 significantly different compared to control. Means without *are not significantly different compared to control**

4.8 Phytochemical analysis of aqueous, ethanol and chloroform extracts of healthy and infected *Panicum maximum* florets

Preliminary phytochemical analysis of the three extracts revealed the presence of alkaloids, tannins, saponins and flavoniods. Percentage of alkaloids and tannins were more in infected aqueous, ethanol and chloroform extracts than in healthy extracts of *Panicum maximum* florets. However, infected ethanol extract had more alkaloids and tannins than aqueous and chloroform extracts. Further more, flavonoids was absent in chloroform extracts of both healthy and infected *Panicum maximum* florets but present in aqueous and ethanol extracts though at varying quantities (Table 5).

Table 5: Phytochemical composition of aqueous, ethanol and chloroform extracts of healthy and infected *Panicum maximum* florets

Plant Extracts	Alkaloids %	Tannins %	Saponins %	Flavonoids %
Aqueous extract				
Healthy	0.50	0.90	0.33	0.75
Infected	0.98	1.07	0.74	1.01
Ethanol extract				
Healthy	0.31	0.09	1.83	0.78
Infected	1.15	1.03	1.14	0.69
Chloroformextract				
Healthy	0.41	0.19	1.93	--
Infected	1.07	0.92	0.29	--

(--Absent)

4.9 Lethal dose determination and subchronic toxicity of infected ethanol and chloroform extracts

The LD_{50} value for ethanol and chloroform extacts was 9g/kg body weight respectively using Probit analysis method (Tables 6). Doses equal or lower than 15g/kg body weight caused death in animals given the two extracts while animals that received 1.0g/kg body weight survived beyond the 24hours of observation.This result showed a wide safety margin as any LD_{50} value between 5-15g/kg is considered not to give any dose related toxicity. No changes in the behavior and sensory nervous system responses were observed in the animals and the viscera of the animals did not show any macroscopic changes that could point to the cause of the death.The animals did not convulse before dying.

Tables 7a and b show the weekly mean weight changes of rats fed with infected ethanol and chloroform *Panicum maximum* floret extracts for three weeks. The animals fed with 0.5ml 0.5% Tween 80 solution as control gained weight throughout the investigation while those fed with infected extracts initially gained weight but later lost weight in the last week of the study. Visual macroscopic examination of the wet organs viz. Heart, Kidneys, Liver, Brain, Uterus, and Ovary of each rat (both the control and treated groups) showed no colour change suggesting that *Panicum maximum* floret extracts administered to the rats for 21 days had no adverse effects on the organs of the treated rats. However, Kidney weight reduced at the highest dose while at low to moderate doses it increased along with liver weight. Furthermore, uterus and ovary weight significantly ($p<0.05$) increased at low to moderate doses of the extracts (Tables 8a and 8b). Table 9a shows that there was significant ($p<0.05$) increase in level of red blood cells and haemoglobin at all the doses of infected ethanol extract administered. Packed cell volume also increased significantly ($p<0.05$) as the doses of the extract increased. Administration of infected

chloroform extract to the animals as shown in Table 9b also had a similar effect on the haematological parameters.

Table 6: Acute toxicity of Ethanol (Eth.) and Chloroform (Chl.) extracts of Infected *Panicum maximum* floret

Number of mice		Doses (g/kg b.wt)	Log dose	Mortality (no. of dead mice)	Mortality (%)	Probit value	Cumulative death (%)
Eth.	5	1.0	3.00	0	0	---	---
Chl.	5	1.0	3.00	0	0	---	---
Eth.	5	5.0	3.70	1	20	4.16	14.29
Chl.	5	5.0	3.70	1	20	4.16	16.67
Eth.	5	10.0*	4.00	2	40	4.75	42.86
Chl.	5	10.0*	4.00	2	40	4.75	50.00
Eth.	5	15.0	4.18	4	80	5.84	100.00
Chl.	5	15.0	4.18	4	60	5.23	100.00

Values are expressed as Mean±SEM n=5 .Control group received 0.3ml 0.5% Tween 80.

***LD_{50} was determined to be 9.0g/kg**

Table 7a: Body weight variation of rats during three weeks of administration of Ethanol extract of infected *P.maximum* florets.

Group/Treatment		Dose (mg/kg)	Day1	Day7	Day14	Day21
Control (2% Tween80)		0.5ml	146+0.01	172±0.01	172±0.02	181±0.02
Pmf $_{inf.eth}$	I	100	152±0.22	172±0.16	188±0.22*	180±0.18
	II	250	160±0.16	178±0.18	180±0.18*	168±0.16*
	III	500	168±0.15	188±0.22*	170±0.20	165±0.20*
	IV	750	164±0.15	184±0.18*	169±0.18*	159±0.16*

Values are expressed as Mean±SEM. n=5 *P<0.05 significantly different compared to control. Mean values without * are not significant compared to control.
Pmf $_{inf.eth}$—Ethanol extract of infected *Panicum maximum*

Table 7b: Body weight variation of rats during three weeks of administration of Chloroform extract of infected *P.maximum* florets.

Group/Treatment		Dose (mg/kg)	Day1	Day7	Day14	Day21
Control (0.5%Tween 80)		0.5ml	146+0.01	172±0.01	172±0.03	180±0.02
Pmf inf chl.	I	100	152±0.22	172±0.62	188±0.22*	180±0.18
	II	250	160±0.16	178±0.18	180±0.18*	168±0.15*
	III	500	168±0.15	188±0.22*	171±0.20	164±0.20*
	IV	750	164±0.15	184±0.1*8	167±0.18*	160±0.16*

Values are expressed as Mean±SEM. n=5 *P<0.05 significantly different compared to control. Mean values without * are not significant compared to control.
Pmf $_{inf.chl.}$—Chloroform extract of infected *Panicum maximum*

Table 8a: Effect of Ethanol extract of infected *P.maximum* florets on organ weight control and treated rats after three weeks of administration.

Organ	Control(ml)	Infected Ethanol extract(mg/kg)			
	0.5ml (0.5%Tween80)	**100**	**250**	**500**	**750**
Heart	0.15 ± 0.02	0.72±0.02*	0.71±0.07*	0.70±0.02*	0.61±0.05*
Kidney	1.03±0.32	1.99±0.43*	1.02±0.43	1.17±0.43	0.41±0.13*
Liver	6.54±0.57	8.54±0.54*	7.56±0.56*	6.69+0.20	7.41±0.06*
Brain	1.56±0.49	1.53±0.08	1.67±0.68*	1.73+0.75*	1.18±0.62
Uterus	0.14±0.20	0.14±0.01	0.21±0.18*	0.16+0.18	0.12±0.16
Ovary	0.15±0.13	0.14±0.02	0.15±0.14	0.18+0.16*	0.13±0.13*

Values are expressed as Mean ±SEM n=5 control received 0.5ml of 2% Tween 80
***P< 0.05 significantly different compared to control. Means without * are not significantly different compared to control**

Table 8b: Effect of Chloroform extract of infected *P.maximum* florets on organ weight of control and treated rats after three weeks administration.

Organ	Control(ml)	Infected Chloroform extract (mg/kg)			
	0.5 (0.5%Tween80)	100	250	500	750
Heart	0.15 ± 0.02	0.71±0.08*	0.62±0.02*	0.68±0.07*	0.63±0.08*
Kidney	1.03±0.32	1.05±0.24	1.96±0.44*	1.35±0.14*	1.15±0.12
Liver	6.54±0.57	4.77±0..70*	6.88±0.52	7.29±0.57*	5.98±0.46
Brain	1.56±0.49	1.65±0.602	1.68±0.56*	0.55±0.66*	1.72±0.64*
Uterus	0.14±0.20	0.13±0.15	0.16±0.35*	0.26±0.21*	0.12±0.15
Ovary	0.15±0.13	0.17±0.14	0.20±0.13*	0.18±0.13*	0.15±0.14

Values are expressed as Mean±SEM.n=5, **Control received 0.5ml of 0.5% Tween 80**
***P< 0.05 significantly different compared to control. Means without * are not significantly different compared to control**

Table 9a: Effect of ethanol extract of infected *P. maximum* florets on Haematological parameters of rats after three weeks of administration

Parameters	Control(ml)	Infected Ethanol extract (mg/kg)			
	0.5(0.5% Tween80)	100	250	500	750
WBC (mm^3)	9.57+0.23	7.20±0.058	8.23±0.07	5.97±0.09*	9.27±0.09
RBC (mm^3)	6.15±0.07	7.94±0.023*	8.07±0.03*	8.53±0.02*	7.80±0.01*
Hb (g/dL)	11.30±0.32	13.77±0.15*	13.57±0.88*	16.50±0.12*	14.23±0.15*
Packed Cell Volume (%)	40.89±0.60	44.27±0.74*	43.57±0.09*	50.23±0.15*	47.27±0.15*

Values are expressed as Mean±SEM. Control received 0.5ml of 2% Tween 80
***P< 0.05 significantly different compared to control. Means without * are not significantly different compared to control**

WBC = White blood cell
RBC = Red blood cell
Hb = Haemoglobin

Table 9b: Effect of chloroform extract of infected *P. maximum* florets on Haematological parameters of rats after three weeks of administration.

Parameters	Control(ml)	Infected Chloroform extract (mg/kg)			
	0.5 (2% Tween80)	100	250	500	750
WBC(mm^3)	9.57+0.23	6.77±0.09*	8.17±0.09*	11.10±0.06*	13.17±0.12*
RBC(mm^3)	6.14±0.07	7.22±0.12*	7.84±0.023*	8.12±0.012*	7.86±0.032*
Hb(g/dL)	11.30±0.32	12.97±0.09*	13.50±0.15*	14.50±0.02*	14.80±0.12*
Packed Cell Volume (%)	40.89±0.60	30.30±0.12*	30.43±0.12*	31.13±0.09*	30.37±0.32*

Values are expressed as Mean±SEM,.n=5 **Control received 0.5ml of 2% Tween 80**
***P< 0.05 significantly differently different compared to control. Means without *are not significantly different compared to control**

WBC = White blood cell
RBC = Red blood cell
H b = Haemoglobin

Table 10a: Effect of Ethanol extract of infected *P.maximum* florets on Biochemical Profile of rats after three weeks of administration

Parameters	**Control (ml)**	**Infected Ethanol extract (mg/kg)**			
	0.5(0.5%Tween 80)	**100**	**250**	**500**	**750**
AST(i.u/L)	224.50±0.22	156.40±0.90*	138.20±9.01*	176.00±3.35*	224.40±18.62
ALP(i.u/L)	314.60±0.19	79.72±0.31*	84.02±0.74*	99.30±1.24*	145.4±2.51*
ALT (i.u/L)	101.90±0.58	76.40±1.19*	58.00±4.42*	43.47±2.03*	223.73±3.45*
T. BIL (mg/L)	0.52±0.02	0.52±0.01	0.52±0.01	0.75±0.13	0.51±0.03
Creatinine (mg/L)	45.15±0.10	44.00±1.06	44.01±0.96	24.51±6.79*	46.16±0.86*
Urea(mg/dL)	10.97±0.15	7.53±0.47*	8.07±0.18*	9.43±0.33*	10.00±0.12
Albulmin(g/dL)	33.83±0.20	40.63±0.09*	38.60±0.07*	38.80±1.29*	31.53±0.77*
T.Protein (g/dL)	83.03±0.58	83.43±0.18	79.00±2.84	73.30±2.84	66.55±3.84*
HDL-chol. (mg/dL)	0.90±0.58	0.90±0.58	0.97±0.09	1.00±0.06	0.43±0.04*
LDL-chol.(mg/dL)	0.25±0.03	0.23±0.01	0.20±0.03	0.23±0.01	0.22±0.04
CHOL (mg/dL)	1.52±0.02	1.15±0.00*	1.28±0.01*	1.31±0.01*	1.30±0.01*
Triglycende (mg/dL)	0.66±0.02	0.55±0.01	0.90±0.01*	0.90±0.01*	0.73±0.01*

Values are expressed as Mean±SEM.*P<0.05 significantly different compared to control. Means without *are not significantly different compared to control

AST: Aspartate aminotransferase, ALP: Alkaline phosphate, ALT: alanine amino transferase, HDL-chol: high -density lipoprotein cholesterol, LDL-chol: low-density lipoprotein cholesterol;T.BIL-Total bilirubin; T.protein-Total protein.

