# PREVALENCE OF NON-DERMATOPHYTIC MOLDS ASSOCIATED WITH CUTANEOUS MYCOSES IN CATTLE IN ABIA AND IMO STATES, NIGERIA

**BY**

**NWOFOR, CHIOMA NNENNA**

**(NAU/PG/PhD/2015487006P)**

# DEPARTMENT OF APPLIED MICROBIOLOGY AND BREWING

# FACULTY OF BIOSCIENCES

# NNAMDI AZIKIWE UNIVERSITY, AWKA

# MAY, 2021

# PREVALENCE OF NON-DERMATOPHYTIC MOLDS ASSOCIATED WITH CUTANEOUS MYCOSES IN CATTLE IN ABIA AND IMO STATES, NIGERIA.

BY

**NWOFOR, CHIOMA NNENNA**

**(NAU/PG/PhD/2015487006P)**

**A DISSERTATION SUBMITTED TO THE DEPARTMENT OF APPLIED MICROBIOLOGY AND BREWING, FACULTY OF BIOSCIENCE, NNAMDI AZIKIWE UNIVERSITY, AWKA, ANAMBRA STATE, NIGERIA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD) IN MEDICAL MICROBIOLOGY.**

**SUPERVISOR: PROF. C A. OYEKA**

## CERTIFICATION

The research work embodied in the dissertation titled “Prevalence of non- dermatophytic molds associated with cutaneous mycoses in cattle in Abia and Imo States, Nigeria” was carried out by Nwofor, Chioma Nnenna with registration number NAU/PG/PhD/2015487006P under the supervision of Prof. A. C. Oyeka is original and has not been submitted in part or full for the

award of any degree or diploma in this university or any other university.

……

……

Nwofor, Chioma Nnenna

Date

(Student)

# APPROVAL

This dissertation tittled “ Prevalence of non-dermatophytic molds associated with cutaneous mycoses in cattle in Abia and Imo States

Nigeria', carried out by Nwofor, Chioma Nnenna with registration number NAU/PG/PhD/2015487006P, has been approved after meeting the requirements for the award of the degree of Doctor of Philosophy( PhD) in Medical Microbiology, in the Department of Applied Microbiology and Brewing, Faculty of Bioscience, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

…….

…….

Prof. A. C. Oyeka

Date

(Supervisor)

……

……..

Prof, E. I. Chukwura

Date

(H.O. D, Applied Microbiology and Brewing)

……

……..

Prof . C. A. Ekwunife

Date

(P. G. Sub-Dean, Faculty of Biosciences)

……

…….

Prof. S.C. Udedi

Date

(Dean, Faculty of Biosciences)

……

……..

Prof. V.O. Nwugo Date

(External Examiner)

……

……..

Prof, P, K, Igbokwe

Date

(Dean, School of Postgraduate Studies)

# EDICATION



This work is dedicated to Almighty God

# ACKNOWLEDGEMENT

I acknowledge Almighty God for his mercies, favours and grace upon my life throughout the period of my research work in Nnamdi Azikiwe University Awka, Anambra State. Special thanks goes to my supervisor, Prof. ( Mrs) A.C. Oyeka,

who through her motherly love and advice, painstakingly read through my work and made corrections, I say may God bless her abundantly. My appreciation also goes to the head of department Prof. E.I. Chukwurah for her effort and encouragement, may God equally bless her accordingly.

My appreciation goes to all my lecturers at Nnamdi Azikiwe University Awka, Anambra State,, Prof. C. O. Anyamene, Prof. R. A. Nwobu, Prof. F. J. C. Odibo, Dr. (Mrs) Ify Okonkwo, Dr (Mrs) C. C. Ekwealor, Dr. S. C. Onuorah, Prof. J. J Okeke, Mr Onyi Udemezuem for all their efforts and encouragement during the course of this study, may God equally bless them. To all the laboratory technologist especially the head of

unit, Mrs Anyaoha, and all other staff of the laboratory unit of the department of Applied Microbiology and Brewing, Nnamdi Azikiwe University Awka and the Department of Microbiology, Imo State University Owerri, I say may God bless them abundantly. My sincere appreciation also goes to all veterinary doctors in charge of all cattle markets visited during the course of this research for their assistance. I also appreciate Prof .Y.M. Tatfeng, of department of Medical Laboratory Science of Niger Delta University Willberforce Island where I did part of my molecular analysis. My sincere gratitude also go to my uncle Prof. B.E.B. Nwoke, of department of Animal and Environmental Biology of Imo State University Owerri, Dr.

Nathaniel. E. Onyenwe and Mr. Fajana, Akibu of department of Pharmarcy Igbenedion University Benin, Mr Galaxy, of department of Biochemistry of Imo State Polythenic Umuagwo for their professional assistance.

My humble regard goes to my husband, Prof. Okechukwu. K. Nwofor, our children, who through their support and encouragement has helped in the commencement and completion of my study. I also wish to show my gratitude to my parents, siblings and inlaws who prayed and desired that this journey of mine was fruitful and successful.

Finally my immense gratitude goes to Mr Chidi Orji and Prof. F. Nwobi of Statistic

Department, Imo State University who did the statistical analysis.



# ABSTRACT

Cutaneous mycoses is a communicable disease that leads to the destruction of the superficial layer of the skin of man and animals. Dermatophytes are mostly incriminated, although studies have shown that non-dermatophytes are also capable of causing similar lesions. A total of 451 skin samples from both infected and asymptomatic animals were collected from six cattle markets in Abia and Imo States, Nigeria between January and August, 2018. Sixty soil samples and Sixty air samples were also collected

from the environment for fungal analysis within the same period. These samples were analysed and identified for fungal isolates and keratinophilic isolates using cultural, hair bait method, microscopic, polymerase chain reaction methods and DNA sequencing techniques. Pathogenecity test on the isolates were carried out using albino mice. Isolates were screened for enzyme secretion using standard plate method. Antibiogram activity against the isolates were determined by oxoid disc diffusion method using four conventional drugs (ketoconazole, voriconazole, fluconazole and amphotericin B) for this study. Four plant leaves (*Occimum grastissium*, *Euphorbia hirta*, *Mitracarpus scaber* and *Jatropha multifida*) were screened for phytochemical compounds and antifungal activity using disc diffusion method. Minimum Inhibitory Concentration (MIC) and minimum fungicidal concentration (MFC) against the isolates using the plant extracts were also carried out using tube dilution method followed by subculturing relative samples. The data was analysed using multiple comparison and one way analysis of variance

(ANOVA). A total of 16 non-dermatophytic molds were obtained from this study at different frequency of occurrences from the cattle skins, air and soil samples which includes *Penicillum citrinum* (3.0%), *Aspergillusfumigatus* (3.6%), *Aspergillus terreus* (2.7%). *Aspergillus welwitschiae* (13.5%), *Aspergillus flavus* (10.0%), *Aspergillus aculeatus* (9.0%), *Aspergillussydowii* (5.0%), *Fusarium solani* (3.2%), *Fusarium lichenicola* (17.9%), *Fusarium succisae* (12.0%), *Fusarium oxysporum* (2.0%), *Curvularia kusanol* (0.6%), *Cladosporiumtenuissimum* (4.9%), *Pestalotiopsismicrospora* (0.1%), *Talaromyceskendrickii* (0.1%) and *Absidia* specie (12.9%). There was a significant difference in the frequency of occurrence from soil samples within markets in Abia State at (P= 0.05). Pathogenecity studies showed that *Aspergillus welwitschiae*, *Cladosporium tenussimum* and *Absidiacorymbifera* were highly virulent. The highest keratinase (65mm), amylase (86mm), protease (60mm), lipase (60mm) and cellulase (86mm) activities was observed on *P.microspora*, *A.welwitschiae*, *C.tenuissimum*, *A.welwitschiae*

and *A.welwitschiae* respectively. Amongst the conventional drugs, ketoconazole was highly effective on the isolates with inhibition zone ranging from 10–53mm. Phytochemical analysis revealed presence of active ingredients steroids(35.7-35.86), saponin (27.84 - 27.94), alkaloids (0.3502- 0.6122), tannin(0.026- 0.1606), phenol(0.0085- 0.0127), glycoside(0.232-0.744) and flavonoids(-0.310 - -6.68) mg/100g. Antifungal sensitivity test at concentration range (6.25 -200mg/ml) showed that *Mitracarpus scaber* and *Euphorbia hirta* were highly effective on the isolates at 200mg/ml while all isolates were resistant to *Jatropha multifida* at all concentrations. The concentration range of Minimum inhibitory concentration (MIC) for methanolic extract of the plant leaves against the fungal isolates tested was 6.25 -200mg/ml. Statistical analysis shows that there was a significant difference in the activity of these plant herbs against the fungal isolates at ($P<0.05$). Minimum fungicidal concentration (MFC) ranged from 50 - ≥200mg/ml respectively. The most significant MFC (50mg/ml) was observed in

extracts of *Mitracarpus scaber* Statistical results shows a significant difference in activity amongst the extracts at ($P < 0.05$). This study highlights the antifungal potentials of some local plants present in our environment as this can also be harnessed and used in production of drugs which can be used in treatment of some of these skin infections.

## TABLE OF CONTENTS

# CHAPTER ONE



# CHAPTER TWO

**CHAPTER THREE**

# LIST OF TABLES

**Table: Titie**

**Page**

# LIST OF PLATES

# LIST OF FIGURES

**Figure:** **Title** **Page**

**12:** Frequency occurrence of non-dermatophyte molds from soil samples from cattle markets in Imo State using hair bait technique.



# APPENDIX

**Title**
**Appendix**
**page**

**1**. Analysis of variance (Anova) to determine the freouency of occurrence of non-dermatophytic molds isolated from air within the market in abia state.

164

2 analysis of variance ( Anova) to determine the frequency occurrence of non-dermatophytic molds isolated from air within the market in Imo State.

165

**3.** Analysis of variance (Anova) to determine the frequency occurrence of non-dermatophytic molds isolated from soil within market in Abia State**.** 166

**4**. Analysis of variance (Anova) to determine the frequency occurrence of non-dermatophytic molds isolated from soil within the market in Imo State. 167

**5.** Analysis of variance ( Anova) to determine the mic's of the four selected plant ( leaves) extract against 12 non—dermatophytic molds isolated from the study.
168

**6.** Analysis of variance (Anova) to determine MFC of four selected plant (leaves) extract against 12 non- dermatophytic molds isolated from the study. 169

isolated from Abia State and Imo State.

181

**11.** Frequency of occurrence of non – dermatophytic molds from cattle skin in Abia State and Imo State.

182

**12.** Frequency of occurrence of non – dermatophytic molds from cattle skin in Abia State.

183

**13.** Frequency of occurrence of non – dermatophytic molds from cattle skin in Imo State.

184

technique. 192

**22.** Some laboratory equipments used during this research. 193

**23**. Showing hair bait technique plate , agar plate exposed at strategic point within the market, some lesion elicited on albino mice used for pathogenecity test.

**24.** Some local plants used in this study; *euphorbia hirta* , *occimum gratissimum* , *jatropha multifida* .

196

**25.** Result from nano drop 1000 spectrophotometer 197

**26.** Equipment and glassware:

198

# CHAPTER ONE

## 1.0 INTRODUCTION

Cutaneous mycosis is a wide spectrum of fungal infections caused by a group of fungal agents called dermatophytes (dermatophytosis), *Candida* (candidiasis) and *Malassezia* (pityrasis versicolor) (Zarei, 2011).These groups of fungi are capable of infecting the superficial keratinized tissues of the skin and appendages of its host (Emenuga and Oyeka, 2013).They comprise of three genera, *Trichophyton*, *Epidermophyton and Microsporum* species. They are limited to the nonviable skin because of its inability to penetrate the viable tissues of immune competent hosts (Jawetz, *et al*., 2010). They are also capable of

causing mild to severe lesions on their hosts with the help of virulent factors such as acid protienases, elastase and keratinases (Emenuga and Oyeka, 2013).

Cutaneous mycosis in cattle is a worldwide zoonotic infection which is usually responsible for economic losses in the farm (Didier, 2017).It is a highly contagious skin infection all over the world especially in the tropics (Africa, India) (Modupeola, *et al*., 2011) and it is known to be caused by a dermatophyte *Trichophyton verrucossum* (El-diasty *et al*., 2013; Agnetti *et al*., 2014; Akbarmehr, 2011).

However,it has been observed that non-dermatophytic molds (NDMs) are fast replacing the dermatophytes in causing cutaneous mycoses

(Ravinder, *et al*., 2015; Vyas, *et al*., 2013; Pietro, *et al*., 2014), which includes the *Aspergillus* species, *Penicillum* species, *Fusarium* species, *Cladosporium* species, *Alternaria* species.These non-dermatophytic molds are known to be mere environmental contaminants that inhabit animal fur (Tampieri, 2004). They are also known to be good secretors of enzymes such as amylase, protease, cellulase, keratinase, lipases (Luis, *et al*., 2006).

In Nigeria, cattle rearing is an occupation of the Northerners. These cattle rearers (nomads) move their animals from place to place in search of grazing lands for their animals. In the past two to three years, there has been a high influx of cattle from the North to the South eastern part of

Nigeria which might be as a result of the growing aridity in the North as this has raised a lot of controversies within the country. In the search for food, these animals could incure trauma on their skin which might predispose them to these superficial infections (Bakheshwain, 2011). As these animals are on transit, they usually lay on the soil and as such disperse their fur to the soil thereby encouraging the proliferation of keratinophilic organisms in those areas (Moallaei, *et al*., 2006). They also disperse some of these saprophytic organisms embedded in their fur to the atmosphere thereby populating the atmosphere with fungal aerosols that could be a threat to public health (Ajoudanifar, *et al*., 2011).

Due to poor personal hygiene by the animal handlers, they also are at higher risks of this infection since they are at close contact with these animals (Songer and Post, 2005; Zarei, 2011). It can also be transferred from animal to animal (Songer and Post, 2005).

In the treatment of these cutaneous mycoses, azoles are usually recommended (Moriarty *et al*., 2012).This is due to the drug's higher affinity for egosterol, which is an active component of the plasma membrane of fungi. Azoles has the ability of blocking the cytochrome P-450-dependent enzyme lanosterol dimethylase, which is a precursor of ergosterol in fungi.

Although before now, the use of herbal plants have been common as this can be referred to as

botanical medicine or phyto medicine (Bent, 2008; Gini & Jothi, 2015). This could be attributed to the use of plant parts such as the seeds, roots, leaves, bark, flowers and berries for medicinal purposes (Falodun, 2010).

Considering the indispensable nature of cattle in Nigeria and the world at large, it becomes imperative that we identify these non-dermatophytic molds responsible for this cutaneous mycoses associated with cattle in Abia and Imo States, Nigeria, considering the fact that its contagious nature, prolonged duration of treatment and poor management will be of public health importance.

## 1.2 STATEMENT OF PROBLEM

Among livestock, cattle serve as a major protein source to the populace. Despite its importance, a lot of diseases are known to be associated with cattle. Although much attention has been given to cattle diseases but our present understanding of causative mechanisms, epidemiology and management still remain very limited (Miller *et al*.,2013) and this hamper our capacity to adequetly addresses challenges posed by the growing incidences of emerging and re-emerging diseases (Gibbs, 2005; Woolhouse *et al*., 2005). Over 1600 human pathogens are being established with an average of three new diseases reported every 2yrs and a new infecting pathogen being published every week (Tomley and Shirley, 2009). Reports have shown that about 60% of

these emerging diseases and their infectious agents are known to be zoonotic (Jones *et al*., 2008). It has also been established that 75% of re-emerging infections, originated from animals 25years ago (King *et al*., 2006; Siembieda *et al*., 2011). It is therefore evident that increases in the risk of these zoonotic infections will continue if adequate precautions are not taken (Tomley and Shirley 2009).

On the other hand, studies have shown that the global human population is expected to rise above 9 billion in the year 2050 (UNDP, 2008) with more than one billion of this increase occurring in Africa. This will certainly impose grave pressure on the demand for agricultural production generally (Fresco, 2009) including livestocks,

especially in developing countries where increase in household incomes due to urbanization drives demand for meat and dairy products (Jones and Thornton, 2009). In such circumstances diseased animals cannot be used to produce human food, it cannot be sold or delivered to diary plants.

Due to complex benefits of livestock, it is necessary to study the epidemiology, transmission and maintenance of infectious diseases of livestock as this will go a long way in breeding healthy animals and reducing premature deaths.

## 1.3 JUSTIFICATION

Considerable research on livestock has been carried out in many parts of Nigeria mostly in the Northern part with very little done in the Southern part. Considering the large influx of cattle into the

Southern part occasioned by growing aridity in the North which can be linked to climate change.It becomes important that more studies on cattle health in the Southern part of the country be intensified especially with regards to calls to establish grazing reserves within the country. Passing of grazing laws and sponsoring of bills in both State and Federal assemblies across the country have been met with stiff opposition and resistance by some States especially the States within the Southern part of the country. Locally the establishment of these reserves of animal population close to human population may have very serious health implication one of which has to do with cutaneous mycotic infections. Since cattle has

been a major protein source for the entire country, it is therefore necessary to conduct specific studies on cutaneous mycotic infections that are associated with cattle in some States within the Southern parts of the country as this will go a long way in assessing problems that might arise from this action better and also aid in developing different forms of intervention.

## 1.4 AIM OF THE RESEARCH

The aim of this research is to study the prevalence of non-dermatophytic molds associated with cutaneous mycoses in cattle in Abia and Imo states, Nigeria.

## 1.5 OBJECTIVES OF RESEARCH

The specific objectives of this study are to:

1. determine the non-dermatophytes of cattle origin in the zones
2. determine the frequency of occurrence of non-dermatophytic molds associated with cutaneous mycoses with cattle in Abia State and Imo State, Nigeria.
3. evaluate the presence of non-dermatophytic molds present in the cattle market environment in the study area.
4. determine the pathogenecity of these non-dermatophytic molds.
5. screen some of the non-dermatophytic molds for enzyme production.

6. determine the plant leaves for qualitative and quantitative analysis of phytochemical compounds.
7. determine the susceptibility pattern of some non-dermatophytic molds isolated using some conventional drugs and some local herbs extracts.

# CHAPTER TWO

## 2.0 THEORETICAL AND LITERATURE REVIEW

Pathogens that live on animal as their host are known as zoophiles. Usually these pathogens depend on these animal host for their food and shelter. It is also known that man can acquire these pathogens from these animal host. Infection caused by these zoophiles are known as zoonotic

diseases. Reports have shown that more than 60% of infectious diseases are caused by pathogens present in both wild and domestic animals (Karesh *et al.*, 2012) and these diseases are responsible for cases of death annually. Emergence of new pathogens are also growing and this has been a major concern in the past years. Fungi is an example of a zoophile.

Fungal infection will be discussed under these headings:

**2.1 SUPERFICIAL MYCOSES**:

These are superficial cosmetic fungal infection that is limited to the outermost layers of the skin and hair shaft. It does not invade living tissue and as a result the host do not respone to It (Prohic, *et al*., 2016).

### 2.1.1. Pityriasis vesicolor

It is caused by *Malassezia* species e.g *Malassezia globosa*, *M restricta* and other members *M. furfur*. It usually affect the skin of young people especially the chest, back and appear as mascular patches of discoloured skin which may enlarged but scaling inflammation and irritation may be reduce. It can also cause folliculitis. This fungi can also cause seborrheic dermatitis and dandruff. It is known as malasseziosis or tinea versicolor. *Malassezia* species are lipophilic yeasts and as a result require lipid in medium to grow (Maria *et al*., 2013).

**Diagnosis**: It can be confirmed by direct microscopic examination of scrapings of infected skin area stained with 10-20% KOH (Potassium

hydroxide) or calcofluor white ( Mahesh, *et al*., 2019).

Appearance of sphaghetti and meat balls that are oval to round budding yeasts as well as short, septate and sometimes branched hyphae shows signs of positive result.

**Treatment**: It is treated with selenium sulfide or ketoconazole shampoos or systemic/topically applied azoles (ketoconazole or itraconazole), terfinafine (Crespo and Delgado, 2002).

### 2.1.2 Tinea nigra

It is a chronic, asymptomatic infection of the stratum corneum. It is caused by a fungus species

called *Hortaea (Exophiala) Werneckii.* It is characterized by non-scaly brown to gray patches on the palms or soles of feet. It is predominant around warm coastal regions and among young women in tropical and sub tropical zones (Sarangi, *et al.*, 2014).

**Diagnosis**: Microscopic examination of scrapping around the infected skin region with treatment with 10% KOH will reveal pigmented hyphae and budding yeast cells with melanized cell wall.

**Treatment**: Application of keratolytic solutions, salicylic acid or azole antifungal drugs (Ramon, 2018).

### 2.1.3. Black piedra

This particular infection affects the hair shaft. It is caused by a fungus specie called *Piedraia hortae.* It causes shafts to break. It is commonly found in tropical countries and rare in Europe. It is characterized by black concretions in scalp hair. It affect humans and other primate (Azulay *et al*., 2011; Kupiec, *et al*., 2017).

### 2.1.4. White Piedra

It is caused by *Trichosporon* specie. It also infects hair shaft but presents a larger, softer and yellowish nodules of hair.

Both Piedra can also extend to pubic, beard and scalp hair infection. Piedra is more endemic in tropical underdeveloped countries (Bonifaz, *et al*., 2019).

**Diagnosis**: Microscopic examination of scrapped lesions of infected area should reveal hyphae and arthroconidia.

**Treatment**: Scrapping of infected areas and application of antifungal agents.(Swapna, *et al*., 2013; Sampato and Rivitti, 2008).

## 2.2 CUTANEOUS MYCOSES:

### 2.2.1 Dermatophytosis

The causative fungi are usually referred to as dermatophytes or keratophilic fungi. These fungi are capable of producing extracellular enzymes keratinase which are capable of hydrolyzing keratin. These fungi also have the ability of infecting only keratinized tissue (skin, hair and nails) (Jamal, *et al*., 2015). This infection is usually referred to as tineas which means

“ringworm” or moth-like. Dermatologists use the term to refer to a variety of lesions of the skin or scalp. The dermatophytes are known to be the major causative agents. Dermatophytes are a large group of fungi that are grouped under three genera: *Trichophyton*, *Microsporum* and *Epidermophyton.* Most of them do not grow at 37°C so they are usually restricted to the nonviable skin. Although they may be troublesome and persistent but not debilitating or life-threatening. Many species of this group have the ability to release enzymes like keratinases, elastases that enables them evade host defenses. Some species reproduce sexually and they produce ascospores and as such belong to the sexual group (teleomorphic genus) Arthroderma.

Dermatophytes which means skin plants causing human infections have different natural sources and modes of transmission.

**Anthropophilic:** This is the greatest associated human infections. Its transmission is acquired from man to man as a result of close contact or through contaminated objects.Examples of anthropophillic species; *Epidermophyton floccosum, Trichophyton mentagrophytes var interdigitale, T. rubrum.*

**Zoophilic:** These infections are usually acquired from animals. Transmission to man is by close contact with animals (cats, dogs, cows) or by contaminated products. Examples of zoophilic species and their host; *Microsporum nanum* (pigs), *Microsporum canis* (dogs and

cats), *Microsporum gallinae* (fowl), *Trichophyton verrucosum* (cattle), *Trichophyton equinum* (horses).

**Geophilic:** This is found in the soil and are transmitted to man by direct exposure. Knowledge of mode of transmission of dermatophytes is important as this will go a long way in providing proper treatment strategies and control of the source by patient. Infection of zoophilic and geophilic organisms may lead to inflammatory disease in man. Examples of geophilic species that cause human infection; *Microsporum gypseum.*

Dermatophytes are widely distributed but some species have geographic limited distribution.

**Diagnosis**: Microscopic examination of skin samples with KOH will reveal presence of hyaline, septate, branching hyphae or chains of anthroconidia.

Culture on diagnostic media is difficult to identify since most species are closely related. They are differentiated based on their colonial differences and microscopic morphology as well as requirements of vitamins.

Human genetics may also play a major role in predisposing some families to some fungal infections. Other factors may also include; composition of sebum, specific skin chemistry, moisture, warmth, youth and heavy exposure. Dermatophyte infection commences immediately after trauma and skin contact. It is

more prevalent in hot, humid climates and under crowded environment. Wearing of shoe provides warmth and moisture thereby predisposing foot to infection, virulence of the infecting specie and some environmental factors (Jawetz, 2010).

**Clinical Manifestation of Dermatophytosis**

1). Tinea barbae: also known as tinea sycosis is an infection of the beard hair. It appears as edematous and erythematous lesions. It can present as mild or severe pustular folliculitis which can be mistaken to be a *Staphylococcus aureus* infection. This infection can be caused by zoophilic dermatophytes such as *Trichophyton verrucosum*, *Trichophyton mentagrophytes* and at times *Trichophyton*

*erinacei*.Anthropophilic specie, *Trichophyton rubrum* might also be responsible (Bonifaz, *et al*., 2014).

2). Tinea capitis**:** This is referred to as ring worm of the scalp. It is caused by *Microsporum* or *Trichophyton* species (Niema, *et al*., 2018). Its causal species is determined by the animal contacts or contacts associated with travel history or local infection prevalence. In tinea capitis, spores are produced within the hair shaft (endothrix). These hair do not fluoresce instead they are usually weakened and break easily at the follicular opening. The infection is characterized by mild scaling lesions, highly inflamed reaction with folliculitis, scaring and alopecia. This is referred to as kerion. The

position of the spores in the hair shaft can be used in diagnosing the infecting species.The terms normally used are:

Ectothrix: This is used when the arthroconidia (spores) are formed on the outside of the hair shaft.

Endothrix**:** This is used when spores are formed within the hair shaft.

Ectoendothrix**:** This is used when spores formed around and within the hair shaft.

Favus**:** This is used when hyphae and air spaces are formed within the hair shaft. This is usually caused by the specie *Tschoenleinii* .

3). Tinea corporis**:** It is also referred to as ringworm of the body. It affects the trunk,

shoulders and limbs. It might range from mild to severe. The infection gives rise to annular scaly lesions surrounded by sharply defined, raised, erythematous vesicular edges. These dermatophytes grow only within dead keratinized tissues but can penetrate viable tissues of the epidermis to cause lesions. Infections caused by zoophilic and geophilic dermatophytes produce more irritants than anthropophilic species. As the hyphae get older they form chains of arthroconidia. Specimen for diagnosis are usually collected at the periphery of lesion since active hyphae are found there (Alok & Rahul, 2016).

4). Tinea cruris**:** this is infection of the groin or jock itch. It is common among adult male. Its

causative agent is *T.rubrum* and *Epidermophyton floccosum.* It is characterized by erythematous lesions covered with thin, dry scales. Lesions can extend to the inner thighs with a raised defined border that might possess small vesicles (Alok and Rahul, 2016).

5). Tinea manuum**:** This is ringworm of the palms and in between the fingers. It presents itself as a diffused hyperkeratosis. It is caused by *T.rubrum*, *Trichophyton*and *Microsporum* species.

Zoophilic and geophilic dermatophytes are usually associated with this infection of the hands. Its lesions are usually inflammatory. It is easy to spread to other body sites through contagious spread and scratching.

*Neoscytalidium* species can also cause infection of the finger webs. Due to the involvement of the finger webs, this infection might be susceptible to *candida* infection.

6). Tinea pedis (athlete's foot): This infection is the most predominant of all dermatophytes. It affects mostly the toe webs and soles of the feet especially in between the fourth and fifth toes. It is characterized by the maceration, peeling and fissuring of the skin. Other varieties is a chronic, squamous, hyperkeratotic type with fine silvery scales covering the pink areas of the soles, heels and side of feet (moccasin foot). It starts with itching in between the toes, development of small vesicles with a discharge of a thin fluid. At this point the skin of the toes

web becomes mascerated and starts peeling. Thereafter cracks starts appearing and this can make way for secondary bacterial infection. The causative agents are *T.rubrum*, *T. interdigitale* and *E. floccosum.*

*T. mentagrophytes* can cause an acute inflammatory condition accompanied with vesicles, pustules and bullae. *Neoscytalidium* species can also cause dry hyperkeratoic appearance on the soles and toes. *Candida* species can also cause infection of the toe web (Alok and Rahul, 2016).

7).Tinea unguium /Onychomycosis: this is an infection of the nail plate by dermatophyte fungi. Initially an infection by non-dermatophyte was referred to as

onychomycosis, however this term is now used as a general name for any fungal infection of the nail (Nourchene, *et al*., 2017). There are different onychomycosis manifestation:

i). **Distal and lateral subungal onchomycosis:** it is caused by *T.rubrum*, and is characterized by invasion of the hyponychium which is seen under the nail bed and sides of the nail and finally to the nail plate. It is the most common form of this infection.

ii). **Proximal subungual onychomycosis:** it can also be known as proximal white. It is uncommon. Its causative agent is *T. rubrum.* It infects the nail through the cuticle. It is more predominant among HIV/AIDS patients.

iii) **White superficial onychomycosis:** it affects the upper layers of the nail plate. It is characterized with delineated white islands on the nail. It is caused by *T.interdigitale.*

iv) **Total dystrophic onychomycosis:** this is the last stage of nail disease. It might be the end point of the previous disease discussed.

HIV infection is associated with finger nail disease *Candida* species especially *Candida albicans* are strong causative agents of nail infection. It causes paronychia. Nail infection is also associated with Raynaud's disease.

v) **Tinea imbricata:** This is a chronic infection which appears like tinea corporis. It is seen mainly in pacific islands. It is characterized by

concentric rings of overlapping scales. It is caused by *Trichophyton concentricum.*

**Diagnosis:** It is observed by direct microscopy of scrapping from both skin and nail or hair depending involved area in 10-20% KOH with or without calcoflour white. In skin or hair, branched hyphae or chains of arthroconidia (arthrospores) are seen. In hair, *Microsporum* sp. form spores around the hair. Species like *J. tonsurans* and *T. violaceum* produce anthroconidia inside hair shaft (Shiu, *et al*., 2014).

For culture, dermatophtye species are inoculated on sabourand's agar containing cycloheximide and chloramphenicol to inhibit mold and bacteria growth. After 1-3 weeks, species are identified

using colonial morphology, nutritional requirement.

Trichophytid Reaction: Some individuals can develop hypersensitive reactions to dermatophytosis. This might be a way of reacting to some of the fungal products. This allergic manifestations may be referred to as dermatophytids. It usually appears as vesicles elsewhere in the body especially in the hands. Such individuals when subjected to trichophytin test will indicate positive (Jawetz, 2010).

Trichophytin on the other hand is a crude antigen that is used to detect dermatophytic antigen (both in immediate and delayed-type hypersensitivity) in patients–suffering from dermatophytosis infection.

It is observed that most patients who develop chronic and non-inflammatory dermatophyte infections posses poor cell mediated immune responses to dermatophyte antigen. In healthy individuals, immunity to dermatophytes is dependent on the degree and duration of infection, the site and species of the fungus causing the infection.

**Treatment of some tinea types** (Moriarty *et al*., 2012).

Corporis – This is treated with topical allylamines and azoles.

Cruris – Topical agents are applied but in severe cases griseofulvin is used.

Unguium – Doses of fluconazole, itraconazole or terbinafine are applied systemically.

Capitis –Drugs like griseofulvin, fluconazole, itraconazole or terbinafine are applied systemically.

## 2.3 OPPORTUNISTIC MYCOSES

Opportunistic fungi also known as secondary pathogens are widely distributed in nature and are normal floras of the body both in man and animals. It can only cause infection in immunocompromised individuals or in animals that have been receiving an immune suppressed therapy for a long period. They are not true pathogens but can only become pathogenic when they gain access into the host body either through the respiratory tract, alimentary canal, intravascular devices or through a traumatic inoculation. At times this infection may

degenerate from mere primary infections to invasive or even systemic infections. Some of these opportunistic infections include the genus *Candida* and non-dermatophytic moulds belonging to the genus *Aspergillus*, *Fusarium*, *Penicillum* and *Scopulariopsis* (Naveed, 2009; Jamal *et al*., 2015)**.**

### 2.3.1 Candidiasis

*Candida* species are widely distributed and are harmless commensals to man. They usually inhabit the mucosal surfaces of gastrointestinal and genitourinary tracts and the human skin. These organism usually become an opportunistic pathogens in immunologically weak or immunosuppressed individuals. These opportunistic pathogens are capable of causing

local mucosal infection, disseminated infection and systemic infection (Claudi & Dario, 2013). This systemic infection can be fatal especially among immunosuppressed patients like AIDS patients. Of all the disseminated fungal infections candidiasis is the most common one. Its causative agents include *C. albican*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. guilliermondic* and *C. dubliniesis* with *C. albican* causing the most invasive infection (Spinello, *et al*., 2016) and also the most common (Dabas, 2013). Candidiasis can also be referred to as thrush, yeast infection, candidiosis, moniliasis and odiomycosis (Spinello, *et al.*, 2016). The outcome of its pathogenicity is dependent on the host immune status.

According to Dabas, (2013) candidiasis was discussed under different heading: Mucosal candidiasis: This is the colonization of non-sterile mucosal surface like the oropharyngeal and vulvuvaginal candidiasis.

(i) Oropharyngeal candidiasis (OPC): Candida is part of the normal oral flora. In about 30% to 50% of individuals inhabit candida in their mouth (Dabas, 2013). The ability of this organism to cause disease is based on some factors like immune status of the host, the severity of the particular strain and the oral mucosal environment. This accounts the more prevalence of oropharyngeal candidiasis among AIDS patients. Some other factors of the host can also encourage

the organism to colonize and cause infection and this include: reduction of saliva secretion, epithelial change as a result of local mucosal disease, changes in normal floras, denture wearing and high consumption of carbohydrate. Oropharyngeal candidiasis can also be presented at different clinical levels. This include acute pseudomembranous, acute atrophic, chronic hyperplastic, chronic atrophic, median rhomboid glossitis, denture stomatitis and angular cheilitis a (Swidergall and Filler, 2017).

(ii) Vulvovaginal candidiasis (VVC): *C. albican* is the major causative agent of this type of infection although reports have

shown cases of other species of candida that can cause this infection and they include *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. parapsilosis* (Claudia and Dario, 2013). A new specie has also been reported to be associated with candidiasis *C. dubliniensis* (Dabas, 2013). It is a disease of the reproductive tract of females (Hubertine, et al., 2020).

**Cutaneous candidiasis**: This is a secondary infection that is common among diabetic patients; it is a localized infection of the skin and nail or around sites of body folds around the skin. This infection is mostly seen around moist or creased area of the body. Cutaneous candidiasis is likely

seen in intertrigo candidiasis, candida folliculitis, otomycosis etc.

**Invasive candidiasis:** This is the invasion of *C. albicans* to spread into any organ in the host body and a major causes of mortality and morbidity. Its cases are increasing due to the increasing complexity of the population (Arora *et al.*, 2011; Spinello, *et al*., 2016).

**Systemic/Disseminated Candidiasis:** This is the dissemination of ◦candidemia. Among immunocompetent individuals, the organism is been mounted upon by the immune system while in immunosuppressed patients it may develop occult lesions in some organs like the kidney, skin, heart, eyes. This can be associated with mortality (Yan *et al.*, 2013). Apart from *C.*

*albicans* other species of candida can also cause this infection.

## Morphology and Identification

*Candida* species appear as oval, yeast like cells that replicate by budding. Its buds grows in chains forming pseudohyphae. *C. albicans* is dimorphic, it also produces hyphae at 25◦C and yeast at 37◦C.This property differentiates it from the other species. On agar media, it's usually creamy in colour and posses a yeasty odor. On a less nutritional media it is usually large with chlamydospores (Jawetz, 2010).

## Diagnostic Laboratory Test

**Specimens:** This includes swabs, blood, spinal fluid and scrapping from body lesions.

Direct microscopy of specimen should be carried out with 10% KOH or lactophenol blue or calcofluor white. Gram stain can also be prepared. Under the microscope, pseudohyphae and budding cells can be seen. For culture, sabouraud dextrose agar is used. It should be incubated at 37°C. There are no established method for serology examination. It does not give specific or sensitive results. Agglutination (EIA) lacks sensitivity but is specific. PCR can also trace little amount of candida species in blood samples.

## Treatment

Topical azoles can be used to treat oropharyngeal candidiasis. For vulvovaginal candidiasis, topical

azoles like nystatin can be applied. Oral itraconazole can also be taken as complement. Topical antifungal agents like clotrimazole, ketoconazole can also be used in the treatment of cutaneous candidiasis in the case of systemic or disseminated candidiasis, amphotercin B therapy may be employed. Fluconazole can be used to complement amphotericin B in the case of candidemia (Dabas, 2013).

### 2.3.2 Aspergillosis

This is a pathogenic, opportunistic species that belong to the genus *Aspergillus*. There are more than 200 species but only few have been associated with human or animals infection and they include *A. fumigatus*, *A. flavus*, *A. niger*, *A.*

*terreus* and *A. lentulus* (Songer and Post, 2005). They are capable of causing invasive pulmonary, allergic disorders, otitis externa and aspergilloma. Aspergillosis is distributed worldwide. They are saprophytic in nature (Chabi, *et al*., 2015). They also have the ability to contaminate can food products, and also cause nosocomial infections. Ordinarily when the conidia is inhaled, it gets to the lungs where the alveolar macrophages are supposed to engulf it but in cases of immunosuppressed individuals or animals that are undertaking corticosteroid treatment, their macrophages finds it difficult to mount defence on them. This will then lead to:

(a) **Allergy:** This is common among atopic individuals that develop raised

immunoglobin (IgE) due to *Aspergillus* conidia. These conidia grows, producing hyphae that colonize the bronchial tree without affecting the lungs. This disease is called allergic bronchopulmonary aspergillosis which is the major cause of asthma, pulmonary eosinophilia (Aleksandra, *et al*., 2019). Most patients produce sputum that can be used for diagnosis.

(b) **Aspergilloma:** This is referred to as a fungus ball. When the conidia is inhaled it colonizes the existing cavity, grows there and forms a compact ball of mycelium which is finally surrounded by a dense ball. Most times patients are asymptomatic and

usually produces small sputum and cough. Patients that have previous cavitary disease are at higher risk. Some of its symptoms include weight loss, weakness, hemoptysis (Jawetz, 2010).

(c) **Invasive aspergillosis:** This is more severe among immunocompromised individuals suffering from leukemia, lymphoma. This infection may or may not disseminate. *A. fumigatus* is the causative agent of this infection (Chabi, *et al.*, 2015). Its initial clinical presentation includes an unremitting fever, there is usually no respiratory tract symptoms. It can disseminate to the central nervous system, lungs, blood vessels which may lead to thrombosis and infarction.

Invasive aspergillosis may not really end well.

(d) **Sinusitis:** This is usually caused by *A. flavus* and *A. fumigatus.* The conidia when inhaled may colonize the paranasal sinuses. (Agarwal, *et al*., 2013). This infection may disseminate to the bone and orbit of the eye and brain. It is more common among immunosuppressed individuals than immunocompetent individuals.

## Morphology and Identification

These species of organism grow very fast, producing long hyphae with a terminal vesicles that produce chains of conidia.

## Diagnostic Laboratory Test

**Specimen:** Sputum, lung biopsy, respiratory tract specimen.

In potassium hydroxide preparation of sputum, aspergillosis appears as a non pigmented septate hyphae with uniform width and branched dichotomously. On culture, species are identified by their morphology and colonial structures and are usually grown on sabouraud agar at 25-37°C for few days. Skin test is usually applicable for allergic aspergillosis diagnosis. For cutaneous aspergillosis, immunological methods can be employed. EIA (Enzyme Immunoassay) gives a specific and sensitive results (Oritiz *et al.*, 2012) Polymerase chain reaction (PCR) can also be used in-situ to access the organism at molecular level (Pasqualotto, 2010).

**Treatment**

Amphotericin B can be given intravenously in cases of cutaneous and invasive aspergillosis. Some azoles (itraconazole) can also be given. Allergic forms of aspergillosis can be treated using corticosteroids.In cases of hemoptysis surgical excision can be employed (Rohit and David, 2019).

### 2.3.3 Mucormycosis (Zygomycosis)

Mucormycosis can also be known as zygomycosis. It is caused by a saprophytic mouldy fungi that is widely distributed all over the world. It inhabits warm and humid climate. They are usually isolated from decomposing organic matter or fruits. Its characteristic feature in its broad, aseptate hyphae with a sporangium

possessing a numerous asexual spores which develops at the tip of the hypha. Its portal of entry could be oral infection or cutaneous inoculation. Studies have shown that USA records 1.7 million of cases every year and 500 new cases crop up yearly (Petrikkos, *et al.*, 2012). The most prevalent causative agent is *Rhizopus oryzae*. Some of the predisposing factors to this infection includes – acidosis (as a result of diabetes mellitus), Leukemia, lymphoma, premature birth, severe burns, malnutrition, corticosteroid therapy, renal damage, nosocomial infection (Ortiz *et al.*, 2012).