Table 10b: Effect of Chloroform extract of *P.maximum* florets on Biochemical Profile of Rats after three weeks of administration

Parameters	**Control(ml**	**Infected Chloroform extract(mg/kg)**			
	0.5 (2%Tween80)	**100**	**250**	**500**	**750**
AST(i.u/L)	224.5±0.22	157.1±4.12*	187.1±1.78*	144.6±4.65*	223.00±18.00
ALT(i.u/L)	101.97±0.58	52.70±1.21*	55.33±0.24*	95.40±4.98*	68.40±0.06*
ALP(i.u/L)	314.6±0.19	99.60±0.97*	155.6±4.66*	141.1±1.87*	202.8±2.77*
T. BIL(mg/L)	0.52±0.02	0.53±0.01	0.55±0.01	0.74±0.13*	0.63±0.05
Creatinine (mg/L)	45.15±0.10	28.61±1.72*	34.51±1.06*	32.18±10.39*	46.98±1.21
Urea(mg/dL)	10.97±0.15	8.30±0.12*	8.57±0.15*	9.33±0.18*	9.88±0.23
Albulmin(g/dL)	33.83±0.20	40.30±0.05*	37.27±0.40	40.35±0.55*	38.03±0.15*
T.Protein (g/dL)	83.03±0.59	67.90±2.30*	82.77±0.24	75.67±1.56	74.10±0.44*
HDL-chol. (mg/dL)	0.90±0.58	1.23±0.03	1.30±0.06	1.13±0.03	1.07±0.03
LDL-chol. (mg/dL)	0.25±0.03	0.24±0.02	0.42±0.01*	0.21±0.06	0.35±0.01
CHOL (mg/dL)	1.52±0.02	1.37±0.02*	1.61±0.00*	1.28±0.04*	1.33±0.00*
Triglyceride (mg/dL)	0.66±0.02	0.54±0.05*	0.47±0.01*	0.39±0.03*	0.76±0.01*

Values are expressed as Mean±SEM*P<0.05 significantly different compared to control. Means without * are not significantly different compared to control.
AST: Aspartate aminotransferase, ALP: Alkaline phosphate, ALT: alanine amino transferase, HDL-chol: high -density lipoprotein cholesterol, LDL-chol: low-density lipoprotein cholesterol; T.BIL-Total bilirubin; T.protein-Total protein.

Histopathological study of the rats treated with ethanol and chloroform extracts of infected *Panicum maximum,* showed some adverse alteration in the anatomicals architecture of some organs –brain, kidney, liver, ovary and uterus, heart though at varying doses (Plates5-15).

The normal brain showed Neuron cell bodies displayed on a fibrillary background. No Peri neuronal and peri vascular halos or clearing (Virchow Robbins spaces) were seen (Plate6a) while the rats treated with 250mg/ml and 750mg/ml of Infected ethanol extract showed neuron cell bodies displayed on loose fibrillary background. Peri neuronal and peri vascular halos or clearing (Virchow Robbins spaces) are seen. (Plate 6b).

The normal kidney showed normocellular glomerular tufts displayed on a background containing tubules. There is no necrosis (Plate6c) while the rats treated with 250mg/ml infected ethanol extract were not affected. However treatment with extract concentration of 750mg/ml resulted in congestion where the vascular channels within the kidney are distended with blood (Plate 6d).

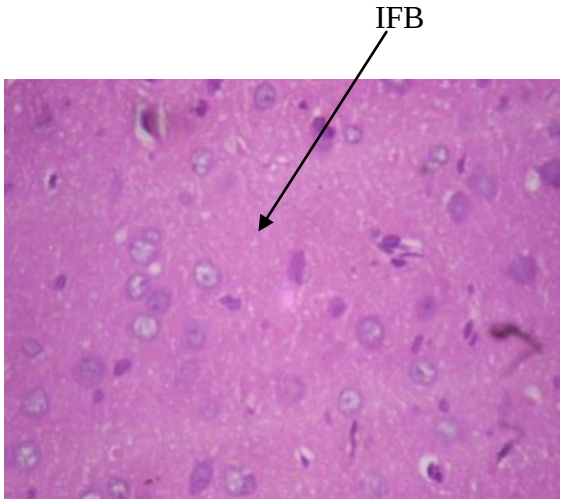
PLATE 6: A-D Photomicrographs of brain and kidney (Haematoxylin and Eosin stain x400)

Plate A: Photomicrograph of brain of a normal rat showing neuron cell bodies (NCB) on fibrillary background (FBG)

Plate B: Photomicrograph of brain of a rat treated with a high dose of infected ethanol extract showing loose fibrillary background (LFB) and distended blood vessel (CBV), and Perivascular halos (PVH)

Plate C: Photomicrograph of kidney of a normal rat showing cellular glomerular tufts (G) with tubules (T)

Plate D: Photomicrograph of kidney of rat treated with high dose of ethanol extract showing blood vessel distended with blood (congestion) (C).



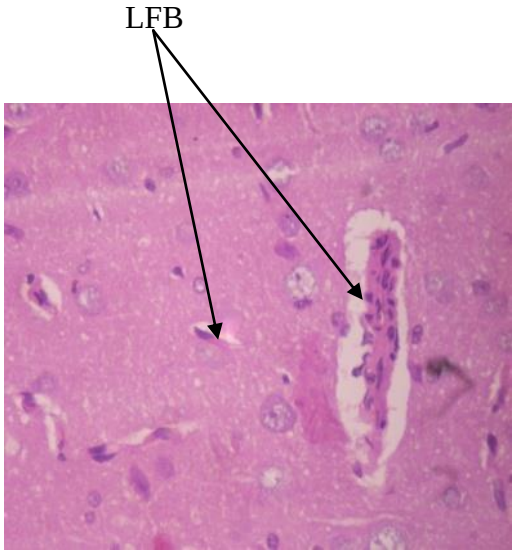
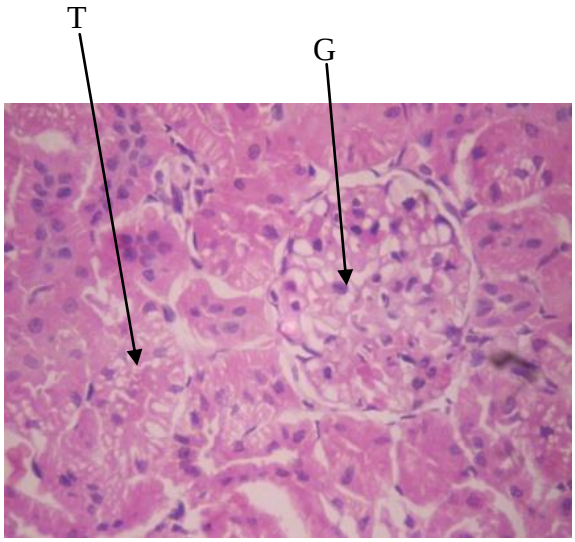


Plate6A: **Photomicrograph of brain of a normal rat showing neuron cell bodies fibrillary background(IFB)**

Plate6B:Photomicrograph of brain of rat treated with 750mg of ethanol extract on intact showing loose fibrillary background



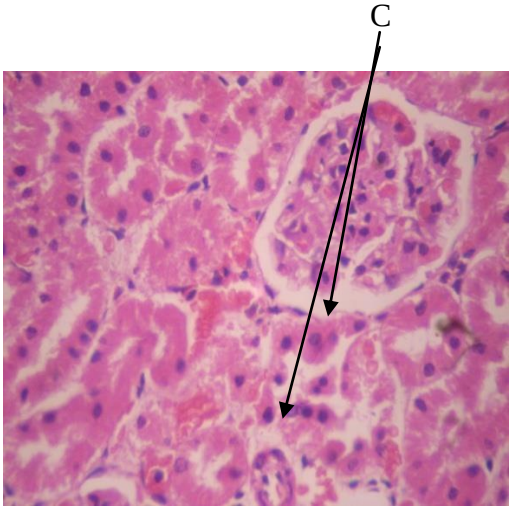


Plate6C: Photomicrograph of kidney control group showing glomerulus and tubules

Plate6D: Photomicrograph of kidney of of rat treated with high doses of Ethanol extract showing congestion

The healthy liver showed hepatocytes arranged as parallel plates, and extend from the portal tracts to the central veins. The sinusoids are not congested. No areas of necrosis hemorrhage or inflammation is seen. No intracellular inclusions are present (Plate7a).But the treated rats with 250mg/ml infected ethanol extract showed mild sinusoidal congestion where the sinusoids are engorged with blood. Treatment with 750mg/ml of these extract caused severe congestion (Plate 7b).

The healthy ovary showed follicles at various stages of development as well as corpora lutea, displayed on a fibro cellular stroma (Plate 7c).The rats treated with 250mg/ml and 750mg/ml of infected ethanol extract showed proliferation of follicles at various stages of development as well as hyperplastic corpora lutea, displayed on a fibrocellular stroma (Plate 7d) The normal Uterus showed myometrial wall composed of smooth muscle cells in parallel array (Plate8a).The rats treated with 250mg/ml and 750mg/ml of infected ethanol extract showed myometrial wall composed of smooth muscle cells disposed in interlacing fashion. The wall is thickened, and there is both hyperplasia and hypertrophy of muscle fibres (Plate 8b).The normal heart showed cardiac myocytes arranged in interlacing and parallel array. Their nuclei are spindle shaped and elongated .This organ was not affected by the extracts (Plate 8c & d).

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PLATE 7A-D: Photomicrograph of liver and ovary (Haematoxylin and Eosin stain x400)

Plate A: Photomicrograph of liver of a normal rat showing normal hepatocytes (H) bounded by portal tracts, no congestion of sinusoids, no necrosis nor degeneration.