Mucormycosis can be rhinocerebral acute, pulmonary intestinal, cutaneous and disseminated. Among all these clinical forms,

rhinocerebral mucormycosis is the most frequent. This starts with germination of the sporangiospores in the respiratory tract which leads to the colonization of the blood vessels by the developed hyphae. This results to thrombosis, infraction and necrosis. The rhinocerebral infection are common among ketoaciditic diabetic patient. The pulmonary infections are seen in cases of neutropenia while the disseminated infection can be found in both cases (Brown, 2005).

Cutaneous mycormycoses are not usually common and its portal of entry is the skin and can be found at the limbs and hardly on the upper trunk. At chronic stages infection can result to necrotizing fasciitis which will eat up the muscle,

tendon and bone. There are also cases of disseminated or rhinocerebral mucormycosis. This is categorized as secondary mucormycosis. Some of its clinical features includes sinusitis, unilateral peri orbital edema, a bloody nasal exudates, orbital cellulitis and the patient develops edema of the involved facial area (Skiada, *et al.*, 2009). Cutaneous mucormycosis shows a single fistula as its initial clinical sign which is usually painful this may become necrotic. This infection can be fatal in cases of convulsion.

## Diagnostic Laboratory Tests

**Specimen:** nasal discharge, sputum, exudates, macerated biopsy.

Direct microscopy with 10-20% KOH reveals broad hyphae with irregular branching, uneven thickness without septate hyphae.

Culture is grown on a sabouraud dextrose agar and potato dextrose agar for one week at 25-28°C. It is characterized with an abundant gray white cottony colonies. PCR tests gives a specific and sensitive results almost 100% (Spellberg *et al.*, 2005).

Histological examination of biopsies gives a positive result (Bonifaz *et al.*, 2014).

**Treatment**

This involves surgical debridement of all necrotic area in cases of cutaneous mucormycosis. It can also be accompanied with Amphotericin B. For secondary cutaneous cases amphotericin B

desoxycholate or lipid is applied. For concomitant cases posaconrazole is employed (Skiada, *et al.*, 2018).

### 2.3.4 Phaeohyphomycosis

This infection is caused by a dematiaceous filamentous fungi that posses melanin in its cell wall. Phaeohyphomycosis is different from chromoblastomycosis and mycetoma that is also caused by a dematiaceous fungi. Most of its causative agents are opportunistic pathogens. Its major predisposing factor is immunosuppressed patient. This infection is attributed to less than hundred species but its most common causative agent is *Scedosporium prolifican* (Revankar *et al.*, 2002). A new specie *Bipolaris spicifera* was associated with this disease in a patient that under

went heart transplant (Revankar *et al.*, 2002). This infection is characterized by fever, skin manifestation including rash, ulcers, respiratory and central nervous system complaint was observed. The less common symptoms were gastrointestinal symptoms, sepsis, this is mainly as a result of *S. prolificans* eosinophila which was mainly caused by *Bipolaris* species, *Curvularia*, *Wangiella* species and *Lecythophara* species (Revankar SG *et al.*, 2002).

Phaeohyphomycosis is classified under four different types (Oritz *et al.*, 2012)

Superficial, subcutaneous, cerebral and disseminated or systemic infections. The site of infection of phaeohyphomycosis is the blood, lung, heart, skin, brain, kidney, liver, spleen,

lymphonodes, bones and joints while the muscle was a less reported site (Revankar *et al.*, 2002). Its mortality rate is high both for immunocompetent individuals and more for immunocompromised patients. Both men and women are affected. Some of the virulence factors associated with the causative organism is the production of melanin (dihydroxynaphthalene melanin) and other enzymes like proteases, peptidases and hyaluronidases.

At superficial level it causes tinea nigra Palmaris which affects the palms or soles producing brown pigmented plagues. Tinea pedis is also caused by *Neoscytalidium dimidiatum* and *Scytalidium hyalinum.*

In immunosuppressed patients subcutaneous nodular dissemination are common. Its initial site of attack is the upper and lower limbs before disseminating. A subcutaneous tumor which is a mycotic cysts is also found. Cerebral phaeohyphomycoses is common among immunosuppressed patients and it is usually caused by dematiaceous fungi (Jaffar and Ali, 2011).

**Laboratory Diagnostic Test**

Specimen: Skin lesions, serum, spinal fluid.

Direct microscopy with 20% KOH reveals pigmented, septate hyphae of less than 1µm in diameter at times with blastoconidia. The organism is cultured using a sabouraud agar

which is incubated at 25-28°C it produces a black yeast like or mold like colonies are seen. For subcutaneous infections histology are very useful. It reveals a pigmented, thick, septate hyphae at times with blastoconidia which lie in the granulomatous infiltrates with lymphocytes, histiocytes, fibroblasts and giant cells (Ana, *et al*., 2012).

**Treatment**

For superficial infections topical kerotolytics and topical azoles like bifonazole, clotrimazole can be used.

For subcutaneous infections, surgical debridement is the step to the right direction which will be followed by amphotericin B.

For systemic infections voriconazole and posaconazoles can be employed (Revankar *et al.*, 2002).

## Epidemiology and Control

The causative agents of phaeohyphomycosis are widely distributed all over the world and its natural habitat is the soil. Its prevalence is found in North America, Europe, Australia, South America and Asia although majority of the reports revealed Spain and Australia as the most endemic areas (Revankor *et al.*, 2002).

## 2.3.5 Pneumocystis Pneumonia

This is a localized infection that is caused by *pneumocystis jiroveci.* It causes pneumonia in immune compromised patients (AIDS). It was initially known as a protozoan until a DNA

analysis was carried out and results showed that it was more of a fungi than protozoa. It is also self-limiting. Its morphology is similar to that of protozoa. Its life cycle starts with being a cysts then development of a sporozoite outside the cyst. All these stages take place in the alveoli of the lungs. With the help of PCR it has been shown that man becomes subclinically infected during childhood and with high immune system the disease will be contained while clinical manifestation is dependent of extent of immune suppression. This can be characterized with fever, non productive cough, dyspnoea and shortness of breath. Apart from pneumonia, pneumocystis infection may spread to the lymphnodes, liver, spleen, bone marrow, adrenal gland, intestines

and meninges. For advanced AIDS patients and those undertaking prolonged prophylaxis pulmonary disease may occur. Pneumocystisspecies are found in the lungs of some animals like rats, mice, dogs, cats, ferret, rabbits (Jawetz, 2010).

## Morphology

*P. jiroveci* poses thin wall trophozoites and cysts that has thick wall and is spherical in shape. It contains four to eight nuclei. In the host, cyst & trophozoite growth appears as a tight mass. In the sera of an acutely ill patient *P.jiroveci* is covered with glycoprotein (Joseph, *et al*., 2015).

## Laboratory Diagnostic Test

Specimen: Lung biopsy tissue or induced sputum.

Direct microscopy, tissue can be stained with silver stain, calcofluor white and observation reveals octonucleate cysts or trophozoites.

Immunofluorescent stain can also be used in detecting organism using induced sputum. PCR can also be used.

**Treatment**

Cell mediated immunity plays a major role in resistance to disease. But in cases of already acquired infection clotrimoxazole or intravenous pentamidine is the drug of choice. In acute cases trimethoprim-sulfamethoxazole or pentamidine isethionate. Aerosolized pentamidine can also be a daily prophylaxis (Jawetz, 2010).

## 2.3.6 Hyalohyphomycosis

A state of immunocompromise predisposes one to lots of infections. The most causative fungal agents in this group includes*Fusarium* species, *Pseudallescheria boydii* and *Scopulariopsis brevicaulis.* Organisms in this group posses septate hyaline hyphae. They are also capable of causing increasing opportunistic infections (Tortorano, *et al*., 2014).

(i) Fusariomycosis

*F.solani*, *F. oxysporum* and *F. verticillioides* are the various species that cause hyalohyphomycosis. Thesefungi is widely distributed in the soil and are capable of causing disease in plants (phytopathogens). Impaired health status are the predisposing factor to this infection. Its portal of entry is the respiratory tract

or through skin trauma in the cases of cutaneous forms. *Fusarium* spp are capable of causing other disease especially in immune competent individuals. It is capable of causing invasive or disseminated infections, cutaneous lesions, pulmonary mycoses and disseminated fusariomycoses (Ortiz *et al.*, 2012).

In direct microscopy with 10% KOH reveals septate hyaline. In histology specific results are not found.

Culture with sabouraud medium would show fleecy white colonies after 3-5 days. Under the microscope this colonies reveals micro and macro conidia. Amphotericin B is the drug of choice. In cases of systemic infections azoles are recommended.

### *(ii) Pseudallescheria boydii*

This organism causes localized and disseminate infection. Immunosuppresed patient are also the most predispose. Its portal of entry could be the respiratory tract, skin, subcutis and blood (Makino *et al.*, 2011). *Pseudallescheria boydii* is also known as *Scedosporium apiospermum* in its asexual state. Its clinical manifestation resembles that of Aspergillosis. Some of its risks factors includes involvement of the lungs (Fungal balls), nasal sinuses, meningitis, sverrucous skin lesions, ulcers, arthritis and asteomyelitis and fungemia.

Microscopically when stained reveals septate or atimes arranged in groups of hyphae. It is grown using sabouraud dextrose agar with or without antibiotics. After two or three weeks a white

colonies that later change to brown are seen. Under the microscope the conidia appear as pear or lemon-shaped.

Treatment of choice are amphotericin B, azole derivatives (Bonifaz, 2012)

*(iii) Scopulariopsis brevicaulis*

This organism is ubiquitous in the soil and environment. It causes different fungal infections of the nail.

Direct microscopy reveals long septate filaments. It can also appear as clusters of lemon-shaped conidia when using nail as specimen. Culture on sabouraud agar help distinguishes them from tinea. It can also cause infections of the lungs, subcutaneous abcesses, peritoneal infections, sinusitis, otomycoses, keratitis mycotica (Oritz *et*

*al.*, 2012). Studies has shown special lesions associated with this organism in AIDS patients (Salmon *et al.*, 2010).

Its mode of treatment is dependent on the site of infection. Amphotericin B is effective, some triazoles are also good.

A novel specie has been introduced and its name is *Roussoella percutanea.* It is an opportunistic pathogen that is capable of causing subcutaneous mycoses (Ahmed *et al.*, 2014). It is characterized by slow production of pycnidial conidiomata. The work also reported that this strain is resistant to echinocandins and flucytosine and sensitive to azoles and amphotericin B.

## 2.4 CATTLE IN NIGERIA

Cattle are large bodied ruminants that feed on pastures and forages or fodder. They belong to the:Family: Bovidae, Class:Mammalia, Genus: BOS, Species: Taurus (for exotic breeds) and indicus (for local breeds).

In Nigeria, cattle rearing is an old occupation which is practiced traditionally by Fulanis and Shwa Arabs in Northern Nigeria with few local villages in Southern Nigeria also involved These mammals are a major protein providers to Nigerian populace and the world at large. The population of cattle in Nigeria is about 13.9 million (Lawal, 2012). Specifically, 90% of this population are concentrated in the Northern region of the country and this can be as a result of low rainfall duration, lighter sandy soils and

longer dry season peculiar to the area (Lawal, 2012). Cattle can serve for other purposes like, multiplying and selling it for income, it can be used for the production of hide and skin, agricultural manure, source of milk and cheese, means of transportation for the movement of products and using bones and blood for the production of feeds (Barnes, *et al*., 2012).

### 2.4.1 Dermatophytes on Cattle

Ringworm is a fungal and zoonotic infectious disease that is caused by different species of dermatophyte. Its lesions are usually found on the head, muzzle, ears, neck and more specifically around the eyes of an infected animals. Dermatophytes are also common in Africa

probably because of the hot and humid environmental conditions.

Ringworm in cattle is a highly zoonotic contagious skin infections all over the world. It is responsible for high economic losses in farms especially during winter (Didier, 2017). Its causative agents are a group of keratinophilic filamentous fungi that belong to the genera *Trichophyton*, *Microsporum* and *Epidermophyton.*

Reports have shown that *Trichophyton verrucosum* is the most etiological agent of cattle dermatophytosis (El Diasty *et al.*, 2013; Agnetti *et al.*, 2014; Akbarmehr, 2011; Shams-Ghahfarokhi *et al*., 2009; Subha G *et al*., 2017).

**Mode of Transmission**

Animals are usually infected by coming in contact with athrospores (spores formed asexually in the parasitic stage of hyphae) or conidia (spores formed sexually or asexually in the environment). Animals can be predisposed to infection by contact with a symptomatic or asyptomatic host (Murray *et al.*, 2005). According to Al-Ani *et al.*, (2002) housing animals in close range to each other for long periods in the presence of infectious debris was responsible for the high prevalence of the disease during winter period.

## Clinical Features

In cattle, its typical lesion presents as patches of hair loss which covers about 10-50mm area, crust formation and desquamation (Cam *et al.*, 2007).

This lesions are usually seen in the head and neck. At times when seen on other parts of the body appears as a grayish-white crust that is raised above the skin and itsusually heavy. It is usually more prevalence among the young calves than the adult cattle (Cam *et al.*, 2007; Akbarmehr, 2011).

## 2.5 POSSIBLE CAUSATIVE AGENTS OF CUTANEOUS MYCOSES

A lot of research have been carried out in this area of interest both within and outside the country, some have worked on animals while some worked on man. Those who worked on dermatophytosis obsevered *Trichophyton verrucosum* to be the major causative agent of this infection, Akbarmehr (2011) worked on the

prevalence of cattle ringworm in native dairy farms of sarab city in Iran, El-Ashmawy *et al*., (2015) also isolated *Trichophyton verrucosum* as the most prevalent agent after which he subjected it to an in-vitro antifungal assay and observed that the isolates were sensitive to bergamont oil. Shams-Ghahfaroki *et al*., (2009); Mohammed and Syed in 2009; El- Diasty *et al*., 2013 all isolated the same organism in their study. Some studies have also shown that cutaneous infections can infect man and might also be caused by this same organism. Mary, (2000); Seyed, (2011);Swai and Sanka, (2012) isolated *Trichophyton verruccosum* as a major causative agent of human infections especially from cattle and diary farmers.

There was also cases of co-infections were other dermatophytes were also isolated. According to Dalis *et al*., (2014); Falah Al-Ani *et al*., (2002); Golah Ham (2012); Hadi *et al*., (2014); Arue Lund *et al*., (2013); Pu Xiong Ming *et al*.,(2006), evaluation of cutaneous mycoses from both man and animals revealed isolation of *Trichophyton* species and *Microsporum* species.

On the contrary, recent studies have shown that non-dermatophytic molds and yeast might also be capable of expressing clinical manifestation of dermatophytosis infact they might be replacing the dermatophytes in causing this infections. Trying to distinguish between molds as relevant pathogens and mere colonization is now a diagnostic challenge (Vyas, *et al*., 2013; Pietro, *et*

*al*., 2014). This was observed by Ravinder, in 2015, when he surveyed the epidemiology and mycological profile of superficial mycoses in North India and his result showed that non-dermatophytic molds were the most common isolates followed by dermatophytes and yeasts. *Aspergillus niger*, was the most common isolate, followed by *Aspergillus teresus* and then *Rhodotorula* specie. According to Wu, *et al*., (2011), human pathogenic fungi in china and the emerging trends from ongoing National survey for 1986, 1996 and 2006 revealed that ten (10) species of dermatophytes decreased markedly while molds and yeast especially *Candida* species increased gradually during the past three decades also Igor, *et al*., (2007) examined the mycology

and skin swabs from dogs with clinical symptoms and isolated *Aspergillus* species, *Penicillum* species, *Alternaria* species, *Mucor* species and *Fusarium* species as the most prevalent isolates which he attributed to be the cause of systemic mycoses and allergies in animals and human. On the other hand Gabreab, *et al*., (2015) in their study revealed *Aspergillus* species as the most common non-dermatophytic mold isolated when he determined the prevalence of dermatophytosis. Sadhna, *et al*., (2013), also isolated species of *Aspergillus* isolates when he examined an immunocompetent farmer who presented generalized nodules and plaques, mimicking erythema nodosum leprosum which turned out to be cutaneous aspergillosis. He concluded that

colonization of burn eschars by *Aspergillus* is common and correlated primary cutaneous infection in immunocompetent host in association with agricultural trauma.

Bakheshwain, *et al*., (2011) sampled skin and nails suspected of dermatophytosis and isolated *Aspergillus* species as the leading genus represented by three species followed by *Alternaria* also represented by three species, while *Fusarium solani*, *Alternaria alternate* and *Exophiala jeanselmei* were the only fungi which were isolated from both samples. He also stated that mold flora has the ability to utilize keratin and therefore its ability to cause superficial cutaneous infection should not be ignored and can cause similar lesion that are produced by true

dermatophytes. Another type of mold was isolated by Rafai, *et al*., (2012), when he worked on the characteristics and taxanomy of *Cladosporium* fungi and observed that this fungi is common in the world and that its spores can be found in the air, soil and water and can cause opportunistic infections in immunocompromised host. Although reports about infections on healthy people has been recorded and this may present as lesions, Ponnusamy *et al*., (2018) isolated *Curvularia* species from skin infections in goat while Ravinder, *et al*., (2017), isolated *Aspergillus* spcies, *Fusarium* species and *Penicillium* species as the major non-dermatophytic molds from patients suffering from onychomycosis showing that these molds are not

mere contaminants but are capable of causing cutaneous infections. Another study by Adane, (2018) revealed that almost the same number of dermatophytes and non-dermatophytic molds were isolated from patients suffering from capitis while Adefemi, *et al*., (2011), isolated majority of non-dermatophytic molds than dermatophytes from children suffering from dermatophytosis. Both dermatophytes and non-dermatophytes were also revealed by Ndako, *et al*., (2012) when he investigated the prevalence of cutaeneous dermatophytosis in school children in Kano metropolis. Chanu, *et al*., (2017) also concurs with this findings.

## 2.6 ENZYME

Enzymes are biological catalysts that speed up biochemical reactions (Gudynaite and White, 2016). Living things use enzymes in different ways. Some use enzymes in their digestive system for the degradation of macromolecules which will be absorbed into their cells while some like microorganism use it to achieve their pathogenic process (Fabiana, *et al*., 2005). It can also be applied in the industries for commercial purposes.

For pathogenic process to commence, microorganism need to colonize a host, invade it, cause infection and evade the host immune system. To achieve this, microorganism need to secrete enzyme (Livia, *et al*., 2017; Vermout, *et al*., 2008), As a virulent factor in cutaneous

mycoses, enzymes are usually initiated after the organism must have gained access into the stratum corneum cells and nutrients (Chinnapun, 2015). Studies have shown that the ability to understand the association of these virulent factors and its substrate in-vitro will go a long way in understanding host- pathogen interaction (Elangovan, *et al*., 2017) and as such this study analyses the ability of some of these non-dermatophytic molds to produce some of these virulent factors in-vitro.

### 2.6.1 Examples of some enzymes that are involved in pathogenecity

1) **Amylase:** these are enzymes that hydrolysed starch. It is found in man, plants, animals and microorganisms. This enzyme is

used to break down bonds in starches, polysaccharides and complex carbohydrate into simple sugar (Sundarrram and Murthy, 2014). In man it is an important enzyme in the salivary gland. Industrially it is used in the conversion of starch to sugar syrups, it is used in the production of drinks, baking, cyclodextrins in the pharmaceutical industries (Pandey, *et al*., 2000). Some microorganisms that can produce amylase include *Aspergillus*, *Rhizopus*, *Penicillum* (Sunitha, *et al*., 2012).

2) **Proteolytic enzymes:** these enzymes hydrolyze the protein components of the skin, hair which might pave way to stratum corneum (Nalu, *et al*., 2010).

a) **Protease:** this is another important enzyme that hydrolyzes the peptide bonds of proteins into peptides and amino acids. It is necessary for cell differenciation and growth (Paula, *et al*., 2015). Animal skin especially cattle are known to posses gelatin and as such this makes it easy for this enzyme to hydrolyse the animal skin once it gains access. It has also been identified to play an important role in infection process (Mercer and Stewart, 2019) and as such tagged a virulent factor. Industrially it is being applied in various sectors such as food, laundry detergent, pharmaceutical industries. Some microorganisms known to produce protease include *Aspergillus*, *Candida* species (Gropp, *et al*., 2009). Protease produced by *Aspergillus*

have been associated with fungal immune evasion by cleaving complement proteins in the human/ animal host thereby promoting inflammation (Shende, *et al*., 2018).

b) **Keratinases:** these are enzymes that hydrolyze protein rich in keratinous wastes which normally result in environmental pollution and health harzards (Amit, *et al*., 2016). Keratin is a protein normally present in animal feathers, horn, nails, skin (Poopathi, *et al*. 2016). Keratinase achieves its hydrolyses by degrading the keratin present in the host tissue into oligopeotides or amino acids which are usually absorbed by the fungus (Nalu, *et al*., 2010). For industrial purposes it is used in the production of leather, biofuel, detergent

addictives (Amit, *et al*., 2016). For public health interest it can be used in the degradation of hair, feathers and during waste management (Gampa, *et al*., 2013). Examples of keratinolytic fungi include, *Aspergillus*,*Curvularia*,*Fusarium*,*Cladosporium* species, during pathogenecity they use this enzyme to invade hair and stratum corneum thereby facilitating infection (Beti and Masa, 2018).

3) **Cellulase:** This is another enzyme used to hydrolyze cellulose. It is being secreted by plants, animals, microorganisms of which fungi is one of them (Lynd, *et al*., 2008; Zhang, *et al*., 2009; Bayer,*et al*., 2007). It hydrolyses the β-1, 4 linkages in the cellulose

chains into sugar (Lin and Tanaka, 2006). Cellulase is a hydrolytic enzyme that plays an important role in pathogenecity by facilitating fungal penetration through cell wall of mainly plants (Olubukola, 2010). Filamentous fungi that are usually producers of these enzymes are *Aspergillus*, *Trichoderma reesei* (He Jun., *et al*., 2011). Animals can be infected by these organisms by coming incontact with contaminated debris in the barn or through direct incision or traumatization by some infected plants or leaves in the bushes during grazing.

4) **Lipase:** This is used for full digestion of fats into smaller fatty acid components by living things (Sundarram and Murthy, 2014).

The cattle skin contains fat although in small quantity. Lipase are capable of membrane distruption during host cell invasion. Apart from hydrolysis it also plays a role in the synthesis of acylglycerides (Reis, *et al*, 2009). . Lipases are known to be a potential virulent factor of some pathogenic fungi such as *Candida albicans*, *Aspergillus fumigatus* (Hube, *et al*., 2000). It achieves pathogenesis, by cleaving phospholipids, then destabilize the membrane and cell lysis ensues. (Warner, *et al*., 2001) while phospholipases target the membrane of phospholipids.This enzyme can also be found useful in the industries for yogurt and cheese

fermentation,baking,laundry addictives (Guo and Xu.,2005).

## 2.7 SOIL

Soil is defined as a mantle of weathered rock which contains minerals and nutrients (Farid and Nareen, 2012). It supports a range of microorganisms of which fungi a one of them. It is one of the most complex microbial habitat (Pahare and Shukla, 2014). Geophilic fungi are group of fungi that could be found in the soil (Deshmukh and Verekar, 2006) and it includes both dermatophytes and non-dermatophytes and as such, this makes the soil a source of infection to human and animals (Seyed *et al*., 2012).These fungus are usually distributed in the soil that is

rich in creatinine, keratin (Pahare, *et al*., 2018). Factors such as pH of the soil and geographical location also affect their distribution. Some of these fungi such as dermatophytes are known to cause cutaneous infections such as athletes foot (Jawetz *et al*., 2010). Due to close contact of animals to the soil, it therefore makes the soil a reservoir for animal infection.

Some researchs have shown the presence of some dermatophytes and non-dermatophytic molds in the soil, Keyvan, *et al*., (2013), identified 22 fungal isolates out of 41 isolated of which *Fusarium* species were highly isolated another by Shokohi, *et al*., (2005), isolated 15 species of which *Fusarium* species were also mostly isolated from soil samples they analysed. Farid and

Nareen in 2012, studied the seasonal distribution of soil borne fungi and their results revealed *Aspergillus* species as the most frequently isolated fungi during the four seasons, followed by *Penicillum* species. On the other hand Subhash, *et al*., (2017), isolated 15 species of fungal isolates when they analysed soil samples, also Seyed, (2012), isolated a total of 222 fungi when he analyzed 200 soil samples collected from parks of municipality districts of Tetran for keratinophilic fungi using hair baiting method while Nosratabadi, (2017), isolated 130 fungal isolates from 60 soil samples using similar method. Another researcher Nouf, *et al*., (2018), isolated 10 different filamentous fungi from soil samples while Raja, *et al*., (2017), isolated 25

fungal isolates and identified 13 from soil samples analysed from Loyola College Campus, Chennai, India.

## 2.8 CHEMOTHERAPEUTIC AGENTS

An antifungal medication is a pharmaceutical agent used to treat and prevent fungal infections such as athlete foot, ringworm, candidiasis (thrush) (Baginski and Czub, 2009). These antifungal agents can be fungicidal or fungistatic and they are known to selectively eliminate fungal pathogens from a host with minimal or no toxicity to the host.

## CLASSIFICATION OF ANTIFUNGAL DRUGS

There are different classes of antifungal drugs with their perculiar mode of action on fungi. We

have the polyene antifungal drugs, azoles, allylamines, echinocandins. In this study we will concentrate on polyenes and azoles.

**2.8.1 Polyenes**: this molecule has multiple conjugated double bonds. It has a macrocyclic polyene with a heavily hydroxylated region on the ring opposite the conjugated system. This composition makes polyene amphiphilic.They are products of *Streptomyces* species.

**Mode of action:** polyenes interact with sterols in cell membrane to form channels through the membrane, which causes the cells to become leaky.This changes the transition temperature (Tg) of the cell membrane.

This reduces the fluid in the cell thereby increasing the crystalline state. This can be

attributed to the loss of monovalent ions ( $K^+$, $Na^+$,$H^+$, and $Cl^-$) and small organic molecules the cell dies.

Some of the polyenes include nystatin, amphotericin B and primaricin. Amphotericin B is nephrotoxic when administered intravenously. It causes glumerular tubuloglomerular feedback (Jawetz, *et al*., 2010). Polyenes can bond to animal membrane cholesterol when there is a reduction in the hydrophobic chain (Baginski and Czub, 2009). Amphoterincin B are usually used mainly in the treatment of life threatening mycoses and for most other mycoses (Kyrickidis *et al*., 2017). Amphoterincin B was discovered by Gold in 1956 and it was reffered to as the gold starndard. It is known to be effective against

most medically important molds and yeasts including dimorphic mold pathogens *Coccidiodes immitis*, *Histoplasma*, *Blastomyces dermatitidis* except *Fusarium*species (Tzar, *et al*., 2016) It can also be used to treat opporturnistic mycoses caused by *Aspergillus* species, *Zygomycetes* and *Cryptococcus neoformans*. Resistance to this drug is rare.

Nystatin was the first successful antifungal antibiotic to be developed which is still in general use. It is usually applied topically and it is usually active against yeasts such as *Candida* species.

Primaricin (natamycin) is another polyene that is applied topically to treat superficial mycotic infections for the eye. Its activity is against both yeasts and moulds (Borkow, 2014).

**2.8.2 Azoles:** This antifungal membrane have five membrane organic rings that has either two or three nitrogen molecules. They are grouped into the imidazole and triazoles respectively. The imdazoles include clotrimazole, miconazole and ketoconazole (Gupta and Lyons, 2015). The triazoles include the itraconazoles and fluconazoles. Most molds or filamentous fungi show higher susceptibility to voriconazole than the other antifungal drugs (Karina, *et al*., 2013; Nickie, 2003).

**Mode of action:** The azoles inhibit the P450-dependent enzymes 14-alpha dimethylase which is involved in the biosynthesis of the cell membrane sterols by converting lanosterol to ergosterol.

Among the azoles ketoconazoles set the pace of orally administered antifungal azoles (Hope, *et al*., 2017). It can be applied orally or tropically and are usually active against infections caused by *H.capsulatum* and *B. dermatitidis*, it is also used in immunocompromised patients, mucosal candidiasis and a variety of cutaneous mycoses (dermatophyte infections, pityriasis versicolor). It is not normally recommended for the treatment of aspergillosis or of systemic infections caused by yeasts.

The triazoles (fluconazole, itraconazole) have become the standard of the azoles and have replaced Amphotericin B for treating certain forms of the systemic infections. Fluconazole is now preferable for the treatment of candidemia in

neuropeenic hosts. It is also gaining acceptance for cryptococeosis and some selected forms of coccidioidomycosis. Itraconazole has been proven to be effective against histoplasmosis, blastomycosis, sporotrichosis, coccidioidomycosis and certain forms of aspergillosis. Fluconazole can be administered orally or intravenously although still undergoing study (Divya and Ravi, 2017). Its side effect is a life threatening liver toxicity which can arise after a long term use. Other symptoms include nausea and vomiting.

### 2.8.3 ALLYLAMINES

The allylamines also inhibit squalene epoxidase, which is another enzyme required for ergosterol synthesis. In the pathway of ergosterol morpholene which is a derivative of amorolfine is being inhibited. Examples include amorolfin, butenafine, naftifine and terbinafine (Cappellentry and Eiseltein, 2007).

## 2.8.4 ECHINOCANDINS

A lot of antifungals are coming into use. An example is the caspofungin which is the first of the echinocandins to be produced.

**Mode of action**: its mode of action is by inhibiting beta- 1- glucan synthase, which is necessary for the formation of fungal cell wall. It is usually not absorbed well in the gastro-intestinal tract. It is being used only

intravenously. It is usually applied against refractory aspergillosis and as an adjunct to amphotericin B. Caspofungin is fungicide and has relatively few side effects which include fever, infusion site reaction or headache. For patients that experience hepatic insufficiency elevation of serum transminase should be monitored (Wellington, 2001).

## OTHER ANTIFUNGAL AGENTS

Griseofulvin is an antifungal antibiotic produced by *Penicillin griseofulvum.* It is usually preffered for dermatophytes and has been the drug of choice for chronic infections caused by this fungus *Trichophyton rubrum*. It is orally administered especially in cases where azoles are challenging (Oliver, *et al*., 2016).

**Mode of action**: Griseofulvin inhibits mitosis in fungi. Potassimum iodide which is given orally is no longer in use to treat cutaneous and lymphocutaneous sporotrichosis (Sutton, *et al.*, 2017) although it is not effective against *Sporothrix schenckii* in-vitro.It appears to act by enhancing the transepidermal elimination process in the infected host.

## 2.9 MEDICAL PLANTS

The use of medicinal plants in the treatment of diseases are of immense importance in the health of people. It is closely linked with conventional medicine as it is been used now in curing both emerging and re-emerging infections (Bent, 2008; Gini and Jothi, 2015). Herbs derived from different parts (leaves,roots,seeds,berries,bark or

flowers) of plant extracts have gone a long way in treating a wide range of clinical diseases (Falodun,2010). These plant parts possess certain chemicals known as primary and secondary metabolites.The primary metabolites includes amino acids, sugar, purines and primidines while the secondary metabolites includes alkaloids, glycosides, phenols, terpenoids. The distribution and concentration of these chemicals differ from one plant to another and from one plant part to another. Chemical compounds such as alkaloids and glycosides are known to be poisonous if present in large quantities but can be useful and harmless if used in smaller quantity (Amir, *et al*., 2011). About three quarter of the world today relie on plants and their extracts for their

healthcare (Jachak and Saklani, 2007).These extracts are now known as potential sources for the development of chemotherapeutic agents (Micheal, *et al*., 2014).

In this research four different types of plants will be used in testing its sensitivity on the isolated organism and they include *Mitracarpus scaber*, *Jatropha multifida*, *Occimum grastissimum* and *Euphorbia hirta.*

### 2.9.1. *Mitracarpus scaber*

This plant belongs to the family Rubiaceae and it is popularly known as the madder family. It belongs to the Gentianales order which is now called Rubiales order. This family consists of approximately 500 genera and about 6,000 species which can be found all over the world.

Some of them can be seen as tropical trees and shrubs (erect, struggling or twining) while some are found as herbs (erect or decumbent).

*Mitracarpus scaber* is a herb that is about 30cm in height or less and possess rough leaves. In Nigeria, different tribes have different names for it. The Igbos' call it Obuobwa, Hausas' call it Gududal while the Yorubas' call it Irawo Ile (Abere *et al.*, 2007).

Its leaf extract can be used in treating a lot of diseases traditionally like headache, toothaches, hepatic diseases, veneral diseases as well as leprosy. It is also believed to possess both antibacterial and antifungal activities (Hemandez *et al.*, 2000).

In Nigeria, its leaf extract have been used in treatment of some skin diseases like, eczema, ringworm, lice, craw-craw, lice. It can be used in dressing cuts, ulcers and wounds (Abere, *et al.*, 2007). Due to the immense importance of this plant alot of scientific studies have been carried out to determine the antibiotic effectiveness of its leaf extracts.

In a study by Hemandez *et al.*, (2000), crude extracts of *Mitracapus scaber* "zucc" showed that *Candida albicans* were inhibited by the formulation at a minimum inhibitory concentration of 75mg/ml. Another study by Karaye (2017) evaluated the antimycotic activity of crude methanolic extracts of *Mitracarpus scaber* on *Candida albicans* and *Trichophyton*

*mentagrophytes* and results showed that both organism were sensitive to *Mitracarpus scaber* at 4mg/ml while Nystatin did not inhibit the growth of *Candida albicans* but inhibited the growth of *T.mentagrophytes* at 7mg/ml. Yet another study by Anejionu *et al.*, (2012) revealed that in-vitro antifungal activity of the ethanol extract of *Mitracarpus scaber* (50μgml$^{-1}$) showed that the clinical isolates were sensitive to the herbal extracts but were more sensitive to *O. gratissium* oil extract ( MIC range of 0.8 – 1.25 μgml$^{-1}$) than to ketoconazole (MIC range of 0.31-5.00 μgml$^{-1}$). Another perspective was employed by Thes, (2011), in his work, he produced a soap by combining plant oils for the treatment of skin infections (*Mitracarpus scaber*, *Cassia alata* and

*Mareya micrantha).*This soap he subjected to an in-vitro test on *Trichophyton mentagrophytes* and clinical trials on infected patients and results from his work showed that the soap was active in-vitro on *T. mentagrophytes* and its application provides total cure for ringworm.

### 2.9.2 *Jatropha multifida*

The genus name of this plant Jatropha is a greek word *jatros* (doctor) and trophie (food) which means medicinal uses (Kumar and Sharma, 2008). This plant belongs to the family Euphorbiaceae. It can be seen as a shrub or a tree that is drought resistant. It can be found in the wild or semi-cultivated areas in central and South America, Africa, India and South East Asia (Martinez, *et al.*, 2006).

Different parts of the Jatropha (seeds, leaves, bark etc) are known to be of great importance in traditional medicine and for veterinary purposes (Prasad, *et al*., 2012). On the other hand *Jatropha* is a non edible oil seed plant, whole extracts from different parts is known to be toxic. This toxic effect of the extracts is as a result of the presence of a toxic ingregient called phorbol ester which is known to contain moluscicidal, piscicidal, insecticidal, rodenticidal, anti-microbial and cytotoxic properties. Its adverse effects on animals including rats, poultry and ruminants are also well established (Rakshit, *et al*., 2010).

A study by Jati and Dian (2017), showed that *Jatropha multifida* latex (whole plant) can be used to cure wounds but toxic when they

surveyed the ethanomedical of the plants. It has also been observed that the watery sap of *Jatropha multifida* obtained by decoction can be used to treat stomach pain Wongsatit, (2005). Another study by Michael, *et al*., (2014) revealed the antibacterial and antifungal activities of *Jatropha multifida* (Ogege) sap against some pathogens, agar well diffusion and broth dilution methods were used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) at concentration of 1050mg/ml to 2mg/ml and results showed that all the organisms were susceptible to the sap. Antimicrobial activity of this plant was also observed by Olapej, *et al*., (2008), in their study hexane, ethyl acetate and

methanol extracts of the plant and chromatographic fractions were screened against seven pathogenic organisms comprising gram positive and gram negative bacteria and fungi and the results showed potent antimicrobial activity against the isolates.

### 2.9.3 *Occimum gratissimum*

This is a shrub that belongs to the family Lamiaceae. It is popularly known as scent leaf or dove basil. It is usually found in tropical countries. Although Africa and Asia are the two continents where most variants of the plant exists (Matasyoh, *et al.*, 2007). In Nigeria, different tribes have different names for it. In Igbo it is called Ncho-anwu, Ahuji, in Yoruba it is called Efinrin, in Hausa it is called Daidoya while Edo

people calls it Aramogbo (Koche, 2012). *Occimum gratissimum* has been known to possess a lot of antibacterial and antifungal properties and as a result has been of great importance to the medical field (Mbakwem *et al*., 2012). It has been proven that *O.gratissimum* is very useful in the medication for people living with Human Immunodeficiency virus (HIV) and Acquired Immuno Deficiency Syndrome Virus (AIDS) (Nwinyi, *et al*., 2009). It is also useful in the treatment of gonorrheal infection, vaginitis, vaginal douches for metritis and mental sickness (Nwinyi, *et al*., 2009).

*Occimum gratissimum* have also been known to be active against some pathogenic bacteria like *Escherichia coli*, *klebsiella pneumonia*,

*Pseudomonas aerugnosa*, *Proteus vulgaris*, *Streptococcus vivadians* (Koche, *et al.*, 2012) and some fungi like *Trichophyton rubrum*, *Crytococcus neoformans*, *T. mentagrophytes*,*Candida albicans* (Mbakwem, *et al.*, 2012). Traditionally it is used in the treatment of ailments like urinary tract, wound, skin and gastrointestinal infections (Nweze and Eze, 2009). The plant is an erect small plumb with many barnacles usually not more than 1m high (Nweze and Eze, 2009). Phytochemical evaluation of *Occimum gratissimum* reveals that it is rich in alkaloid, tannis, phytates, flavonoids and oligosaccharides (Ijeh, *et al.*, 2004). Around the coastal area of Nigeria, this plant is used in the treatment of epilepsy (Ladipo, *et al.*, 2010).

Mbakwem, (2012) studied the effects of *Occimum gratissimum* leaves on common dermatophytes and *Malassezia furfur* and results showed a significant inhibitory effect of *Occimum gratissimum* at five different concentrations of 250mg/ml, 200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml used. Another study by Silva, *et al*., (2005) reported the antifungal activity of *Occimum gratissium* towards some dermatophytesand *Occimum gratissimum* extracts (hexane, chloroform fractions, the essential oil and eugenol) were investigated, results showed that hexane and eugenol were the most active although hexane inhibited the growth of all isolates 100% at the concentration of 125µml$^{-1}$. Yet another study by Nweze and Eze (2009)

justified the use of *Occimum gratissium* as a herbal medicine when they evaluated its interaction with disc antibiotics on some clinical isolates and results were positive although there were varying in-vitro activities against all the isolates used. In another study, the antibacterial properties of *Occimum gratissimum* was evaluatedagainst some selected entero-bacteriacea by Ladipo *et al*., (2010), water and ethanol were used for the extraction of the active constituents of the plant and results revealed that water extracts of the plant was not as active as the ethanolic extracts against the tested organisms.

### 2.9.4 *Euphorbia hirta* (Leaves)

*Euphorbia hirta* (linn) belongs to the family Euphorbiaceae. It is a small annual plant

commonly found in tropical countries. Its height is usually about 40cm. Its stem is slender and often reddish in colour. Its young ones are covered with a yellowish bristly hairs. Its leaves are oppositely arranged and lanceolate (Chitra, *et al.*, 2011). When plucked its stem and leaves produces whitish or milky juice (Chitra, *et al.*, 2011). Its leaves are known to treat dysentery, cough, asthma, worms and vomiting. The white latex is used as eye drops to cure conjunctivitis, it is applied on swellings, piles and boils (Jueriyah, *et al.*, 2016). Its main components are flavonoids, terpenoids and phenols (Huang *et al*, 2012).

The whole plants or its powder have been shown to be used traditionaly in veterinary medicine

such as in treatment of gastritis diarrhea in pigs, cattle, horse, sheep and fish (Huang *et al*., 2012).

So many antimicrobial works have been carried out using extracts from different parts of *Euphobia hirta* plant.

Several studies have been carried out on this plant, in a work by Saravanan, *et al*., (2012) showed that ethanol extracts of *Euphorbia hirta*, prepared at different concentrations against some clinical important bacteria species reavealed that the ethanol extracts had potentially deleterious effects on the microorganisms than petroleum ether extracts. Some studies have also evaluated the different parts of this plant such as the leaf, flower, stem and root for their antibacterial and antifungal activity and brine shrimp lethality and

results showed that leaves extract inhibited the growth of all tested organisms including the yeast (*Candida albicans)* with large zone of inhibition Rajah, *et al.*, (2010). On the contrary, another study by Singh and Kumar (2011), in their work extracted and tested the antimicrobial activity of alkaloids of *Euphorbia hirta* against four bacteria and four fungi and results showed that all micro organisms were sensitive against all the tested extracts from different parts (leaf, stem, root and fruits) of *Euphorbia hirta.* Another study testedthe efficacy of different species of *Euphorbia* plants used to treat skin infections by Tabassum and Hamdani (2014) amongst *Euphorbia* species tested *Euphorbia hirta* showed to have the highest antioxidant activity.

## 2.10 PHYTOCHEMICALCOMPOUNDS

These are chemical compounds that occur naturally in plants. They are present in plants or plant parts and are reffered to as non-nutritive plant chemicals that have protective or disesase preventive properties (Molyneux, *et al*., 2007; Shouchuang, *et al*., 2019).They can achieve this functions alone or in combination or in conjunction with other nutrients.These compounds are applied in the production of useful commodities like antimicrobials and herbal remedies. There are so many phytochemicals which includes flavonoids, alkaloids, tannin etc. These chemicals are divided into primary and secondary metabolites.The primary metabolites such as sugars, fats are found in smaller range and

serve more specific functions while secondary methabolites are toxins use to deter predation and produce pheromones used to attract insects for pollination. It is these metabolites that have therapeutic actions in man and can be refined to produce drugs (Scossa, *et al*., 2018). Compounds like steroids and sterols which are products of terpenoids provide many functions to plants like caroteniod pigments, production of chlorophyll and gibberellins hormones. Probably due to their numerous importance, they are usually applied in traditional medicine. Alkaoids protect plants from insects and herbivores (Okwu, 2004). Flavonoid provides mechanical support, defense against herbivores or pathogens. The also attract pollinator to plants (Silva, *et al*., 2016). Tannins

are phenolic compounds that act as defense mechanism in plants against pathogens and hostile environmental conditions. The also posses properties that hasten the healing of wounds and inflamed mucous membrane. Phenols also functions as antimicrobial compounds produced by some plants (Cheynier, *et al*., 2013) and have alot of effect on cell DNA. Saponin prevent disease invasion of plants by parasitic fungi. It is also used in the production of drugs, the also have the ability to lower cholesterol and inhibit chances of developing colon cancer (Valko, *et al*., 2006).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

## 3.1 STUDY AREAS

This study was carried out in the major cattle markets in Abia and Imo States. Both States are situated in Southeastern Nigeria. Abia State is bounded on the North and North East by Anambra, Enugu and Ebonyi States. To the West is Imo State and the East and South East by Cross River and Akwa lbom State and to the South is River State. lt comprises of three geopolitical zones: Abia North, Abia South and Abia Central. It iies between latitude 5° 06' 23° 69' N and

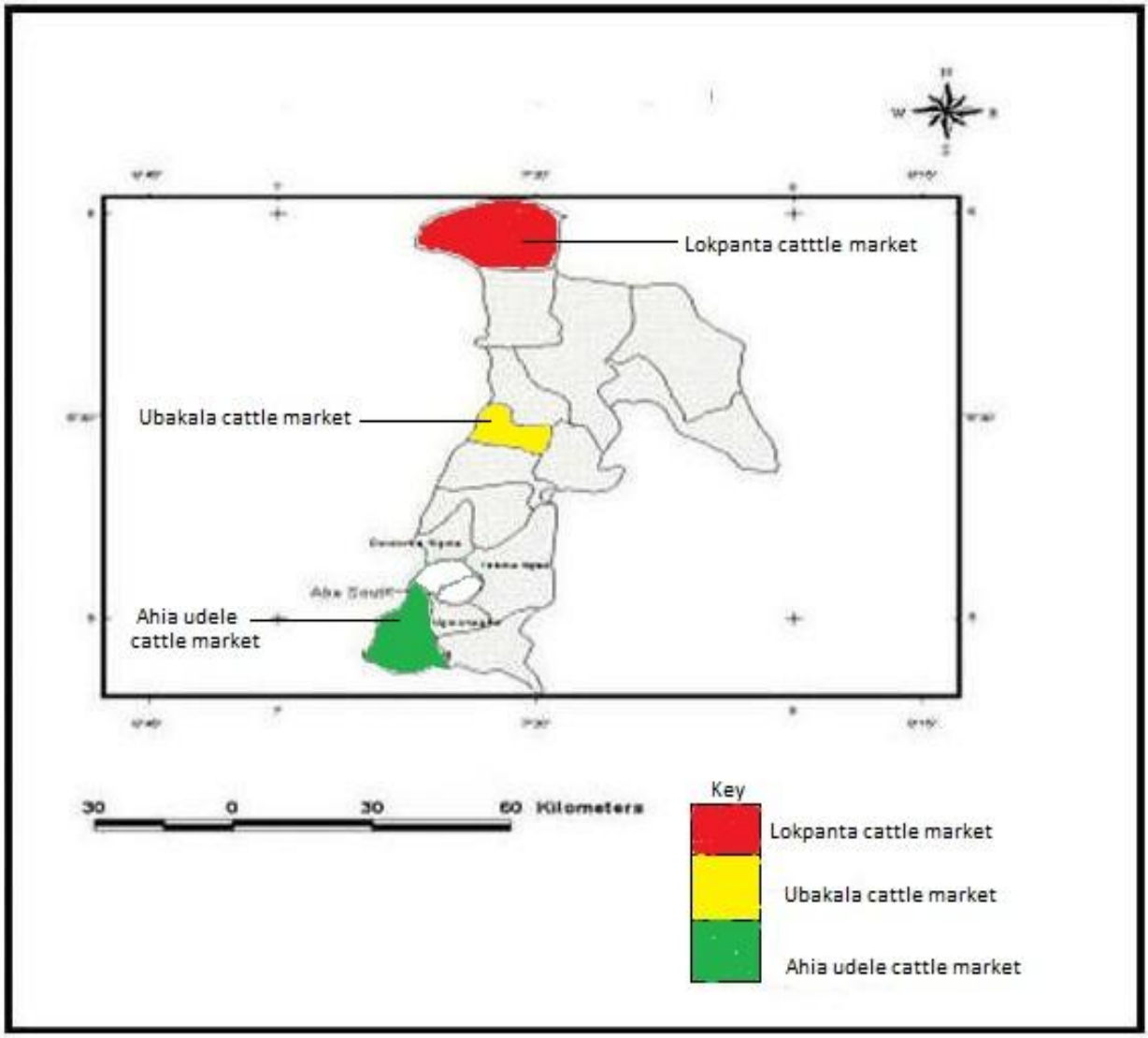
longitude 7˚2' 0 .01' E. The average annual temperature is 26. 9˚C / 80.4˚F. Percipitation is about 2193mm /86.3 inch per year. The southern part of the state experiences heavy rainfall which is usually intense during the month of April through October (Hoiberg, 2010).

Imo State is bounded on the West by Delta State, on the North by Anambra State, on the South by Rivers State and on the East by Abia State. It comprises of three geopolitical zones: Orlu, Owerri and Okigwe. The state lies within latitude 4˚45' N and 7˚15' N and longitude 6˚50' E and 7˚25' E. The average annual temperature is 26.4˚C/ 79.6˚F. Abia State and Imo State are densely populated by men and women of all ages engaged

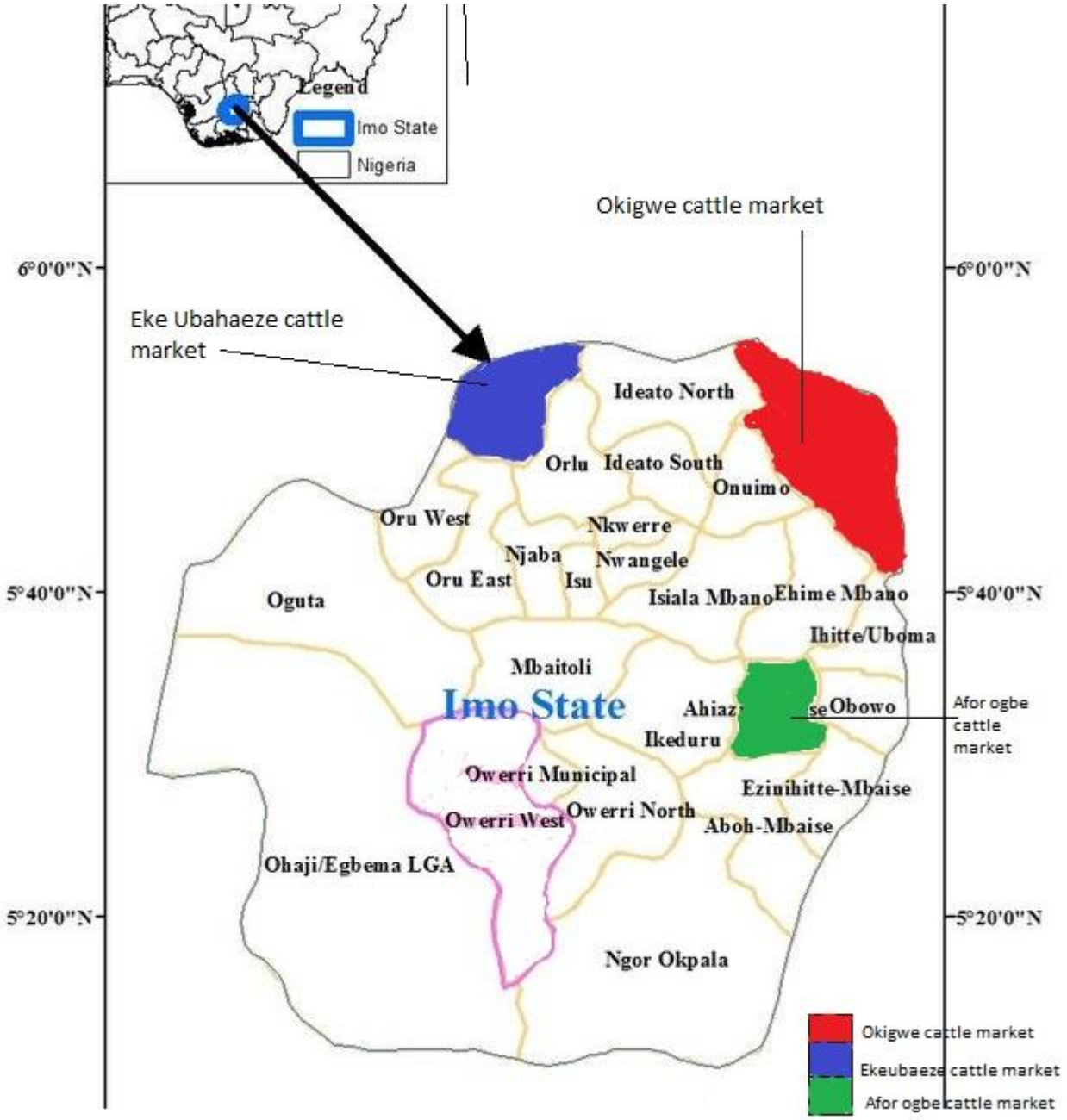
in different walks of life. One cattle market was selected from each of the geopolitical zones in the states bringing to six cattle markets in total (Chukwu, 2011).

## Ethical Permit

An introductory letter from the Ministry of Veterinary Services in Abia and Imo States was obtained and shown to the head of each cattle market association visited and owners of the animals for their consent (see appendix 8 and 9).


Lokpanta catttle market
Ubakala cattle market
Ahia udele
cattle market
30 0 30 60 Kilometers
Key
Lokpanta cattle market
Ubakala cattle market
Ahia udele cattle market

**Map of Abia state highlighting the cattle markets from the three geopolitical zone (adapted from John, *et al*., 2013)**


Legend
Imo State
Nigeria
Okigwe cattle market
Eke Ubahaeze cattle market
6°0'0"N
5°40'0"N
5°20'0"N
Ideato North
Orlu
Ideato South
Onuimo
Oru West
Nkwerre
Njaba
Nwangele
Oru East
Isu
Oguta
Isiala Mbano
Ehime Mbano
Ihitte/Uboma
Mbaitoli
Imo State
Ahiaz
se
Obowo
Afor ogbe cattle market
Ikeduru
Owerri Municipal
Ezinihitte-Mbaise
Owerri North
Owerri West
Aboh-Mbaise
Ohaji/Egbema LGA
Ngor Okpala
Okigwe cattle market
Ekeubaeze cattle market
Afor ogbe cattle market

**Map of Imo state highlighting the cattle markets from the three geopolitical zone (adapted from Adeyemi, *et al*., 2015)**

### 3.1.1 Experimental plan

Each state is made up of three geopolitical zones and each of the zones posses at least one cattle market giving a total of six different cattle markets selected from both states. They include Ubakala cattle market (Abia central), Ahia udele cattle market( Abia south) and Lokpanta cattle market(Abia north) from Abia State while Afor ogbe cattle market(Owerri zone), Eke ubahaeze cattle market(Orlu zone) and Okigwe cattle market(Okigwe zone) were selected from Imo state. Ten soil samples and ten air samples were collected from each of the selected markets respectively, from different points giving a total of sixty soil samples and sixty air samples.

Each of these markets had matured cows for sale and a population of between 50 to 100 cattle in the market.

### 3.1.2 Determination of sample size

Our study population is a migrant population and because the cattle markets do not keep records of number of cows that are brought into the market throughout the year so it becomes important that the sample size is now determined on the day the sampling was carried out.

To determine sample size, the systematic random sampling method (every k sampling) (Cochran, 1977) was employed where we divide total population encountered by desired sample size which is 5. First selection was by random

selection and then followed by a fixed periodic interval and this as follows:

$$K = \frac{N}{n}$$

where N = total population size

n = desired sample size

Thus in Abia State 1115 cattle were found in the markets and in Imo state 1140 were found during the period of the study. Applying the above formular for sample size (k) the number of sample was 451.

### 3.1.3 Method of Sample Collection

A survey of the markets was made and each had matured cows for sale and a population of between 50 to 100 cattle. The veterinary doctors

were recruited as field assistants in the course of this work and were instructed on how to collect samples of skin scrapings from different parts of the cattle skin. A total of 451 cattle were sampled. These areas were cleaned with disposable alcohol swab pad and allowed to evaporate. A sterile tooth brush was used to brush the surface of the required areas of the cattle skin. Four hundred and fifty-one (451) skin samples were collected in a sterile white paper packet and placed in a sterile bottles. Hand gloves were worn and used in collecting sixty (60) soil samples randomly from different points within the markets. Air samples were collected using settled plate technique. Sixty (60) sabouraud agar plates supplemented with chloramphenicol (20mg) were opened at different

locations within the markets for air sample collection. Each collected sample was labeled based on location /zone where it was collected, name of the specimen and date of collection. The sample containers were wrapped with aluminum foil and taken to Imo State university microbiology laboratory for analysis.

## 3.2 MICROSCOPIC EXAMINATION OF SAMPLES

The 451 cattle skin samples were examined by direct microscopy as described by Chesbrough, (2010) and then cultured on the selected media.

**PROCEDURE**

- A drop of 10% KOH was placed on a clean glass slide.
- A small piece of the cattle skin sample was placed on the drop of KOH
- It was covered with coverslip and allowed to clear.
- It was examined microscopically using x10 and x40 objectives for the appearance of hyphae from cattle skins.
- For infected samples, both arthrospores and hyphae were checked for and note was taken of whether infection is located within or outside.

## 3.3 CULTURE TECHNIQUE

Sabouraud dextrose agar and Malt extract agar was prepared according to the manufacturals

instruction. Each sample was cultured directly on the plates of sabouraud dextrose agar and malt extract agar by spreading skin scrappings. The sterile brushes which was used for sample collection, was smeared on the plates containing 20mg of chloramphenicol each to inhibit bacteria growth. This was later kept in the incubators at 37 ℃ for 1-2 weeks.

## 3.4 DILUTION OF SOIL SAMPLE

One gram of each soil samples were suspended in 10ml distilled water in different sterile sample bottles shaked vigorously and allowed to stand for 10 mins. For each suspended sample with a sterile syringe 1ml aliquot was collected and diluted in 9ml distilled water . From which 1ml

was also collected and smeared on the sabouraud dextrose agar media supplemented with chloramphenicol for culture at 37˚C for 1-2 weeks (Sujatha & Swethalatha, 2017).

## 3.5 PRESERVATION OF ISOLATES

Discrete pure colonies of each isolate was inoculated on Sabouraud dextrose agar slants and incubated at 37ºC for 1 week. The slants containing the pure cultures were stored in the refrigerator until required for further studies (Cheesbrough, 2010).

## 3.6 SLIDE CULTURE

Slide culture was carried out to examine the microscopy features of isolated non-dermatophytic molds.

**PROCEDURE**

- 6mm square block of sabouraud dextrose agar was cut and placed on a sterile glass slide.
- With a sterile wire loop, pure culture of isolates was inoculated at the four edges of the sabouraud dextrose agar block.
- The inoculated agar blocks was covered with a coverslip.
- The whole slide was placed in a Petri dish supported under with sterile glass slide.
- A blotting paper were soaked in glycerol water and placed in the bottom of the Petri dish to

prevent drying, this was incubated for one week at 27˚C.

- The coverslip was removed, when there was clearly visible growth from the block, and mounted in a drop of lactophenol cotton blue.
- The block was discarded by dipping it into a disinfectant and a drop of lactophenol cotton blue was added to the growth on the slide and covered with a coverslip.
- Both preparations were examined microscopically using x10 and x40 objectives.

## 3.7 HAIR BAIT TECHNIQUE

1. Hair bait technique of Sandeep and Geeta (2016) as in vanbreuseghem (1952) was employed to isolate and detect the presence of

keratinophilic fungi from soil samples. The moistened soil samples were dispensed on sterile petri dish , after which sterilized defatted human hair were sprinkled over the soil surface. This was incubated at 27℃ in a dark cupboard for 1week. Hair showing signs of fungal growth was removed and viewed under the microscope by placing it in a drop of lactophenol cotton blue to observe for hair strand peforation while some were dropped in sabouraud agar plates containing cloramphenicol.

## 3.8 IDENTIFICATION OF ISOLATES

The isolates were identified using standard methods as in Cheesbrough (2010). The observations from slide culture (surface and

reverse side) of the isolates were compared with standard mycology atlas for identification (David, *et al*., 2007). The identification was based on growth rate, colonial and microscopic morphology.

## 3.9 MOLECULAR STUDIES ON THE ISOLATES

Molecular analysis was also carried out on the isolates at Niger Delta University Bayelsa Wilberforce Island Bayelsa State Nigeria using DNA extracting technique and was later sent to South Africa for DNA Sequencing technique.

### DNA extraction

Extraction was done using a ZR fungal DNA mini prep extraction kit supplied by Inqaba South Africa. A heavy growth of the pure culture of the fungal isolates was suspended in 200 microlitres of isotonic buffer into a ZR bashing bead Lysis tubes, 750 microlitres of lysis solution was added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tube were centrifuged at 10,000x*g* for 1 minute.

Four hundred (400) microlitres of supernatant was transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000x*g* for 1 minute. One thousand two hundred (1200) microlitres of fungal DNA

binding buffer was added to the filtrate in the collection tubes bringing the final volume to 1600 microlitres, 800 microlitres was then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000x*g* for 1 minute, the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) microlitres of the DNA Pre-Wash buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000x*g* for 1 minute followed by the addition of 500 microlitres of fungal DNA Wash Buffer and centrifuged at 10,000x*g* for 1 minute.

The Zymo-spin IIC column was transferred to a clean 1.5 microlitres centrifuge tube, 100

microlitres of DNA elution buffer was added to the column matrix and centrifuged at 10,000x*g*microlitres for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20 degree for other downstream reaction ( Osama, *et al*., 2011).

**DNA quantification**

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal.

The DNA concentration was measured by clicking on the “measure” button (Osama, *et al*., 2011).

**Internal Transcribed Spacer (ITS) Amplification**

The ITSregion of the rRNA genes of the isolates were amplified using the ITS 1F:5'-CTTGGTCATTTAGAGGAAGTAA 3' and ITS4: 5' TCCTCCGCTTATTGATATGC-3’, at the medical laboratory department NigerDelta University Wilberforce Island, Bayelsa State. Primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the

primers at a concentration of 0.8µM and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 53ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 25 minutes and visualized on a blue transilluminator (Osama, *et al*., 2011).

**Sequencing**

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of

10ul, the components included 0.25 ul BigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing condition were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min.

**Phylogenetic Analysis**

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were obtained from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbousr-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500

replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method as in (Kaplan and Risko, 1983).

## 3.10 PATHOGENECITY TEST OF ISOLATES ON ALBINO MICE

Twenty mice were bought from the Department of Biochemistry Imo State Polythenic Umuagwo and examined for skin infection. The mice skin were cleaned with distilled water and were fed for two weeks before introducing the 10 isolates recovered from this study on them. These mice were paired in twos and placed in different cages giving ten different cages. The cages were

labeled against the name of the isolate applied. For each pair, the isolate was rubbed on one mice while the other pair served as the control.A fragment of the isolates were diluted in 5ml of distilled water containing beads to break the fungal strands after which it was then rubbed on the skin of the mice with a sterile swab stick (McEvoy, *et al*., 2005).

## 3.11 ENZYME SCREENING TEST

### Keratinase screening test

Chicken feathers were the substrate used as source of keratin. It was precipitated in acetone allowed to air dry and then ground before it was added to a sterile agar medium as the only source of carbon and nitrogen. The agar medium consists

of ($g/l^{-1}$): $MgCl_2.6H_2O$ (0.1), NaCl (0.5), $K_2HPO_4$ (0.4), $KH_2PO_4$ (0.4), $MgCl_2.6H_2O$ (0.1), yeast extract (0.1) chicken feather (10), 1 liter of distilled water, agar (15) and pH 7.5. The isolates were inoculated at the center of the medium and then incubated for 6 days at room temperature. Keratinase activity of the fungus was then detected as a clear zone around the colony and the diameter measured using a transparent milimeter rule (Korkmaz and Diyncer, 2004).

**Amylase screening**

The amylolytic activity was carried out on fourteen isolates. This was determined by using starch agar plate method described by Bertrand *et al*., (2004). This was done by inoculating the organism individually into potatoes dextrose agar

medium which was supplemented with 1g of starch. The agar plates were then incubated at 30℃ C for 5days. After the incubation period, lugols iodine solution was added to the culture plate to destain and observe zone of clearance around the cultures. The diameter of hydrolysis formed after the introduction of iodine solution was measured to represent the amylolytic activity using a transparent milimeter rule.

**Protease screening test**

Isolates were screened for protease activity by inoculating them on agar medium incorporated with gelatin. The agar medium used was composed of (g/l): 5g peptone powder, 3g beef extract, 3g NaCl, 15g Agar, 1% gelatin, 1 liter of

distilled water, pH 6.0 as described by (Mitchell, *et al*., 2007). After incubation at 35° C for 5-6 days. Clear zones around the colony was an indication of protease production and this was observed by flooding with aqueous saturated solution of mercuric chloride reagent (15g $HgCl_2$ dissolved completely in 20ml 7M conc. of Hcl ,then raised to 100ml with sterile water) .The diameter of zone of clearance using millimeter rule indicates the protease activity.

**Lipase screening test**

Lipase screening test was determined by using plate screening medium according to Singh *et al*., (2006) lipolytic assay. In this method the medium was prepared using olive oil as the lipidic

substrate and phenol red as the indicator. To obtain the basal medium : peptone -5g/l, yeast extract-2g/l, agar-15g/l ,olive oil -10ml/l and phenol red 10g/ls as the indicator . Agar blocks from four days old culture were inoculated on the basal media plates. These plates were incubated at 25$^{\circ}$C for 5 days. Phenol red that has an end point at pH 7.3-7.4 when the color is pink will turn to a yellow colouration when the pH decreases to 7.0 - 7.1. The presence of lipolysis activity was indicated by the yellow colouration. The diameter of hydrolysis was measured using millimeter rule.

**Cellulase screening test**

The cellulose screening test of the fungal isolates were determined by using plate screening

medium according to Mendel (1975) mineral salt per liter (g/l) solution which is composed of : urea -0.3, $(NH4)_2SO4$ -1.4, $KH_2PO$ -2.0, CaCl – 0.3, $MgSO_4$ -0.3, yeast extract -0.25 and protease peptone - 0.75 with $10gl^{-1}$ of carboxymethyl cellulose (CMC) and$17.5gl^{-1}$ agar (Mandels, 1975). Agar blocks (8mm in diameter) from one week old fungal colony were cut and inoculated at the centre of the basal media plates . These plates were incubated at 25℃ for 5 – 7 days. Cellulolytic strains were selected based on the diameter of the

cellulolytic hydrolyses zone surrounding the colonies after flooding plates with 1% congo red dye ( 0.5-1h) followed by destaining with 1M NaCl solution for 15-20 minutes.

## 3.12 COLLECTION, IDENTIFICATION AND PREPARATION OF PLANT MATERIALS

Fresh leaves of *Mitracarpus scaber*, *Jatropha multifida*, *Occimum gratissimum* and *Euphorbia hirta* plants were collected from the Federal research institute of Ibadan, Nigeria.They were identified and authenticated at the Department of Botany Igbenedion University Okada, Edo State by a taxonomist Fajana Akibu. To prepare the plant material (leaves) for analysis, the leaves were washed and rinsed with distilled water, dried initially at room temperature and finally in a

thermostatically controlled hot air oven at 40˚C until constant weight is maintained (Mbata, *et al*., 2009).

The leaves were ground into fine powder in an electric blender (Binatone blender BLG -452). The powdered materials were stored in screw capped bottles and kept in a refrigerator at 4℃ until required for use (Ameh, 2010).

## 3.13 PHYTOCHEMICAL SCREENING OF THE PLANT LEAVES

### Test for Saponins

To test for saponin, 3g of plant powder was boiled in 10ml of water, warmed and filtered. 2 ml of filtrate was mixed with 5ml of distilled water and then warmed on the water shaken

vigorously for a stable persistent froth. The presence of frothing which lasted for which lasted for 3 – 5 minutes indicated the presence of saponins (Khandelwal, 2006).

**Test for phenolic compounds**

To test for presence of phenol, 5g of the sample was also boiled with 25ml of methanol, then warmed on water bath and filtered. 2ml of the filtrate was mixed into 2ml of 1% ferric chloride solution. The formation of brownish- green precipitate was taken for the presence of condensed tannin while bluish-black precipitate was taken for the presence of hydrolysable tannin (Khanelwal, 2006).

**Test for flavonoids**

Another 2ml of filtrate obtained from methanol extract of the plant samples was added to 2ml dilute ammonia solution. The appearance of a yellow colour was taken for the presence of flavonoids (Khandelwal, 2006).

**Test for steriods**

To test for steroids, 2ml of acetic anhydride was added to 0.5g methanol extract of sample in the presence of 2ml $H_2SO_4$. Colour change from violet to blue or green indicates the presence of steroids (Khandelwal, 2006).

**Test for terpenoids**

To test for terpenoid, 0.5g of methanol extract was mixed in 2ml of chloroform. 1ml of

concentrated $H_2SO_4$ was carefully added to form a lower layer. A reddish brown coloration at interface was taken for the presence of steroids (Khandelwal, 2006).

**Test for alkaloids**

A quantity of 0.1g of the ground samples were boiled with 5ml of 2% hydrochloric acid on a steam bath. This was filtered and 1ml portion of the filtrate reacted with 2 drops of the following reagents (Trease and Evans, 1983).

a. Wagners reagent (Iodine in potassium iodide solution), and observed for reddish brown precipitate.

b. Meyers reagent (potassium mercuric iodide solution), and observed for creamy coloured precipitate.

**Test for phlobatannin**

To test for presence of phlobatannin, 0.5g of methanol extract was added 3ml of 1% HCL and warmed on water bath at 90˚C for 15 minutes. The formation of red residue at the base of test-tube was taken for the presence of tannin (Khandelwal, 2006).

**Test for Cardiac glycosides (Keller-Killani)**

The presence of cardiac glycoside (five membered lactone, characteristics of cardiac glycoside) was tested by adding of 1ml methanol

extract of the plant samples with 1ml of kedde's reagent and 1ml of dilute sodium hydroxide, the formation of violet colour was taken as presence of cardiac glycosides (Khandelwal, 2006).

## 3.14 QUANTITATIVE ANALYSIS OF PLANT LEAVES

### Alkaloid determination

The plant leave extracts (1mg) was dissolved in dimethyl Sulphoxide (DMSO), 1ml of 2N Hcl was added and filtered. 1ml of the filtrate was mixed with 1ml of bromocresol green solution (BCG), 2ml of chloroform and was later diluted with 6ml of water.

A standard solution of atropine was also prepared in the same manner as described earlier. The

absorbance of test and standard solution was determined against the reagent blank at 470nm wavelength using the UV spectrophotometer (Harbone, 1973).

**Phenol determination**

The phenolic content of the plant leaves was determined using Folin-Denis reagent. 10ml of the sample filterate was mixed with 1ml of Folin-Denis reagent and 1ml of sodium carbonate. The solution was kept at room temperature for 5minutes.

For standard solution gallic acid was prepared (5g of gallic acid was dissolved in 100ml of water and mixed with 1ml of sodium carbonate).

This was used to measure absorbance for the test at 750nm using spectrophotometer (Harbone, 1973).

**Glycoside determination**

Glycosidic content was measured by solubilizing 1gram of sample into 50ml of water and filtered. 1ml of the filtrate was mixed with 2ml of dinitricsaliculic acid (10g of sodium hydroxide was dissolved in 250ml of water, 60ml of this solution was then mixed with 2grams of saliculic acid) and allowed to stand for some minutes.

For standard solution, glucose solution was prepared and used to determine total glycosidic

content for test at 540nm wavelength (Harbone, 1973).

## Saponin determination

Saponin content was determined by dispensing 0.5g of sample into 40ml of 50% methanol in a conical flask. This was heated for 3hours after which the solution was filtered. 1ml of filterate was mixed with 5ml of magnesium carbonate and 1ml of 5% ferric chloride. The absorbance of saponin content was determined using spectrophotometer at wavelength at 380nm (Harbone, 1973).

## Flavonoid determination

The flavonoid content was determined by dissolving 1g of sample in 50ml of 50% methanol. 1ml of extract was mixed with 200 microliter sodium nitrate (100ml,5%) , 200microliter of 10% aluminum chloride and allowed to stand for 5 mins after which 1ml sodium hydroxide was added and allowed to stand for 10minutes. The absorbance was of the test was measured using spectrophotometer at 510nm (Bohn and Kocipai-Abyazan, 1994).

**Tannin determination**

To determine concentration of tannin, 1ml of sample filtrate was mixed with 0.5ml of Follin-

Dennis reagent and 5ml of 5% sodium carbonate. This was kept undisturbed for about 30mins.

The standard solution of 1mg/ml tannin acid was prepared by dissolving 100mg stock solution of tannic acid in water. 1ml of tannic acid was measured and mixed with 0.5ml of Folin -Denis reagent and 5ml sodium carbonate solution. The tannin content for the test was measured by reading at 760nm against blank reagent using spectrophotometer (Polshettiwar, *et al*., 2007).

**Steriod determination**

To determine the concentration of steroid, 0.5 gram of sample was weighed and mixed with 50ml of 50% methanol which was heated for 30minutes and then filtered. The filterate was

mixed with 2ml of 2mole solution of sulphuric acid, 2ml of 10% ferric chloride and 0.5ml of ferric cyanide. The absorbance was read at 750nm wavelength using UV spectrophotometer (Harbone, 1973).

## 3.15 SUSCEPTIBILITY TESTING OF ANTIFUNGAL AGENTS

For the chemotherapeutic agents, Ketoconazole, Fluconazole, Voriconazole and Amphotericin B were tested on the isolates using Kirby Bauer disc diffusion method. A cell suspension of the organisms (Inoculum) equivalent to 0.05% Mcfarland standard was employed (Kirby and Bauer, 1996).

## Inoculum Preparation

The growth method was performed as follows:

1. Colonies of fungi were selected from a one week agar plate culture. The top of each colony was touched with a sterile loop, and the growth was transferred into a tube containing 4ml of normal saline and compared with 0.05% McFarland standard (0.6ml of 1%barium chlorie solution to 99.4ml of 1% sulphuric acid).
2. The turbidity was adjusted with sterile saline to obtain a turbidity optically comparable to that of Mcfarland standard (Adriana and Sandra, 2015).

## Inoculation of Test Plates

1. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, 0.1ml of the suspension was dispensed on the dried surface of the sabouraud dextrose agar plate.
2. The dispensed innoculum on the dried surface of the sabouraud dextrose agar plate was evenly spread on its surface using a hockey stick (Adriana and Sandra, 2015).

## Application of Discs to Inoculated Agar Plates

1. The oxoid brand antimicrobial discs made in United Kingdom (Ketoconazole 10µg (0.01mg), Fluconazole 25µg (0.025mg), Amphotericin B 20µg (0.02mg), Voriconazole 1µg (0.001mg) were dispensed onto the surface of the inoculated agar plate. Each disc

was pressed down to ensure complete contact with the agar surface. Drug diffuses almost instantaneously, a disc was not relocated once it came into contact with the agar surface.

2. The plates were inverted and placed in an incubator set to 28°C within 15 minutes after the discs were applied.

3. After 72 hours of incubation, each plate was examined for zone of clearance which was measured

4. The drugs that inhibited growth of isolates around it was taken as been sensitive while drugs that allowed growth of isolates on it was taken as been resistant.

## 3.16 EXTRACTION OF ACTIVE COMPONENTS FROM PLANT MATERIALS

The active principle of the plant (leaves) were extracted, using soxhlet extractor and rotary evaporator in the microbiology laboratory of Igbenedion University Okada, Edo State. One kilogram of the powered materials, was extracted in 250ml of 95% methanol to extract the active principle using soxhlet apparatus as described by Mbata *et al*., (2009). The crude extract was allowed to evapourate to dryness using rotary evaporator (Mbata, *et al*., 2009).

## 3.17 ANTIFUNGAL SUSCEPTIBILITY TESTING OF EXTRACTS

Antifungal susceptibility test was carried out on 12 test isolates using the crude extracts of the plant leaves. This was carried out, using disc diffusion method. In this method, 1ml of the test isolate in broth was diluted in 9ml of distilled water. 0.2ml of the dilution was spread evenly on the surface of the solidified sabouraud dextrose agar (SDA) plates, using a sterile bent glass rod spreader (Hockey stick) before introducing the discs.

## 3.18 DILLUTION OF PLANT EXTRACTS

Crude extracts of the plant (leaves) was allowed to evapourate to dryness. The solvent used for all plant extracts in this study was 99% dimethyl sulfoxide (DMSO). Two grams (2g) of the dried

extract was dissolved in 10ml of 99% dimethlyl sulphoxide (DMSO) to obtain the stock of 200mg/ml. Briefly two-fold serial dilution was carried out from the stock to obtain the following concentrations 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. These were applied on already prepared discs (Maurice, *et al*., 2013).

**PREPARATION OF DISC**

Sterile paper discs (made of Whatman No.1 filter paper) measuring 6mm were used to impregnated with 20µl of the different dilutions (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml) of the plant extract and then air dried. This discs were transferred onto the solidified sabouraud dextrose agar media inoculated with

the isolate. 20µl of 99% DMSO was also impregnated on a separate disc and delivered as a control and labeled accordingly. The discs were kept on the bench for 40minutes for pre-diffusion of the extract. After diffusion, the discs were aseptically introduced on the surface of the medium with the aid of a sterile forceps and allowed for 10-15 mins, before incubating at 35℃ for 72hours and examined for fungal growth inhibition. The diameter zone of growth inhibition on the different plates was measured in millimeters, using a transparent milimeter ruler.

## 3.19 DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION

## (MIC) OF THE PLANT EXTRACT USING TUBE DILUTION METHOD

The tube dilution method of broth was used to determine the MIC of the test extract, using two fold serial broth dilution method. The selected plant leaves extract were *Occimum gratissimum*, *Euphorbia hirta*, *Jatropha multifida* and *Mitracarpus scaber.*

Sabouraud dextrose broth was used to prepare the stock (5ml of 99% DMSO was used to dissolve 2g of the extract initially and then raised to 10ml using 5ml sabouraud dextrose broth) to give 200mg/ml. This stock was diluted serially by introducing 5ml of the stock aliquot into another 5ml of sabouraud dextrose broth to obtain 100mg/ml. Similarly dilutions up to $10^{-4}$ was

carried out to obtain 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml respectively. The serially diluted test extracts were then inoculated each with 0.2ml of $10^{-2}$ dilutions of an overnight broth culture of the test isolates. All tubes were incubated for 72 hours and later inoculated onto sterile sabouraud dextrose agar plates and incubated. The least drug concentration that showed least growth was taken as the minimum inhibitory concentration (MIC) for the particular plant extract while absence of growth indicates a fungicidal concentration; hence the Minimum Fungicidal Concentration (MFC) as described by Clinical Laboratory Standards Institute, CLSI, (2008).

## 3.20 METHOD OF ANALYSIS OF DATA

The data obtained from this study were analyzed statistically using multiple comparison and one way analysis of variance (ANOVA) as in Martins and Igwemma (2000).

# CHAPTER FOUR

## 4.0 RESULTS

The results of the total population of cattle sampled from Abia state were given in Table 1. Out of 223 cattle sampled from Abia State, 54 were sampled from Ubakala cattle market representing Abia central zone, 71 were from Ahia Udele cattle market representing Abia South Zone and 98 were sampled from Lokpanta cattle market representing Abia North Zone. Out of the 223, 24 cattle had lesion. Out of the 24 that had lesion, 1 was recovered from Ubakala cattle market, 10 was recovered from Ahia Udele cattle market while 13 was recovered from Lokpanta cattle market. The infections were observed to be encountered more amgonst cattle from Ahia udele

cattle market (14.1%) while cattle from Ubakala cattle market had the least encounter of infection (1.9%) while in Imo state a total of 228 cattle were sampled from cattle markets in Imo State as shown in table 2. Out of the 228 cattle sampled, 78 were sampled from Afor Ogbe cattle market representing Owerri zone, 70 were sampled from Eke Ubahaeze cattle market representing Orlu zone and 80 from Okigwe cattle market representing Okigwe zone. Out of the 228 cattle sampled, 29 had skin lesion. Out of the 29 cattle encountered with lesion, 10 was recovered from AforOgbe cattle market, 8 was recovered from Ekeubahaeze cattle market while 11 was recovered from Okigwe cattle market. The infection were observed to be encountered more

with cattle from Okigwe cattle market (13.8%) while cattle from Eke ubahaeze cattle market had the least prevalent of (11.4%).

**TABLE 1: Total population of cattle sampled from different cattle market/zone including cattle with lesion in Abia state, Nigeria.**

| CATTLE MARKET/ZONE | TOTAL POPULATION | POPULATION WITH LESION (%) |
|---|---|---|
| Ubakala Cattle market /Abia central | 54 | 1(1.9) |
| Ahia Udele Aba/Abia South | 71 | 10(14.1) |
| | | 13(13.3) |

| | | |
|---|---|---|
| Lokpanta/Abia North | 98 | |
| TOTAL | 223 | 24 |

**TABLE 2: Total population of cattle sampled from different cattle market /zone including cattle with lesion in Imo state, Nigeria**

| CATTLE MARKET/ZONE | TOTAL POPULATION | POPULATION WITH LESION (%) |
|---|---|---|
| Ogbe cattle market/ Owerri Zone | 78 | 10(12.8) |
| | 70 | 8 (11.4) |

| | | |
|---|---|---|
| Eke Ubahaeze Cattle market/Orlu Zone<br><br>Okigwe cattle market/Okigwe zone | 80 | 11(13.8) |
| **TOTAL** | **228** | **29** |

The distribution of samples collected based on anatomical sites including sites with lesion was shown in table 3. A total of 31 samples were sampled from the head of cattle but no lesion was observed there. 58 samples were sampled from the leg region and 10 had lesion. 15 were sampled from the ear region and 3 had lesion, 16 were sampled from the neck region and no lesion was observed. 8 were sampled from the groin region and 6 had lesion. 285 were sampled from the abodomen and 27 had lesion while 38 were sampled from the tail and 7 had lesion. From the

result, most of the lesion were sampled from the abdomen n= 27 (51.0%) followed from the Leg n= 10 (18.9%), tail n= 7 (13.2%), groin n= 6(11.3%) while the least was sampled from the ear n= 3 (6.0%). Plates 1- 5 shows some of lesions encountered from different parts of the cattle skin during the course of this study.