Plate B: Photomicrograph of liver treated with highest dose of ethanol extract showing severe sinusoidal congestion (SC)

Plate C: Photomicrograph of ovary of a normal rat showing follicles (F) and corpora lutea (CL) displayed on fibro cellular stroma

Plate D: Photomicrograph of ovary of rat treated with low and high doses of ethanol and chloroform extracts showed proliferation of follicles and hyperplastic corpora lutea (HCL)

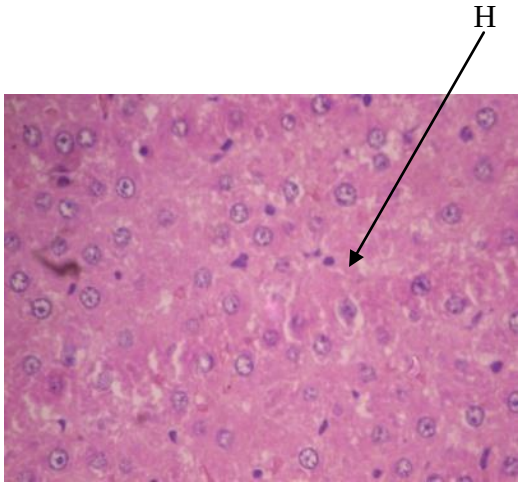


Plate7A: Photomicrograph of liver of control group showing normal hepatocytes with out congestion

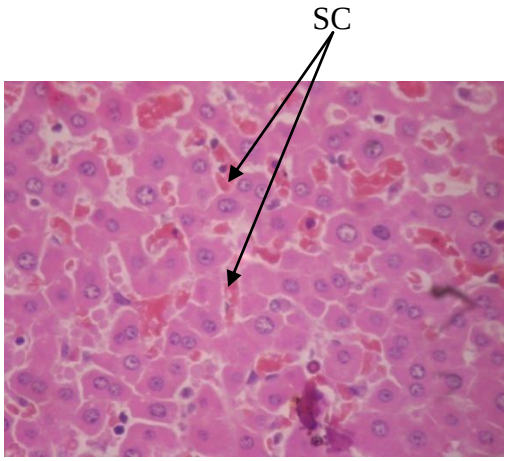


Plate7B:Photomicrograph of liver of treated group(high dose of ethanol extract) showing sinusoidal congestion

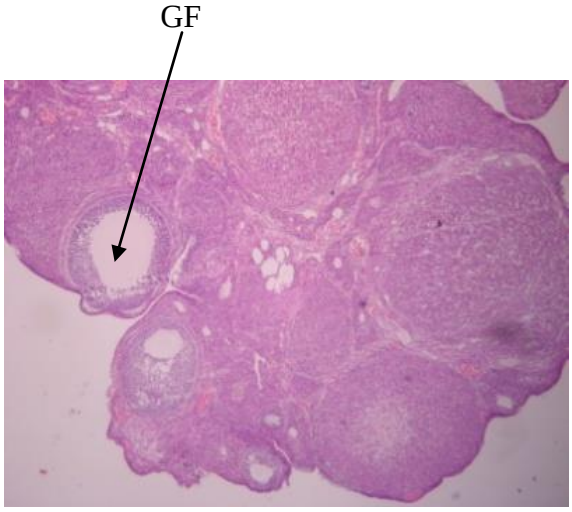


Plate7C:Photomicrograph of ovary of control group showing Graffian Follicles

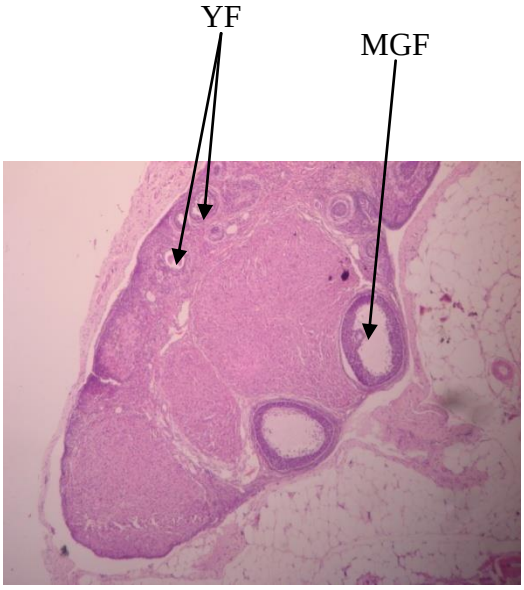


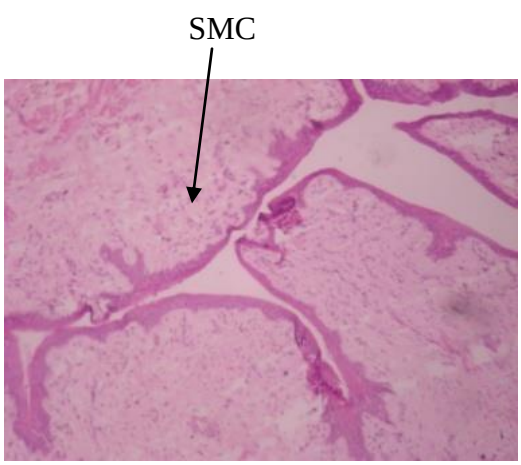
Plate7D:Photomicrograph of ovary of treated group showing proliferation of follicles

PLATE 8A-C: Photomicrograph of uterus and heart (Haematoxylin and Eosin stain x400)

Plate A: Photomicrograph of uterus of a normal rat showing smooth muscle cells in parallel array (SMC)

Plate B: Photomicrograph of uterus of rat treated with low and high doses of ethanol and chloroform extract showing wall hypertrophy and hyperplasia of muscle fibres and interlacing smooth muscles cells.

Plate C&D: Photomicrograph of heart of normal and treated rat showing cardiac myocytes (CM) arranged in interlacing parallel array. It was not affected by the extracts.



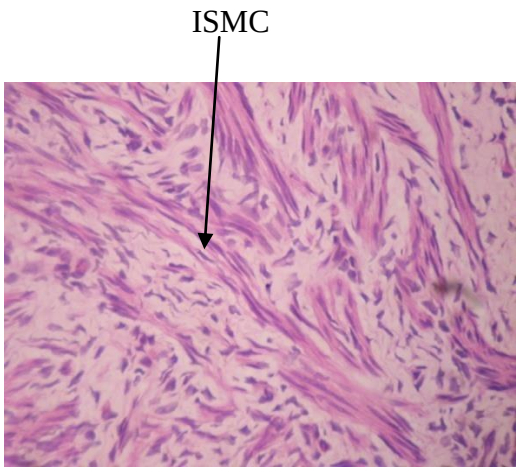
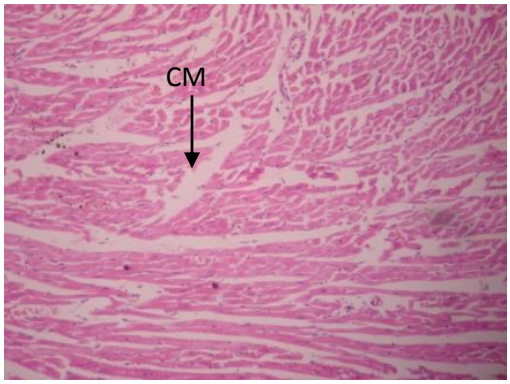


Plate8A:Photomicrograph of uterus of control group with smooth muscles

Plate8B: Photomicrograph of uterus of treated group showing thickened uterine muscles



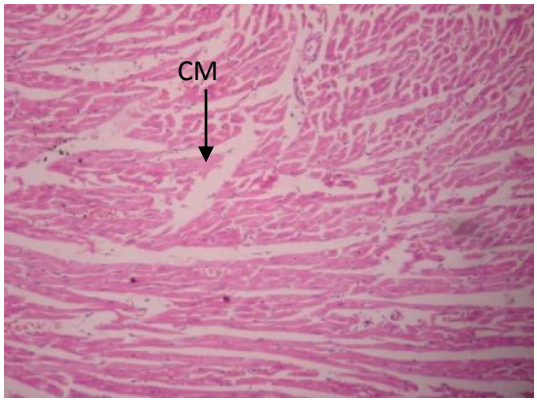


Plate8C: Photomicrograph of heart of control group showing cardiac myocytes

Plate8D: Photomicrograph of heart of treated group showing normal cardiac myocytes

CHAPTER FIVE

5.0 DISCUSSION

In this study, the high disease incidence observed in the three sites from June – November in Nigeria during the four year duration of the study (2007-2010) could be as a result of presence of adequate environmental factors such as rainfall, relative humidity and temperature in those sites during the months of study. These factors may have influenced disease development in the field.

This agrees with the finding of Arohkesi (1997) who reported high incidence of the disease on *Panicum maximum* floret from June to November. Several workers have reported influence of disease incidence in the field by environmental factors. Bock, (1964) reported temperature of 13^{o}C as optimum for production of infection hyphae, promycelia and sporidia in sugar cane smut disease in Kenya. There was also increase in the severity of this disease in the presence of rainfall and high temperature. Maximum smut development was also recorded to occur at 23^{o}C temperature with reduction at 15^{o}C and much lower temperature (Waller 1969; Dean, 1969; Bockhout *et al*., 2009). Adejumo and Ikotun (2003) in their study on appearance of leaf smut in cowpea plant in the field concluded that the incidence was high at rainfall of 5.50 – 103.00mm and progressed under 35-54% relative humidity. However, the significant smut incidence in Ikorodu site when compared to Akoka and Isolo sites (non-significant smut incidence), may be attributed to the nature of ecosystem in these sites. Ikorodu site (Ewuelepe village) is in rural area with stable and undisturbed ecosystem while Akoka and Isolo sites were in the city (urban) with unstable ecosystem. Furthermore, it was observed that the pathogenecity plot with covered inoculated florets smutted while the uncovered florets did not smut probably due to difference in microclimate created.

The smut symptoms observed in this study were similar to the smut diseases of *Panicum maximum* inflorescence observed by Tar (1955) and Arohkesi; (1997). Some florets on the panicle were smutted and these appeared pinkish, larger than normal florets and emitted grey spores from the infected ovary. This colour change was probably due to loss and/ or alterations in plant pigments. Singh (1978) suggested that pigment changes occurred in plant organs during disease and physiological processes which resulted in colour change in the affected organs. Esele, (1995) in his work on foliar and head diseases of sorghum, reported series of colour

change in foliar and head of the infected sorghum which could be a response to the presence of pathogen in the plant. Alteration of physiological processes of infected plant especially photosynthesis where the chloroplast was destroyed has been reported by Mehrotra and Aggarwal (2003). This also agrees with the findings of Petit *et al.* (2006) whose study on alteration of physiology in grapevine affected by Esca disease showed that foliar symptoms were associated with both stomatal closure and alteration of photosynthetic apparatus.

The increase in size of the ovary of the infected floret could be due to the presence of spores (teliospores, sterile cells, globose and "Y" shaped conidia) which replaced ovarian contents. This replacement of ovarian contents has been reported by several workers. Abdalla and El-Tayeb (2009) reported colonization of cotton balls between first and second week following flower opening under Sudanese field conditions. The balls are made up of spores of the pathogen. Chandra and Huff (2008) found that ovaries of buffalo grass flowers became sterile because their contents were replaced with teliospores to form "spore balls". Komolong *et al.* (2003) in their study of sorghum ovary colonization by *Claviceps africana* observed that the diameter of the infected ovaries were 1.5 – 2 times larger than those free from the pathogen. Ingold (1987) has the same view.

Presence of balls of conidia especially "Y" shaped ones, sterile cells and teliospores is a unique morphology peculiar to *Tilletia ayresii* (Berk). This character is however uncommon to other species of *Tilletia* such as *T. tumefasciens* H sydow & sydow which infects *Panicum antidotale,Tilletia indica* and *T.controversa* which infect wheat (Trione,1982; Smilanick *et al.,* 1994; Bonde *et al.,*1999).

Nine genera from thirteen fungal species were isolated from infected *Panicum maximum* floret collected from five sampling sites (Akoka, Ikorodu, Isolo, Ifo and Enugu) studied. The genera include *Aspergillus, Curvularia, Fusarium, Mucor, Paeciliomyces, Penicillium, Syncephalastrum and Tillietia* species. *Tilletia ayresii* which was isolated from intact sori was found to be responsible for the loose smut of *Panicum maximum* florets. This agrees with the findings of Vanky and Bauer (1992), Arohkesi (1997), who reported that *Tilletia ayresii* incited smut in *Panicum maximum* florets. It was observed that *Aspergillus niger, A.fumigatus and Tilletia* ayresii were the most frequent in occurrence. These isolates could be regarded as commensals or associated fungi. Abundance is the term chosen for the average specimen of species group occurring in a unit area (Cooke, 1979). According to Kalamee (1971), it is not correct to treat fungi group in biocoenoses as independents communities. He suggested that the role of fungal grouping in biocoenoses can best be characterized as consortia that are functional structural elements, the relationships being largely trophic or nutritional. From a syneaological point of view, no direct relationship seems to exist between fauna and micro-population. Abundance could be a response to environmental and intrinsic factors. This is also supported by Cooke (1979).