Some of the morphological characteristics of some non-dermatophytic molds isolated from this study includes *Aspergillus welwitschiae* which expresses a dusty black with white egde and a striated creamy to brown reverse while microscopically it has a large conidiophores with a globular vesicle that is brown to black with biserate phialides that cover the entire surface of the vesicle forming a radiate head as shown on

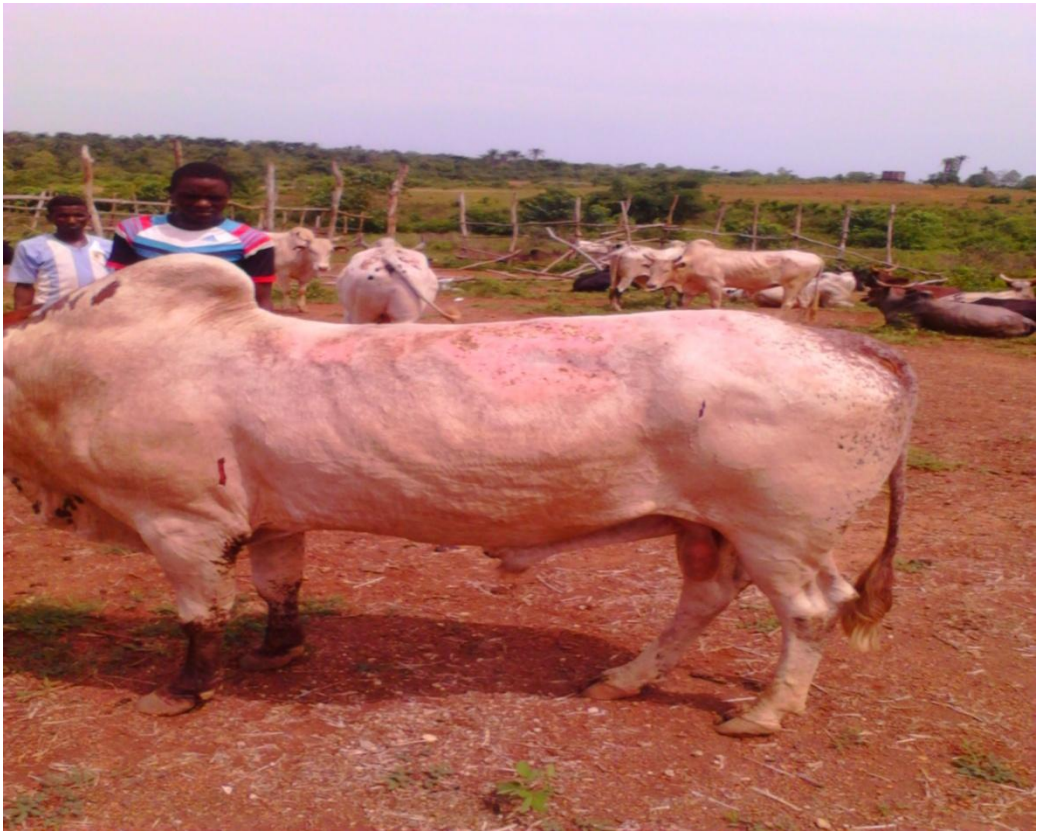
plate 6. *Absidia corymbifera* which expresses colonies that grow rapidly, filling the petri dish. It is usually woolly white but turns gray with age. The reverse is also white and turns grey-black with age. Microscopically it produces few rhizoids with sporangiophore that grows at a point on the stolon as shown on plate 7. *Penicilliumcitrinum* expresses morphologically a folded blue colour with a shiny surface. Its reverse is rough with a creamy –yellowish colour. Microscopically it shows a fruiting head. Its conidia are oval in shape with a conidiophores that posses 3-5 metulae as shown on plate 8. *Fusarium oxysporum* expresses a woolly white growth that tends to turn purple at the centre. Microscopically shows macroconidia that are

slightly curved and are pointed at the centre as shown on plate 9. *Cladosporium tenuissimum* phenotypically present black mold with dark black cracking reverse. Microscopically the hyphae are dark and septate with branches. The conidiophors are usually elongatrd and produce chains of ellipsoid as shown in plate 10.

**TABLE 3: Distribution of samples collected based on anatomical sites including sites inflicted with lesions**

| ANATOMICAL SITES | TOTAL SAMPLES COLLECTED FROM ANATOMICAL SITES | TOTAL SAMPLES COLLECTED FROM ANATOMICAL SITES WITH LESION | % OCCURRENCE OF ANATOMICAL SITES WITH LESIONS |
|---|---|---|---|
| HEAD | 31 | 0 | 0.0 |

| | | | |
|---|---|---|---|
| LEG | 58 | 10 | 18.9 |
| EAR | 15 | 3 | 6.0 |
| NECK | 16 | 0 | 0.0 |
| GROIN | 8 | 6 | 11.3 |
| ABDOME | 285 | 27 | 51.0 |
| N | 38 | 7 | 13.2 |
| TAIL | | | |
| **TOTAL** | 451 | 53 | |



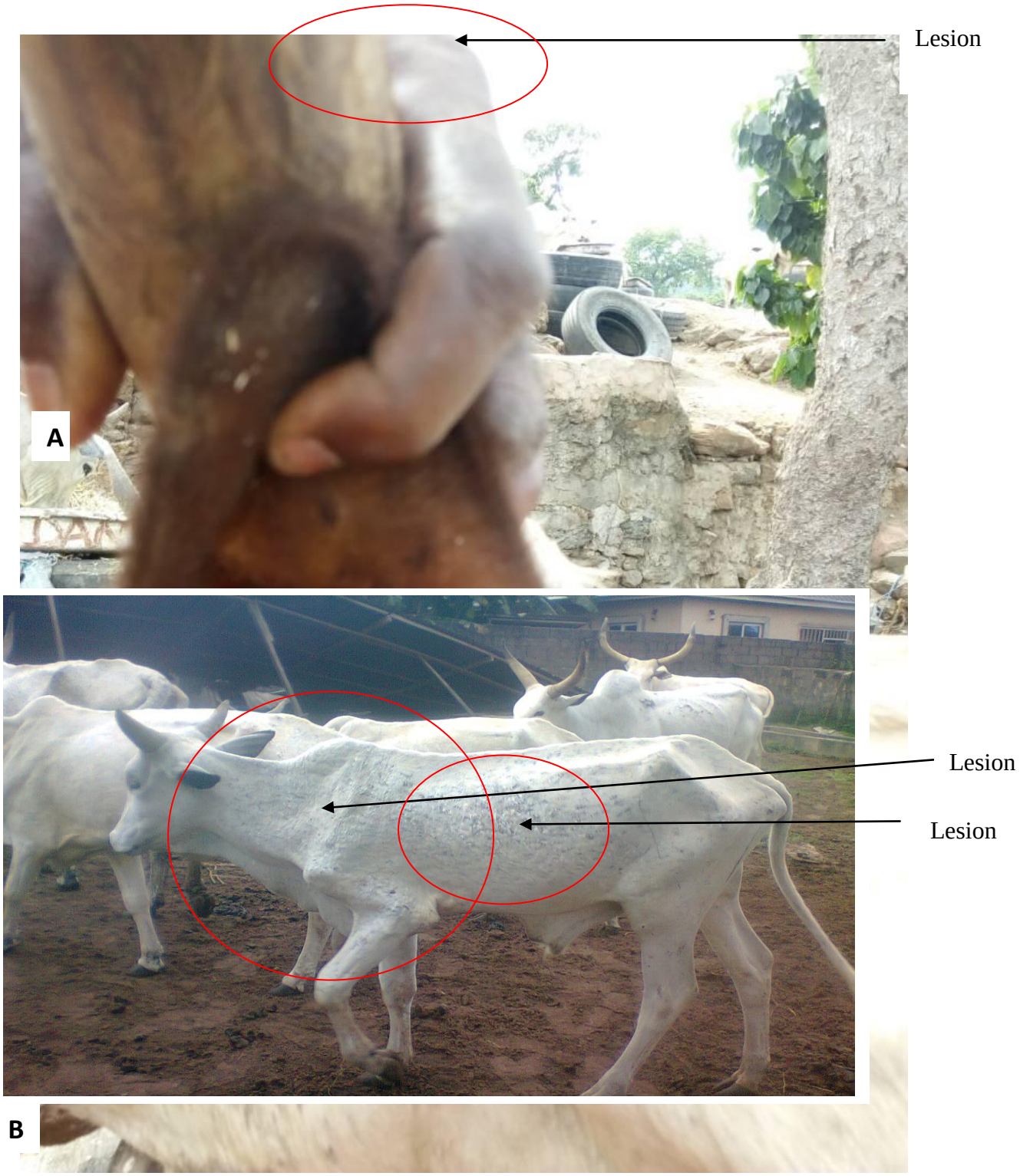



Plate 1;  lesions on the abdomen (A and B)

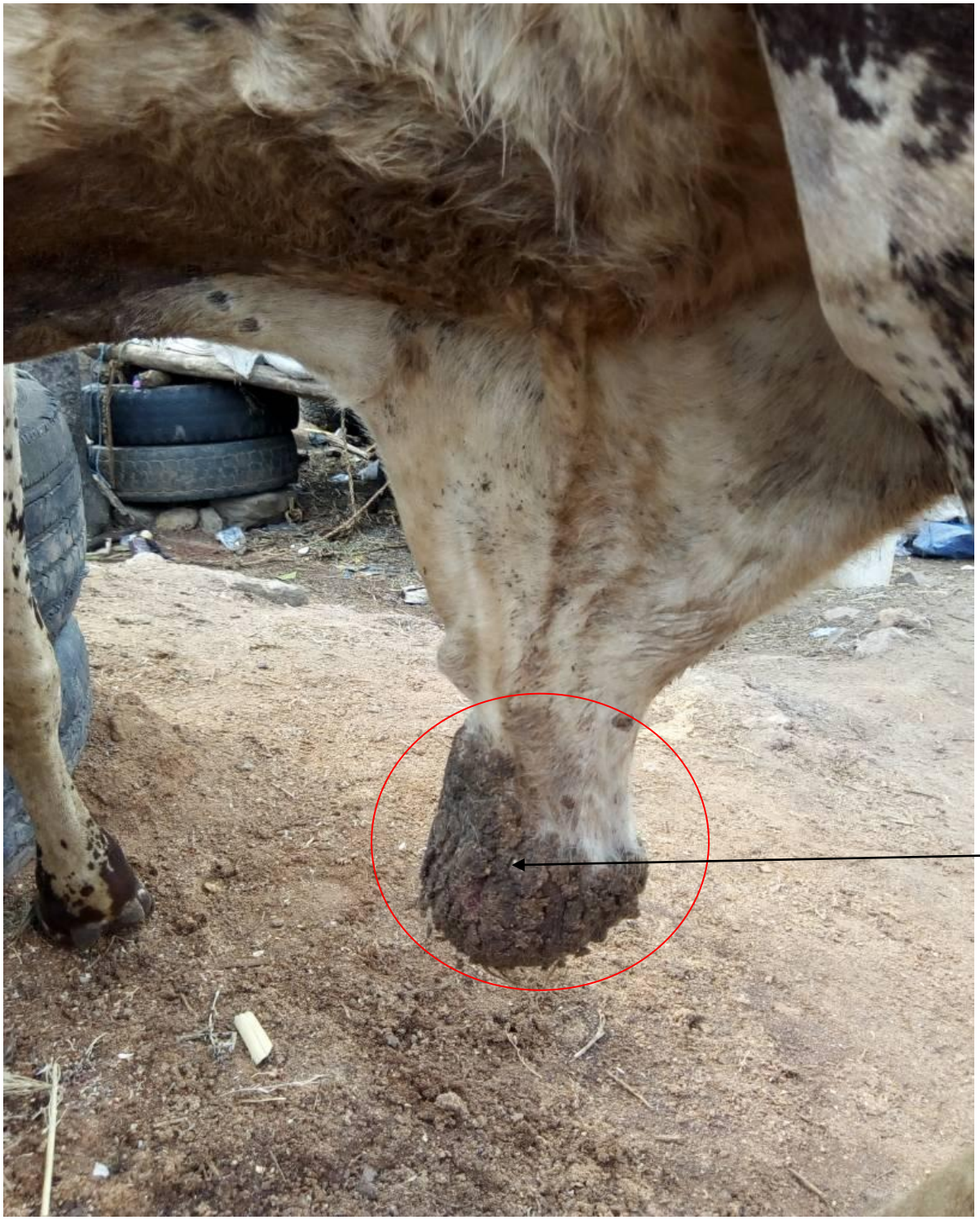



Plate 3;  lesion on the cattle groin

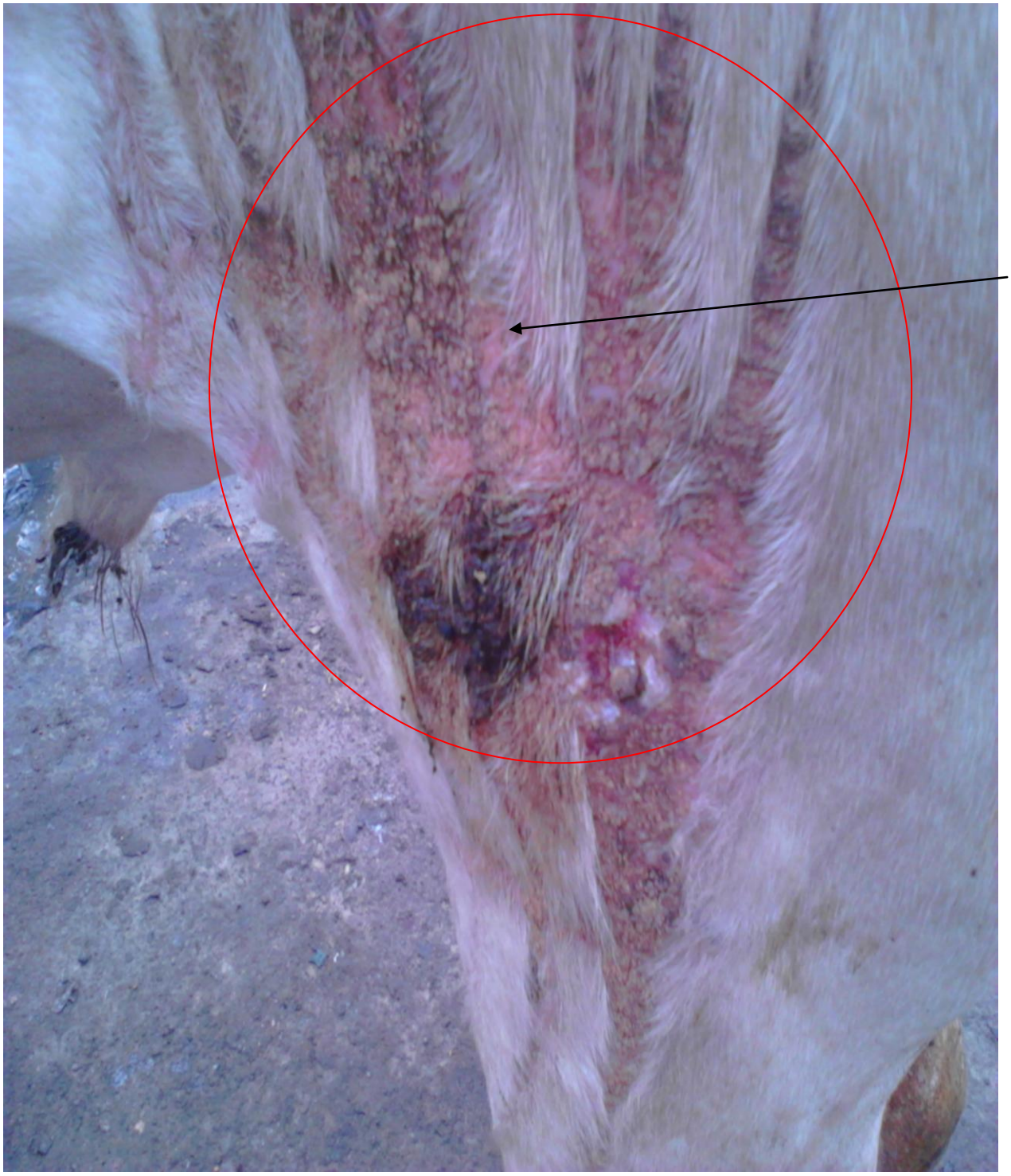
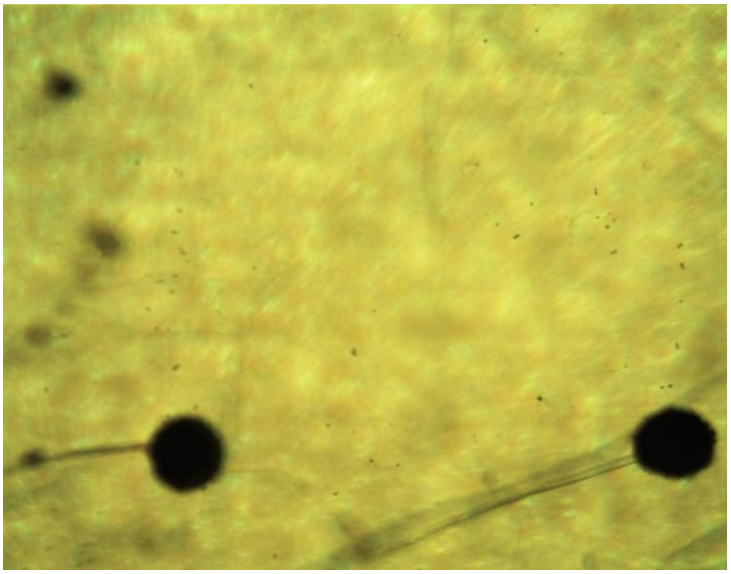



Plate 4:  lesions on the leg


Plate 5: lesion on the tail


**A** Dusty black colour

**B** Straited creamy brown

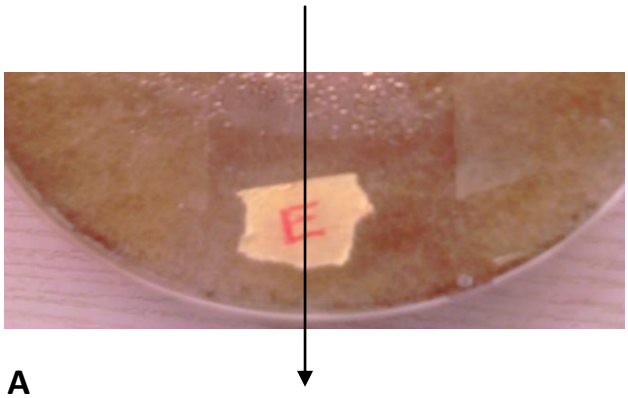


**C**

**C**

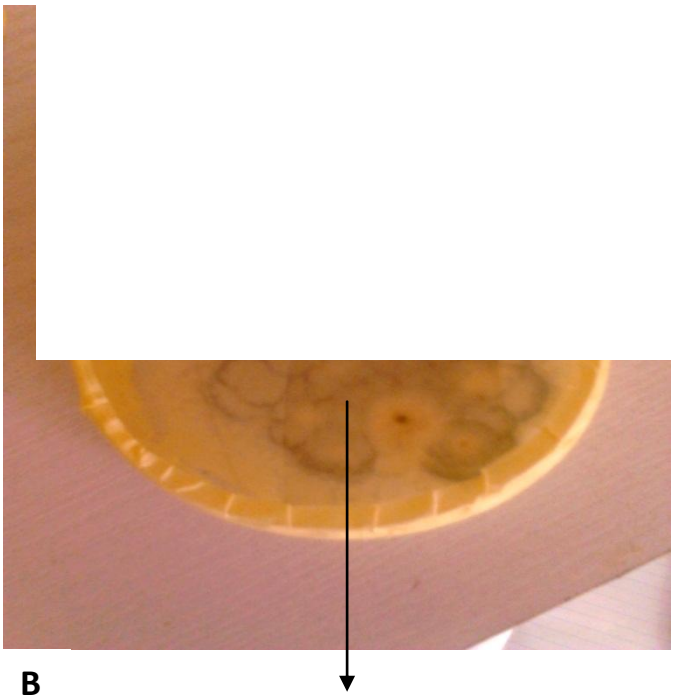
Microscopic view showing black phialides that covers the entire surface forming a radiate head.

Plate 6; front view of *Aspergillus welwitschiae* showing dusty black colour (A), reverse view showing a striated creamy brown colour(B) and microscopic view showing large conidiophores with a globular vesicle with brown black phialides that cover the entire surface forming a radiate head (C)



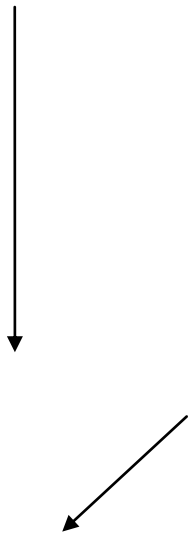
**A**

Wolly white colour that turns gray with age



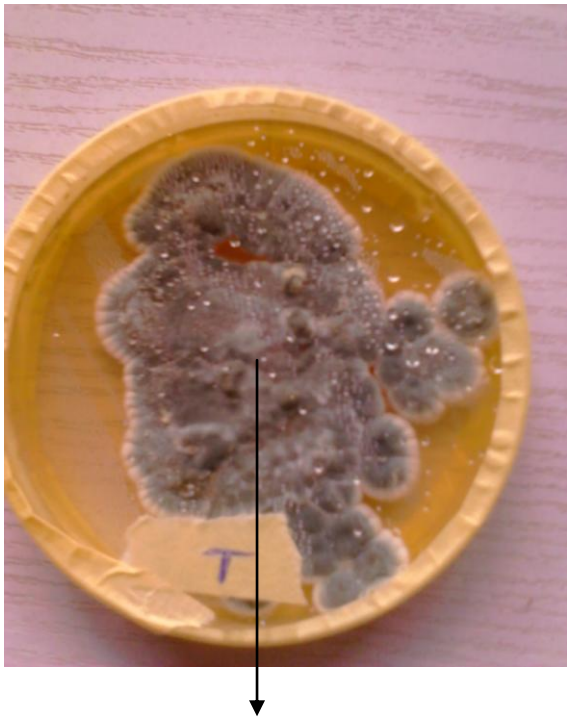
**B**

Reverse view of *Absidia corymbifera*



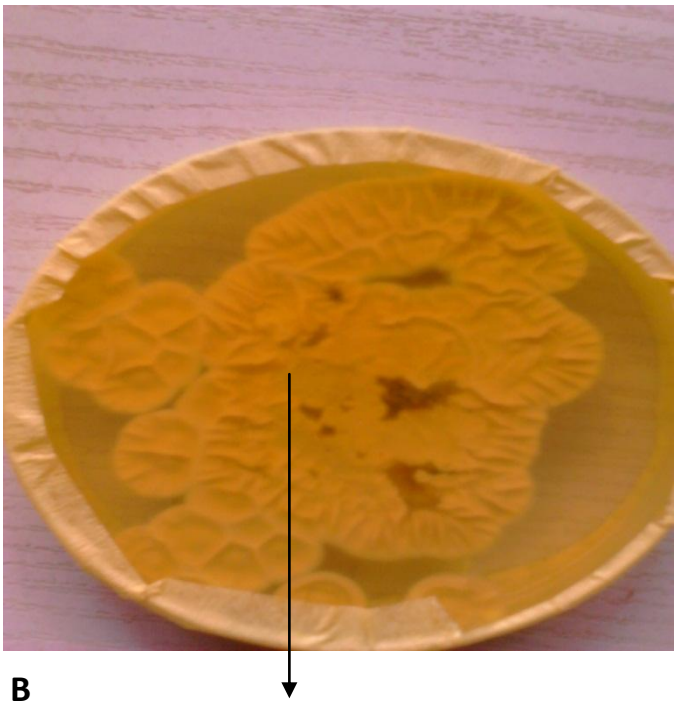
**C** Microscopic view showing sporangiophore that grows at a point

Plate 7; front view of *Absidia corymbifera* with its wolly white colour that turns grey with age (A), reverse view (B) and microscopic view showing rhizoids with sporangiophore that grows at a point(C)



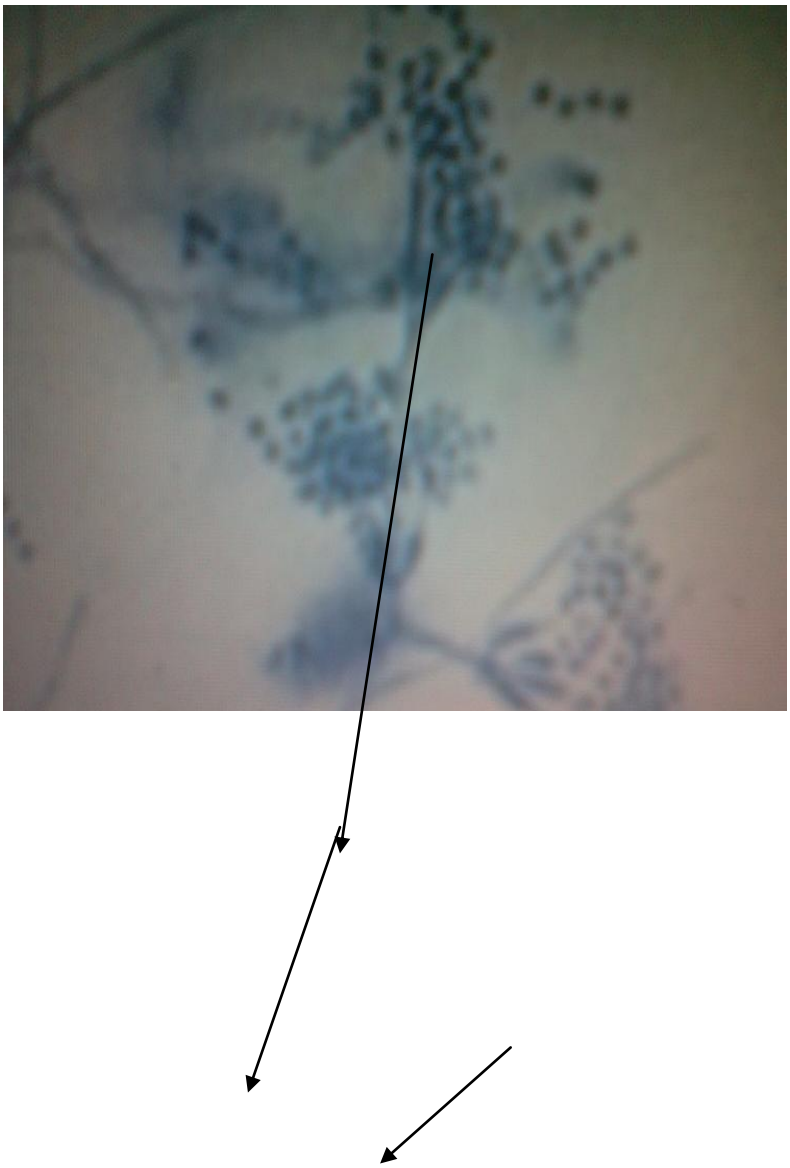
A

Folded blue with shiny surface



B

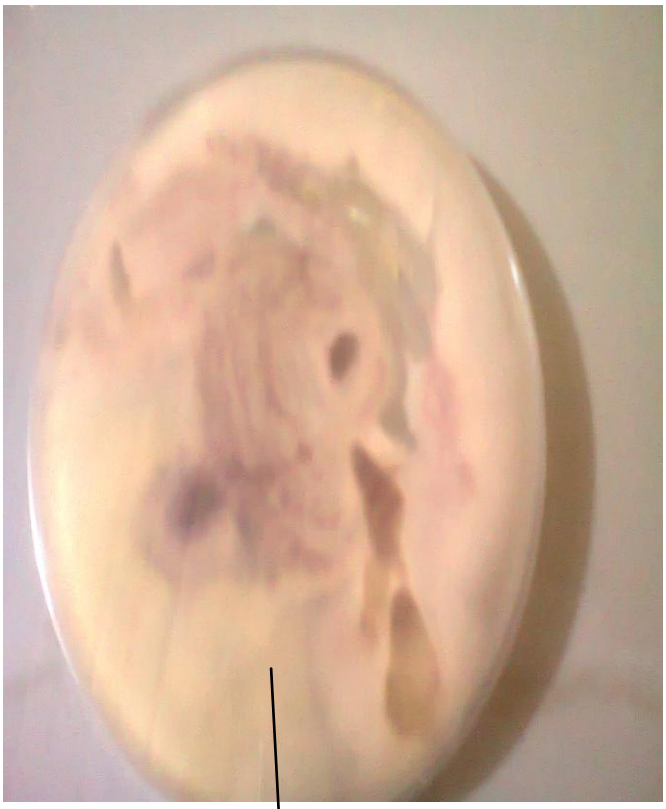
Showing rough creamy reverse



**C**

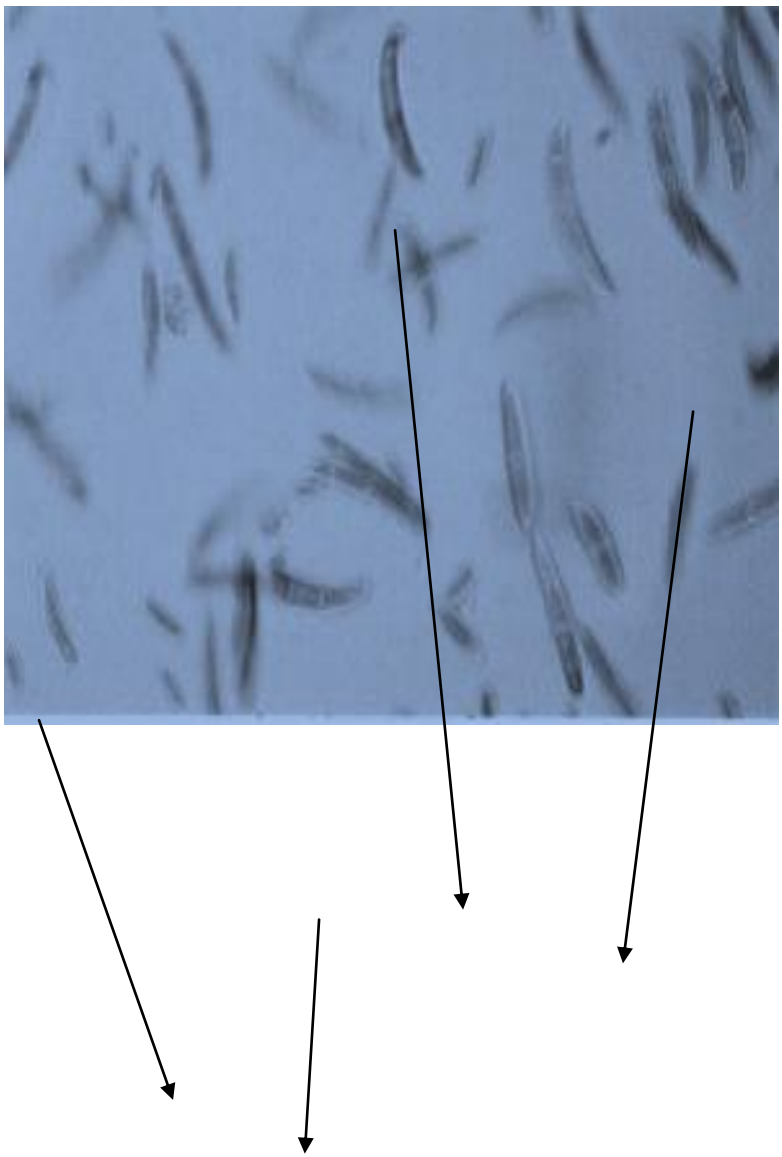
Microscopic view showing Fruiting heads with 3-4 metulae

Plate 8; *Penicillium citrinum* showing folded blue with shiny surface (A), reverse side showing rough creamy reverse (B) and microscopic features showing fruiting heads with 3-4 metulae. Conidia are oval with smooth conidiophores (C).

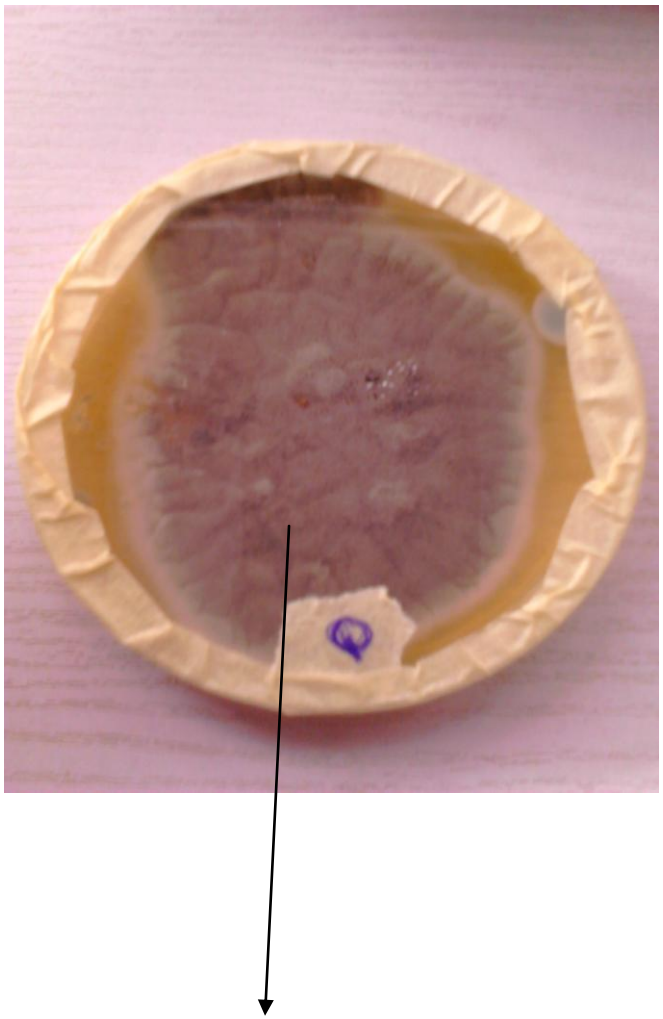
**A** Wolly white which turns purple at the center

**B** Wolly white which turns purple at the center (Reverse side)



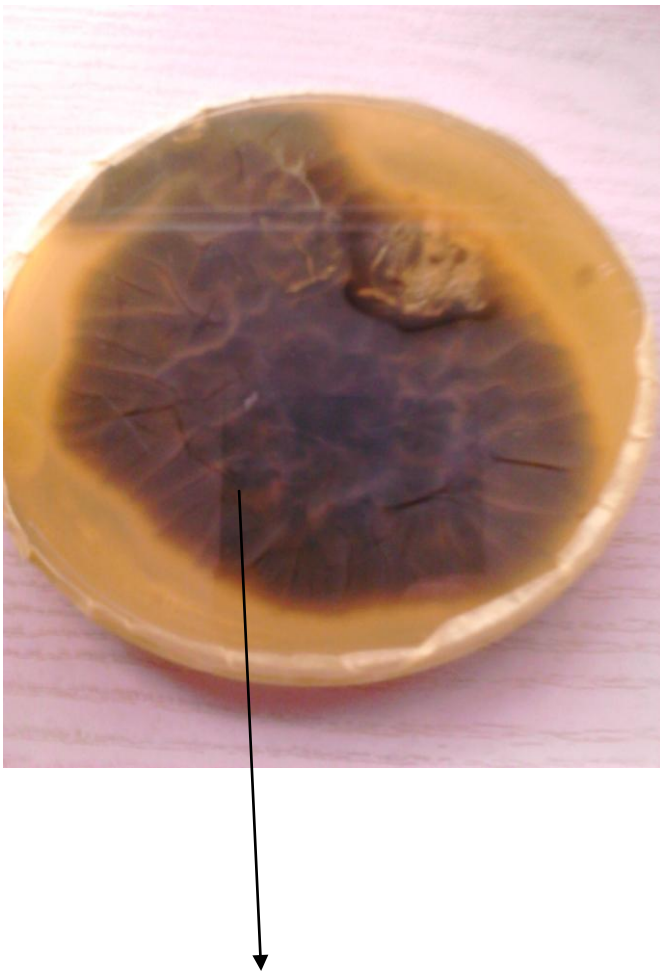
**C** Microscopic view showing macroconidia that are slightly curved with pointed tips

Plate 9; *Fusarium oxysporum* showing woolly white colour which turns purple at centre at both front and reverse side (A and B) and microscopically showing macroconidia that are slightly curved with pointed tips (C).



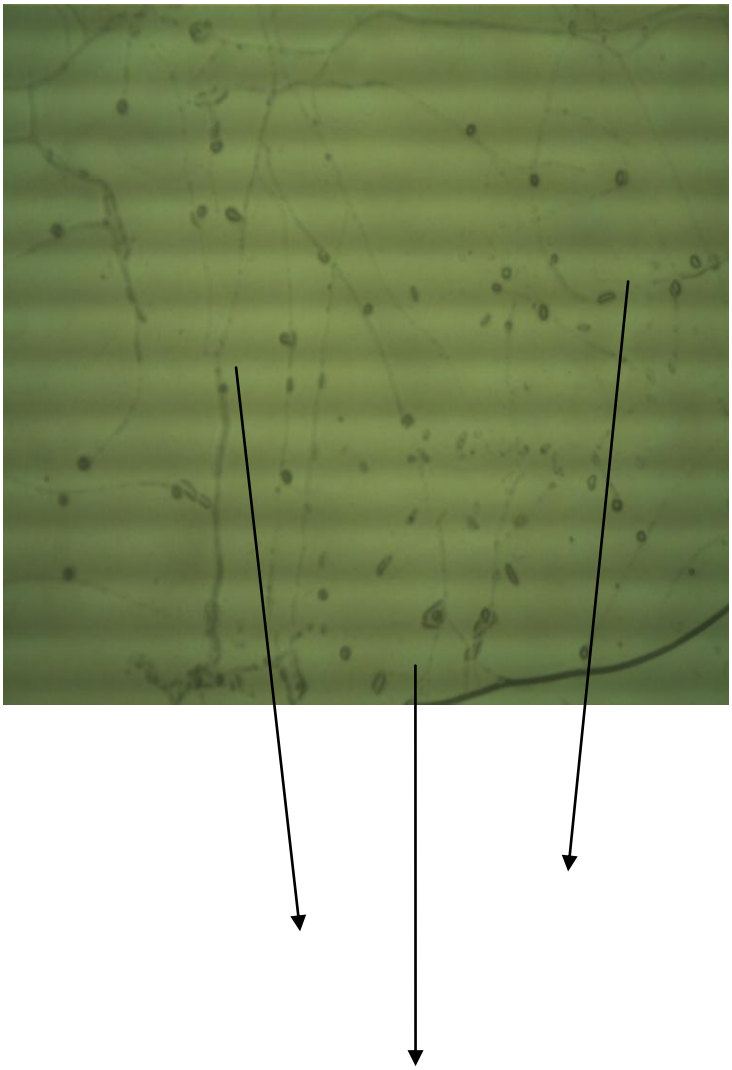
**A**

The front side showing black mold



**B**

Dark cracking reverse side



**C**

Microscopic view showng one celled conidia

Plate 10; s *Cladosporium tenuissimum* showing black mold on the front side (A) with a dark cracking reverse side (B). Microscopically the conidia are one celled (very occsssionally two-celled), pale brown smooth wall, ellipsoid to oblong- ellipsoid and are 2-3 x 4-7 µm in size. The conidiophores are more or less distinct from the vegetative hyphae(C).

The ıgal isolates visualized under the blue transilluminator was shown in plate 11. Isolates from lanes 1, 2, 4,

6-11 represents ITS bands at 600bp while lane 3 represents 500bp. L represents the 100bp molecular ladder.

Plate 12 shows results obtained from ITS sequence from the isolate which produced an exact match during the megablast search for highly similar sequences from the NCBI non reductant nucleotide (nr/nt) database. The ITS of the isolates showed 99-100% similarity to other species. The evolutionary distances which was computed using the Jukes-cantor method were in agreement with the phylogenetic placement of ITS of C18 within the *Penicillum* sp. and revealed a closely relatedness to *Penicillum citrinum* (MH990629) other than any other *Penicillum* sp., C9 and C10 revealed a closely

relatedness to *Aspergillus fumigatus* (MK461083), C14 and C16 to *Aspergillus terreus* (MK418744), C6 and C13 revealed a closely relatedness to *Aspergillus welwitschiae* (MG576117), C7, C11, C12 is related to *Aspergillus aculeatus* (MK461093), C3 is closely related to *Aspergillus flavus* (Mk 299130), C2 and C19 is related to *Fusarium succisae* (Mk 418691), C17 is closely related to *Aspergillus sydowii* (Mk 396475), C23 is related to *Talaromyces kendriikii* (kko 98055), C 25 closely related to *Curvularia kusanol* (MG975624), C15 is related to *Cladosporium tenuissimum* (MK 357638), C22 is related to *Pestalotiopsis microspora* (MK 224482), C24 was also found to be related to *Fusarium solani*

(MH517359), CI to *Fusarium lichenicola* (KH921661) and C20 was found closely related to *Fusarium oxysporum* (KM203578).

From the study, *Aspergillus* genera were the most isolated species having a total frequency of occurrence of 338(43.6%), followed by *Fusarium* genera with a total frequency of occurrence of 269 (34.7%), *Absidia* genera with a total frequency of occurrence of 100 (12.9%), *Penicillium* genera with a total frequency of occurrence of 23 (3.0%), *Cladosporium* genera with a total frequency of occurrence of 38(4.9%), *Curvularia* genera with a total frequency of occurrence of 5(0.6%) and *Pestalotiopsis* genera with a total frequency of occurrence of 1( 0.1%), *Talaromyces* genera with a total frequency of

occurrence of 1(0.1%) which was the least occurring isolate from this study as shown in figure 1.

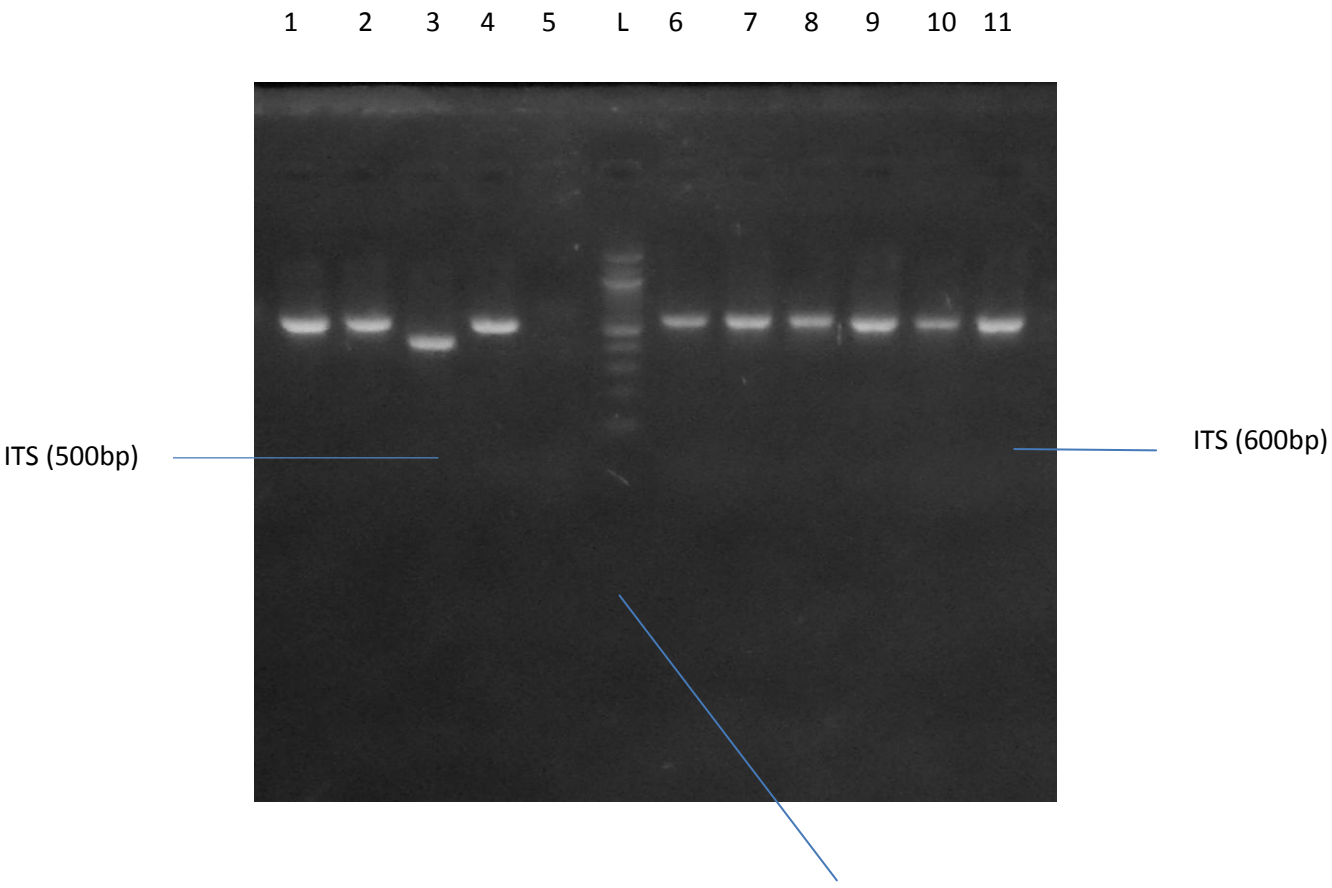


Plate 11: **Agarose gel electrophoresis showing the amplified ITS fragment of the fungal isolates.**


MK393940 Penicillium sp.
C18 Penicillium sp.
MH990629 Penicillium citrinum
MK460917 Aspergillus fumigatus
MK461083 Aspergillus fumigatus
C10 Aspergillus fumigatus
C9 Aspergillus fumigatus
C14 Aspergillus terreus
C16 Aspergillus terreus
MK418744 Aspergillus terreus
MK418545 Aspergillus sp.
MK367515 Aspergillus sp.
C6 Aspergillus sp.
MG576117 Aspergillus welwitschiae
C13 Aspergillus welwitschiae
MK461093 Aspergillus aculeatus
MK461090 Aspergillus niger
C3 Aspergillus flavus
MK299130 Aspergillus flavus
MK397504 Aspergillus sp.
C2 Fusarium succisae
MK418691 Fusarium succisae
MK461562 Aspergillus flavus
C11 Aspergillus aculeatus
MH656795 Aspergillus aculeatus
C7 Aspergillus aculeatus
MK271293 Aspergillus aculeatus
C12 Aspergillus aculeatus
MK418753 Aspergillus aculeatus
MK280716 Aspergillus aculeatus
C17 Aspergillus sydowii
MK396475 Aspergillus sydowii
MH580299 Aspergillus sydowii
KF741976 Talaromyces kendrickii
KX098055 Talaromyces kendrickii
C23 Talaromyces kendrickii
C25 Curvularia kusanoi
MG975624 Curvularia kusanoi
MG975620 Curvularia kusanoi
C15 Cladosporium tenuissimum
MK311328 Cladosporium tenuissimum
MK357638 Cladosporium tenuissimum
MK120574 Pestalotiopsis microspora
MK224482 Pestalotiopsis microspora
C22 Pestalotiopsis microspora
C24 Fusarium solani
KY776027 Fusarium solani
MH517359 Fusarium solani
C1 Fusarium lichenicola
KP132217 Fusarium lichenicola
KM921661 Fusarium lichenicola
C2 Fusarium sp.
MH582470 Fusarium sp.
MH455292 Fusarium sp.
MK130961 Fusarium succisae
MK130960 Fusarium succisae
C19 Fusarium succisae
C20 Fusarium oxysporum
KU170716 Fusarium oxysporum
KM203578 Fusarium oxysporum

plate 12; **The phylogenetic tree of the result from the internal transcriber spacer (its) obtained from the isolate from this study**

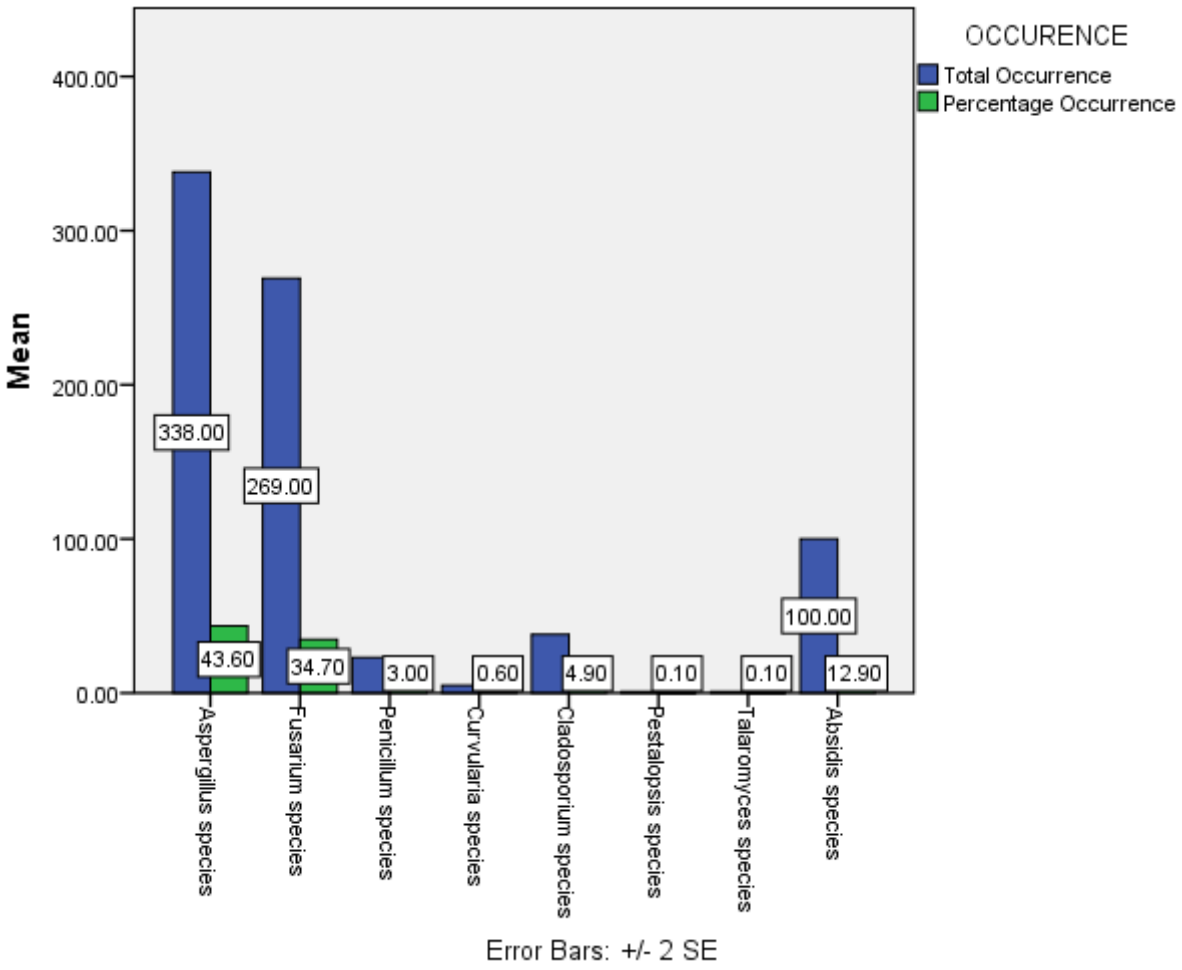



**Figure 1: Frequency of occurrence of non-dermatophytic molds isolated from cattle in Abia State and Imo State Nigeria.**

Out of 451 cattle skin sampled, 16 non-dermatophytic molds species were tsolated belonging to 8 different genera, *Aspergillus* was the highest occurring genera 338(43.6%), followed by *Fusarium* genera 269(34.7%), *Absidia* genera 100(12.9%), *Cladosporium* genera 38(4.9%), *Penicillum* genera 23(3.0%), *Curvularia* genera 5(0.6%), *Talaromyces* genera 1(0.1%) and *Pestalotiopsis* genera 1(0.1%) were

also observed as shown in figure 1 (appendix 10). Out of the 16 non-dermatohytic molds isolated *Fusarium lichenicola* had the highest frequency of occurrence of 87 in Abia State and 52 in Imo State giving a total of 139(17.9%), followed by *Aspergillus welwitschiae* with 67 in Abia State and 38 in Imo State giving a total of 105(13.5%), *Absidiacorymbifera* with 63 in Abia State and 37 in Imo State giving a total of 100 (12.9%), *Fusarium succisae* with 56 in Abia State and 34 in Imo State giving a total of 90(12.0%), *Aspergillus flavus* with 50 in Abia State and 26 in Imo State giving a total of 76(10.0%), *Aspergillus aculeatus* with 33 in Abia State and 36 in Imo State giving a total of 69(9.0%), *Aspergillus sydowii* with 16 in Abia State and 23 in Imo State

giving a total of 39(5.0%), *Cladosporium tenuissimum* with 20 in Abia State and 18 in Imo State giving a total of 38(4.9%), *Aspergillus fumigatus* with 21 in Abia State and 7 in Imo State giving a total of 28(3.6%), *Fusarium solani* with10 in Abia State and 15 in Imo State giving a total of 25(3.2%), *Penicillum citrinum* with 18 in Abia State and 5 in Imo State giving a total of 23(3.0%), *Aspergillus terreus* with 8 in Abia State and 13 in Imo State giving a total of 21(2.7%), *Fusarium oxysporum* with 0 in Abia State and 15 in Imo State giving a total of 15(2.0%), *Curvularia kusanol* with 0 in Abia State and 5 in Imo State giving a total of 5(0.6%), *Talaromyces kendrickii* with had 0 in Abia State and 1 in Imo State giving a total of 1(0.1%) while

*Pestalotiopsis microspora* had 0 in Abia State and 1 in Imo State giving a total of 1 (0.1%) as shown in figure 2 (appendix 11).

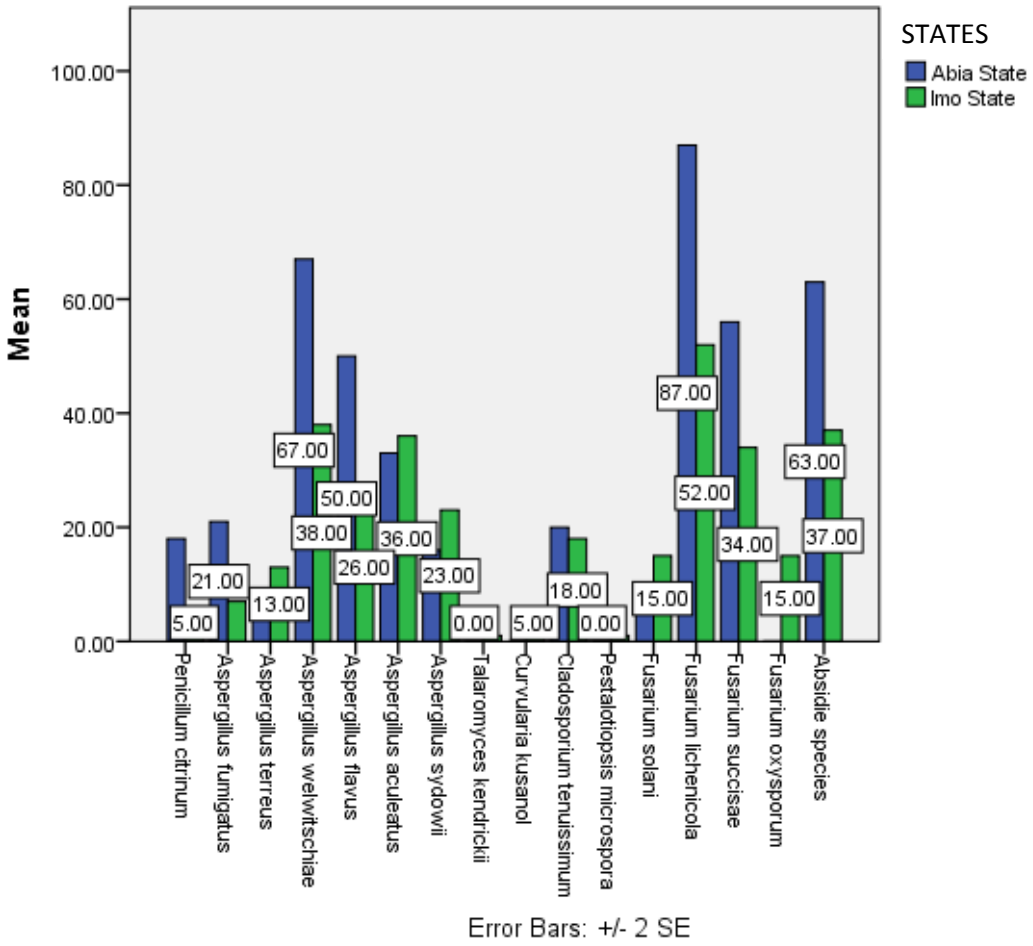



Figure 2: **Frequency of occurrence of non dermatophytic molds species isolated from cattle skin in Abia state and Imo state, Nigeria.**

Out of the 223 cattle skin sampled from Abia state, a total of 12 non-dermatophyttic molds were isolated. *Fusarium lichenicola* had the highest frequency of occurrence of 87(19.4%), of which 26 were isolated from Ubakala market, 29 were isolated from Ahia udele and 32 were isolated from Lokpanta market, followed by *Aspergillus welwitschiae* which had a total frequency of occurrence of 67(15.0%), of which 13 were isolated from Ubakala market, 21 from

Ahia udele and 33 from Lokpanta market, *Absidia corymbifera* which had a total frequency of occurrence of 63(14.0%), of which 19 were isolated from Ubakala market, 20 from Ahia udele and 24 from Lokpanta, *Fusarium succisae* had a total frequency of occurrence of 56 (12.5%), of which 5 were isolated from Ubakala market, 27 from Ahia udele and 24 from Lokpanta market, *Aspergillus flavus* had a total frequency ofg occurrence of50 (11.1%),of which20 wereisolated fromUbakala market, 7 from Ahia udele market and 23 from Lokpanta market, *Aspergillus aculeatus* had a total frequency of occurrence of 33 (7.3%), of which 11 were isolateded from Ubakala market, 14 from Ahia udele market and 8 from Lokpanta market,

*Aspergillus fumigatus* had a total frequency of occurrence of 21(4.7%) of which 11 were isolated from Ubakala market, 7 from Ahia udele and 3 from Lokpanta market, *Cladosporium tenuissimum* had a total frequency of occurrence of 20(4.5%), of which 5 were isolated from Ubakala market, 9 from Ahia udele market and 6 from Lokpanta market, *Penicillium citrinum* had a total frequency of occurrence of 18 (4,0%), of which 9 were isolated from Ubakala market, 7 from Ahia udele market and 2 from Lokpanta market, *Aspergillus sydowii* had a total frequency of occurrence of 16(3.6%), of which 7 were isolated from Ubakala market, 0 from Ahia udele market and 0 from Lokpanta market while *Fusarium solani* had total frequency of

occurrence of 10(2.2%) were isolated of which 4 were isolated from Ubakala market, 6 from Ahia udele market and 0 from Lokpanta market as shown in figure 3 (appendix 12).

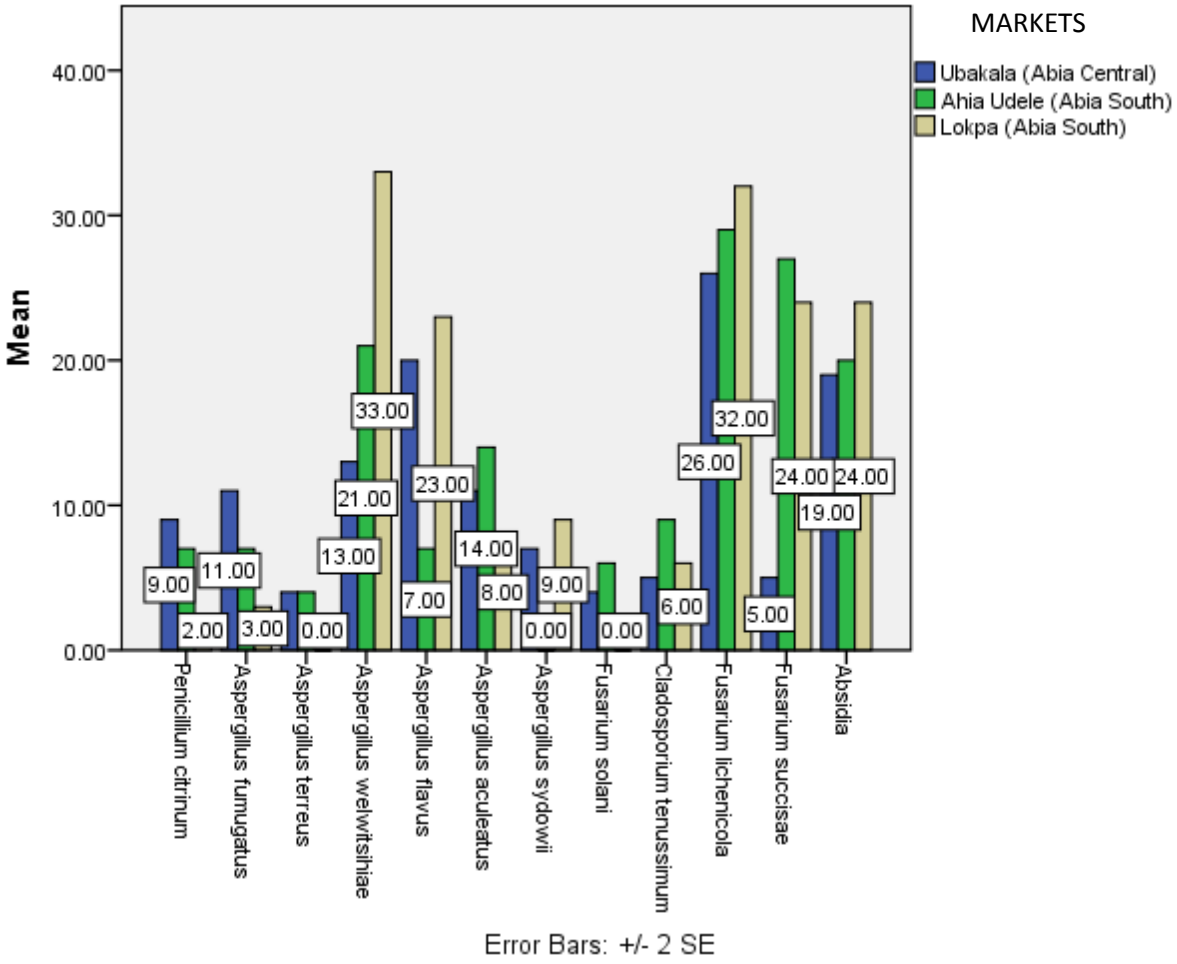



FIGURE 3: **Frequency of occurrence of non dermatophytic molds from cattle skin within markets in Abia State Nigeria.**

Out of the 228 cattle skin sampled from Imo state a total of 16 non-dermatophytic molds were isolated. *Fusarium lichenicola* had a total frequency of occurrence of 52(16.0%) of which 16 were isolated from Afor Ogbe market, 16 from Ekeubahaeze market and 20 from Okigwe market (20) followed by *Aspergillus welwitschiae* which had a total frequency of occurrence 38(12.0%) of which 12 were isolated from Afor Ogbe market, 16 from Ekeubahaeze market and 10 from Okigwe market, *Absidiacorymbifera* had a total frequency of occurrence of 37(11.3%) of which 14 were isolated from Afor Ogbe market, 7 from

Ekeubahaeze and 16 from Okigwe market, *Aspergillus aculeatus* had a total frequency of occurrence of36(11.0%), of which 13 were isolated from Afor Ogbe market, 6 from Ekeubahaeze market and 17 from Okigwe cattle market, *Fusarium succisae* had a total frequency of occurrence of 34(10.4%), of which were 14 were isolated from Afor Ogbe market, 8 from Ekeubahaeze and 12 from Okigwe market (12), *Aspergillus flavus* had a total frequency of occurrence of26(8.0%)of which 16 were isolated from Afor Ogbe market, 0 from Ekeubahaeze and 10 from Okigwe market, *Aspergillus sydowii* had a total frequency of occurrenceof 23(7.1%), of which 4 were isolated from Afor Ogbe market, 19 from Ekeubahaeze market and 0 from Okigwe

market (0) , *Cladosporium tenuissimum* had a total frequency of occurence of 18(5.5%), of which 10 were isolated from Afor Ogbe market, 0 from Ekeubahaeze and 8 from Okigwe market, *Fusarium solani* had a total frequency of occurrence of15 (4.6%), of which 9 were isolated from Afor Ogbe market, 6 from Ekeubahaez market and 0 from Okigwe market, *Fusarium oxysporum* had atotal frequency of occurrence of 15(4.6%) of which 10 were isolated from AforOgbe market, 0 from Ekeubahaeze market and 5 from Okigwe market, *Aspergillus terreus* had a total frequency of occurence of13(4.0%), of which 10 were isolated from Afor Ogbe market, 3 from Ekeubahaeze market and 0 from Okigwe market (0) while *Talaromyces kendrickii* had a

total frequency of occurrence of1(0.3%)of which 1 were recovered from Afor Ogbe market, 0 from Ekeubahaeze market and 0 from Okigwe market, *Pestalotiopsis microspora* had a total frequency of occurrence of 1(0.3%) of which 1 was isolated from Afor Ogbe market, 0 from Ekeubahaeze and 0 from Okigwe market, *Curvularia kusanol* had a total frequency of 5(1.5%), of which 5 were isolated from Afor Ogbe market, 0 from Ekeubahaeze and 0 from Okigwe, *Penicillum citrinum* had a total frequency of occurrenceof 5(1.5%), of which 5 were isolated from Afor Ogbe market, 0 from Ekeubahaeze and 0 from Okigwe market and *Aspergillus fumigatus* had a total frequency of occurrence of 7(2.1%), of which 0 were isolated from Afor Ogbe market, 3

from Ekeubahaeze and 4 from Okigwe market as shown in figure 4 (appendix 13).

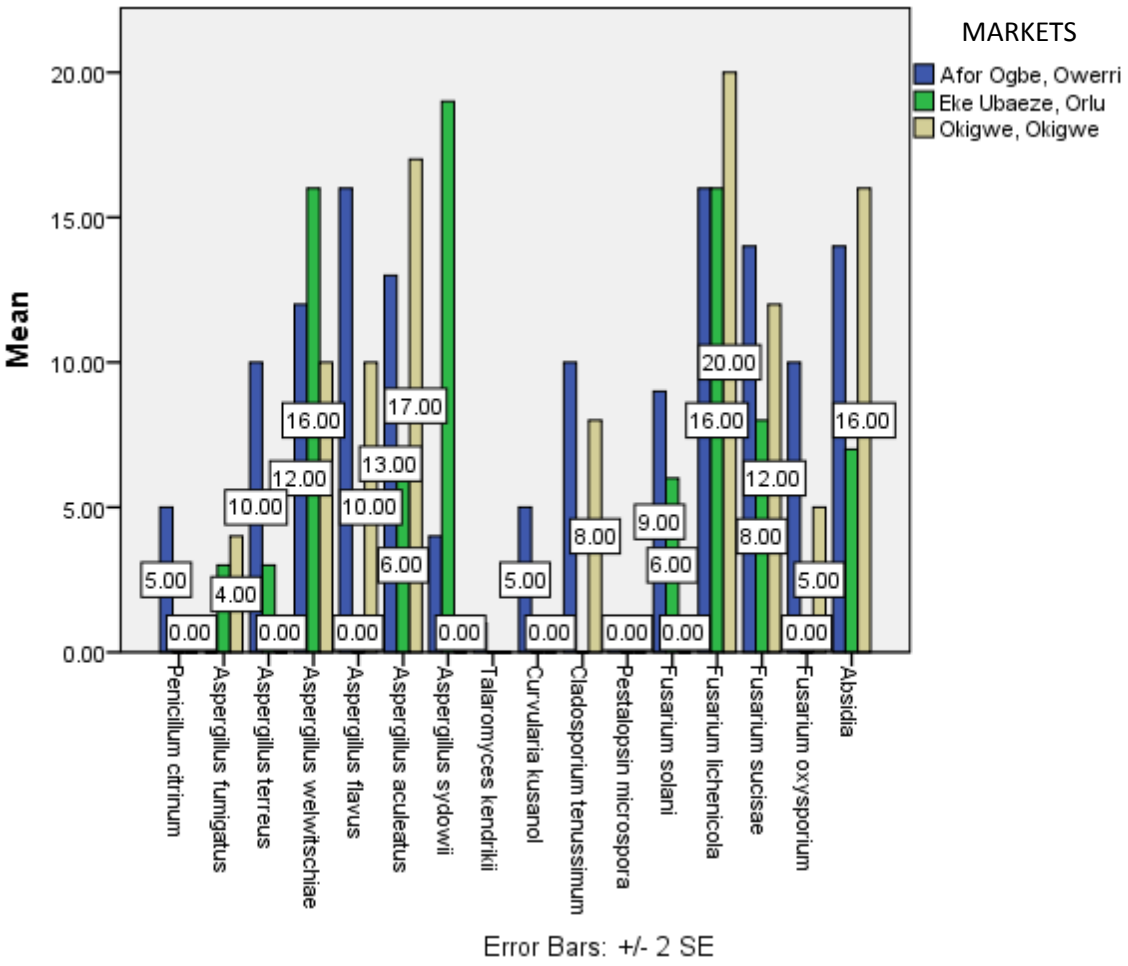


FIGURE 4: **Frequency of occurrence of non dermatophytic molds from cattle skin within markets in Imo State Nigeria.**

A total of 10 non-dermatophytic molds were isolated from cattle skin with lesion only from the cattle market representing the three geopolitical zones in Abia state. Among the *Aspergillus* species recovered, *Aspergillus welwitschiae* had

the highest frequency of occurrence of 13 (21.3%) of which 0 were isolated from Ubakala market, 7 from Ahiaudele market and 6 from Lokpanta market followed by *Aspergillus aculeatus* which had a total frequency of occurrence of 3(5.0%), of which 1 were isolated from Ubakala market, 2 from Ahiaudele market and 0 from Lokpanta market, *Aspergillus flavus* had a total frequency ofoccurrence of3(5.0%),of which 0 were isolated from ubakala market, 1 from Ahiaudele market and 2 from Lokpanta market, *Aspergillus fumigatus* had a total frequency of occurrence of 1(2.0%), of which 0 were isolated from Ubakala market, 1 from Ahiaudele market and 0 from Lokpanta market, *Aspergillus sydowii* had a frequency of

occurrence of 2(3.3%), of which 0 were isolated from Ubakala market, 0 from Ahiaudele market and 2 from Lokpanta market, *Fusarium succisae* had a total frequency of occurrence of 12 (20.0%), of which 0 were isolated from Ubakala market, 7 from Ahiaudele market and 5 from Lokpanta market, *Fusarium lichenicola* had a total frequency of occurrence of8 (13.1%) of which 2 were isolated from Ubakala market, 2 from Ahiaudele market and 4 from Lokpanta market, *Absidia corymbifera* had a total frequency of occurrence of 12(20.0%)of which 1 were isolated from Ubakala market, 7 from Ahia udele market and 4 from Lokpanta market, *Cladosporium tenussimum* had a total frequency of occurrence of 4(7.0%), of which1 was isolated

from Ubakala market, 2 from Ahiaudele market and 1 from Lokpanta market and *Penicillum citrinum* had a total frequency of occurrence of3(5.0%)of which 0 was isolated from Ubakala market, 3 from Ahiaudele market and 0 from Lokpanta market as shown in figure 5 (appendix 14).

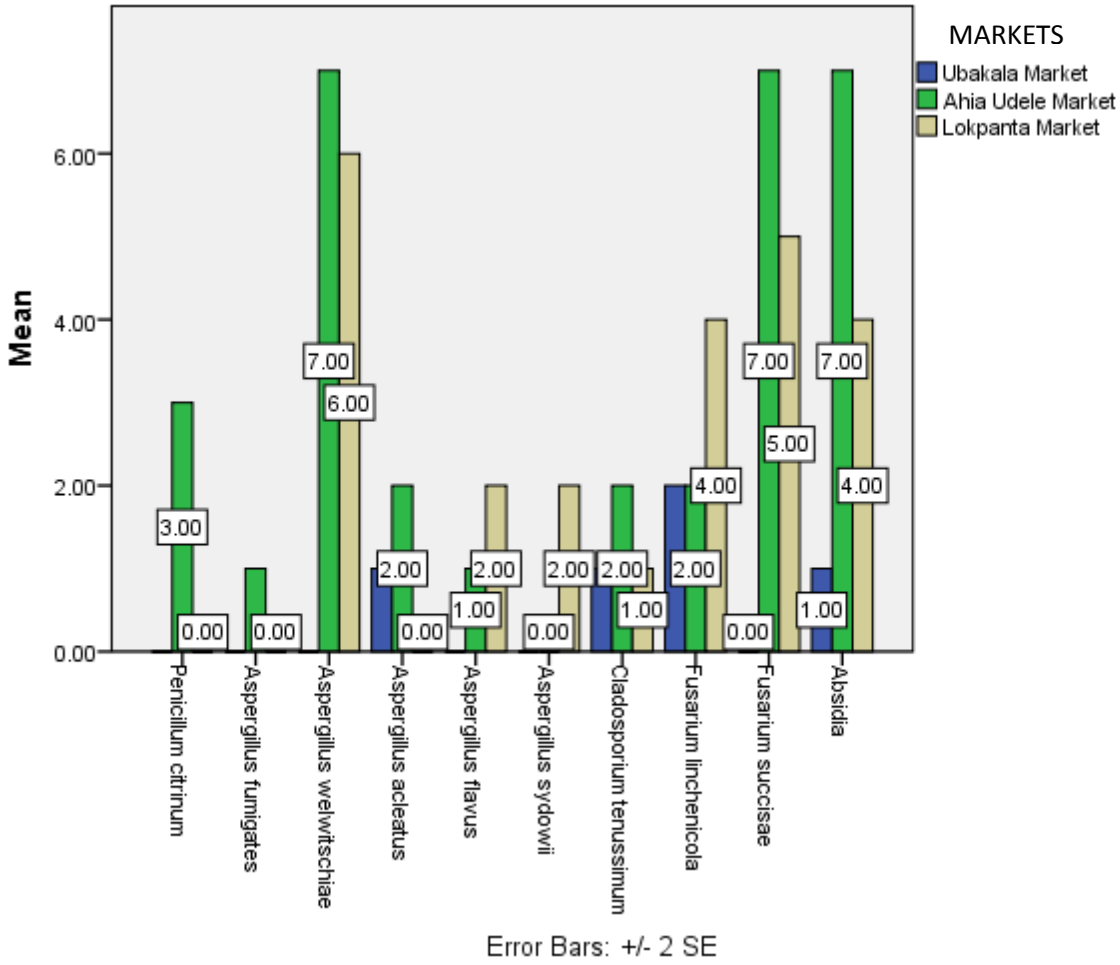



FIGURE 5: **Frequency occurrence of non dermatophytic molds on cattle skin with lesion only in Abia State, Nigeria.**

A total of 10 non-dermatophytic molds were isolated from cattle skin with lesion only from the cattle markets representing the three geopolitical zones in Imo State. *Aspergillus welwitschiae* had a total frequency of occurrence of 20(28.2%) of which 5 were isolated from Afor Ogbe market, 4 from Ekeubahaeze market, 11 from Okigwe market, *Aspergillus aculeatus* had a total frequency of occurrence of 11(15.5%),of which 7

were isolated from Afor Ogbe, 4 from Ekeubahaeze, 0 from Okigwe market, *Aspergillus sydowii* had a total frequency of occurrence of 6(8.5%) of which 2 were isolated from Afor Ogbe market, 4 from Ekeubahaeze, 0 from Okigwe market, *Aspergillus flavus* had a total frequency of occurrence of 1 (1.4%), of which 0 was isolated from Afor Ogbe market, 0 from Ekeubahaeze and 1 from Okigwe market, *Fusarium lichenicola* had a total frequency of occurrence of 10(14.1%), of which 6 were isolated from which Afor Ogbe, 3 from Ekeubahaeze and 1 from Okigwe, *Fusarium succisae* had a total frequency of occurrence of 7 (9.9%)of which 0 was isolated from Afor Ogbe market, 1 from Ekeubahaeze market and 6 from

Okigwe market, *Absidiacorymbifera* had a total frequency of occurrence of 10(14.1%) of which 4 were isolated from Afor Ogbe market, 2 from Ekeubahaeze market and 4 from Okigwe market , *Cladosporium tenussimum* had a total of 4(5.6%) of which 4 were isolated from Afor Ogbe market, 0 from Ekeubahaeze and 0 from Okigwe market, *Talaromyces kendrickii* had a total frequency of 1(1.4%)of which 1 was isolated from Afor Ogbe market,0 from Ekeubahaeze market and 0 from Okigwe market and *Pestalotiopsis microspora* had a total frequency of occurrence of1(1.4%)of which 1 was isolated from Aforogbe market, 0 from Ekeubahaeze market and 0 from Okigwe market as shown in figure 6 (appendix 15).

MARKETS

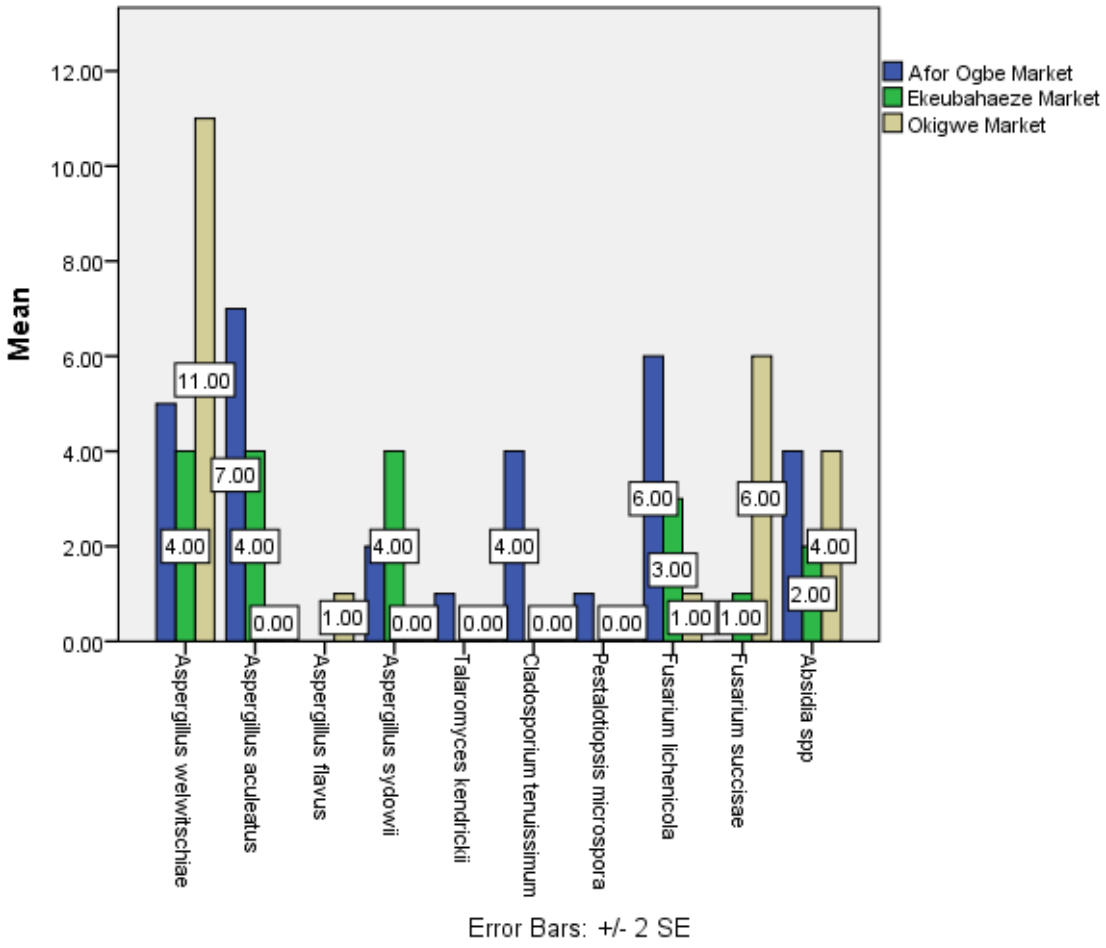



FIGURE 6: **Frequency occurrence of non-dermatophytic molds from cattle skin with lesions only in Imo State, Nigeria.**

Out of 30 (10 from each market) air samples analysed within the cattle markets in Abia State Nigeria. The highest isolated specie was *Aspergillus welwitschiae* with the total frequency of occurrence of12(20.0%),of which 3 was from Ubakala market, 3 from Ahiaudele market and 6 was from Lokpanta market, *Absidia corymbifera* hadthe total frequency of occurremce of 12(20.0%)of which 2 was isolated from Ubakala market, 6 was from Ahiaudele market and 4 was from Lokpanta market, *Penicillum citrinum* had a total frequency of occurrence of 10(16.7%). of

which 2 was isolated from Ubakala market, 3 was from Ahiaudele market and 5 was from Lokpanta market, *Aspergillus flavus* had a total frequencyof occurrence of 8(13.3%),of which 2 was isolated from Ubakala market, 2 from Ahiaudele market and 4 was from Lokpanta market, *Aspergillus aculeatus* had a total frequency of occurrence of 6(10.0%),of which 2 was from Ubakala market, 2 was from Ahiaudele market and 2 from Lokpanta market, *Fusarium lichenicola* had a totalfrequency of occurrence of 6(10.0%), of which 2 was isolated from Ubakala market, 2 was from Ahia udele market and 2 was from Lokpanta market, *Cladosporium tenussimum* had a total frequency of occurrence of (6.7%), of which 0 was isolated from Ubakala market, 2 from

Ahiaudele market and 2 from Lokpanta market and *Aspergillus sydowii* had a total frequency of occurrence of 2(3.3%), of which 0 was isolated from Ubakala market, 2 from Ahiaudele market and 0 from Lokpanta market as shown in figure 7 (appendix 16). Statistical analysis showed that there was no significant difference in the frequency of occurrence of non- dermatophytic molds isolated from air within the markets in Abia State at P = 0.05 as shown in appendix 1.