This present report shows that *Tilletia ayresii* (Berk) infected healthy *Panicum maximum* floret while the other 12 isolates were non-pathogenic. Artificial inoculation of inoculum from spore mass of this organism caused smuts on healthy *Panicum maximum* florets. This pathogenic effect of *Tilletia ayresii* agrees with the report of Arohkesi (1997). Angos *et al*. (2003) who in their evaluation of genetic resistance of *Tilletia ayresii* in *P. maximum* stated that smut caused by these organism substantially reduced seed production of *P. maximum* due to high incidence and severity in the inflorescence of the diseased plant. The panicles or florets covered with sterile

polythene bags after inoculation were easily susceptible to the disease. This was probably because of favorable microclimate created by covering of the inoculated florets. This view was also reported by Whitney (1989) in pathogenicity studies of *Tilletia barclayana*, the causal organism of kernel smut of *Oryza sativa* (rice). The plot with uncovered inoculated florets showed no sign of smut. This could be due to lack of microclimate which was created in the covered florets. Furthermore, some of the spores in the inoculum may have been blown or washed away from the florets thus preventing germination and penetration of the spores on the stigma and style of the floret which are two important stages of disease development. Agrios (1997) reported earlier that *Ustilago tritici* infects wheat by first germinating on stigma and penetration through the style and then to inner parts of the plant where infection is established.

Mehrotra and Aggarwal (2003) are of the opinion that the process of pathogenesis which is chain of events by which disease development takes place involves spore germination and growth (prepenetration), entry of germ tube (penetration) and intercellular or intracellular ramification of the host by the pathogen (colonization). Each stage of the event precedes the other. Furthermore, host penetration and infection is often determined by a complex of interacting factors such as temperature, relative humidity, moisture, susceptibility of plant tissues and so on. Arohkesi (1997) also got similar result after inoculating teliospore suspension obtained from intact smutted florets on healthy florets and covering with sterile polythene bags.

Maximal radial growth of most of the isolates obtained on potato dextrose agar (PDA) and malt extract agar (MEA) could be as a result of high nitrogen and carbon content in PDA and MEA. This agrees with the findings of Ibrahim *et al.* (2002) who reported that growth medium with rich sources of carbon and nitrogen can provide optimal growth of microorganisms. They also

observed that the rapid germination of fungal spores was linked to the high protein content of the inoculums. Khattab (2006) stated that optimal pH and temperature for growing most microorganisms are 5 - 6.5 and 25^{o}C -28^{o}C. High radial growth of *Trichoderma* sp and *S. racemosa* could be due to the ability of these organisms to degrade and absorb the components of the media faster than others. Minimal increases in radial growth diameter of the organisms in water agar may be due to lack of nutrient required for optimal growth.

Growth studies of *Tilletia ayresii* on potato dextrose broth, malt yeast peptone broth and carrot dextrose broth showed that the organism grew in all the three broth media used though at varying rates. This potential to grow on all the media indicates that the species investigated is able to decompose the major part of the substrate. Also, ability of the organism to grow at pH 6.5 shows that it is acid tolerant. Kok *et al*. (1992) reported similar findings. The increase in optical density of the potato dextrose broth indicates high turbidity which suggests increase in population of the organism in the medium. The high optical density on potato dextrose both in comparism with the other two media indicates that *Tilletia ayresii* degraded the components of the medium and absorbed them much more rapidly than others. Potato dextrose broth is a good carbon source due to the glucose content for growth of fungal organisms. In their studies on screening of nutritional parameters for production of protease from *Aspergillus oryza,* they reported that glucose is the best source of carbon for fungal growth and thus may be the reason for more sporulation of the organism on it.

The aqueous, ethanol and chloroform extracts of healthy *Panicum maximum* caused significant reduction in the force and frequency of contraction. Several workers have reported relaxation of uterine muscles by some plant extracts. Kamanji and Etta (1992) reported that aqueous extracts

of *Carica papaya* simulteonously inhibited contraction. Olatunji-bello *et al.* (2000) also reported that ethanol and chloroform extracts of *Cajanus cajan* produced relaxation on the uterine muscles. It is known that contraction induced by agonists is mainly due to calcium influx through the voltage-gated calcium channels opened directly or indirectly by agonist receptor (Ruttner *et al.,* 2000; Ruttner *et al.,* 2002). It is thus speculated that the three floret extracts investigated might have inhibited uterine contraction by interrupting with the influx of calcium probably through voltage-gated channel. This action could be due to the presence of uterine relaxants in the healthy florets extracts.

Clinically, drugs used to induce labour contract uterine muscles. Some of these drugs are oxytocin and ergometrine.The use of ergometrine in obstetrics for induction of labour to facilitate delivery of placenta and prevent bleeding after childbirth through vasoconstriction has been reported (Vandongen and Groot, 1995; Hudler, 1998; Boicheuko *et al.,* 2001; Lee, 2009). In the present study different doses of aqueous, ethanol and chloroform extracts of infected *P. maximum* florets produced progressive increase in uterine contraction. Similar pattern of dose-dependent increase in contraction was recorded when ergometrine agonist was tested on spontaneous contraction of uterine muscle.

The extract despite being crude exhibited good potential as an oxytocic agent. Small doses of the extracts increased amplitude of spontaneous uterine contraction while large doses sustained it. The onset of action exhibited by the extracts could merely reflect the high concentration of active compound present. However, ethanol extract showed higher contraction than the aqueous and chloroform. This could be due to its ability to extract more active component than others.This result suggests the presence of ergometrine-like substances in the crude infected *Panicum*

maximum extract. Furthermore, a production of a similar pattern of contraction on uterine muscles when ethanol fraction was tested on unstimulated and ergometrine-stimulated uterine muscles is an indication that the fraction contains an active component similar to that of ergometrine. The presence of three zones detected on TLC plate suggests presence of three components in the pure fraction.

Phytochemical screening of healthy and infected *Panicum maximum* florets extracts (Table5) showed the presence of alkaloid, tannin, saponin and flavonoid in varying quantities. Alkaloids have been credited with wide fascinating pharmacological activities, and hence its been the source of several drugs. Studies have shown that ergot alkaloids are used for prevention of bleeding after child birth, treatment of migraine and other types of headache, hypertension particularly in aged patients, Parkinson's disease and mammary carcinoma (breast cancer). Kubmarawa *et al.,* (2007) reported its importance in formulation of various antibiotics used in treating common pathogenic strains. Furthermore they have been found to possess antimicrobial activity against organism such as *Klebsielle preumonia, Mycobacterium sinegmatis* and *Candida albican* (Addae-Mensah, 1992).

Saponins have been credited with many health benefits. Studies have shown that saponins have beneficial effects on blood cholesterol level, cancer, bone health, sexual function and stimulation of immune system (Rao and Sung, 1995). Saponin–containing plant extracts have protective activity against human diseases (Plock *et al.* 2001). Tannins have protein precipitating and vasoconstriction (astringent) effect and could be advantageous in preventing ulcer development (Aguwa and Nwankwo, 1985).

Growth inhibition of all the fungal isolates except *Tilletia ayresii* by crude ethanol and chloroform extracts suggests that there is a substance in the plant which enhanced the growth of this organism. However the reason for the inhibition of other organisms was not investigated in this study. Furthermore, inhibition of *Tilletia ayresii* by the orthodox plant fungicide (Benlate) and not by animal fungicide (keta conazole) suggests strongly that it is a plant pathogen. Benlate can therefore be used for the control of loose smut disease incited by this organism.

In the acute toxicity study of the ethanol and chloroform extracts of infected *Panicum maximum* florets, the fact that animals did not convulse before dying is an indication that the extracts did not have any toxic effect on the nervous system of treated mice (Ogwai-Okeng *et al.* 2003). According to Prascae (1983), Ghosh (1984) and Klassan *et al.* (1995), the extracts can be classified as being slightly toxic since the LD_{50} was found to be between 5-15.0g/kg translating to 630g equivalent dose in a 70kg human adult. This is very high value making the extract relatively safe for use. However, the lethality at higher doses of the extracts observed in this study is similar to the earlier observations of RosaKutttyl *et al*. (2010).

Significant ($p<0.05$) increase in weights of treated rats as the dose increased up to 500mg/kg and a significant ($p<0.05$) reduction at the highest dose of 750mg/kg could be interpreted as the ability of the infected *Panicum maximum* florets extracts to stimulate appetite of the animals at the onset of their feeding on them and when the toxic effects accumulated at the highest dose, there was rejection of food and thus weight reduction. These could be as a result of saponin in the extract whose bitter taste reduced the palatability of the extract and thus its intake. Shi *et al.*, (2004) reported similar effect of saponin on immune system. Oyewole *et al.* (2007) suggested that decrease in body weight could be due to effect of extracts on internal organs or general

discomfort which led to low feeding rate in the treated animals. Furthermore in rodents, a decrease in food and water consumption is an important sign of deterioration or an indicator of poor health which generally results in loss of body weight (Ullman– Cullere and FoHz, 1999). Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Hilahy *et al.* 2004). Arohkesi, (1997) also reported reduction in body weight of guinea pig fed with *Panicum maximum* florets infected with *Tilletia ayresii* at later stage of their feeding on infected grass. This is probably due to persistence sneezing experienced by the animals resulting to rejection of food.

The observed significant increase in red blood cells (RBC), haemoglobin (Hb) and packed cell volume (PCV) suggests that RBC was not lysed by the extract which is often indicated by reduction in RBC (Mahluli, 2003). Increase in Hb might also be due to the increased absorption of iron.

The macroscopic examination of the organs of animals treated with various doses of the extracts did not show any changes in colour compared with the control. Also there were significant changes in some of the organ weights of the animals at varying doses compared with control. This report differs from that of Arohkesi, (1997) who recorded colour change of liver from smooth and maroon to hard and black, pink colour of heart to black. The white blood cells (WBC) serves as scavengers that destroy micro-organisms at infection sites, removing foreign substances and debris that results from dead or injured cells (Miller and Harley, 1996; Guilermino, 1998; Mahluli, 2003). This increase of white blood cells at the highest doses of ethanol and chloroform extracts in the treated animals suggests that the two extracts were not toxic to the immune system at the appropriate doses. The improved immunity might be due to saponin content of the extract which supports the evidence that oral administration of saponin

may stimulate the immune system and increased resistance to disease challenge (Rao and Sung, 1985). An increase in packed cell volume (PCV) indicates that the red blood cell (RBC) and white blood cell (WBC) in the blood were not destroyed by the extracts. The presence of tannins in the extract which binds to protein and carbohydrate which are components of erythrocyte membrane and prevented breakdown of erythrocyte membrane may have contributed to non-lysing of RBC. The increase in PCV is an indication of haemoconcentration which may be due to increased RBC and Hb mass. This result suggests that the ethanol and chloroform extract of infected *Panicum maximum* floret did not have any adverse effect on haemoglobin metabolism since the RBC was not affected. Arohkesi (1997) also reported non-significant ($p>0.05$) effect of infected *P. maximum* floret fed on guinea pig on the haemoconcentration of the animal. Nwimuka et al. (2008) reported similar findings in their studies of effect of aqueous extract of *Mangifera indica* stem bark on haematological parameters of normal albino rats. Furthermore a non significant reduction in WBC at doses 100-500mg/kg body observed in this study was also reported by Ikpi and Nku (2008) in their studies on effect of ethanolic extract of *Dennettia tripetala* fruit on haematological parameters in Wistar rats. Collectively it could be said that the results suggest that despite small differences between groups, the bioactive components of infected ethanol and chloroform extracts can safely be introduced into medicine for drug formulation.