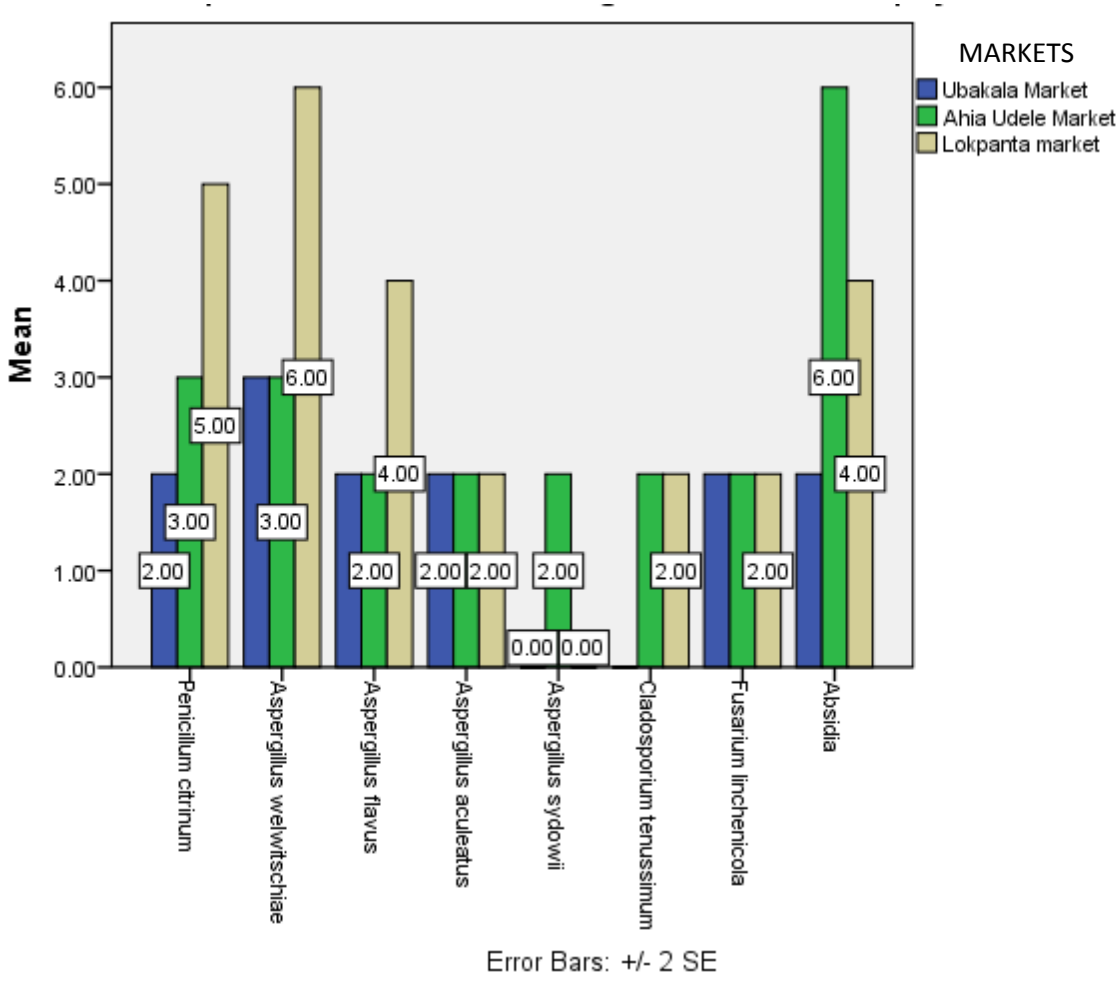



FIGURE 7: **Frequency of occurrence of non dermatophytic molds isolated from air within the cattle markets in Abia State Nigeria.**

Out of 30 (10 from each market) analysed from air environment within cattle markets in Imo State *Fusarium lichenicola* had frequency of occurrence of 14(20.6%), of which 5 was isolated from AforOgbe market, 5 from Ekeubahaeze market and 4 from Okigwe market, *Absidiacorymbifera* had frequency of occurrence of 14(20.6%), of which 5 was isolated from Afor Ogbe market, 4 from Ekeubahaeze market and 5 from Okigwe market, *Aspergillus welwitschiae* had a total frequency of occurrenceof 12(17.6%), of which 5 was isolated from Afor Ogbe market, 3 was from Ekeubahaeze market and 4 from

Okigwe market, *Aspergillus flavus* had a total frequency of occurrence of8(11.8%),of which 5 was isolated from Afor Ogbe market, 3 was from Ekeubahaeze market and 0 from Okigwe market, *Aspergillus aculeatus* had a totalfrequency ofoccurrence of 8(11.8%),of which 4 was isolated from Afor Ogbe market, 1 was from Ekeubahaeze market and 3 was from Okigwe market, *Penicillum citrinum* had a total frequency of occurrenceof 5(7.4%), of which 3 was isolated from Afor Ogbe market, 2 was from Ekeubahaeze market and 0 was from Okigwe market, *Fusarium oxysporum* had a total frequency of occurrence of 5(5.4%), of which 5 was isolated from AforOgbe market, 0 from Ekeubahaeze market and 0 from Okigwe market and *Cladosporium tenussimum*

of which had the total frequency of occurrence of 2(3.0%), of which 1 was isolated from Afor Ogbe market , 0 from Ekeubahaeze market and 1 was from Okigwe market as shown in figure 8 (appendix 17). Statistical analysis showed that there is no significant difference in the frequency of occurrence of non- dermatophytic molds isolated from air within markets in Imo State at P= 0.05 as shown in appendix 2.

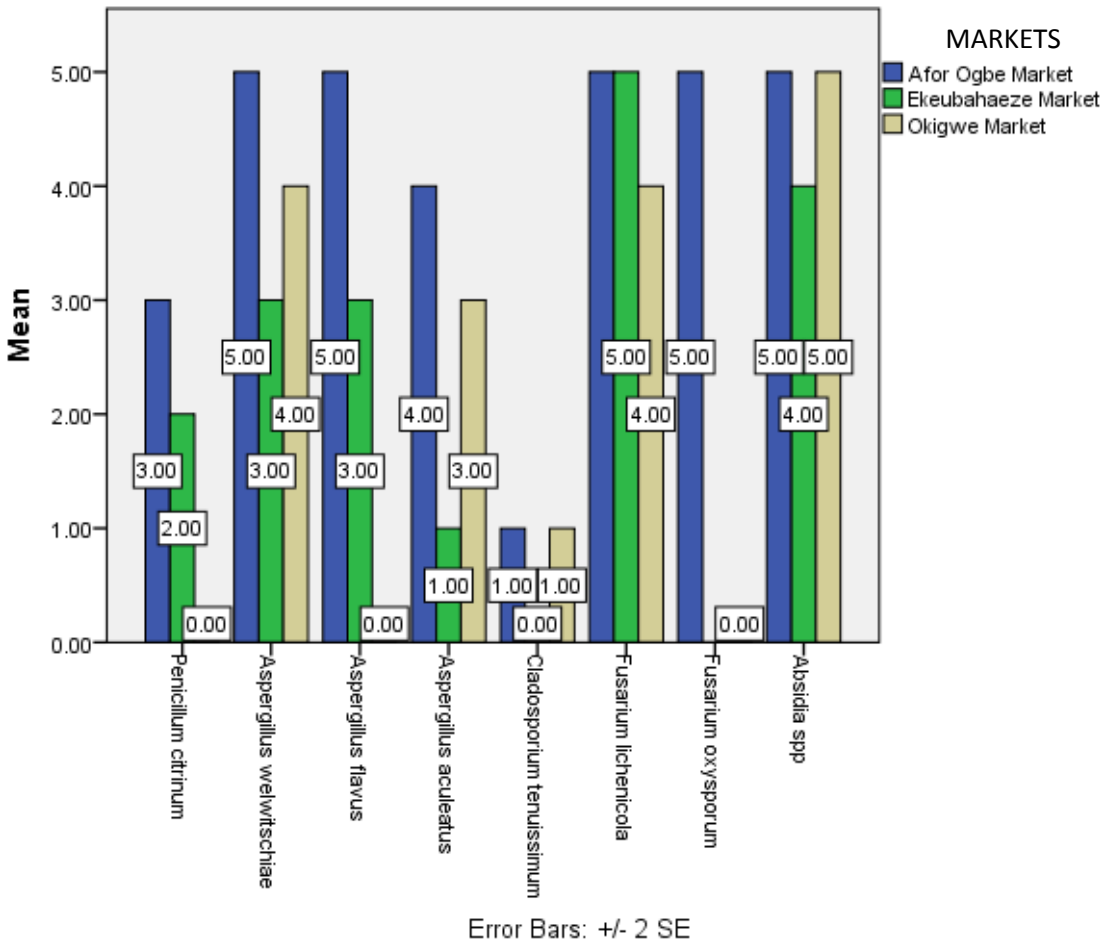

MARKETS
Afor Ogbe Market
Ekeubahaeze Market
Okigwe Market
Mean
5.00
4.00
3.00
2.00
1.00
0.00
3.00
2.00
0.00
5.00
3.00
4.00
5.00
3.00
0.00
4.00
1.00
3.00
1.00
1.00
0.00
5.00
4.00
5.00
5.00
0.00
5.00
4.00
5.00
Penicillum citrinum
Aspergillus welwitschiae
Aspergillus flavus
Aspergillus aculeatus
Cladosporium tenuissimum
Fusarium lichenicola
Fusarium oxysporum
Absidia spp
Error Bars: +/- 2 SE

FIGURE 8: F**requency of occurrence of non dermatophytic molds isolated from air samples within the cattle markets in Imo State Nigeria.**

Out of 30 soil samples (10 from each market) analysed within cattle markets in Abia State, a total of 6 fungal species were identified of which

*Aspergillus welwitschiae* had a total frequency of occurrence of 13((0.0%), of which 6 was isolated from Ubakala market, 4 from Ahiaudele market and 3 from Lokpanta market, *Aspergillus flavus* had a total frequency ofoccurrence of 12(18.2%) of which 6 was isolated from Ubakala market, 0 from Ahiaudele market and 6 from Lokpanta market, *Aspergillus sydowii* had a total frequency of occurrence of 2(3.0%), of which0 was isolated from Ubakala market, 0 from Ahiaudele market and 2 from Lokpanta market, *Absidia corymbifera* had a total frequency of occurrence of 19(29.0%),of which 13 was isolated from Ubakala market, 11 from Ahiaudele market and 5 from Lokpanta market, *Fusarium lichenicola* had a total frequency of occurrence

ofof which 14(21.2%), of which 9 was isolated from Ubakala market, 1 from Ahiaudele market and 4 from Lokpanta market, *Fusarium succisae* had a total frequency of occurrence of 6(9.1%), of which 3 was isolated from Ubakala market, 0 from Ahiaudele market and 3 from Lokpanta market as shown in figure 9 (appendix 18). Statistical analysis shows that there is a significant difference in the frequency of occurrence of non-dermatophytic molds from soil samples within markets in Abia State at P = 0.05 as shown in appendix 3.

Out of 30 (10 from each market) soil samples analysed within cattle markets in Imo State, a total of 8 fungal species were identified of which *Aspergillus flavus* had a frequency of occurrence

of 16(21.1%), of which5 was isolated from AforOgbe market, 5 from Ekeubahaeze market and 6 from Okigwe market, *Fusarium lichenicola* had a total frequency of occurrence 16(21.1%), of which 6 was isolated from AforOgbe market, 5 from Ekeubahaeze market and 5 from Okigwe market (5), *Absidia corymbifera* ohad a total frequency occurrence of 15(20.0%), of which 6 was isolated from AforOgbe market, 4 from Ekeubahaeze market and 5 from Okigwe market, *Aspergillus welwitschiae* had a total frequency of occurrence of 14(18.4%), of which 8 was isolated from Afor Ogbe market, 4 from Ekeubahaeze market and 2 from Okigwe market, *Aspergillus sydowii* had a total frequency of occurrence of 2(3.0%), of which 0 was isolated from Aforogbe

market, 2 was from Ekeubahaeze market, 0 was from Okigwe market, *Fusarium succisae* had a total frequency of occurrenceof 10(13.2%), of which 4 was isolated from AforOgbe market, 3 from

Ekeubahaeze market and 3 from Okigwe market, *Fusarium solani* had a totalfrequency of occurrence of 2(3.0%),of which 2 was isolated from Afor Ogbe market, 0 from Ekeubahaeze market and 0 from Okigwe market and *Cladosporium tenussimum* had the total frequency of occurrence of 1(1.3%), of which1 was isolated from Afor Ogbe market, 0 from Ekeubahaeze market and 0 from Okigwe

as shown in figure 10 (appendix 19) . Statistical analysis showed that there was no significant

difference in the frequency of occurrence of non-dermatophytic molds from soil samples analysed from cattle markets in Imo at P= 0.05 as shown in appendix 4.

Out of 15 (5 from each market) soil samples analysed for the presence of keratinophilic non-dermatophytic molds, a total of 6 isolates were identified of which *Penicillium citrinum* which had a total frequency of occurrence of 2(6.0%), of which 0 was isolated from Ubakala market, 0 from Ahiaudele market and 2 from Lokpanta market, *Aspergillus flavus* had a total frequency of occurrence of 9(26.0%),of which 3 was isolated from Ubakala market, 2 was from Ahiaudele market and 4 from Lokpanta market, *Absidia corymbifera* had a total frequency of

occurrenceof 9(26.0%), of which 3 was isolated from Ubakala market, 3 from Ahiaudele market and 3 from Lokpanta market, *Aspergillus welwitschiae* had a total frequency ofoccurrenceof 7(20.0%), of which 4 was isplated from Ubakala market, 3 from Ahiaudele market and 0 from Lokpanta market, *Fusarium lichenicola* had a total frequency of occurrence of6(17.1%), of which2 was isolated from Ubakala, 2 from Ahiaudele market and 2 from Lokpanta market and *Aspergillus aculeatus* had a total frequency of occurrenceof 2(6.0%), of which 0 was isolated from Ubakala market, 0 from Ahiaudele market and 2 from Lokpanta market as shown in figure 11 (appendix 20).

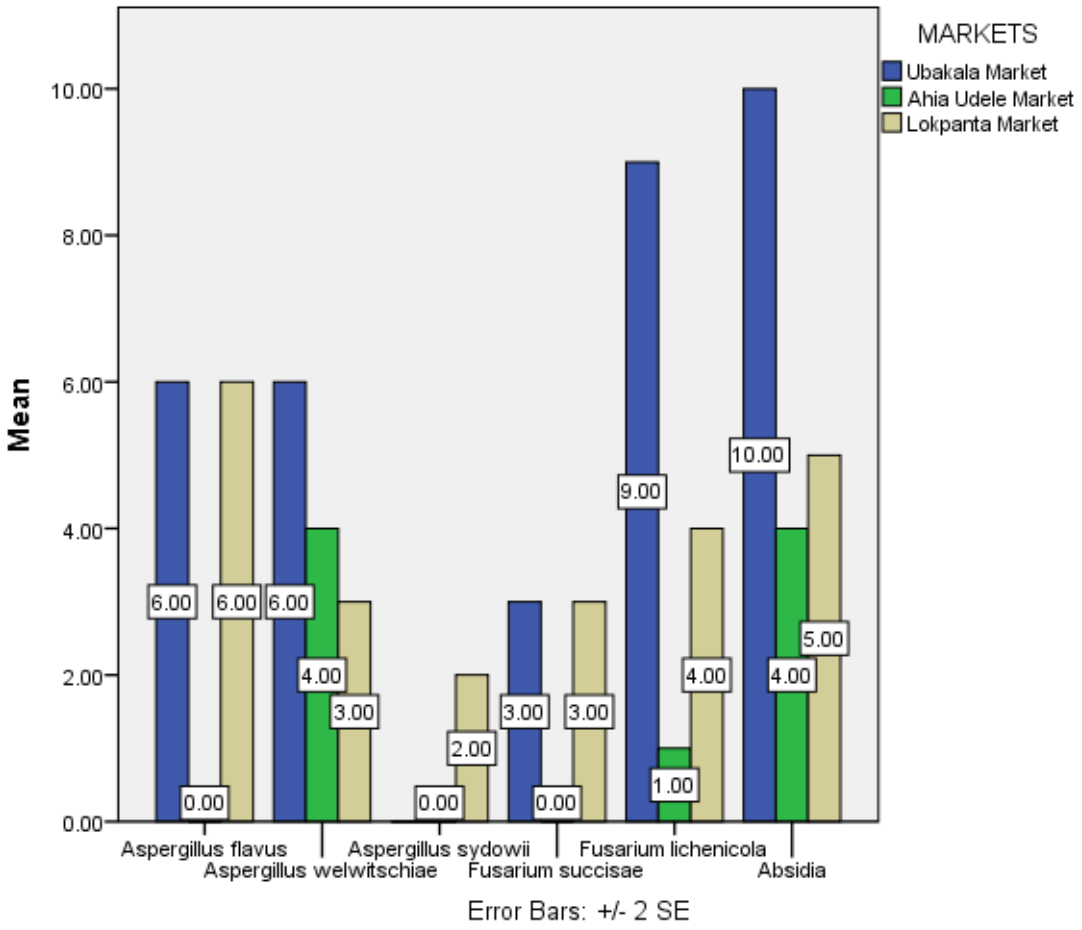



FIGURE 9: **Frequency of occurrence of non dermatophytic molds from soil samples within the cattle markets in Abia state, Nigeria.**

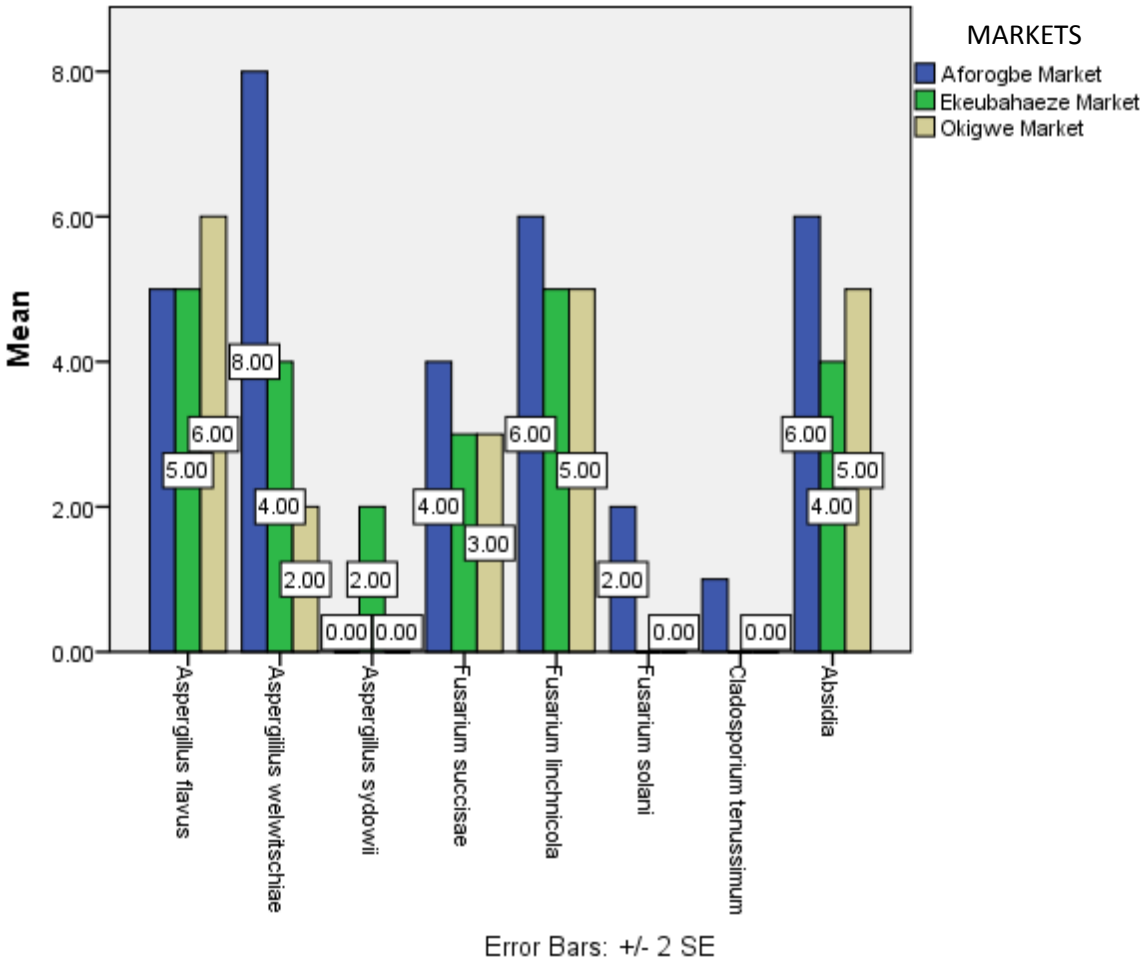

MARKETS
Aforogbe Market
Ekeubahaeze Market
Okigwe Market
Mean
8.00
6.00
4.00
2.00
0.00
5.00
6.00
8.00
4.00
2.00
0.00
2.00
0.00
4.00
3.00
6.00
5.00
6.00
2.00
0.00
0.00
6.00
4.00
5.00
Aspergillus flavus
Aspergillus welwitschiae
Aspergillus sydowii
Fusarium succisae
Fusarium linchnicola
Fusarium solani
Cladosporium tenussimum
Absidia
Error Bars: +/- 2 SE

FIGURE 10: **Frequency of occurrence of non-dermatophytic molds from soil samples within the cattle markets in Imo State, Nigeria.**

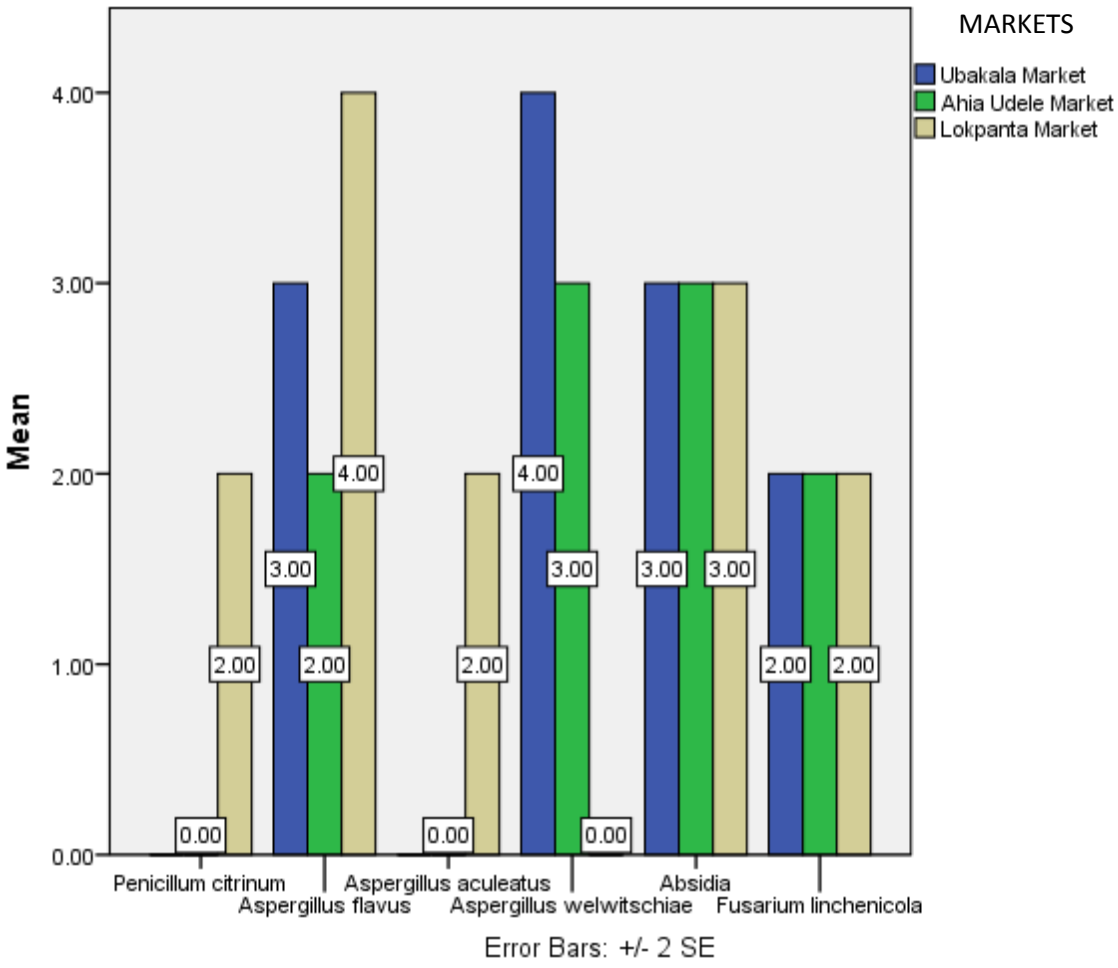



FIGURE 11: **Frequency of occurrence of keratinophilic non-dermatophytic molds from soil samples within cattle markets in Abia State using hair bait technique**

Out of 15 (5 from each market) soil samples analysed for keratinophilic non-dermatophytic molds within cattle markets in Imo State. A total of 10 fungal isolates were identified of which *Aspergillus welwitschiae* had the total frequency of occurrence of 9(20.0%),of which 2 was isolated from Afor Ogbe market, 4 from Ekeubahaeze market and 3 from Okigwe market, *Aspergillus flavus* had a totalfrequency of occurrence of 7(16.0%), of which 3 was isolated

from AforOgbe market, 2 from Ekeubahaeze market and 2 from Okigwe market, *Absidia corymbifera* had a total frequency of occurrence of 7(16.0%), of which 3 was isolated from Afor Ogbe market, 4 from Ekeubahaeze market and 0 from Okigwe market, *Fusarium lichenicola* ohad a total frequency of occurrence of 7(16.0%), of which 2 was isolated from Afor Ogbe market, 3 from Ekeubahaeze market and 2 from Okigwe market, *Fusarium succisae* had a total frequency of occurrence of5(11.1%), of which 2 was isolated from Afor Ogbe market, 1 from Ekeubahaeze market and 2 from Okigwe market, *Aspergillus fumigatus* had a total frequency of occurrence of 2(4.4%), of which 0 was isolated from Afor Ogbe market, 0 from Ekeubahaeze

market and 2 from Okigwe market, *Aspergillus terreus* had a total frequency of occurrence of 2(4.4%),of which 0 was isolated from Afor Ogbe market, 0 from Ekeubahaeze market and 2 from Okigwe market (2), *Penicillum citrinum* had a total frequency of occurrence of 1(2.2%), of which 0 was isolated from Afor Ogbe market, 0 from Ekeubahaeze market and 1 from Okigwe market, *Aspergillus aculeatus* had a total frequency of occurrence 4(9.0%), of which 2 was tsolated from Afor Ogbe market, 0 from Ekeubahaeze market, and 2 from Okigwe market and *Cladosporium tenussimum* had a total frequency of occurrence of 1(2.2%), of which 0 was isolated from Afor Ogbe market, 0 from

Ekeubahaeze market and 1 from okigwe market respectively as shown in figure 12 (appendix 21).

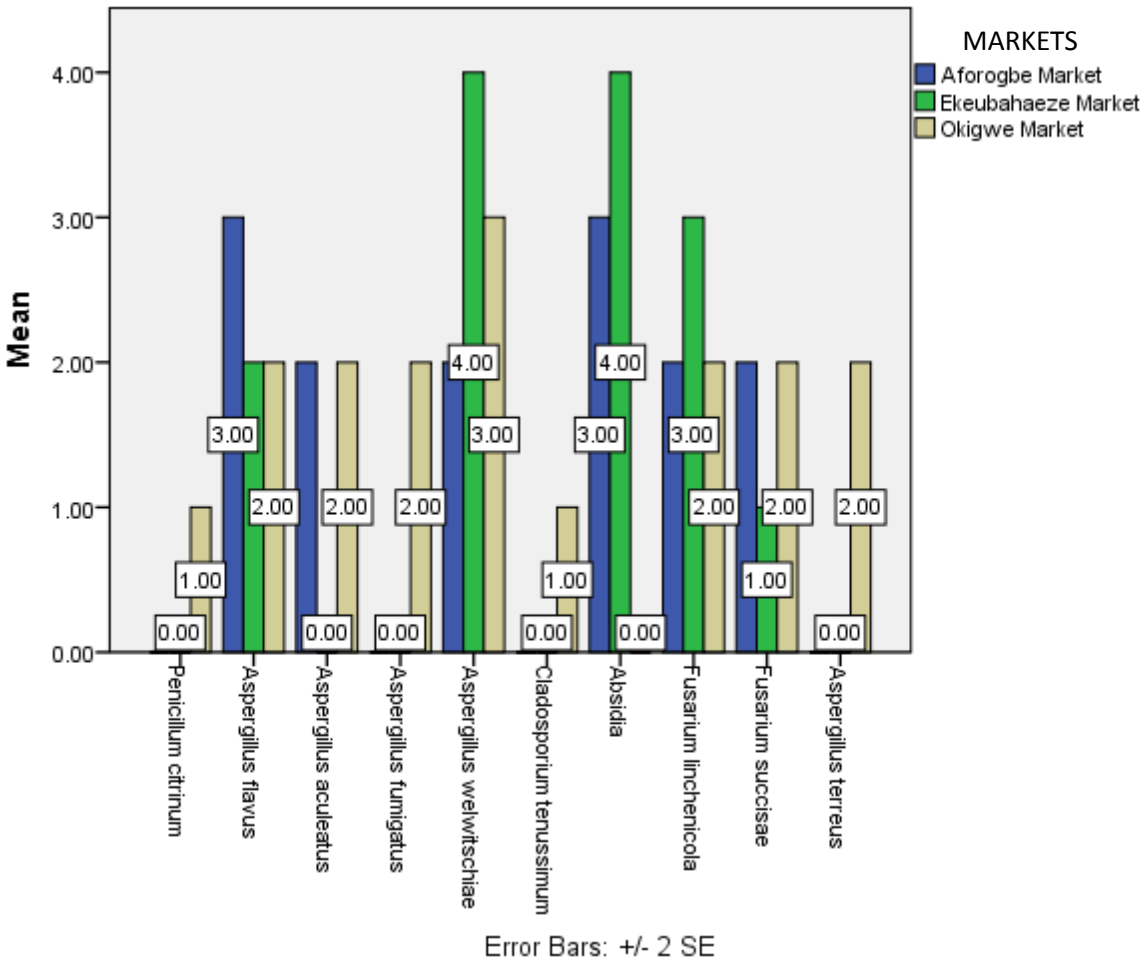



FIGURE 12: **Frequency occurrence of keratinophilic non-dermatophytic molds from soil samples within cattle markets in Imo State using hair bait technique**

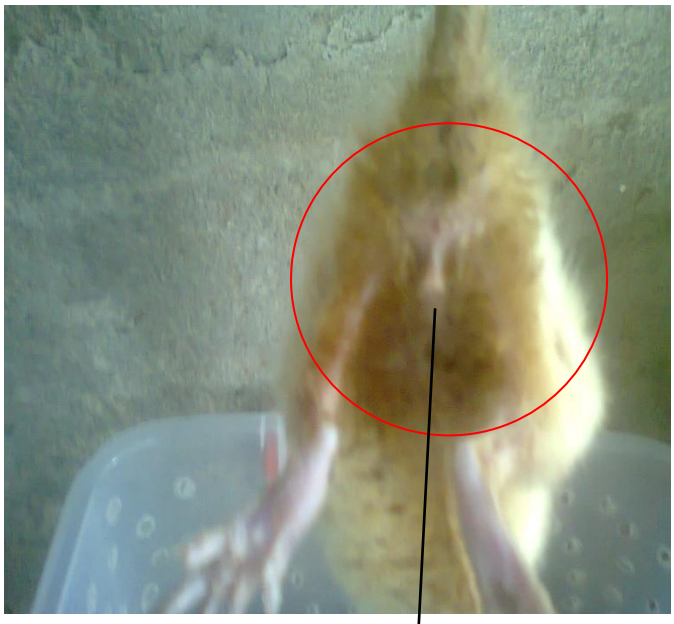
The pathogenecity test was carried out on albino mice using 10 selected non- dermatophytic molds isolated from this study. Three (3) isolates which includes *Absidia corymbifera*, *Cladosporium tenuissimum* and *Aspergillus welwitschiae* out of the 10 selected estabilished clinical symptoms suggesting cutaneous mycoses such as discolouration of fur, lesion production and alopecia respectively while the other isolates which includes *Aspergillus fumigatus*, *Aspergillus aculeatus*, *Fusarium linchenicola*, *Talaromyces kendrickii*,

*Pestalotiopsis microspora*, *Aspergillus sydowii* and *Curvularia kusanol* did not express any clinical symptoms suggesting cutaneous mycoses within the one month period of observation as shown in plate 13A, 13B and 13C.

The keratinase enzyme screening test carried out on 13 non-dermatophytic molds isolated from this study was analysed. From the analysis the result showed that 9 isolates hydrolysed the keratin present while 4 was not able to hydrolyze. From the result as shown in table 4, *Penicillum citrinum* grew moderately on medium and hydrolysed keratin at a diameter 50mm, *Aspergillus welwitschiae* grew moderately on medium and hydrolysed keratin at a diameter 55mm, *Aspergillus aculeatus* showed a no growth on

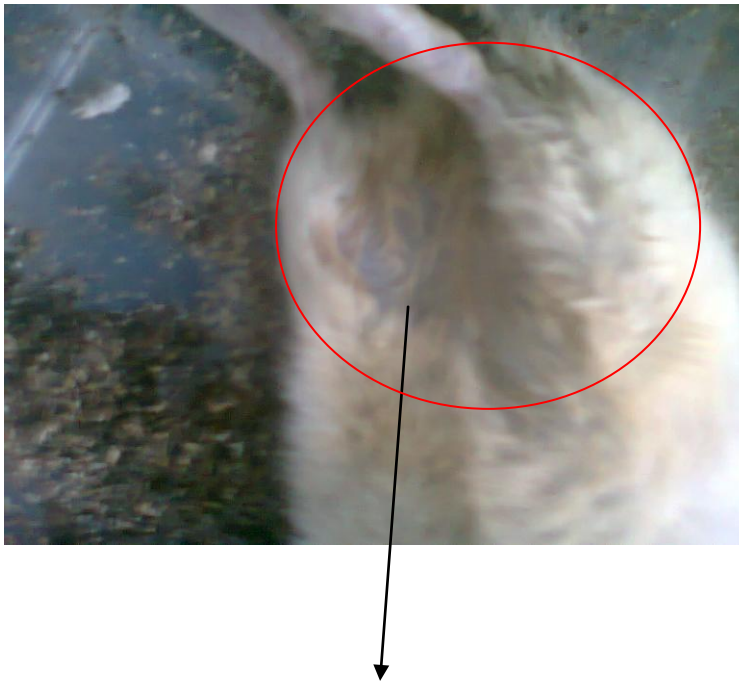
medium and no activity was observed, *Curvularia kusanol* grew moderately and showed a hydrolysis diameter at 60mm, *Cladosporium tenussimum* showed a low growth on medium and hydrolysed keratin at diameter 20mm, *Pestalotiopsis microspora* grew moderately on medium and diameter showed hydrolysis at 65mm, *Fusarium oxysporum* grew moderately on medium and hydrolysed keratin at diameter 55mm, *Fusarium lichenicola* and *Absidiacorymbifera* had a no growth on medium and showed no activity, *Aspergillus fumigatus* showed a moderate growth on medium and showed keratin hydrolysis at diameter 46mm, *Aspergillus flavus* grew moderately on medium and hydrolysed keratin at diameter 60mm,

*Aspergillus sydowii* showed no growth on medium and no activity was observed while *Fusarium succisae* grew moderately on medium and showed hydrolysis of keratin at diameter 50mm as shown in table 4 as in plate 14A.



**A** Discolouration of the albino mice skin

**B** Lesion appearance on the albino mice skin



**C**

Alopecia - (loss of fur) around the leg region

Plate 13; showing clinical symptoms observed on albino mice used for pathogenecity test. Discolouration on the skin rubbed with *Absidia corymbifera* (A), lesion appearance on the skin rubbed with *Cladosporium tenuissinum* (B) and alopecia observed on the skin rubbed *with Aspergillus welwitschiae (C).*

## TABLE 4: Enzyme screening test

Keratinolytic activity on the non-dermatophytic molds using chicken feathers.

| **Fungal Isolates** | **Growth on chicken feather agar** | **Clear Zone** | **Diameter zone (mm)** |
|---|---|---|---|
| *Penicillum* | ++ | + | 50 |
| *citrinum* | ++ | + | 55 |
| *Aspergillus* | - | - | 0 |
| *welwitschiae* | ++ | + | 60 |
| *Aspergillus* | + | + | 20 |
| *aculeatus* | ++ | + | 65 |

| | | | |
|---|---|---|---|
| *Curvularia* | ++ | + | 55 |
| *kusanol* | - | - | 0 |
| *Cladosporium* | - | - | 0 |
| *tenussimum* | ++ | + | 46 |
| *Pestalotiopsis* | | | |
| *microspora* | ++ | + | 60 |
| *Fusarium* | - | - | 0 |
| *oxysporium* | ++ | + | 50 |
| *Fusarium* | | | |
| *lichenicola* | | | |
| *Absidia* | | | |
| *corymbifera* | | | |
| *Aspergillus* | | | |
| *fumigatus* | | | |
| *Aspergillus* | | | |

| | | | |
|---|---|---|---|
| *flavus*<br>*Aspergillus*<br>*sydowii*<br>*Fusarium*<br>*succisae* | | | |

Key: +++, ++, +, - represents heavy growth, moderate growth, low growth and no activity respectively

Amylase screening test of some non-dermatophytic molds isolated from this study using starch agar as source of carbon and nitrogen source was carried out. 13 isolates were screened and only 10 were able to hydrolyze the starch present. *Penicillum citrinum* grew moderately on the medium and hydrolysed the substrate at diameter 60mm. *Aspergillus welwitschiae grew* heavily on medium and hydrolysed starch completely at diameter 86mm, *Aspergillus aculeatus* grew heavily on medium and hydrolysed starch completely at diameter 86mm, *Curvularia kusanol* showed a low growth on medium and no activity was observed. *Cladosporium tenuissium* grew moderately on medium and hydrolysed starch with a diameter

75mm. *Fusarium oxysporum* grew moderately on medium and hydrolysed starch at diameter 64mm, *Absidiacorymbifera* grew on medium moderately and hydrolysed starch at 69mm, *Aspergillus fumigatus* showed a slow growth on medium and hydrolysed starch at diameter 30mm, *Aspergillus terreus* showed a low growth on medium and hydrolysed starch at diameter 37mm, *Aspergillus flavus* grew heavily on medium and hydrolysed starch completely at diameter 86mm, *Fusarium succisae* grew moderately on medium and hydrolysed starch at diameter 75mm while *Pestalotiopsis microspora* and *Fusarium lichenicola* did not grow on medium and no activity was observed as shown in table 5 and plate 14B .

Proteolytic enzyme activity using gelatin agar was carried out on 11 non-dermatophytic molds isolated from this study, only 7 showed zone of hydrolysis. *Penicillium citrinum* showed a slow growth on medium and hydrolysed substrate at diameter 35mm. *Aspergillus welwitschiae* grew moderately on medium and hydrolyse substrate at diameter 50mm. *Aspergillus aculeatus*, *Curvularia kusanol* showed no growth on medium and no hydrolysis was observed. *Cladosporium tenuissimum* showed a heavy growth on medium and expressed the highest zone of clearance 80mm followed by *Fusarium succisae* that grew moderately on medium but hydrolysed substrate at diameter 60mm, *Absidiacorymbifera* grew moderately on substrate

and hydrolysed substrate at diameter 50mm, *Aspergillus terreus* showed a slow growth on medium and hydrolysed substrate at diameter 30mm, *Aspergillus flavus* grew moderately on medium and hydrolysed substrate at diameter 55mmwhile isolates like *Fusarium oxysporium*, *Pestalotiopsis microspora* did not grow at all and as such showed no activity on the agar plates as shown in table 6 and plate 14C.

**TABLE 5: Amylase activity on the non-dermatophytic molds using starch agar plates**

| Fungal Isolates | Growth on starch agar | Clear Zone | Diameter zone (mm) |
|---|---|---|---|
| *Penicillum* | ++ | + | 60 |
| *citrinum* | +++ | + | 86* |
| *Aspergillus* | +++ | + | 86* |
| *welwitschiae* | + | - | 0 |
| *Aspergillus* | ++ | + | 75 |
| *aculeatus* | - | - | 0 |
| *Curvularia* | ++ | + | 64 |
| *kusanol* | - | - | 0 |
| *Cladosporium* | ++ | + | 69 |

| | | | |
|---|---|---|---|
| *tenussimum* | + | + | 30 |
| *Pestalotiopsis microspora* | +++ | + | 86* |
| *Fusarium oxysporium* | ++ | + | 75 |
| *Fusarium lichenicola* | | | |
| *Absidia corymbifera* | | | |
| *Aspergillus fumigatus* | | | |
| *Aspergillus terreus* | | | |
| *Aspergillus flavus* | | | |
| *Fusarium* | | | |

| | | | |
|---|---|---|---|
| *succisae* | | | |

**Key:** +++, ++, +, –, * represents heavy growth, moderate growth, low growth, no activity and complete hydrolysis respectively.

**TABLE 6 : Proteolytic activity on non-dermatophytic molds using gelatin agar**

| Fungal Isolates | Growth on gelatin | Clear Zone | Diameter zone (mm) |
|---|---|---|---|
| *Penicillum* | + | + | 35 |
| *citrinum* | ++ | + | 50 |
| *Aspergillus* | - | - | 0 |
| *welwitschiae* | - | - | 0 |
| *Aspergillus* | +++ | + | 80 |
| *aculeatus* | ++ | + | 50 |
| *Curvularia* | + | + | 30 |
| *kusanol* | ++ | + | 60 |
| *Cladosporium* | - | - | 0 |
| *tenussimum* | - | - | 0 |
| *Absidia* | ++ | + | 55 |

| | | | |
|---|---|---|---|
| *corymbifera*<br>*Aspergillus terreus*<br>*Fusarium succisae*<br>*Pestalotiopsis microspora*<br>*Fusarium oxysporium*<br>*Aspergillus flavus* | | | |

**Key:** +++, ++, +, -, represents heavy growth, moderate growth, low growth, and no activity respectively.

Eleven (11) non-dermatophytic molds isolated from this study were screened for lipolytic activity using olive oil as substrate and phenol red

as indicator. Table 7, shows how the non-dermatophytic molds grew on the media and the rate of activity with diameter zones of hydrolysis. From our result *Penicillum citrinum* showed a slow growth on medium, without hydrolyzing the substrate. *Aspergillus welwitschiae* grew moderately, hydrolysed the substrate with diameter at 60mm, *Aspergillus aculeatus* did not grow on the medium and did not hydrolyse the substrate. *Curvularia kusanol* did not grow on medium and showed no activity, *Cladosporium tenuissium* showed a slow growth on medium with no activity. *Pestalotiopsis microspora* showed no growth on medium and no activity was observed, *Fusarium lichenicola* grew moderately on medium and hydrolysed substrate

at a diameter of 50mm. *Absidiacorymbifera* showed a slow growth on medium and hydrolysed substrate at a diameter of 35mm. *Aspergillus fumigatus* did not grow on medium and no activity was observed. *Aspergillus flavus* grew heavily on medium, hydrolysed substrate at diameter 60mm while *Fusarium succisae* grew moderately on medium with diameter 50mm as shown in table 7 and plate 15A.

Cellulolytic enzyme activity and diameter zones of hydrolysis of non-dermatophytic mold isolates on cellulose agar plate using carboxymethyl cellulose as source of carbon and nitrogen was tested. From the result it was revealed, out of 12 isolates analyzed, 10 showed zone of hydrolysis. *Penicillum citrinum* showed a slow growth on the

medium and hydrolysed the substrate at a diameter (30mm), *Aspergillus welwitschiae* grew heavily on the medium and hydrolysed the substrate at a diameter (86mm), *Aspergillus aculeatus* showed slow growth on media and hydrolysed the substrate at diameter (35mm), *Curvularia kusanol* expressed *a s*low growth on medium and hydrolysed substrate at a diameter (30mm), *Cladosporium tenuissimum* had a low growth on medium and hydrolysed substrate at a diameter (20mm), *Absidiacorymbifera* grew moderately on medium and hydrolysed substrate at a diameter (60mm), *Aspergillus succisae* grew heavily on medium and hydrolysed substrate at a diameter (70mm), *Pestalotiopsis microspora* grew moderately on mediumandhydrolysed

substrate at a diameter (65mm), *Fusarium oxysporum* grew moderately on the medium and showed positive cellulolytic activity at a diameter (65mm), *Fusarium lichenicola* had a slow growth on medium at a diameter (10mm), while *Aspergillus flavus* and *Aspergillus fumigatus* showed a no growth on media respectively on the media but showed no sign of cellulolytic activity on the media as shown in table 8 and plate 15B.

**TABLE 7: Lipase activity on non-dermatophytic molds using olive oil with phenol red agar**

| **Fungal Isolates** | **Growth on olive oil with Phenol red agar** | **Clear Zone** | **Diameter zone (mm)** |
|---|---|---|---|
| *Penicillum* | + | - | 0 |
| *citrinum* | +++ | + | 60 |
| *Aspergillus* | - | - | 0 |
| *welwitschiae* | - | - | 0 |
| *Aspergillus* | + | - | 0 |
| *aculeatus* | - | - | 0 |
| *Curvularia* | ++ | + | 50 |
| *kusanol* | + | + | 35 |
| *Cladosporium* | - | - | 0 |
| *tenussimum* | +++ | + | 60 |

| | | | |
|---|---|---|---|
| *Pestalotiopsis microspora*<br>*Fusarium lichenicola*<br>*Absidia corymbifera*<br>*Aspergillus fumigatus*<br>*Aspergillus flavus*<br>*Fusarium succisae* | ++ | + | 50 |

**Key: +++, ++, +, -**represents heavy growth, moderate growth, low growth and no growth respectively.

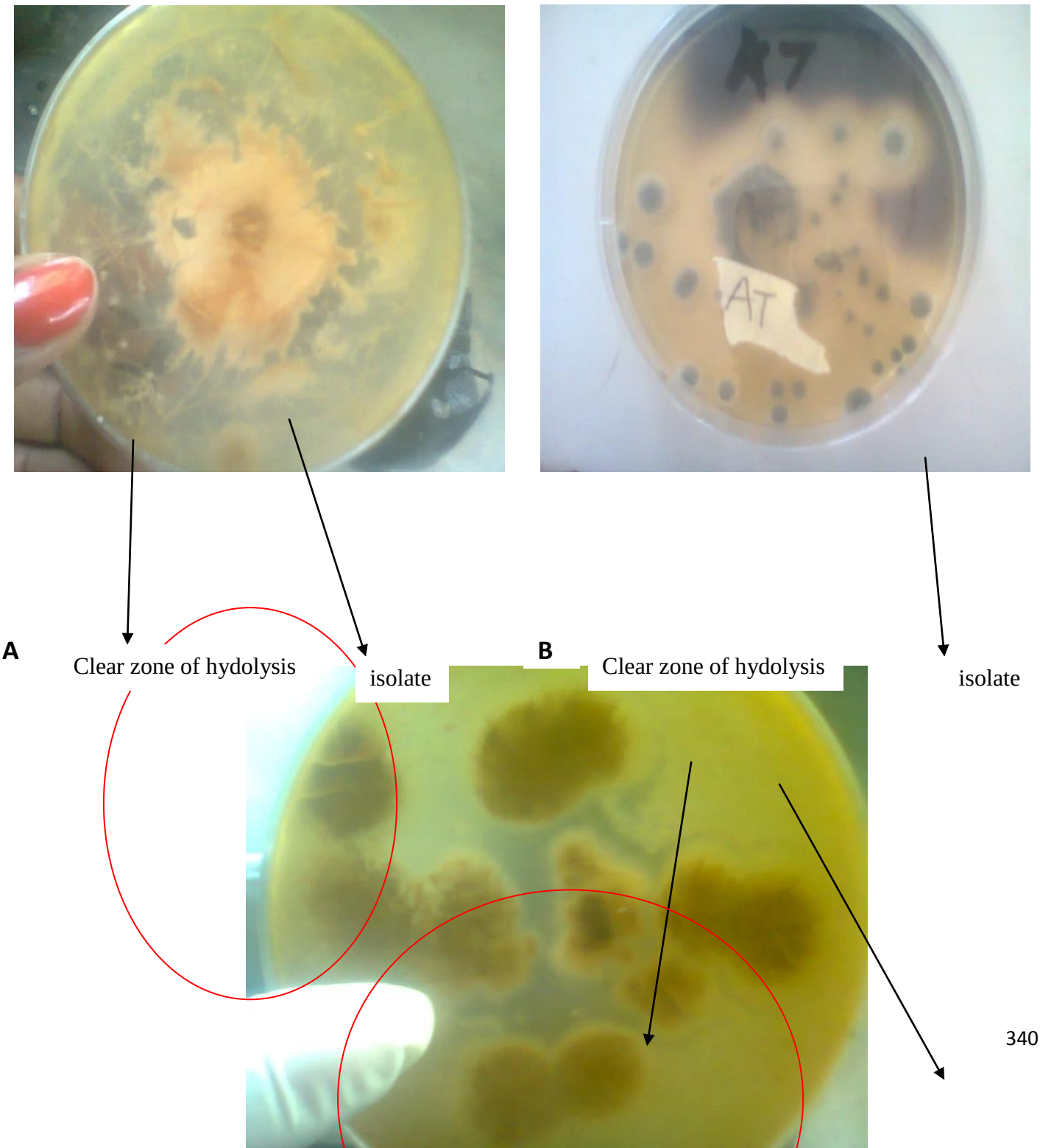
**TABLE 8: Cellulolytic activity on non-dermatophytic molds using carboxymethyl cellulose**

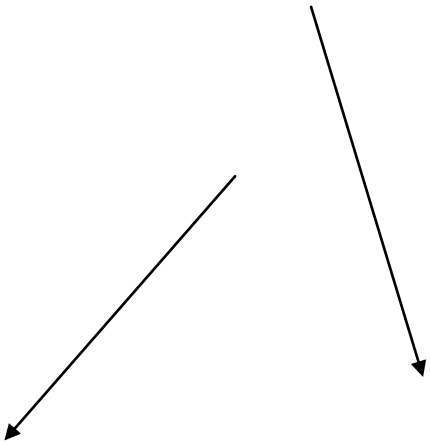
| Fungal | Growth | Clea | Diamete |
|---|---|---|---|

| **Isolates** | **Carboxymethyl cellulose** | **r Zone** | **r zone (mm)** |
|---|---|---|---|
| *Penicillum* | + | + | 30 |
| *citrinum* | +++ | + | 86 |
| *Aspergillus* | + | + | 35 |
| *welwitschiae* | + | + | 30 |
| *Aspergillus* | + | + | 20 |
| *aculeatus* | ++ | + | 60 |
| *Curvularia* | +++ | + | 70 |
| *kusanol* | ++ | + | 60 |
| *Cladosporiu* | ++ | + | 65 |
| *m* | - | - | 0 |
| *tenussimum* | - | - | 0 |
| *Absidia* | + | + | 10 |
| *corymbifera* | | | |
| *Aspergillus* | | | |

| | | | |
|---|---|---|---|
| *succisae*<br>*Pestalotiopsis microspora*<br>*Fusarium oxysporium*<br>*Aspergillus flavus*<br>*Aspergillus fumigatus*<br>*Fusarium lichenicola* | | | |

**Key:** +++, ++, +, -, represents heavy growth, moderate growth, low growth, and no activity respectively.



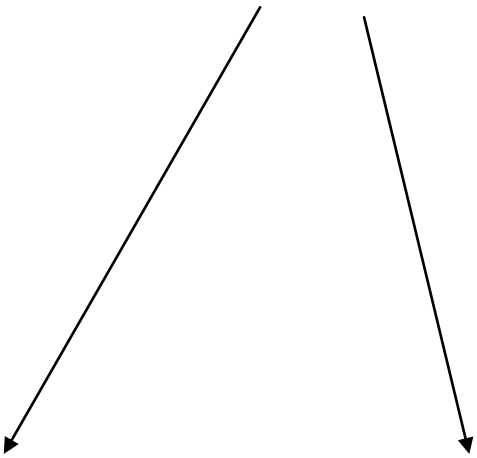


C isolate

Clear zone

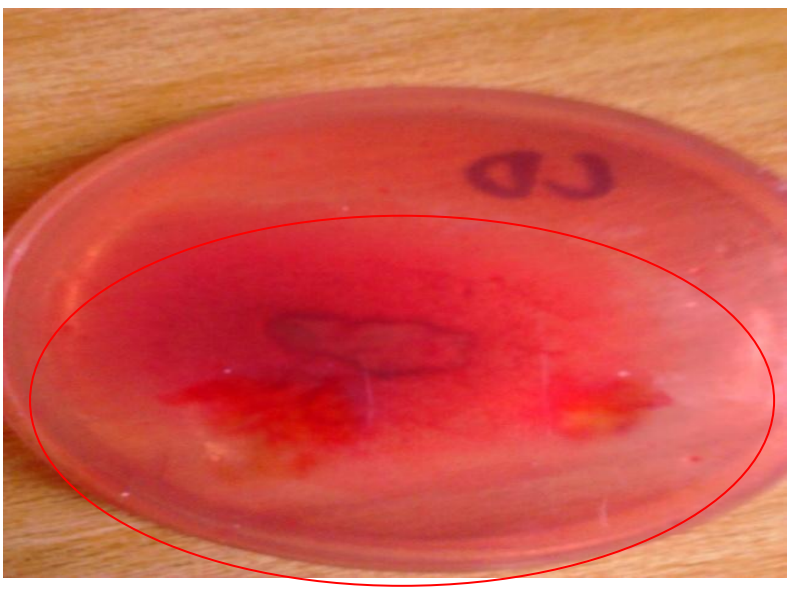
Plate 14: result of enzyme screening test, hydrolysis of keratin by *Curvulariakusanol*

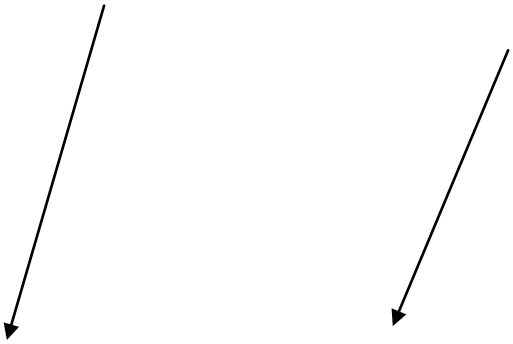
hydrolysis of starch by *Penicillium citrinum* (B) and hydrolysis of protein by *Fusarium succisae* (C).



**A**

Clear zone isolate





B

Clear zone

isolate

Plate 15: enzyme screening test, hydrolysis of lipid by *Aspergillus welwitschiae* (A) and hydrolysis of cellulose by *Absidia corymbifera* (B).

Different inhibition zones were obtained from some antifungal conventional drugs. For this study four antifungal drugs were tested,

Ketoconazole, Voriconazole, Fluconazole and Amphotericin B.

From the analysis the inhibition zone of clearance was measured in millimeter. Ketoconazole showed strong sensitivity on most non-dermatophytic molds of which *Penicillum citrinum* showed an inhibition zone (IZ) of (30mm), *Aspergillus fumigatus* (10mm), *Aspergillus welwitschiae* (18mm), *Fusarium succisae* (30mm), *Curvularia kusanol* (45mm), *Cladosporium tenuissimum* (53mm), *Pestalotiopsis microspora* (34mm), *Fusarium solani* (50mm), *Fusarium lichenicola* (49mm), *Absidiacorymbifera* (10mm), except on *Aspergillus aculeatus*t hat was resistant. Voriconazoleweakly sensitive on the isolates

completely of which *Aspergillus fumigatus* showed an inhibition of ($16^{RM}$mm), *Aspergillus welwitschiae* ($20^{RM}$mm), *Aspergillus aculeatus* ($30^{RM}$mm), *Fusarium succisae* ($20^{RM}$mm), *Curvularia kusanol* ($40^{RM}$mm), *Cladosporium tenuissimum* ($35^{RM}$mm), *Pestalotiopsis microspora* ($10^{RM}$mm) while *Penicillum citrinum*, *Fusarium solani*, *Fusarium lichenicola* and *Absidia* specie were completely resistant. All the isolates were resistant to Fluconazole. Amphotericin B showed minute inhibition zone, *Aspergillus fumigatus* had inhibition zone of (10mm), *Aspergillus welwitschiae* (9mm), *Fusarium succisae* (12mm), *Curvularia kusanol* (7mm), *Pestalotiopsis microspora* (15mm), *Fusarium lichenicola* (12mm), *Absidia* specie

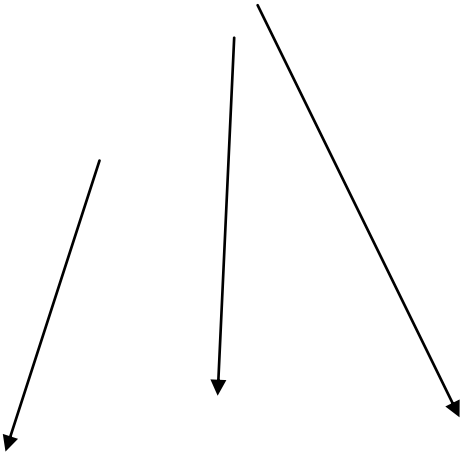
(7mm) while *Penicillum citrinum*, *Aspergilluus aculeatus*, *Cladosporium tenussimum* and *Fusarium solani* showed no inhibition zone as shown in table 9 and plates 16A, 16B, 16C and 16D.

**TABLE 9: Antifungal sensitivity test on the non-dermatophytic molds**

| **Fungal Isolates** | **Antibiotics and Diameter Zone of Clearance (mm)** | | | |
|---|---|---|---|---|
| | **Keto** | **Vori** | **Fluco** | **Ampho** |
| *Penicillum* | 30 | 0 | 0 | 0 |
| *citrinum* | 10 | 16$^{RM}$ | 0 | 10 |
| *Aspergillus* | 18 | 20$^{RM}$ | 0 | 9 |
| *fumigatus* | 0 | 30$^{RM}$ | 0 | 0 |
| *Aspergillus* | 30 | 20$^{RM}$ | 0 | 12 |
| *welwitschiae* | 45 | 40$^{RM}$ | 0 | 7 |
| *Aspergillus* | 53 | 35$^{RM}$ | 0 | 0 |
| *aculeatus* | 34 | 10$^{RM}$ | 0 | 15 |
| *Fusarium* | 50 | 0 | 0 | 0 |

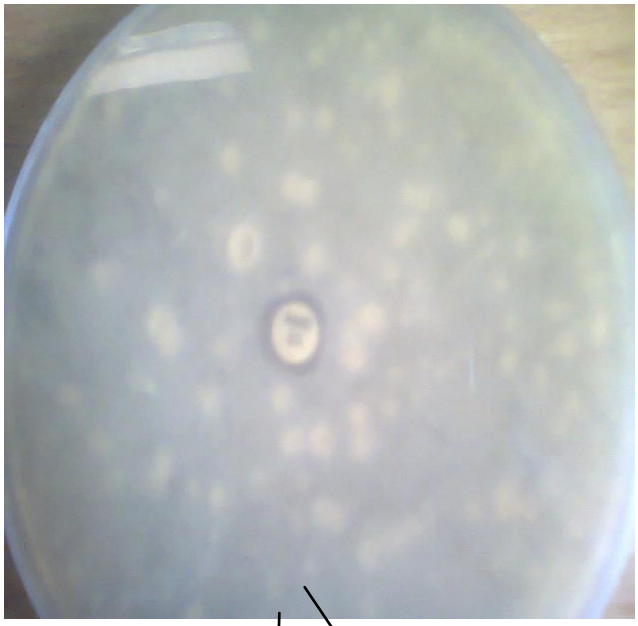
| | | | | |
|---|---|---|---|---|
| *succisae* | 40 | 0 | 0 | 12 |
| *Curvularia kusanol* | 10 | 0 | 0 | 7 |
| *Cladosporium tenussimum* | | | | |
| *Pestalotiopsis microspora* | | | | |
| *Fusarium solani* | | | | |
| *Fusarium lichenicola* | | | | |
| *Absidia corymbifera* | | | | |

**Key** = Keto - Ketoconazole, Vori – Voriconazole, Fluco – Fluconazole, Ampho – Amphotercin B, RM - Resistant mutant (fungi static).



B Isolate Unclear zone Drug

Isolate Clear zone Drug



D

C

Isolate Drug Isolate Clear zone Drug

Plate 16: antibiotic sensitivity test using some antifungal conventional agents; ketoconazole on *Fusarium succisae* (A), voriconazole on *Aspergillus welwitschiae* (B), fluconazole on *Aspergillus fumigatus* (C) and Amphotericin B on *Aspergillus fumigatus* (D)

Phytochemical analysis was carried out on the four plant leaves used for this study. Seven phytochemical compounds were tested for which included Phenolic compounds, Flavonoids, Saponin, Anthraquinone Glycosides, Steroids,

Terpenoids, Alkaloids and Phlobatannin. The four Plants analysed were *Occimum gratissimum*, *Euphorbia hirta*, *Mitracarpus scaber* and *Jatropha multifida*. From the results, *Occimum gratissium* showed presence of phenolic compounds, flavonoid, alkaloid, saponin and steroids. Glycosides and terpenoid, were in abundance while tannin and alkaloid were absent.

*Euphorbia hirta*, showed presence of flavonoid, alkaloid and saponin. It also showed abundance of glycosides, terpenoid and steroids while tannin, phenol and alkaloid in the presence of mayer were absent.

*Mitracarpus scaber*, showed presence of flavonoid, alkaloid. Glycoside, terpenoid, tannin,

alkaloid in the presence of wagner and steroid were in adundance while phenol were absent.

*Jatropha multifida*, showed presence of glycoside, tannin, flavonoid, phenol, alkaloid in the presence wagner were present. Saponin and steroids were in abundance while alkaloid in the presence of mayer was absent as shown in table 10.

**TABLE 10: Qualitative phytochemical analysis**

| **TEST** | **PLANTS** | | | |
|---|---|---|---|---|
| | ***Occimum gratissimum*** | ***Euphorbia hirta*** | ***Mitracarpus scaber*** | ***Jatropha Multifida*** |

| | | | | |
|---|---|---|---|---|
| Saponin | + | + | + | ++ |
| Phenol | + | _ | _ | + |
| Tannin | _ | _ | ++ | + |
| Flavonoids | + | + | + | + |
| Steriods | + | ++ | ++ | ++ |
| Terpenoids | ++ | ++ | +++ | + |
| Alkaloids | | | | |
| Wagner | _ | + | ++ | + |
| Mayer | + | _ | + | _ |
| Glycoside | ++ | ++ | +++ | + |

Key: (+) present, (-) absent and (++) abundance

Futhermore, quantitative phytochemical analysis of the four plant leaves were carried out.

*Occimum gratissium* expressed the presence of steroid (35.7 mg/100g) as the most maximum active compound followed by saponin (77.94mg/100g), alkaloid (0.6122mg/100g), glycoside (0.232mg/100g), tannin (0.0262mg/100g), phenol (0.0085 mg/100g) and flavonoid (-2.04mg/100g).

*Euphorbia hirta* expressed the presence of steroid (35.86 mg/100g) as the most abundant active compound followed by saponin (27mg/100g), glycoside (0.744mg/100g), alkaloid (0.3502 mg/100g), tannin (0.1606mg/100g), phenol (0.0023mg/100g) and flavonoid (-6.68mg/100g) as shown in table 11.

*Mitracarpus Scaber* expressed the presence of steroid (35.7mg/100g) as the most abundant compound followed by saponin (27.84mg/100g), glycoside (0.600mg/100g), alkaloid (0.369 mg/100g), tannin (0.054mg/100g), Phenol (0.0127 mg/100g) and flavonoid (-0.310mg/100g).

*Jatropha multifida* expressed the presence of steroid (35.9mg/100g) as the most abundant followed by saponin (27.9mg/100g), glycoside (0.510 mg/100g), alkaloid (0.383mg/100g), tannin (0.030 mg/100g), phenol (0.0023 mg/100g) and flavonoid (-2.030mg/100g) as shown in table 11.

# TABLE 11: Quantitative phytochemical analysis of the plant leaves

| **TEST** | **PLANTS (mg/100g)** | **(mg/100g)** | **(mg/100g)** | **(mg/100g)** |
|---|---|---|---|---|
| | ***Occimum gratissium*** | ***Euphorbia hirta*** | ***Mitracarpus scaber*** | ***Jatropha Multifida*** |
| Saponin | 27.94 | 27.94 | 27.84 | 27.9 |
| Phenol | 0.0085 | 0.0023 | 0.0127 | 0.0023 |
| Tannin | 0.0262 | 0.1606 | 0.054 | 0.030 |
| Flavonoids | -2.04 | -6.68 | -0.310 | -2.030 |
| Steriods | 35.7 | 35.86 | 35.7 | 35.9 |

| | | | | |
|---|---|---|---|---|
| Alkaloids | 0.6122 | 0.3502 | 0.369 | 0.383 |
| Glycoside | 0.232 | 0.744 | 0.660 | 0.510 |

Antifungal susceptibility test of the plant (leaves) extracts were carried out on 12 non-dermatophytic molds isolated from this study which includes *Fusarium solani*, *Fusarium succisae*, *Fusarium linchenicola*, *Aspergillus flavus*, *Aspergillus welwitschiae*, *Aspergillus fumigatus*, *Aspergillus aculeatus*, *Cladosporium tenuissimum*, *Curvularia kusanol*, *Pestalotiopsis microspora*, *Penicillium citrinum* and *Absidiacorymbifera*. It was observed that all the molds were restistant to *Jatropha multifida* at concentrations ranging from 6.25mg/ml to 200mg/ml except *Fusarium solani* that was resistant mutant (RM) at 200mg/ml.

For *Occimum gratissium* plant leaves extract, *Fusarium solani*, *Fusarium succisae*, *Cladosporium tenuissinum* and *Pestslotiopsis microspora* were resistant at concentrations ranging from 6.25mg/ml to 200mg/ml. *Fusarium linchenicola* showed diameter zone of inhibition of 0.5mm at 200mg/ml, *Aspergillus flavus* showed diameter zone of inhibition of 20mm at 200mg/ml and 8mm at 100mg/ml, *Aspergillus welwitschiae* showed diameter zone of inhibition of 10mm at 200mg/ml, *Aspergillus fumigatus* showed of diameter zone inhibition of 15mm, 10mm, 10mm and 10mm at 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml respectively. *Aspergillus aculeatus* showed diameter zone of inhibition of 15mm, 10mm at 200mg/ml amd

100mg/ml respectively. *Penicillium citrinum* showed diameter zone of inhibition of 20mm and 15mm at 200mg/ml and 100mg/ml while *Absidiacorymbifera* showed diameter zone of inhibition of 15mm, 10mm and 10mm at 200mg/ml, 100mg/ml and 50mg/ml respectively.

For *Euphorbia hirta* plant leaves extract, *Aspergillus aculeatus* and *Absidiacorymbifera* were resistant at all concentrations ranging from 6.25mg/ml to 200mg/ml. *Fusarium solani*, *Fusarium succisae*, *Aspergillus fumigatus*, *Curvularia kusanol* and *Pestalotiopsis microspora* showed diameter zone of inhibition of 10mm at 200mg/ml respectively but were resistant at lower concentrations. *Aspergillus flavus* and *Cladosporium tenuissinum* showed

diameter zone of inhibition of 15mm at 200mg/ml concentration, *Aspergillus welwitschiae* showed diameter zone of inhibition of 16mm at 200mg/ml concentration, *Pestalotiopsis microspora* showed diameter zone of inhibition of 10mm, 9mm and 8mm at 200mg/ml, 100mg/ml and 50mg/ml concentrations respectively while *Penicillium citrinum* showed diameter zone of inhibition of 15mm, 15mm, 10mm, 10mm at 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml concentrations respectively.

For *Mitracarpus scaber* plant leaves extract, *Fusarium solani*, *Fusarium succisae* and *Absidiacorymbifera* were resistant to the extract at concentration ranging from 6.25mg/ml to 200mg/ml. On *Fusarium linchenicola*, *Curvularia*

*kusanol* and *Penicillium citrinum* showed diameter zone of inhibition of 10mm at 200mg/ml and was resistant at lower concentrations. *Aspergillus flavus* showed diameter zone of inhibition of 10mm and 10mm at 200mg/ml and 100mg/ml concentrations respectively. *Aspergillus welwitschiae* and *Aspergillus fumigatus* showed diameter zone of inhibition of 15mm and 10mm at 200mg/ml and 100mg/ml concentrations respectively. *Pestalotiopsis microspora* shows diameter zone of inhibition of 15mm, 10mm, 10mm, 10mm, 10mm at 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml concentrations respectively as shown in table 12. Plate 17A and 17B shows some of the antifungal sensitivity tests.

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the four plants leaves of *Jatropha multifida*, *Occimum gratissimum*, *Euphorbia hirta* and *Mitracarpus scaber* were testedon 12 non-dermatophytic molds isolated from this study which includes *Fusarium solani*, *Fusarium succisae*, *Fusarium lichenicola*, *Aspergillus flavus*, *Aspergillus welwitschiae*, *Aspergillus fumigatus*, *Aspergillus aculeatus*, *Cladosporium tenuissimum*, *Curvularia kusanol*, *Pestalotiopsis microspora*, *Penicillum citrinum* and *Absidiacorymbifera*.

*Jatropha multifida* showed minimum inhibitory concentration (MIC) above 200mg/ml on all the isolates and minimum fungicidal concentration

(MFC) above 200mg/ml except on *Penicillium citrinum* that expressed MIC at 100mg/ml and MFC at 200mg/ml.

*Occimum gratissimum* showed MIC at 100mg/ml and MFC at 200mg/ml on *Fusarium solani*, *Aspergillus fumigatus*, *Aspergillus aculeatus*, *Curvularia kusanol*, *Penicillum citrinum*. On *Absidia corymbifera* MIC was at 50mg/ml and MFC was at 100mg/ml. On *Aspergillus flavus*, *Aspergillus welwitschiae*, *Fusarium succisae*, *Fusarium lichenicola*, *Cladosporium tenuissimum* and *Pestalotiopsis microspora* had its MIC at 200mg/ml and MFC above 200mg/ml.

*Euphorbia hirta* showed MIC at 200mg/ml and MFC above 200mg/ml on *Fusarium*

*succisae*, *Aspergillus flavus* and *Absidiacorymbifera*, On *Pestalotiopsis microspora* and *Penicillium citrinum* showed MIC at 25mg/ml and MFC at 50mg/ml respectively. On *Fusarium solani*, *Aspergillus welwitschiae*, *Aspergillus fumigatus* and *Cladosporium tenuissimum* showed their MIC at 100mg/ml and MFC at 200mg/ml respectively and *Aspergillus aculeatus* had their MIC at 200mg/ml and MFC above 200mg/ml.

*Mitracarpus scaber* showed MIC at 25mg/ml and MFC at 50mg/ml on *Fusarium solani*, *Fusarium lichenicola*, *Aspergillus flavus*, *Aspergillus welwitschiae*, *Aspergillus aculeatus* and *Curvularia kusanol*. On *Fusarium succisae*, *Aspergillus fumigatus*, *Cladosporium*

*tenuissimum*, *Pestalotiopsis microspora* and *Penicillium citrinum* showed its MIC at 50mg/ml and MFC at 100mg/ml while *Absidacorymbifera* showed its MIC above 200mg/ml and MFC above 200mg/ml as shown in table 13. Plate 17C shows some from the tests.

Based on statistical analysis on MIC and MFC for all the four plants, there were highly significant different on the individual activity of these plant extracts against the non-dermatophytic molds at $P < 0/05$ as shown in appendix 5 and 6.

**TABLE 12: Antifungal susceptibility pattern of 12 non-dermatophytic molds isolated from this study against the four selected methanolic extracts of the plant leaves**

| ISOLATES | *Jatropha multifida* | | | | | | *Occimum gratissium* | | | | | | *Euphobia hirta* | | | | | | *Mitracarpus scaber* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| mg/ml | 200mg/ml | 100mg/m | 50 | 25 | 12.5 | 6.25mg/ml | 200 | 100 | 50 | 25 | 12.5 | 6.25 | 200 | 100 | 50 | 25 | 12.5 | 6.25 | 200 | 100 | 50 | 25 | 12.5 | 6.25 |

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Fusarium solani* | 15 RM | R | R | R | R | R | R | R | R | R | R | R | 10 | R | R | R | R | R | R | R | R | R | R | R |
| *Fusarium succisae* | R | R | R | R | R | R | R | R | R | R | R | R | 10 | R | R | R | R | R | R | R | R | R | R | R |
| *Fusarium lichenicola* | R | R | R | R | R | R | 5 | R | R | R | R | R | R | R | R | R | R | R | R | 10 | R | R | R | R | R |
| *Aspergillus flavus* | R | R | R | R | R | R | 20 | R | R | R | R | R | 15 | R | R | R | R | R | R | 10 | 10 | R | R | R | R |
| *Aspergillus welwitschiae* | R | R | R | R | R | R | 10 | R | R | R | R | R | 16 | R | R | R | R | R | R | 15 | 10 | R | R | R | R |
| *Aspergillus fumigatus* | R | R | R | R | R | R | 15 | 10 | 10 | 10 | R | R | 10 | R | R | R | R | R | R | 15 | 10 | 5 | R | R | R |
| *Aspergillus aculeatus* | R | R | R | R | R | R | 15 | 10 | R | R | R | R | R | R | R | R | R | R | R | 12 | R | R | R | R | R |

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Clasdosporium tenuissimum* | R | R | R | R | R | R | R | R | R | R | R | R | 15 | R | R | R | R | R | 15 | R | R | R | R | R |
| *Curvularia kusanol* | R | R | R | R | R | R | 20 | 20 | 10 | 10 | R | R | 10 | R | R | R | R | R | 10 | R | R | R | R | R |
| *Pestaloptiosis microspora* | R | R | R | R | R | R | R | R | R | R | R | R | 10 | 9 | 8 | R | R | R | 15 | 10 | 10 | 10 | 10 | R |
| *Penicillum citrinum* | R | R | R | R | R | R | 20 | 15 | R | R | R | R | 15 | 15 | 10 | 10 | R | R | 10 | R | R | R | R | R |
| *Absidia corymbifera* | R | R | R | R | R | R | 15 | 10 | 10 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R |

**KEY: RM – Resistant mutant, R – Resistant, Inhibition Zone (IZ) in Millimetres (mm)**

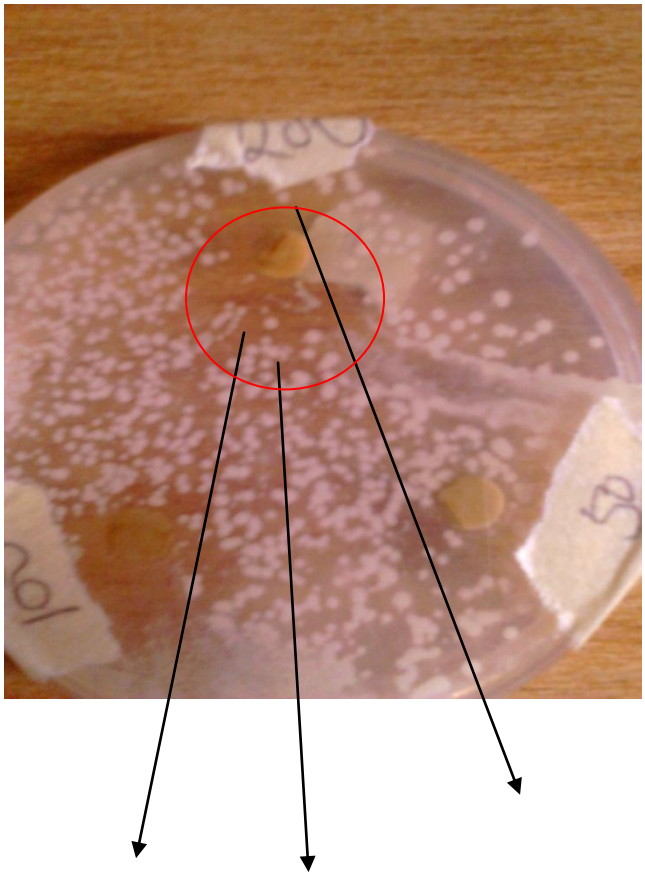
Table 13: **MIC's and MFC's of the four selected plant (leaves) extract against 12 non dermatophytic molds isolated from the study**

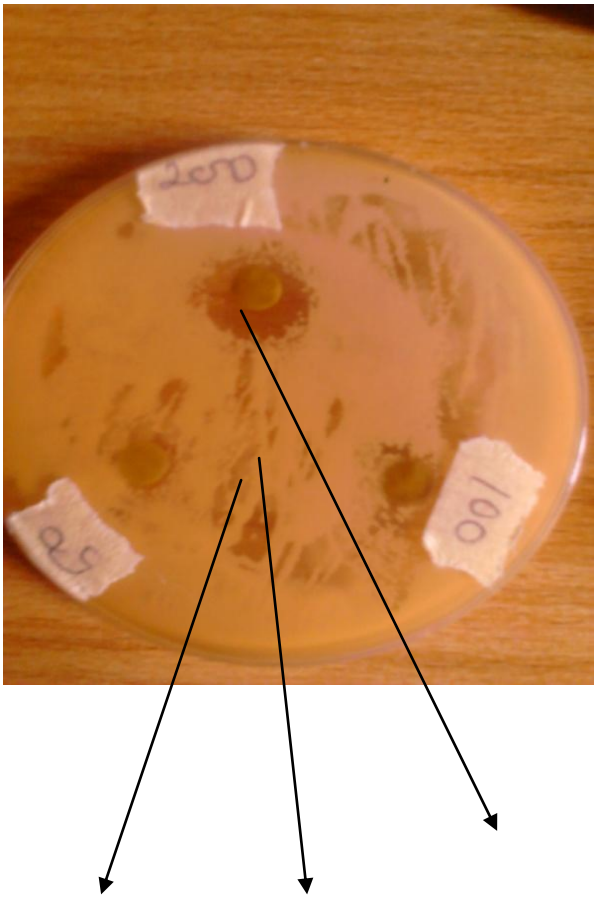
| **ISOLATE** | ***Jatropha multifida*** | | ***Occimum gratissium*** | | ***Euphobia*** |
|---|---|---|---|---|---|
| | **MIC (mg/ml)** | **MFC(mg/ml)** | **MIC(mg/ml)** | **MFC (mg/ml)** | **MIC(mg/m** |
| *Fusarium solani* | >200 | >200 | 100 | 200 | 100 |
| *Fusarium succisae* | 200 | >200 | >200 | >200 | 200 |

| | | | | | |
|---|---|---|---|---|---|
| *Fusarium lichenicola* | >200 | >200 | >200 | >200 | >200 |
| *Aspergillus flavus* | >200 | >200 | 200 | >200 | 200 |
| *Aspergillus welwitschiae* | >200 | >200 | 200 | >200 | 100 |
| *Aspergillus fumigatus* | 200 | >200 | 100 | 200 | 100 |
| *Aspergillus aculeatus* | >200 | >200 | 100 | 200 | >200 |
| *Clasdosporium* | >200 | >200 | >200 | >200 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| *tenuissimum* | | | | | |
| *Curularia kusanol* | >200 | >200 | 100 | 200 | 50 |
| *Pestaloptiosis microspora* | >200 | >200 | >200 | >200 | 25 |
| *Penicillum citrinum* | 100 | 200 | 100 | 200 | 25 |
| *Absidia corymbifera* | >200 | >200 | 50 | 100 | 200 |

**Key: MIC = Minimum Inhibitory Concentration, MFC= Minimum Fungicidal Concentration**

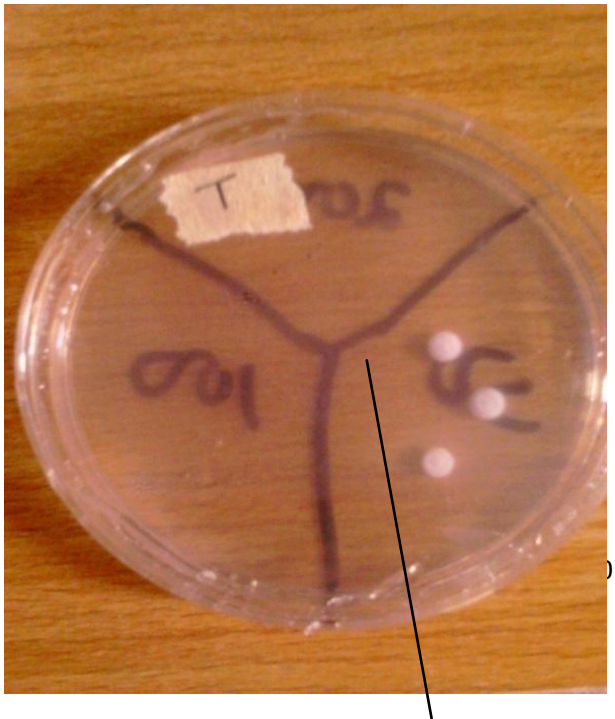




**B**

Clear zone Disc 200mg/ml

ml



**D**

MIC at 100mg/ml MFC at 200mg/ml

25mg/ml 12.5mg/ml 6.25mg/ml

Plate 17 showing antifungal susceptibility test of some plant extract on non-dermatophytic molds: *Mitracarpus scaber* on *Aspergillus fumigatus* (A), *Occimum grastissum* on *Aspergillus fumigatus* (B) and minimum inhibitory concentration (MIC) plates and minimum fungicidal concentration (MFC) on

*Penicillum citrinum* using *Jatropha multiphida* (C)

# CHAPTER FIVE

## 5.0 Discussion

Cutaneous mycoses is an infection caused by keratinophilic fungi. These fungi are capable of producing extracellular enzymes such as keratinase, elastase, protease (Emenga and Oyeka, 2013).