It is known that the liver and kidney play significant roles in various metabolic processes. The liver play important role in xenobiotic function and the kidneys are the main organs involved in drug elimination and therefore, particularly exposed to the toxic effect of exogenous compounds (Bidhe and Ghosh, 2004). The transaminases (AST and ALT) are useful enzymes as biomarkers predicting possible toxicity (Rahman *et al.,* 2001, Ojiako and Nwanyo, 2006). These are two

liver enzymes that are associated to the hepaticellular damage. Although both are common liver enzymes because of their higher concentrations in hepatocytes, only ALT is remarkably specific for liver function since AST is mostly present in myocardium, skeletal muscle, brain and kidneys (McLintyre *et al*., 1987; Witthawaskul *et al.,* 2003). Due to the fact that ALT is located only in the cytoplasm, serum levels tend to be relatively higher than AST, as a result of membrane leakage from the hepatocyctes. Any damage to the parenchymal liver cells will result in elevation in both of these transaminases. On the other hand, AST found in the serum is of both mitochondria and cytoplasmic origin and if it is raised, that can be taken as a first sign of cell damage that lead to the outflow of the enzymes into the serum. The higher the activity of AST, the larger the infarction size. A mild elevation of AST levels has been shown to be associated with liver injury or myocardial infarctions serum (Mahluli, 2003). It can be seen from the result of biochemical analysis obtained in this study that serum AIT and AST levels were lower at doses of 100mg/kg to 500mg/kg body weight of the two extracts but increased significantly ($p<0.05$) at the highest dose (750mg/kg b.wt). This suggests that the extracts may be nephrotoxic at higher doses. This view was similarly reported by Ogbonnia *et al.* (2008). These result also seem to suggest that at higher doses, the infected extracts may adversely affect the cell mitochondria. The reduction in total protein though non-significantly ($p>0.05$) in the treated groups especially at highest dose is an indication of impaired renal function especially the renal filtration mechanism (Wasan *et al.* 2001). Furthermore as there was reduction in the levels of transaminases ALT and creatinine, there was also no significant effect on total bilirubin in the treated groups. This result suggests that liver and kidneys in the treated animals are in good condition (Hilaly *et al.* 2004). Significant ($p<0.05$) reduction of ALP also occurred in the groups

treated with the two extracts as the doses increased thereby strongly indicating non-hepatotoxic effect of the extracts.

There were reduction in LDL-cholesterol and HDL-cholesterol levels of treated groups compared to control indicating reduction in cardiovascular risk factor. Non-significant effect of extracts on HDL could be due to the presence of polyphenols (flavonoids and tannins) in the extracts which have numerous health benefits including lowering of blood lipids. Ogbonnia *et al.* (2008) reported similar findings in their evaluation of subacute toxicity of *Alstonia congensis* and *Xylopia aethopica* fruits mixtures used in treating diabetes. A significant decrease in plasma total cholesterol level might be due to the presence of hypolipidermic agents in the extracts while the increase in the triglyceride levels (TG) could be secondary to a variety of disorders that might be induced by the extracts (Ellefson and Caraway, 1982).

Histopathological studies of six organs (brain, kidney, liver, ovary, uterus, heart) in the treated rats showed that some of the organs were affected in different ways. Low dose administration of the two extracts resulted to mild sinusoidal congestion which did not affect the function of the liver. The transaminases (ALT and AST) remained low since the liver metabolism was not affected at these doses. An increase in the liver function enzymes on administration of higher doses of the extract shows that the liver function was affected at this dose. Arohkesi,(1997) reported loss of liver lobular radiation ,obliterations of the sinusoids and agglutination of cells with clumps of blood after feeding the guinea pigs with *T. ayresii* infected *Panicum maximum*.This could be as a result of haemorrhage in the liver. The kidney congestion only at highest dose of the extract is a sign of renal impairment which could be the reason for the low protein in the blood serum. However low level of urea and creatinine shows that the kidney

metabolism was not affected. The brain also had oedema and congestion only at higher doses of the extract. The heart was not affected. These pathological effects of the extracts suggest that the extracts contains some toxic substances in addition to the phytochemical substances which caused these changes at high doses of the extract where it was assumed that the toxic substances was more. This is strongly substantiated by the fact that the animals fed with healthy extracts which also contains some quantity of phytochemical constituents did not affect the anatomical structures.

Increase in the surface follicles in the ovary could be attributed to high level of estrogen-like substances present in the infected extracts. This also reflected in the slight increase in the weight of ovary and uterus. Several workers have reported similar findings. Murphy and Hendrich, (1998); Mehrotra and Umashanker (2004); Thakur *et al.* (2009) reported that increase in the weight of ovary and uterus indicates high level of estrogen which is also capable of causing thickening of the uterine walls due to hyperplasia and hypertrophy of the muscle fibres. Musa *et al.* (2008) reported that estradiol stimulates the growth of uterine linings, causing it to thicken during pre-ovulatory phase of the cycle.

SUMMARY OF FINDINGS

1. Of all the three sampling sites studied for smut disease incidence, Ikorodu had significantly higher incidence of smut disease than Akoka and Isolo. The height of smut disease incidence was between August and November during the four years of study.

2. Thirteen fungal organisms were isolated from smutted *Panicum maximum* floret and identified appropriately.The identity of some of the fungal species (*Fusarium* sp and *Fusarium fujikuroii*) were confirmed through molecular studies. Furthermore the best media for cultivation of all the organisms isolated were potato dextrose agar, malt extract agar and potato dextrose broth.

3. *Tilletia ayresii* was proved to be pathogenic while the other twelve fungal isolates were non-pathogenic.

4. The crude phytochemical compounds present in the infected *Panicum maximum* floret were alkaloids, tannins, saponins and flavonoids but the percentage of alkaloids and tannins was more in ethanol extract of the infected floret. Partial fractionation and purification of ethanol extract fraction revealed the presence of three compounds with R_f values as-0.20, 0.30 and 0. 90. The compound in the zone with R_f 0.20 was active while compounds in the other zones were inactive.

5. The crude aqueous, ethanol and chloroform extracts of infected *P.maximum* floret produced a dose-dependent contraction of uterine muscle with ethanol extract being more potent than others. Pure ethanol fraction produced a similar pattern of contraction with ergometrine stimulated uterine smooth muscles.

6. The ethanol and chloroform extracts of infected *Panicum maximum* florets have high oral dose toxicity safety. They provoked toxic effects on liver and kidney only at high doses but did not affect these organs and heart at low to moderate doses. The extracts of infected floret caused proliferation of ovarian follicles as well as thickening of uterine walls. Haematological and biochemical parameters were not adversely affected by the floret extracts.

CONTRIBUTIONS TO KNOWLEGDE

1. This study is the first report in Nigeria of the isolation of five spore types of *Tilletia ayresii* from infected *Panicum maximum* floret after consultation with fungal culture centres around the world (a major contribution in mycology).

2. Gene sequence of *Fusarium* sp. and *F. fujikuroii* (*Fusarium* organisms) were documented.

3. The purified bioactive compounds (preliminary identified as alkaloid) in infected *Panicum maximum* floret was isolated and structurally elucidated.

4. *Panicum maximum* floret (tropical plant) infected with *T.ayresii* exhibited contractility effect similar to ergometrine (orthodox drug) used to induce contraction and subsequent control of postpartum haemorrhage and so could serve as an alternative to ergometrine.

5. This work showed that exracts from infected *P. maximum* florets have high LD50, indicating safety even at high doses.

SUGGESTION FOR FURTHER WORK

1. Mechanism of action of the crude /purified extracts of infected *Panicum maximum* floret on uterine smooth muscles of rat.

2. Further studies on alkaloids in the healthy *Panicum maximum* florets even though they caused relaxation of the uterine muscles..

3. Hormonal assay of active extracts to ascertain the level of reproductive hormones present.

4. Antimicrobial activity of n-hexane, ethyl acetate and methanol fractions of ethanol extract on human pathogens.

5. Growth studies of *Fusarium* sp.(IMI396990) to enable the naming to specific level.

6. Further bioassay with n-hexane, ethyl acetate and methanol fractions to check for synergistic effect.

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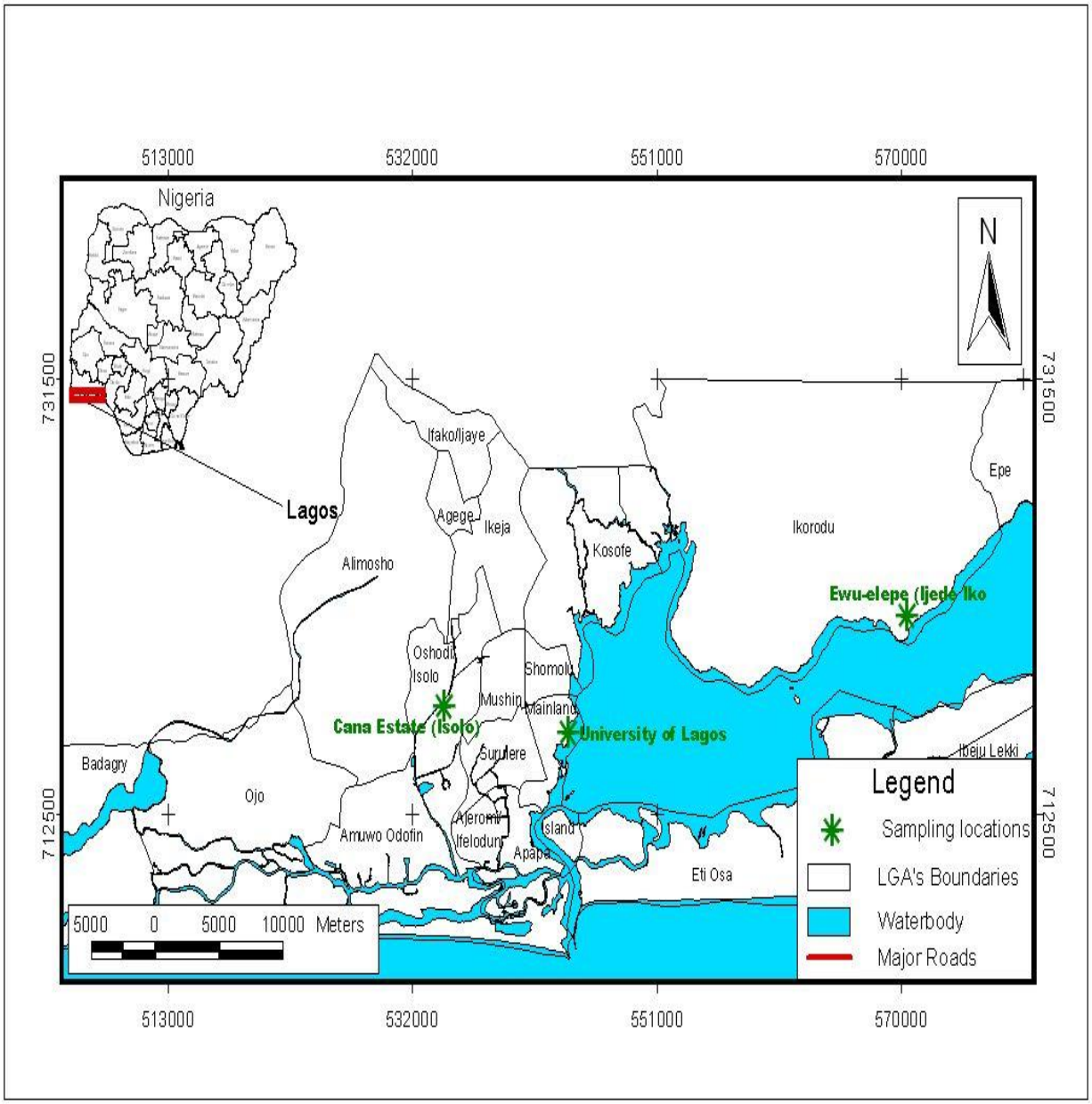
APPENDIX

APPENDIX 1: Map showing the three sites used for smut disease incidence study



APPENDIX 2: RAINFALL AND RELATIVE HUMIDITY DATA OF THE SAMPLED SITES WITHIN FOUR YEARS STUDY OF SMUT DISEASE INCIDENCE IN THE FIELD

STATION	ENVIROMENTAL FACTORS	YEAR	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
ENUGU	**RAINFALL**	**2007**	420.4	104.5	53.2	129.9	98.2	7.1	50.0
		2008	312.0	300.0	200.0	180.0	150.0	152.0	88.0
		2009	300.0	311.00	250.0	230.0	218.0	160.0	100.0
		2010	368.7	131.8	190.6	235.7	300.0	320.2	290.0
LAGOS	**RAINFALL**	**2007**	326.8	329.0	261.9	286.1	265.8	262.0	200.0
		2008	325.0	320.0	251.0	273.0	272.0	250.0	150.0
		2009	312.0	314.0	325.0	270.0	235.0	260.0	200.0
		2010	403.4	132.3	153.2	439.0	320.0	319.0	200.0
IFO	**RAINFALL**	**2007**	324.2	415.3	169.2	176.3	182.2	36.0	50.0
		2008	214.0	162.0	150.0	142.0	141.0	100.0	20.0
		2009	213.7	162.9	56.8	129.6	141.4	88.0	70.0
		2010	51.0	392.9	211.8	206.4	216.2	217.3	205.0

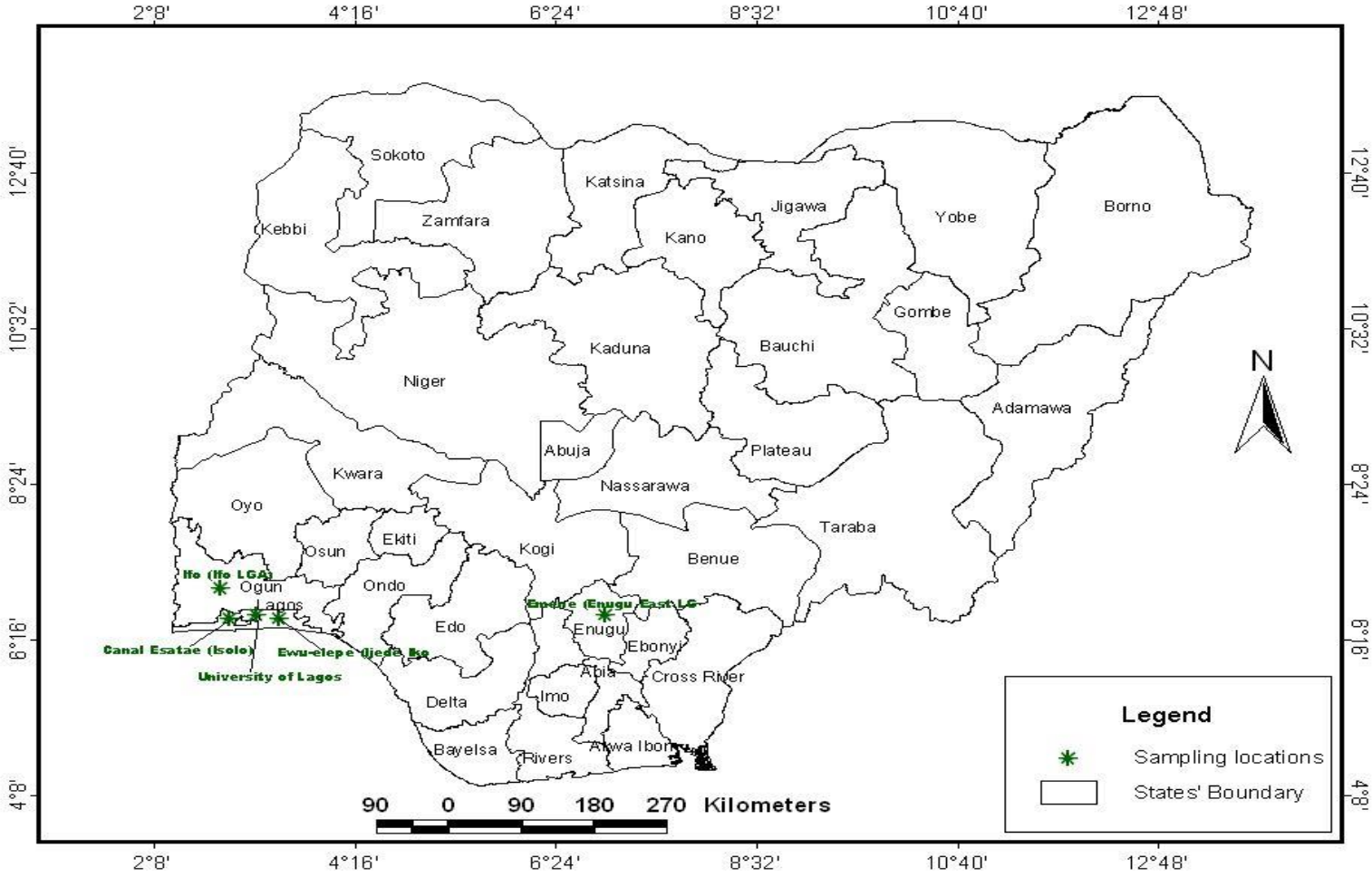
Appendix 2 Contd.

Stations	Environmental factor	Year	June	July	August	September	October	November	December
ENUGU	**RELATIVE HUMIDITY**	**2007**							
		09hrs	83	83	85	86	75	73	70
		15hrs	79	73	76	73	70	65	62
		2008							
		09hrs	86	88	84	84	83	85	87
		15hrs	79	84	80	81	77	74	72
		2009							
		09hrs	84	85	83	84	82	81	86
		15hrs	76	80	79	78	76	72	79
		2010							
		09hrs	87	85	86	86	88	88	87
		15hrs	80	75	77	78	79	81	90
LAGOS	**RELATIVE HUMIDITY**	**2007**							
		09hrs	80	83	85	85	81	73	67
		15hrs	67	70	74	74	64	48	38
		2008							
		09hrs	84	85	84	81	84	76	38
		15hrs	72	73	71	70	50	33	36
		2009				84	80	75	77
		09hrs	84	82	82	65	54	33	54
		15hrs	69	69	71				
		2010							
		09hrs	82	83	82	86	85	79	72
		15hrs	69	92	72	74	70	72	69
IFO	**RELATIVE HUMIDITY**	**2007**							
		09hrs	87	86	86	86	82	79	71
		15hrs	76	76	81	73	64	55	50
		2008							
		09hrs	85	87	88	88	86	82	75
		15hrs	78	75	71	74	57	38	51
		2009							
		09hrs	83	87	87	87	86	86	78
		15hrs	75	71	75	67	56	43	55
		2010							
		09hrs	82	85	86	84	81	80	79
		15hrs	73	74	75	75	70	69	65

APPENDIX 3: SELECTED SAMPLED SITES WITH THEIR GLOBAL POSITION SYSTEMS LOCATION(GPS) COORDINATES

Sampled sites	**Latitude**	**Longitude**	**Specific area sampled in the site**
Akoka (Lagos state)	06^0 30' 774"N	003^0 22' 34"E	University of Lagos
Ikorodu (Lagos state)	06^0 36' 621"N	003^0 33' 640"E	Ewu-elepe (Ijede L. G . A)
Isolo (Lagos state)	06^0 30' 247"N	003^0 19' 165"E	Canal estate (Isolo L. G.A)
Enugu (Enugu state)	06^0 24' 803"N	007^0 30' 092"F	Emene(Enugu east L.G.A)
Ifo (Ogun State)	06^0 48' 716"N	003^0 12' 338"F	Ifo (Ifo L. G.A)

APPENDIX 4: MAP SHOWING SITES USED FOR ISOLATION AND IDENTIFICATION OF FUNGAL ISOLATES



APPENDIX 5: SEQUENCE DATA OF *FUSARIUM FUJIKUROII* (IMI 396989) SHOWING SIGNIFICANT ALIGNMENT WITH OTHER FUNGAL ISOLATES IN GENE BANK

AGTGATGTGTTAATAATAGGATATATAGAACGGAGCAAGAGCGACAACATACCAATGACG

GTGACATAGTAGCGAGGAGTCTCGAACTTCCAGAGAGCAATATCGATGGTGATACCACGCTCACGCTCAGCCTTGAGC
TTGTCAAGAACCCAGGCGTACTTGAAGGAACCCTTACCGAGCTCAGCGGCTTCCTATTGTCGGATGGTTAGTGACTGTT
TGACACGTGACGATGCACTCAATGAGGTTGTGGAATAAAAGAGGGCAAAAAACGCGCCGCTCGAGTGGCGGGGTAAA
TGCCCCACCAAAAAAATTACGGTCGTATCGCAAAATTTTTGGGCTCGAGCGGGGTAGCGGGCACGTTTCGAGTCGTAG
GGAGAAATCGATGGACAAAGGACGCGCGATCGAAGGGAGTGTGACTAACCTTCTCGAACTTCTCGATGGTTCGCTTGT
CGATACCACCGCACTGGTA

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AF160304.1	Fusarium sp. NRRL 25615 translation elongation factor 1 alpha gene, partial cds	826	826	100%	0.0	98%
EF107154.1	Fusarium aywerte isolate F11027 translation elongation factor 1-alpha gene, partial cds	769	769	96%	0.0	97%
EF107153.1	Fusarium aywerte isolate F10108 translation elongation factor 1-alpha gene, partial cds	769	769	96%	0.0	97%
EF107140.1	Fusarium sp. ECYL-2007d isolate F14354 translation elongation factor 1-alpha gene, partial cds	736	736	98%	0.0	95%
EF107139.1	Fusarium sp. ECYL-2007d isolate F14350 translation elongation factor 1-alpha gene, partial cds	736	736	98%	0.0	95%

Appendix 5 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
EF107138.1	Fusarium sp. ECYL-2007d isolate F14357 translation elongation factor 1-alpha gene, partial cds	736	736	98%	0.0	95%
EF107137.1	Fusarium sp. ECYL-2007d isolate F14348 translation elongation factor 1-alpha gene, partial cds	736	736	98%	0.0	95%
AM295810.1	Fusarium sp. IMI 389581 partial tef gene for translation elongation factor 1 alpha, exons 1-4, strain IMI 389581	699	699	93%	0.0	95%
AY662325.1	Gibberella moniliformis strain KSU 12911 translation elongation factor 1a gene, partial cds	686	686	93%	0.0	94%
FJ496268.1	Fusarium sp. MS-493 translation elongation factor 1-alpha gene, partial cds	710	710	96%	0.0	94%
FJ496270.1	Fusarium sp. MS-848 translation elongation factor 1-alpha gene, partial cds	732	732	99%	0.0	94%
EF152427.1	Gibberella moniliformis isolate M-1141 translation elongation factor 1-alpha gene, partial cds	732	732	99%	0.0	94%
EF453022.1	Gibberella moniliformis strain NRRL 43697 translation elongation factor 1-alpha gene, partial cds	732	732	99%	0.0	94%
AM422699.1	Gibberella fujikuroi var. moniliformis partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FvMM2-4	732	732	99%	0.0	94%
AM422698.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM4-1	732	732	99%	0.0	94%
AM422697.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM1-3	732	732	99%	0.0	94%
AM422696.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM1-2	732	732	99%	0.0	94%
AM422695.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM1-1	732	732	99%	0.0	94%

Appendix 5 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AM422693.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM6-1	732	732	99%	0.0	94%
AM422692.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM4-2	732	732	99%	0.0	94%
AM422690.1	Gibberella fujikuroi var. moniliformis partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate M5500	732	732	99%	0.0	94%
AM404155.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, haplotype B	732	732	99%	0.0	94%
AM404154.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, haplotype A	732	732	99%	0.0	94%
AM404150.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv2242	732	732	99%	0.0	94%
AM404147.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv674	732	732	99%	0.0	94%
AM404146.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv2025	732	732	99%	0.0	94%
AM404145.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv2006	732	732	99%	0.0	94%
AM404144.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv1939	732	732	99%	0.0	94%
AM404143.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv1838	732	732	99%	0.0	94%
AM404142.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv1370	732	732	99%	0.0	94%
AM404141.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv1259	732	732	99%	0.0	94%

Appendix 5 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AM404140.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate CB109	732	732	99%	0.0	94%
AM404139.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate A0999	732	732	99%	0.0	94%
AM296115.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fvw86	732	732	99%	0.0	94%
AM296114.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fvo35	732	732	99%	0.0	94%
AM296113.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fvo31	732	732	99%	0.0	94%
AM296112.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fvc23	732	732	99%	0.0	94%
AM296111.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fvc15	732	732	99%	0.0	94%
AM296110.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fvc12	732	732	99%	0.0	94%
AM296109.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fv3a	732	732	99%	0.0	94%
AF160309.1	Fusarium sp. NRRL 26793 translation elongation factor 1 alpha gene, partial cds	732	732	100%	0.0	94%
FJ496271.1	Fusarium sp. MS-433 translation elongation factor 1-alpha gene, partial cds	730	730	99%	0.0	94%
FJ496269.1	Fusarium sp. MS-249 translation elongation factor 1-alpha gene, partial cds	723	723	98%	0.0	94%
FJ496267.1	Fusarium sp. MS-461 translation elongation factor 1-alpha gene, partial cds	723	723	98%	0.0	94%

Appendix 5 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AF160276.1	Fusarium acutatum strain NRRL13308 translation elongation factor 1 alpha gene, partial cds	678	678	92%	0.0	94%
AF160266.1	Fusarium napiforme strain NRRL13604 translation elongation factor 1 alpha gene, partial cds	728	728	100%	0.0	94%
AM422694.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM3-1	726	726	99%	0.0	94%
AM422691.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM6-2	726	726	99%	0.0	94%
AM404157.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, haplotype D	726	726	99%	0.0	94%
AM404156.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, haplotype C	726	726	99%	0.0	94%
AM404149.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv1917	726	726	99%	0.0	94%
AM404148.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Gf2	726	726	99%	0.0	94%
EU220409.1	Gibberella moniliformis isolate MRC826 elongation factor 1 alpha gene, partial sequence	725	725	99%	0.0	94%
AM404138.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv2056	725	725	99%	0.0	94%
EU246586.1	Fusarium oxysporum isolate 1517 translation elongation factor 1-alpha gene, partial cds	723	723	100%	0.0	94%
DQ435353.1	Fusarium oxysporum isolate BW-1517 translation elongation factor 1 alpha gene, partial cds	723	723	100%	0.0	94%
DQ435349.1	Fusarium oxysporum isolate BW-7108 translation elongation factor 1 alpha gene, partial cds	723	723	100%	0.0	94%

Appendix 5 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
EF453009.1	Fusarium sp. NRRL 43682 translation elongation factor 1-alpha gene, partial cds	721	721	99%	0.0	94%
EF452989.1	Fusarium sp. NRRL 43658 translation elongation factor 1-alpha gene, partial cds	721	721	99%	0.0	94%
EF452988.1	Fusarium sp. NRRL 43657 translation elongation factor 1-alpha gene, partial cds	721	721	99%	0.0	94%
EF452987.1	Fusarium sp. NRRL 43656 translation elongation factor 1-alpha gene, partial cds	721	721	99%	0.0	94%
EF452972.1	Gibberella moniliformis strain NRRL 43547 translation elongation factor 1-alpha gene, partial cds	721	721	99%	0.0	94%
AM404153.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Gf16	721	721	99%	0.0	94%
AM404152.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Gf15	721	721	99%	0.0	94%
AM404151.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Gf1	721	721	99%	0.0	94%
AF160267.1	Fusarium ramigenum strain NRRL25208 translation elongation factor 1 alpha gene, partial cds	721	721	100%	0.0	94%
AF273318.1	Gibberella moniliformis strain NRRL28899 translation elongation factor 1 alpha gene, partial cds	721	721	99%	0.0	94%
AF273317.1	Gibberella moniliformis strain NRRL28898 translation elongation factor 1 alpha gene, partial cds	721	721	99%	0.0	94%
AF273316.1	Gibberella moniliformis strain NRRL28897 translation elongation factor 1 alpha gene, partial cds	721	721	99%	0.0	94%
AF273315.1	Gibberella moniliformis strain NRRL28896 translation elongation factor 1 alpha gene, partial cds	721	721	99%	0.0	94%

Appendix 5 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AF273314.1	Gibberella moniliformis strain NRRL28895 translation elongation factor 1 alpha gene, partial cds	721	721	99%	0.0	94%
AF273313.1	Gibberella moniliformis strain NRRL28894 translation elongation factor 1 alpha gene, partial cds	721	721	99%	0.0	94%
AF273312.1	Gibberella moniliformis strain NRRL28893 translation elongation factor 1 alpha gene, partial cds	721	721	99%	0.0	94%
AF160288.1	Fusarium sp. NRRL 28852 translation elongation factor 1 alpha gene, partial cds	680	680	94%	0.0	94%
AY927313.1	Fusarium sp. BVS2005a translation elongation factor 1 alpha gene, partial cds	708	708	98%	0.0	94%
AF160262.1	Gibberella moniliformis strain NRRL22172 translation elongation factor 1 alpha gene, partial cds	717	717	99%	0.0	94%
EU246585.1	Fusarium oxysporum isolate 1537 translation elongation factor 1-alpha gene, partial cds	717	717	100%	0.0	94%
EU246553.1	Fusarium oxysporum isolate 4560 translation elongation factor 1-alpha gene, partial cds	717	717	100%	0.0	94%
EU246552.1	Fusarium oxysporum isolate 4503 translation elongation factor 1-alpha gene, partial cds	717	717	100%	0.0	94%
EU246551.1	Fusarium oxysporum isolate 3613 translation elongation factor 1-alpha gene, partial cds	717	717	100%	0.0	94%
EU246550.1	Fusarium oxysporum isolate 2620 translation elongation factor 1-alpha gene, partial cds	717	717	100%	0.0	94%
DQ435354.1	Fusarium oxysporum isolate BW-1537 translation elongation factor 1 alpha gene, partial cds	717	717	100%	0.0	94%
AF160273.1	Fusarium nygamai strain NRRL13488 translation elongation factor 1 alpha gene, partial cds	717	717	100%	0.0	94%

Appendix 5 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AF160272.1	Fusarium lactis strain NRRL25200 translation elongation factor 1 alpha gene, partial cds	717	717	100%	0.0	94%
EU091074.1	Fusarium subglutinans strain F2 translation elongation factor 1-alpha gene, partial cds	717	717	100%	0.0	94%
EU620633.1	Fusarium andiyazi strain 2001 translation elongation factor 1 alpha (TEF1alpha) gene, partial cds	702	702	98%	0.0	94%
EU620631.1	Fusarium andiyazi strain 2043 translation elongation factor 1 alpha (TEF1alpha) gene, partial cds	702	702	98%	0.0	94%
EU620628.1	Fusarium andiyazi strain 2172 translation elongation factor 1 alpha (TEF1alpha) gene, partial cds	702	702	98%	0.0	94%
DQ837698.1	Fusarium subglutinans isolate BMP1462 translation elongation factor 1 alpha (EF-1alpha) gene, partial cds	712	712	100%	0.0	94%
AF160265.1	Fusarium brevicatenulatum strain NRRL25446 translation elongation factor 1 alpha gene, partial cds	710	710	100%	0.0	94%
AF160271.1	Fusarium pseudocircinatum strain NRRL22946 translation elongation factor 1 alpha gene, partial cds	704	704	99%	0.0	93%
EU620635.1	Fusarium andiyazi strain 1965 translation elongation factor 1 alpha-like (TEF1alpha) gene, partial sequence	697	697	98%	0.0	93%
EU620632.1	Fusarium andiyazi strain 2008 translation elongation factor 1 alpha (TEF1alpha) gene, partial cds	697	697	98%	0.0	93%
EU620626.1	Fusarium andiyazi strain 2221 translation elongation factor 1 alpha (TEF1alpha) gene, partial cds	691	691	98%	0.0	93%
EU620625.1	Fusarium andiyazi strain 2234 translation elongation factor 1 alpha (TEF1alpha) gene, partial cds	691	691	98%	0.0	93%
EU620634.1	Fusarium andiyazi strain 1990 translation elongation factor 1 alpha (TEF1alpha) gene, partial cds	686	686	98%	0.0	93%

Appendix 5 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
EU620630.1	Fusarium andiyazi strain 2102 translation elongation factor 1 alpha (TEF1alpha) gene, partial cds	686	686	98%	0.0	93%
EU620629.1	Fusarium andiyazi strain 2130 translation elongation factor 1 alpha (TEF1alpha) gene, partial cds	686	686	98%	0.0	93%
EU620627.1	Fusarium andiyazi strain 2193 translation elongation factor 1 alpha (TEF1alpha) gene, partial cds	686	686	98%	0.0	93%
AF160268.1	Fusarium sp. NRRL 25221 translation elongation factor 1 alpha gene, partial cds	688	688	98%	0.0	93%

APPENDIX6: SEQUENCE DATA OF *FUSARIUM* SPECIE (IMI 396990) SHOWING SIGNIFICANT ALIGNMENT WITH OTHER FUNGAL ISOLATES IN GENE BANK

AGTGATGTGTTAGTAATAGGATATATAGAACGGAGCAAGAGCGACAACATACCAATGACGGTGACATAGTAGCGAGGAGTCTCGAACTTCCAGAGAGCAATAT
CGATGGTGATACCACGCTCACGCTCGGCCTTGAGCTTGTCAAGAACCCAGGCGTACTTGAAGGAACCCTTACCGAGCTCAGCGGCTTCCTATTGTCGGATGGTTA
GTGACTGTTTGACACGTGACGATGCACTCAATGAGGTTGTGGAATAAAAGAGGGCAAAAAACGCGCTCGAGTGGCGGGGTAAATGCCCCACCAAAAAAAATTA
CGGTCGTATCGCAAAATTTTTGGGCTCGAGCGGGGTAGCGGGCACGTTTCGAGTCGTAGGGAGAAATCGATGGACAAAGGACGCGCGATCGAAGGGAGTGTG
ACTAACCTTCTCGAACTTCTCGATGGTTCGCTTGTCGATACCACCGCACTGGTAGATCAAG

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
Appendix 6 Contd.						
AF160304.1	Fusarium sp. NRRL 25615 translation elongation factor 1 alpha gene, partial cds	870	870	100%	0.0	99%
EF107154.1	Fusarium aywerte isolate F11027 translation elongation factor 1-alpha gene, partial cds	780	780	96%	0.0	97%
EF107153.1	Fusarium aywerte isolate F10108 translation elongation factor 1-alpha	780	780	96%	0.0	97%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
	gene, partial cds					
AM295810.1	Fusarium sp. IMI 389581 partial tef gene for translation elongation factor 1 alpha, exons 1-4, strain IMI 389581	715	715	93%	0.0	95%
EF107140.1	Fusarium sp. ECYL-2007d isolate F14354 translation elongation factor 1-alpha gene, partial cds	741	741	98%	0.0	95%
EF107139.1	Fusarium sp. ECYL-2007d isolate F14350 translation elongation factor 1-alpha gene, partial cds	741	741	98%	0.0	95%
EF107138.1	Fusarium sp. ECYL-2007d isolate F14357 translation elongation factor 1-alpha gene, partial cds	741	741	98%	0.0	95%
EF107137.1	Fusarium sp. ECYL-2007d isolate F14348 translation elongation factor 1-alpha gene, partial cds	741	741	98%	0.0	95%
AM422691.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM6-2	749	749	99%	0.0	95%
AM404157.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, haplotype D	749	749	99%	0.0	95%
AM404149.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation	749	749	99%	0.0	95%

Appendix 6 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
	factor 1-alpha, exons 1-4, isolate Fv1917					
FJ496270.1	Fusarium sp. MS-848 translation elongation factor 1-alpha gene, partial cds	743	743	99%	0.0	94%
EF152427.1	Gibberella moniliformis isolate M-1141 translation elongation factor 1-alpha gene, partial cds	743	743	99%	0.0	94%
EF453022.1	Gibberella moniliformis strain NRRL 43697 translation elongation factor 1-alpha gene, partial cds	743	743	99%	0.0	94%
AM422699.1	Gibberella fujikuroi var. moniliformis partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FvMM2-4	743	743	99%	0.0	94%
AM422698.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM4-1	743	743	99%	0.0	94%
AM422697.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM1-3	743	743	99%	0.0	94%
AM422696.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM1-2	743	743	99%	0.0	94%
AM422695.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM1-1	743	743	99%	0.0	94%

Appendix 6 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AM422693.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM6-1	743	743	99%	0.0	94%
AM422692.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM4-2	743	743	99%	0.0	94%
AM422690.1	Gibberella fujikuroi var. moniliformis partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate M5500	743	743	99%	0.0	94%
AM404155.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, haplotype B	743	743	99%	0.0	94%
AM404154.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, haplotype A	743	743	99%	0.0	94%
AM404150.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv2242	743	743	99%	0.0	94%
AM404147.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv674	743	743	99%	0.0	94%
AM404146.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv2025	743	743	99%	0.0	94%
AM404145.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation	743	743	99%	0.0	94%

Appendix 6 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
	factor 1-alpha, exons 1-4, isolate Fv2006					
AM404144.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv1939	743	743	99%	0.0	94%
AM404143.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv1838	743	743	99%	0.0	94%
AM404142.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv1370	743	743	99%	0.0	94%
AM404141.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Fv1259	743	743	99%	0.0	94%
AM404140.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate CB109	743	743	99%	0.0	94%
AM404139.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate A0999	743	743	99%	0.0	94%
AM296115.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fvw86	743	743	99%	0.0	94%
AM296114.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fvo35	743	743	99%	0.0	94%

Appendix 6 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AM296113.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fvo31	743	743	99%	0.0	94%
AM296112.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fvc23	743	743	99%	0.0	94%
AM296111.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fvc15	743	743	99%	0.0	94%
AM296110.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fvc12	743	743	99%	0.0	94%
AM296109.1	Gibberella moniliformis partial tef-1 gene for elongation factor-1 alpha, exons 1-4, isolate Fv3a	743	743	99%	0.0	94%
FJ496271.1	Fusarium sp. MS-433 translation elongation factor 1-alpha gene, partial cds	741	741	99%	0.0	94%
FJ496269.1	Fusarium sp. MS-249 translation elongation factor 1-alpha gene, partial cds	734	734	98%	0.0	94%
FJ496267.1	Fusarium sp. MS-461 translation elongation factor 1-alpha gene, partial cds	734	734	98%	0.0	94%

Appendix 6 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
FJ496268.1	Fusarium sp. MS-493 translation elongation factor 1-alpha gene, partial cds	715	715	96%	0.0	94%
AM422694.1	Gibberella fujikuroi var. intermedia partial tef-1alpha gene for translation elongation factor 1-alpha, exons 1-4, isolate FpMM3-1	737	737	99%	0.0	94%
AM404156.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, haplotype C	737	737	99%	0.0	94%
AM404148.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Gf2	737	737	99%	0.0	94%
AF160309.1	Fusarium sp. NRRL 26793 translation elongation factor 1 alpha gene, partial cds	737	737	100%	0.0	94%
AF160272.1	Fusarium lactis strain NRRL25200 translation elongation factor 1 alpha gene, partial cds	734	734	100%	0.0	94%

Appendix 6 Contd.

AF160266.1	Fusarium napiforme strain NRRL13604 translation elongation factor 1 alpha gene, partial cds	734	734	100%	0.0	94%
EU091074.1	Fusarium subglutinans strain F2 translation elongation factor 1-alpha gene, partial cds	734	734	100%	0.0	94%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
EF453009.1	Fusarium sp. NRRL 43682 translation elongation factor 1-alpha gene, partial cds	732	732	99%	0.0	94%
EF452989.1	Fusarium sp. NRRL 43658 translation elongation factor 1-alpha gene, partial cds	732	732	99%	0.0	94%
EF452988.1	Fusarium sp. NRRL 43657 translation elongation factor 1-alpha gene, partial cds	732	732	99%	0.0	94%
EF452987.1	Fusarium sp. NRRL 43656 translation elongation factor 1-alpha gene, partial cds	732	732	99%	0.0	94%
EF452972.1	Gibberella moniliformis strain NRRL 43547 translation elongation factor 1-alpha gene, partial cds	732	732	99%	0.0	94%
AM404153.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Gf16	732	732	99%	0.0	94%
AM404152.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Gf15	732	732	99%	0.0	94%
AM404151.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation factor 1-alpha, exons 1-4, isolate Gf1	732	732	99%	0.0	94%

Appendix 6 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AF273318.1	Gibberella moniliformis strain NRRL28899 translation elongation factor 1 alpha gene, partial cds	732	732	99%	0.0	94%
AF273317.1	Gibberella moniliformis strain NRRL28898 translation elongation factor 1 alpha gene, partial cds	732	732	99%	0.0	94%
AF273316.1	Gibberella moniliformis strain NRRL28897 translation elongation factor 1 alpha gene, partial cds	732	732	99%	0.0	94%
AF273315.1	Gibberella moniliformis strain NRRL28896 translation elongation factor 1 alpha gene, partial cds	732	732	99%	0.0	94%
AF273314.1	Gibberella moniliformis strain NRRL28895 translation elongation factor 1 alpha gene, partial cds	732	732	99%	0.0	94%
AF273313.1	Gibberella moniliformis strain NRRL28894 translation elongation factor 1 alpha gene, partial cds	732	732	99%	0.0	94%
AF273312.1	Gibberella moniliformis strain NRRL28893 translation elongation factor 1 alpha gene, partial cds	732	732	99%	0.0	94%
EU220409.1	Gibberella moniliformis isolate MRC826 elongation factor 1 alpha gene, partial sequence	730	730	99%	0.0	94%
AM404138.1	Gibberella fujikuroi var. moniliformis partial tef-1 gene for elongation	730	730	99%	0.0	94%

Appendix 6 Contd.

Appendix 6 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
	factor 1-alpha, exons 1-4, isolate Fv2056					
AF160262.1	Gibberella moniliformis strain NRRL22172 translation elongation factor 1 alpha gene, partial cds	728	728	99%	0.0	94%
EU246586.1	Fusarium oxysporum isolate 1517 translation elongation factor 1-alpha gene, partial cds	728	728	100%	0.0	94%
DQ435353.1	Fusarium oxysporum isolate BW-1517 translation elongation factor 1 alpha gene, partial cds	728	728	100%	0.0	94%
DQ435349.1	Fusarium oxysporum isolate BW-7108 translation elongation factor 1 alpha gene, partial cds	728	728	100%	0.0	94%
DQ837698.1	Fusarium subglutinans isolate BMP1462 translation elongation factor 1 alpha (EF-1alpha) gene, partial cds	728	728	100%	0.0	94%
AF160267.1	Fusarium ramigenum strain NRRL25208 translation elongation factor 1 alpha gene, partial cds	726	726	100%	0.0	94%

Appendix 6 Contd.

AY927313.1	Fusarium sp. BVS2005a translation elongation factor 1 alpha gene, partial cds	713	713	98%	0.0	94%
EU246585.1	Fusarium oxysporum isolate 1537 translation elongation factor 1-alpha gene, partial cds	723	723	100%	0.0	94%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
EU246553.1	Fusarium oxysporum isolate 4560 translation elongation factor 1-alpha gene, partial cds	723	723	100%	0.0	94%
EU246552.1	Fusarium oxysporum isolate 4503 translation elongation factor 1-alpha gene, partial cds	723	723	100%	0.0	94%
EU246551.1	Fusarium oxysporum isolate 3613 translation elongation factor 1-alpha gene, partial cds	723	723	100%	0.0	94%
EU246550.1	Fusarium oxysporum isolate 2620 translation elongation factor 1-alpha gene, partial cds	723	723	100%	0.0	94%
DQ435354.1	Fusarium oxysporum isolate BW-1537 translation elongation factor 1 alpha gene, partial cds	723	723	100%	0.0	94%
AF160273.1	Fusarium nygamai strain NRRL13488 translation elongation factor 1 alpha gene, partial cds	723	723	100%	0.0	94%
EU620633.1	Fusarium andiyazi strain 2001 translation elongation factor 1 alpha (TEF1alpha) gene, partial cds	708	708	98%	0.0	94%
EU620631.1	Fusarium andiyazi strain 2043 translation elongation factor 1 alpha (TEF1alpha) gene, partial cds	708	708	98%	0.0	94%
EU620628.1	Fusarium andiyazi strain 2172 translation elongation factor 1 alpha	708	708	98%	0.0	94%

Appendix 6 Contd.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
	(TEF1alpha) gene, partial cds					
AF160265.1	Fusarium brevicatenulatum strain NRRL25446 translation elongation factor 1 alpha gene, partial cds	715	715	100%	0.0	93%
AF160271.1	Fusarium pseudocircinatum strain NRRL22946 translation elongation factor 1 alpha gene, partial cds	710	710	99%	0.0	93%
EU620635.1	Fusarium andiyazi strain 1965 translation elongation factor 1 alpha-like (TEF1alpha) gene, partial sequence	702	702	98%	0.0	93%
EU620632.1	Fusarium andiyazi strain 2008 translation elongation factor 1 alpha (TEF1alpha) gene, partial cds	702	702	98%	0.0	93%
AF160268.1	Fusarium sp. NRRL 25221 translation elongation factor 1 alpha gene, partial cds	704	704	98%	0.0	93%
AB448731.1	Fusarium foetens gene for translation elongation factor 1 alpha, partial cds	699	699	100%	0.0	93%
AB302888.1	Fusarium foetens EF1-a gene for translation elongation factor 1 alpha, partial cds, strain: MAFF 240182	699	699	100%	0.0	93%
AB302887.1	Fusarium foetens EF1-a gene for translation elongation factor 1 alpha,	699	699	100%	0.0	93%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
	partial cds, strain: MAFF 240181					
AB302886.1	Fusarium foetens EF1-a gene for translation elongation factor 1 alpha, partial cds, strain: MAFF 240180	699	699	100%	0.0	93%
AB302885.1	Fusarium foetens EF1-a gene for translation elongation factor 1 alpha, partial cds, strain: MAFF 240179	699	699	100%	0.0	93%
AY320089.1	Fusarium foetens strain NRRL31947 translation elongation factor 1 alpha gene, partial cds	699	699	100%	0.0	93%
AY320088.1	Fusarium foetens strain NRRL31943 translation elongation factor 1 alpha gene, partial cds	699	699	100%	0.0	93%
AY320087.1	Fusarium foetens strain NRRL31852 translation elongation factor 1 alpha gene, partial cds	699	699	100%	0.0	93%
AY320086.1	Fusarium foetens strain NRRL31850 translation elongation factor 1 alpha gene, partial cds	699	699	100%	0.0	93%