The infection causes the superficial damage of the skin tissue in animals and leads to high losses in the farm and reduction on its economic value (Didier, 2017).

The isolation of sixteen non-dermatophytic molds species from this study was similar to the work by Igor, *et al.*, (2007) who reported that *Aspergillus* species, *Penicillum* specie, *Alternaria* specie, *Mucor* specie and *Fusarium* specie were the most isolated fungi when he examined the mycology of skin swabs from dog skin also EL-Said, *et al.*, (2009) sampled hair from sheep and goat in Libya and reported the prevalence of saprophytes in the frequency order of *Aspergillus*, *Penicillum*, *Emericella*, *Alternaria* and *Cochliobolus.* Paixao, *et al.*, (2001) also isolated non-dermatophytes when he surveyed dermatophytes and saprophytic fungi isolated from dogs and cats in the city of Fortaleza, Brazil. From his study it was

revealed that *Aspergillus* species (37.9%) had the highest saprobe isolated followed by *Penicillum* species (21.4%), *Cladosporium* species (8.7%), *Fusarium* sp (7.8%), *Trichoderma* s (6.8%), *Candida* species (6.8%). *Curvularia* species (3.9%), *Rhizopus* specie (2.9%), *Choetomium* species (1.9%) and *Alternaria* species(1.9%). Ponnusamy, *et al*., (2018) isolated *Curvularia* species when they analyzed skin infection in goat in Cauvery delta region of Tamil Nadu, India.

Ravinder, *et al*., (2015) in their work also reported that non-dermatophytic molds (NDM) were the most isolated when he surveyed the epidemiological and mycological profile of superficial mycoses in North India and stated

that NDM are fast replacing the dermatophytes as a causative agents of dermatomycoses.

From our study it was observed that there were no significant difference between non-dermatophytic molds that were isolated from the cattle skins from both states. This is expected since these livestock are being produced in the northern part of the country and the markets in both states are being fed from this same source. This is an indication that these non-dermatophytic molds are widely distributed in the environment where these cattle were produced (Mahendra and Anil, 2014). In Nigeria cattle rearing is an occupation of the Northerners and considering that they migrate from the same region

following the same route from the North to the South-eastern states, it is most likely that they will be predisposed to the same species of non-dermatophytic molds which as reported by Emenuga and Oyeka, (2013). This was also observed in the highest frequently occurring isolate from both Abia and Imo States. *Fusarium Iichenicola* was the highest frequently occurring non-dermatophyte from both states, Abia state had a frequency of occurrence 87 (19.4%) and Imo State 52(16.0%) respectively.

Out of 451 cattle skin sampled, 53 cattle had lesion. From our study it was observed that similar isolates recovered from cattle without lesion were also isolated from those with

lesions. The highest frequent occurring non-dermatophytic molds isolated from cattle with lesion from Abia State and Imo State were *Aspergillus welwitschiae, followed by Fusarium* species, *Absidia* specie and *Cladosporium* specie. In Abia State, *Penicillium citrinum*, *Aspergillus fumigatus*, *Aspergillus welwitchiae*, *Aspergillus aculeatus*, *Aspergillus flavus*, *Aspergillus sydowii*, *Cladosporium tenuissimum*, *Fusarium lichenicola*, *Fusarium succisae* and *Absidiacorymbifera* were isolated while in Imo State, *Aspergillus welwitschiae*, *Aspergillus aculeatus*, *Aspergillus flavus*, *Aspergillus sydowii*, *Talaromyces kendrickii*, *Cladosporium tenuisimum*, *Pestalotiopsis*

*microspora*, *Fusarium lichenicola*, *Fusarium succisae* and *Absidiacorymbifera* were isolated. These non-dermatophytic molds are also similar to isolates recovered from cattle skin generally from both States. This is in agreement with a study by Jain, *et al*., (2011), showed the current status of *Fusarium* infection in animal and human and concluded that this filamentous fungus is widely distributed in the soil and on plants and can only cause superficial infections on compromise host. Also Suzana and Natasa, (2007) in their study agrees that *Cladosporium* spp. are opportunistic pathogens that are capable of causing cutaneous infections.

Based on anatomical sites that had lesions in this study, it was observed that most lesions were encountered at the abdominal region. This could be attributed to the fact that these animals lay and roll on the soil with their abdomen than other parts of their body thereby predisposing their glabrous skin to contaminated soil. These animal also often lay side by side to each other, thereby coming in contact often with other infected animals in the market. They can also incure trauma on their glabrous skin due to the stacking effect they suffer on transit in the trucks or it can also be as a result of trauma incurred in the bushes during grazing, that paves way for these non-dermatophytic molds to gain access through

the skin. This agrees with the work of Emenuga and Oyeka, (2013) who reported that lesions were mainly observed on the animals (goats and sheep) glabrous skin. This however disagrees with the work of Balogun, *et al*., (2017), on the prevalence and distribution of dermatophytes among domestic horses in Kwara State, Nigeria. They observed that lesions were seen more on the limbs (18.7%) of horses than other parts of the body where samples were collected.

In this study it was observed that among *Aspergillus* species isolated from cattle with lesions from both states *Aspergillus welwitschiae* was the most prevailing specie in Abia State (21.3%) and Imo State (28.2%).

This could be linked to its ability to utilize more virulent factors like production of enzyme such as keratinase, protease, cellulase, lipase. Most mold flora are known to cause superficial or cutaneous infections due to its ability to utilize keratin (Bakheshwain, *et al.*, 2001; Dider Pin, 2017).

The isolation of non-dermatophytic molds from air within the cattle markets in both States has no significant difference in their frequency occurrence as shown in appendix 16 and 17. This finding agrees with study by Ajoudanifar, *et al.*, (2011), they isolated similar non-dermatophytic fungal species from the air when they analyzed indoor and outdoor fungi at poultry and cattle homes in Iran, from

their results it was reveleaed *Cladosporium* (55.3%), Yeast (10.0%), and *Aspergillus* (9.4%) were the most common isolates, Pavan and Manjunath (2014), also reported similar results when they analyzed indoor and outdoor air borne fungi in animal rearing houses in cowshed, in their study the most dominant genera identified were *Cladosporium* sp., *Aspergillus* sp., and *Alternaria alternate*. This also agrees with a study by Sharm and Khade, (2019), when they analysed the aeromycoflora of indoor dairy cattle shed and their study revealed higher frequency prevalence of *Aspergillius* spp., *Ustilago* spp., *Penicillium*, *Alternaria* and *Cladosporium*.

This results could be attributed to the mechanical activities that go on in the market due to man and animal activities, animal to animal activities, man, animal to soil activities, as they can carry some of these molds from loose soils to the atmosphere. Wind movement can also carry some of these loose athrospores from the soil to the atmosphere. These animals fight alot, so they can shake off some of the loose mold embedded on their skin to the atmosphere.

This suspension of air environment within the market makes it an issue of public health concern as this predisposes those working there, buyers and sellers to serious health challenges and this was also reported by

Shakil,(2007) in his work when he analyzed air fungal spores in working environments.

The isolation of non- dermatophytic molds recovered from soil samples within markets in Abia state shows that there was a significant difference at P= 0.05 while in Imo State statistical analysis on frequency of occurrence of non-drmatophytic molds from soil samples within markets did not show significant difference at P= 0.05, this was shown in appendix 18 and 19 respectively. This fimdimg agrees with Farid and Nareen, (2012), when they analyzed soil samples from different areas of Erbil Government for the distribution of soil borne fungi and the result revealed *Aspergillus* specie as the most frequently isolated fungi,

followed by *Penicillum* specie, *Rhizopus*, *Fusarium* specie, also study by Nosratabadi, *et al.*, (2017) showed similar non-dermatophytic molds when they surveyed soil samples from greater Tunb, Abu-Musa and Sirri Islands in the Persian Gulf, Iran, in their study it was revealed that *Chrysoporium tropicum* (18.5%) was the most occurring specie followed by *Aspergillus* species (6.1%), *Penicillum* species (6.1%), *Alternaria* species (4.6%), *Fusarium chlamydosporum* (3.1%). Another study by Marcelo, *et al.*, (2015) reported that *Cladosporium* species can be isolated from the soil and can also be associated with localized superficial lesions in animals. This report also agrees with Rafi, *et al.* (2012) on the fungus

*Cladosporium* where their study stated that this fungus is widely disseminated in the environment and are commonly isolated from public use areas.

This study also observed the cross matches and disparity between non-dermatophytic molds isolated from the soil and cattle skin within the various markets and the implication is that these cattle are brought into these markets with new molds embedded within their fur so possibly if they are allowed to stay for a longer time in the market environment they may establish those molds residing under their skin in the area and may also pick up some new molds within the environment with their skin.

The keratinophilic non-dermatophytic molds fungi from soil samples within cattle markets in both states showed a significant difference in their frequency of occurrence and this agreed with the study by Seyed, *et al.*, (2012), in their work *Fusarium* (11.3%), *Penicillum* (4.0%), *Aspergillus* (3.6%), *Cladosporium* (0.4%), *Alternaria* (2.2%), *Rhizopus* (0.9%) were keratinophilic fungi isolated when they analyzed soil samples from parks of municipal districts of tetran. Also Deshmukh, (2002) in his work stated that most keratinophilic fungi are soil inhabitants than dermatophytes. Another study by Ganaie, *et al.*, (2010) revealed the presence of *Aspergillus species*, *Fusarium* species,

*Rhizopus* species, *Alternaria* species, *Candida* species, *Trichoderma* species and *Penicillum* species when they examined soil from samples Jhansi city for keratinophilic fungi using hair baiting technique, Moallaei, *et al.*, (2006) also isolated keratinophilic fungi from soil samples collected from forests and farm yards and results revealed *Aspergillus* spp. as the second most isolated saprophyte. Another work by Nwadiaro, *et al.*, (2015) isolated *Cladosporium* and *Trichoderma* species from barbers'

Landfill while Malek, *et al.*, (2013) in their study isolated *Aspergillus* species (15.92%) as the highest saprophytic fungi isolated when he analyzed soil samples from park soils in

Gorgan, North of Iran, this studies are in agreement with our study.

From our study and previous studies, it was shown that most keratinophilic fungi are soil inhabitants and considering that the market is an open environment were these animals and human carry out a lot of activities  this will certainly encourage the proliferation of keratinophilic fungi in new frontiers.

Pathogenecity test carried showed clinical signs associated with cutaneous mycoses on the tender mice skin. Three out of the ten non-dermatophytic molds tested showed positive result of lesion production, alopecia and discolouration on the albino mice skin respectively. The positive isolates infective on

the mice skin were *Cladosporium tenuissimum*, *Aspergillus welwitschiae* and *Absidia corymbifera* respectively. The results show that these non-dermatophytic molds are capable of establishimg superficial infections, as reported by Gebreab, (2015), revealing *Aspergillus* species as an important cause of dermatophytosis. Marcelo, *et al.*, (2015) concurs that *Cladosporium* species is capable of infecting animals and human by causing localized superficial lesions while Plancastell, *et al.*, (2009) revealed that *Absidia* species are well established causative agents of disease in animal and man.

Considering results from pathogenecity test on albino mice, out of the 16 non-dermatiophytic

molds from this study, 14 were screened for keratinase, 10 showed zone of clearance of which *Curvularia kusanol*, *Aspergillus welwitschiae*, *Aspergillus flavus*, *Pestalotiopsis microspora*, expressed zone of clearance. Positive results by these isolates is an indication that they can hydrolyze keratinous substances which is one of the hardest substrate to degrade and this can justify the reason why some of these non-dermatophytic molds were able to elicit clinical symptoms on albino mice skin. This result was in agreement with reports of Beti and Masa, (2018); Marcondes, *et al.*, (2008); Saber, *et al.*; (2010). It was revealed that *Aspergillus* species, *Alternaria* species,

*Cladosporium* specie, *Fusarium* specie were keratinophytic fungi. Also out of 13 non-dermatophytic molds screened for amylase enzyme production, *Aspergillus welwitschiae*, *Aspergillus aculeatus*, *Aspergillus flavus*, on starch agar plates showed signs of good producers of amylase while isolates such as *Fusarium lichenicola* and *Pestalotiopsis microspora* are not good producers of amylase. Amylase enzyme is an important enzyme in the hydrolysis of starch which can be found in man and animals. This agrees with study by Luis, *et al.*, (2006); Pandey, *et al.*, (2000) Sasi, *et al.*, (2010) and Ibatsam, *et al.*, (2011) their findings showed that *Aspergillus* species and *Fusarium* species are good producers of

amylase. The inability of some non-dermatophytic molds to grow on the media and show no activity of hydrolysis could be due to its genetic constitution, or it could be that they did not find the environment enabling so could not express their genetic trait.

For protease screening, *Cladosporium tenussimum* and *Fusarium succisae* showed very high zone of clearance indicating that these isolates can secrete protease enzyme while all four *Aspergillus* species screened, showed sign of hydrolysis, although some grew moderately while some had a slow growth on the media. This is an indication that, it has no ability to secrete protease enzyme. The ability to secrete protease enzyme has

been related to fungal pathogenecity (Groop, *et al*., 2009). This certainly justifies the ability of *Cladosporium tenuissimum* to elicit lesion on albino mice. This work agrees with the study by Sudarkodi, *et al*., (2015); Nwadiaro, *et al*., (2015); Muthukrishnan and Mukilarasi, (2016); Suryawanshi and Pandya, (2017) that *Cladosporium* species are good producers of protease.

Out of the eleven (11) non-dermatophytic molds screened for lipase enzyme in this study, the ability of *Aspergillus welwitschiae* and *Aspergillus flavus* to express a very high zone of clearance on the agar, followed by *Fusarium lichenicola* and *Fusarium succisae* suggest their ability to be a good producer of

lipase enzyme. The skin posses sebaceous gland that secrets lipids which are hydrolysed by lipase. This could explain the reason why *Aspergillus welwitschiae* was able to express alopecia on the albino mice skin. This result agrees with work of Charles and James (2011), where *Aspergillus* spp. from palmoil mill effluent dump sites were observed to be lipase producers. Also Razieh, *et al*., (2015), observed that *Cladosporium langeronii* showed the highest lipolytic clear zone on tributyrin agar plates. This was not in line with our findings. In our work *Cladosporium tenuissimum* did not show any positive zone of clearance. On the other hand Suseela and Tabitna, (2017), stated that *Aspergillus* spp.,

*Fusarium* spp., and *Penicillum* spp. were some of the fungal isolates that showed zone of clearance on phenol red agar plate, when they analyzed lipase producing fungi from marine water obtained from Machilipatnam Costal Region. This was in agreement with our study although *Penicillum citrinum* in our work did not show any zone of clearance.

Out of 12 non-dermatophytic molds from this study subjected to cellulase screening, all isolates showed zone of clearance except for some *Aspergillus* species which includes *Aspergillus flavus* and *Aspergillus fumigatus*, observing they did not grow on the media could be attributed to lack of genetic composition to produce cellulase enzyme. This

result agrees with a study carried out by Ibatsam, *et al*., (2011); Ibatsam, *et al*., (2012) and Kluezek-Turpeinen, *et al*., (2005).

This study has shown that majority of the isolates recovered are capable of secreting enzyme. This justifies the ability of some of them to elicit lesions on the albino mice skin.

The effectiveness of ketoconazole on most of the isolates in this study could be as a result of chemical composition of the drugs, this is in agreement with Mohamed et al., 2015; Gupta and Lyoni, 2015, while the resistant pattern of the isolates to most antifungal agents such as voriconazole and fluconazole could be attributed to mutation or other genetic mediating enzymes peculiar to the isolate. For

Voriconazole, it could also be attributed that the isolates have acquired some traits that makes it difficult for the drug to clear it completely. This study agrees partially with the work of Stanislaw, *et al*., (2012). Based on their analysis, they revealed that *Aspergillus* species isolated from dermatological specimen were highly susceptible to Ketoconazole, Voriconazole, Fluconazole and Terbinafine another study by Tzar, *et al*., (2016) revealed that Amphotericin B and Clotrimazole were the most sensitive antifungal agents against all moulds except *Fusarium* species. This is not in accordance with the findings in this study, on the other hand Karina, *et al*., (2013) also observed in their study that Voriconazole

expressed high efficacy on flamentous fungi (*Aspergillus* and *Fusarium*). This also does not apply in this study.

Javier, *et al.*, (2008) in their study revealed that voriconazole showed an excellent in vitro activity against dimorphic fungi and opportunistic moulds (*Aspergillus* spp, *Fusarium* spp*)*, this does not agree with the finding in this study.

Pearce, *et al.*, (2009) in their work also observed that Voriconazole and Miconazole were most significantly effective than Fluconazole and Ketoconazole on *Aspergillus* spp. and *Fusarium* specie respectively. These results is not in line with the findings in this study. A study by Emenuga and Oyeka, (2013)

showed that Fluconazole were more sensitive to *Fusarium* specie and *Aspergillus* species than Ketoconazole while in our study the reverse was the case. Another study by Brooks, *et al.*,(1998) agreed with our findings in this study when they reported that Ketoconazole were more sensitive to the fungal isolates such as *Aspergillus* spp., *Fusarium* spp. and *Penicillum* spp. recovered from ulcerative horses from Florida than Fluconazole. Another study by Keith, *et al*., (2013) showed that amphotericin B expressed activity on *Curvularia* species isolated from clinical samples while voriconazole did not show any activity. This also agrees with our findings.

The methanolic extract of four medicinal plant leaves *Euphorbia hirta*, *Occimum grastissium*, *Mitracarpus scaber* and *Jatropha multifida* subjected to phytochemical analysis reveals the presence and absence of phytochemical compounds at different concentrations respectively. For *Euphorbia hirta*, the abundance of glycosides, terpenoids, steroids and presence of flavonoid, alkaloid in wagner reagent and saponin is an indication of presence of secondary metabolites which might posses antimicrobial qualities. The presence of flavonoids in this study is in agreement with analysis by Geeta and Padma (2013). Another work by Amir, *et al*., (2011) revealed presence of alkaloids, saponin,

tannin, flavonoids, steroids and cardiac glycoside while terpeniod was absence when they carried out phytochemical analysis on *Euphorbia hirta*.

*Occimum grastissium* reveals abundance of glycoside and terpenoid, presence of flavonoid, saponin, phenol, alkaloid with mayer and steroids while terpenoid was absent. This work agrees with study by Afolabi, *et al*., (2007); Ameh, (2010); Gupta, *et al*., (2011) and Pricilla Alexander, (2016) in their work *Occimum gratissium* leaves reveals presence of alkaloids, flavonoids, saponin, tannin and saponin. On the contrary, Orji, *et al*., (2015) when he screened *Occimum grastissium*

leaves, flavoniod was absent while all other compounds were present.

*Mitracarpus scaber* reveals abundance of glycoside, steroids, terpenoid, tannin, alkaloid with wagner and presence of flavoniod, saponin and alkaloid with mayer while phenol was absent. This result agrees with study by Chilaka, *et al*., (2009) and Ouadja, *et al*., (2018) in their study phytochemical analysis of *Mitracarpus scaber* leaves reveals presence of alkaloids, tannin, cardiac glycosides and saponin. In another work by Shinkafi, (2013), the reverse was the case when he analysed the qualitative analysis of *Mitracarpus scaber* leaves, his result revealed abundance of

saponin and flavoniod and presence of tannin, alkaloid, glycosides and steroids.

*Jatropha multifida* reveals abundance of saponin and steroids, presence of glycosides, terpenoid, tannin, flavonoid, phenol, alkaloid with wagner while with mayer reagent alkaloid was absent. This is in accordance with a study by Nwokocha, *et al*., (2011); Sallykutty, (2016), in their work *Jatropha multifida* leaves revealed presence of alkaloid, tannin, saponin, flavonoid and phenol.

Quantitative phytochemical analysis of the total phenolic, flavonoid, tannin and alkaloid present in the plant leaves were determined by UV spectrophotometric method. The total glycosidic compound were found to be in

maximum quantity in *Euphorbia hirta*, followed by *Mitracarpus scaber*, *Jatropha multifida* and then *Occimum gratissium*.The presence of this chemical compound in ascending order of plants could be attributed to its potentiality of possessing antifungal properties. It is also an indication that *Euphorbia hirta* could possibly be a good producer of glycoside. Our analysis also reveals a high content of saponin present in all the four plant leaves. Saponin have been known to posses antifungal, antiyeast and antibacterial properties. This analysis also revealed a trace amount of flavonoid content in all the four plant leaves. This disagrees with study by El-Mewafy Abdou, *et al*., 2016, in

their analysis methanolic extract of *Jatropha multifida* leaves contains significant amount of flavonoid (38.22 ±3.26mg/100mg). Alkaloid compound was observed to be maximum in *Occimum gratissium* leaves than the other plant leaves. Tannin content was observed to be in a very high amount in *Euphorbia hirta* leaves than in the other plants. Tannin has been known to posses antimicrobial activity as reported by Nouioua, *et al*., 2016, when tannin was extracted from the *Phlomis bovei DeNoe* plant, it inhibited spore formation of *Aspergillus flavus* and mycelia growth of *Aspergillus niger*. Our result also reveals presence of phenol although not in a very high quantity. Amongst the plant leaves

understudied in ascending order *Mitracarpus scaber* had the highest content, followed by *Occimum gratissium*, *Jatropha multifida* and *Euphorbia hirta* respectively. This agrees with work by Nwokocha, *et al*., (2011), when they analysed phytochemical compounds of *Jatropha* L species in their study, they reported that phenol had the least concentration (0.18%). This disagrees with the reports of El- Mewafy Abdou, *et al*., 2016, in their study methanolic extract of *Jatropha multifida* expressed highly significant content of phenol (17.09 ± 0.010mg/ 100g).

The antifungal susceptibility analysis carried out on the methanolic extracts of the four plant leaves on 12 non- dermatophytic molds

showed a significant difference in their rate of activity at P< 0.05. The methanolic extract of *Jatropha multifida*, *Occimum grastissimum*, *Euphorbia hirta* and *Mitracarpus scaber* were tested. Results revealed that all isolates were resistant to *Jatropha multifida* in-vitro at all concentrations tested except for *Fusarium solani* that showed a weak sensitivity to *Jatropha multifida* at 200mg/ml and was attributed to be as a result of genetic mutation (RM). To strengthen this, minimum inhibitory concentration(MIC) showed that most isolates had their MIC at concentration > 200mg/ml except *Penicillum citrinum* that expressed its MIC at a concentration of 100mg/ml and minimum fungicidal concentration (MFC) at

200mg/ml, while *Aspergillus fumigatus* and *Fusarium succisae* showed its MIC at 200mg/ml and MFC > 200mg/ml respectively. This could be attributed to antimicrobial potentials of *Jatropha multifida* leaves due to the presence of primary and secondary metabolites. This agrees with a study by Sillma, *et al*., (2014), in their work it was stated that methanolic extract of *Jatropha multifida* leaves inhibited growth of fungus at an MIC of 25µg/L.

Results from this study,revealed that the selected isolates were sensitive to *Occimum grastissimum* at different concentrations and expressed different diameter zones of inhibition while MIC carried out on the

isolates revealed that some of the isolates had MIC at 100mg/ml and MFC at ≥ 200mg/ml while some had MIC at 200mg/ml and MFC >200mg/ml. This result is an indication that *Occimum grastissimum* possess strong antimicrobial potentials .This could be attributed to the presence of phytochemical compounds such as steroids, terpenoids which are known for their numerous importance and use in traditional medicine. This agrees with previous works by Ameh, 2010, in their work antimicrobial activities of leaves of *Occimum grastissimum* were observed. Another by Orji, *et al*., (2015) concords that *Occimum grastissium* leaves posseses antifungal properties. In yet another study by Adekunle

and Uma, (2005) stated that *Occimum grastissium* leaf extract at higher concentration did not support growth of fungi (*Absidia blakslseeana*, *Macrophomina phareolina* and *Fusarium solani*). This is also in agreement with our findings.

Methanolic extract of *Euphorbia hirta* leaves tested for antifungal susceptibility on the isolates revealed that 9 isolates were sensitive to the extract at different concentrations and different diameter zones of inhibition ranging from 10mm to 20mm while 3 were resistance to of the extracts. To strengthen this, MIC of the extract was carried out and it was observed that the 3 isolates that were resistance had their MIC at concentration above 200mg/ml

others that were sensitive had their MIC at concentration between 100mg/ml and 200mg/ml except *Pestalotiopsis microspora* that had its MIC at concentration ≥ 50mg/ml and MFC at concentration ≥ 100mg/ml while *Penicillum citrinum* had its MIC at concentration ≥ 25mg/ml and its MFC at concentration ≥ 50mg/ml. This is an indication that methanolic extract of *Euphorbia hirta* leaves posses antifungal properties that are effective on these isolates and this can be attributed to presence of active ingredients present in the leaves extract. This is not in agreement with a work by Waeem, *et al*., (2017), who observed that ethanolic extract of *Euphorbia hirta* (leaves) showed

no antifungal activity on *Aspergillus fumigatus* and *Aspergillus niger* using dics diffusion method. IIondu and Bosah, (2015) in their study also concurs that leaf extracts of *Euhorbiaceae* posses growth inhibition against fruit rot fungi.

Antifungal sensitivity tests carried out on methanolic extract of *Mitracarpus scaber* leaves were tested for antifungal effects on the isolates and results revealed that the 9 fungal isolates were sensitive to extract at different concentrations and different diameter zones of inhibition ranging from 5mm to 15mm, three (3) of the isolates were resistant to this extract. To strengthen this, minimum inhibitory concentration (MIC) against the fungal isolates

ranged from >200mg/ml to 25mg/ml and MFC from >200mg/mi to 50mg/ml, while *Absidiacorymbifera* had both MIC and MFC >200mg/ml. This is an indication that *Mitracarpus scaber* is highly effective against these molds recovered from this study which could be attributed to presence of active phytochemical compounds although its concentration wasn't high enough to inhibit *Absidia*. This is in agreement with work by Abere, *et al*., (2007), in their study antimicrobial activity of *Mitracarpus scaber* "zucc" were analysed on agar plates and concentration ranging between 25mg/ml and 300mg/ml were used to evaluate minimum inhibitory concentration (MIC). From their

results it was observed that fungal isolates such as *Candida albicans* had MIC at 75mg/ml. Another study by Anejionu *et al*., (2012) analysed the efficacy of ethanolic extract of *Mitracarpus scaber* against fungal isolates ( mould and yeast) and results revealed that clincal isolates showed sensitivity at 50µg/ml$^{-1}$. This is also in agreement with our study. According to Cimanga, *et al*., (2004) in their work *Mitracarpus scaber* expressed antifungal activity against *Aspergillus flavus*, *Microsporum canis* and *Candida albicans* with MIC and MFC <65µg/ml. This also is inaccordance with our study.

## 5.1 CONCLUSION

Infestation of the animals' skin , cattle especially, has drawn alot of global concern as this leads to the production of small-size cow, superficial destruction of animal skin, thereby giving rise to heavy economic losses in the farms and low quality production of hides and skins for leather manufacturing industries. This study has reaffirmed that the non-dermatophytic molds are also capable of estabilishing cutaneous mycoses as was observed when subjected to pathogenecity test and enzymes screening test. Also the study estabilished ketoconazole as an outstanding conventional drug of choice and suggests that some of our local plant leaves can be harnessed as they posses some antimicrobials

potentials due to presence of phytochemical compounds they posses.

## 5.2 SUMMARY OF FINDINGS AND CONTRIBUTIONS TO KNOWLEDGE

Following this research, hence the following may be considered as the study's contribution to knowledge;

1) This study has re-estabilished that the non-dermatophytic molds (NDMs) may infact be the prevailing causative agents of cutaneous mycoses.
2) This study estabilished the effects of cattle rearing and air environment. Agar plates exposed within the cattle market on examination showed substantial quantity

of the non-dermatophytic molds obviously suspended in the atmosphere. The implication of this is that the air quality within the vicinity of the cattle market may be severely compromised

3) Considering also the obvious cross match and disparity between non-dermatophytic molds isolated from the soil and that isolated from the cattle skin from both States, it implies that movement of cattle from one location to another and settlement of cattle colony would definitely lead to introduction of new NDMs in areas thereby predisposing cattle to NDMs through contact with infested soil.

4) This study revealed ketoconazole as an outstanding antifungal chemotherapeutic agent as was observed when it was subjected to antifungal sensitivity test on these non- dermatophytic molds with other antifungal chemotherapeutic counterparts.
5) Amongst the four methanolic plant extract studied *Mitracarpus scaber* was most effective against the fungal isolates tested followed by *Euphorbia hirta* and *Occimum gratissium*.

## 5.3 RECOMMENDATIONS

1) Cattle should be kept in columns in trucks while on transit to reduce the tendency of incurring trauma on the skin,

which paves way for these non-dermatophytic molds gaining access into the tissues.

2) Regular checks by veterinary doctors should be ensured.

3) The cattle markets should be placed on regular fumigation exercise in order to get rid of pollutants in the atmosphere and also decontaminate the soil in order not to predispose buyers and sellers to serious health challenges and cattle to skin infection.

4) Animal handlers should ensure that habits of washing their hands before and after handling these animals are taken

seriously since they come incontact with these animals more often.

5) Some of the local plants leaves studied in this research work should be harnessed as they possess antifungal properties.

## REFERENCES

Abere, TA., Onyekweli, AO and Ukoh, GC (2007). In vitro Antimicrobial activity of the extract of mitracarpus Scaber leaves

formulated as syrup. *Tropical Journal of Pharmaceutical Research 6(1)*: 679-682.

Abere, TA., Onwukaeme, DN and Eboka, CJ (2007). Pharmacognostic evaluation of the leaves of *mitracarpus scaber* Zucc (Rubiaceae). *Tropical Journal of Pharmaceutical Research 6(4)*: 849-853.

Adane Bitwe (2018). Dermatophytosis: Prevalence of Dermatophtes and Non-dermatophyte fungi from patients attending Arsho Advanced Medical Laboratory, Addis Ababa,Ethiopia. Dermatology Reasearch and Practice.Article ID8164757.

Adefemi,SA., Odeigah,LO., and Alabi,KM. (2011).Prevalence of dermatophytosis among primary school children in Oke-Oyi community of Kwara State. *Nigerian Journal of Clinical Practice 14(1)*:23-28.

Adenkunle, AA, and Uma, NU.(2005). Effect of Benlate solution crude leaf extract of Azadirachta Indica and Occimum gratissium on growth of fungi and preservation of melon seeds. *Plant pathology Journal 4(1)*: 29-34.

Adeyemi, AA,. Otiwo, OA. And Ekeada, IS (2015). Status distribution patterns of selected medicinal and food tree species in Owerri West Local Government Area of

Imo State, Nigeria. *Journal of Agriculture, Forestry and the Social sciences (JOAFSS) 13(1)*:44-48.

Adriana Zaputa and Sandra Ramirez-Acros (2015). A comparative study of Mcfarland Turbidity Standards and the Densimat Photometer to determine Bacterial cell Density. Current Microbiology 70: 907-909.

Afolabi Clement Akinmoladun, Emmanuel Afor Efere Martins Obuofor (2007). Phytochemical constituent and antioxidant activity of extract from leaves of *Occimum grastissium.Scientific research and essays 2(5)*: 163-166.

Agarwal, R., Chakrabarti, A., Shab, A., Gupta, JF and Guleria (2013). Allergic bronchopulmonary aspergillosis: review of literature and proposal of a new diagnostic and classification criteria. *Clin. Exp. Allergy 43(8)*: 850- 873.

Agnetti Francesco, Righi Cecilia, Soccia Elenora, Felici Andrea, Crotti Silvi, Moretta Lolanda, Morretti Annabella, Maresca, Carmen, Troiani Lucas and Pappini Manuela. (2014). *Trichophyton vernicosum* Infection in cattle farms of Umbria (Central Italy) and transmission to humans. *Mycosis 57(7):* 400-405.

Ahmed, SA., Stevens, DA., Van de Sande,WJ., Meis, JF and Hoog, GS.(2014). *Roussoella percutanea*, a novel opportunistic pathogen causing subcutaneous mycoses. *Medical Mycology 52*:689-698.

Ajoudanifar, H., Hedayati, M.T., Mayahi, S.,Khosrari, A and Mousari, B.(2011).”Volumetric assessment of airborne indoor and outdoor fungi at poultry and cattle houses in the Mazandaran Province,Iran”. *Arhivza Higijenu iToksikologiju 62(3)*:243-248.

Akbarmehr J. (2011). The prevalence of cattle ringworm in native dairy farms of sarab city

(East Azarbayjan Province), Iran.*African Journal ofMicrobiology Research 5(11):* 1268-1271.

Al-Ani FK., Younes FA and Al-Rawas-hdeh OF.(2002). Ringworm infection in cattle and Horses in Tordan.Acta.*Vet. Brno. 71(1):* 55-60.

Aleksandra Barac, Chris Kosmidis, Ana Alastruey- Izquierdo and Helmut TF Salzer (2019). Chronic Pulmonary Aspergillosis Update: A year in review. *Medical Mycology 57(2)*: 104- 109.

Ali Zarei Mahmoudabadi and Baharea Izadi (2011).Prevalence of cutaneous mycoses among workers.*Turk.J.Med.Sci; 41(2):*291-294.

Alok Kumar Sahoo and Rahul Mahajan (2016). Management of Tinea corporis , tinea cruris and tinea pedis : A comperative review. *Indian dermatology online. Journal 7(2)*: 77- 86.

Ameh,G.I.(2010). Evaluation of the phytochemical composition and antibacterial properties of crude methanolic extract of leaves of *Ocimum gratissimum.J. Nat. Sci. Engr. Tech. 9(1)*:147-152.

Amir Muhammad Khan, Rizwana Aleem Qureshi, Faizan Ullah, Syed Aneel Gilani, Asia Nosheen, Sumaira Sahreen, Muhammad Khan Laghari, Shafia-Ur-Rehman, Ishtiag Hussain and Waheed Murad (2011). Phytochemical analysis of medicinal plants of Margalla Hills and surroundings. *Journal of Medicinalplants Research 5(25)*: 6017-6023.

Amit Verma,Hukum Singh,Shahbaz Anwar,Anirudha Chattopadhyay,Kapil K.Tiwari,Surindar Kaur and Gurpreet Singh Dhilon(2016).Micrbial Keratinases:Industrial enzymes with waste

management potential.*Journal of critical Reviews in Biotechnology 37(4)*:476-491.

Ana Maria Perusquia- Ortiz, Denisse Vazquez-Gonzalez, Alexandro Bonifaz (2012). *Journal derDeutschen Dermatologischen Gesellschaft 10(9)*: 611- 621.

Anejionu Miriam Goodness, Nweze Emeka Innocent, Dibua Esther Uju and Esimone Charles Okechukwu (2012). Efficacy of two commonly used antifungal Herbs in Nigeria against Clinical isolates of Fungi. *Microbiology Journal 2(3)*: 70-8.

Arora, D., Anand, N., Goya, G., Kumar, R.,Gupta, P and Sarita (2011). Prevalence and risk factors of candida in cases of candidemia in a tertiary care hospital.*International Journal of Pharmacy and Pharmaceutical Science 3(1)*:0975-1491.

Arue Lund, Anna Marie Bratberg, Bjorn Naess and Roar Gudding (2013). Control of bovine ringworm by vaccination in Norway.*Journey of veterinary Immunology and Immunopathology 108(1-2)*: 37-45.

Azulay, RD, Azulay DR, Abulafia LA., and Azulay DR Dematologia. 5. Rio de Janeiro:

Guanabara Koogan; (2011). Mycoses superficial: PP. 419-439.

Baginiski, M and Czub, J. (2009). Amphotericin B and its new derivatives mode of action.*Current Drug Metabolism 10(5)*: 459-469.

Bakheshwain, S., Khizzi,N.El, Al-Rasheed,A.M., Al-Ajlan,H.H. and Parvez,S.(2011). Isolation of opportunistic fungi from Dermatophytic samples.*Asian Journal of Dermatology(3)*:13-19.

Balogun, RB., Jegede, HO., Jibril, A., Kwanashie, CN. and Kazeem, HM. (2017).

Prevalence and distribution of dermatophytes among domestic horses in Kwara State, Nigeria. *Sokoto Journal of Veterinary Sciences 15(2)*: 1-6.

Barnes, K., Collins, T., Dion, S., Reynolds, H., Riess, S., Stanzyk, A., Wolfe, A., Lonergan, S., Boettcher, P., Charrondiere, UR and Stadlmayr (2012). Importance of cattle biodiversity and its influence on the nutrient composition of beef, *Animal frontiers 2(4)*: 54- 60.

Bayer, EA.,La,ed,R. and Himmel, ME.(2007).The potential of cellulases and cellulosomes for cellulosic waste

management.*Current Opinion Biotechnology18*:237-245.

Bent S.(2008). Herbal medicine in the United States: review of efficacy, safety, and regulation. *Journal of general internal medicine, 23(6):* 854-859.

Bertrand, T.F., Frederic,T. and Robert,N.(2004). Production and partial characterization of thermostable Amylase from Ascomycetes yeast strain isolated from starely sait. McGraw Hill Inc., New York,USA,pp:53-55.

Beti Vidmar and Masa Vodovnik (2018).Microbial Keratinases:Enzymes with promising Biotechnological Applications:*Food Technology Biotechnology56(3)*:312-328.

Bhikane AU, CS Jaibhaye, AM Syed, AV Bhonsle, SS Ghoke and PS Masare (2015). Epidemiological investigations on dermatomycosis in cattle with special reference to polyherbal therapy.*Inter J Vet Sci*, *4(2)* :92-96.

Bohn, BA, Kocipai- Abyazan, R (1994). Flavonoid and condensed tannins from the leaves of vaccinum raticulation and

*Vaccinum calcyimium*. *Pacific Science48*: 458 -463.

Bonifaz, A., Ramirez-Tamayo, T and Saul, A (2014). Tinea barbae (tinea sycosis): experience with nine cases. *J. Dermatol :30 (12)*:898-903.

Bonifaz, Alexandro, Stchigel, Alberto Miguel and Guarro, Josep, Esther Guevara, Liliana Pintos, Marta Sanchis and Jose Cano (2914). Primary cutaneous mucormycosis produced by new species. *Apophysormyces mexicamus*. *Journal of Clinical Microbiology 52 (12)*: 4428 -4431.

Bonifaz, A., Tirado- Sanchez A, Araiza J, Rodrignez- Leviz A., Guzman- Sanchez D., Gutierrez Mendoza S., and Castrejon L (2019). White piedra: clinical, mycological and therapeutic experience of fourteen cases. *Skin Appendage Discord 9(5)*: 135-141.

Borkow, G.(2014). Using copper to improve the well-being of the skin.*Current chemical biology 8(2)*:89-102.

Brown, J. (2005). Zygomycosis an emerging fungal infection.Am.*J.healthsystpharm 62*:2593-2596.

Cam Y., Gümüssoy KS., Kibar M., Apadin N., Atalay O. (2007). Efficacy of ethlylendiamine dihydriodise for the treatment of ringworm in young cattle.*Vet. Rec. 160(12*): 408-410.

Cappelletty, D., Eiseitein-Mckitrick, K. (2007).“ The Echinocandin”s.*Pharmacotheraphy 27(3)*: 369-88.

Chabi, ML., Goracci, A., Rocha, N., Pugam, A., Lupo, A and Revel, MMP (2015). Pulmonary Aspergillosis. *Diagnostic and Interventional Imaging 96(5)*: 435- 442.

Chanu,Jain, Deepti, Rawat, Ravinder, Kaur(2017). Non-dermatophyte moulds: Not so uncommon agents of Onychomycosis. *International Journal ofScientific Research 6(11)*:204.

Charles Ogugua Nwuche and James Chukwuma Ogbonna (2011). Isolation of lipase producing fungi from palm oil mill effulent (POME) dump sites at Nsukka .*Brazillan Archives of Biology and Technology 54(1*): 113-116.

Cheesbrough, M. (2010): District Laboratory Practice in Tropical Countries. Part 2, second edition,update. Tropical Health

Technology/ Buterworths and Co. Ltd. Cambridge/ sevanaks.

Chynier, V., Comte G., Davies, KM., Lattanzio, V and Martens, S (2013). Plant phenolics: recent advancfs on their biosynthesis, genetics and ecophysiology. *Plant physiology andbiochemistry 72*: 1-20.

Chilaka, KC., Unekwe, PC., Enyi ,JC., Ogamba, JO.(2009). Evaluation of antimicrobial properties of methanol extract of *Mitracarpus scaber* leaves. *Tropical journal of medical research 13(2)*:10-15.

Chukwu, A.O, Onweagba,AE, Nwosu, CS and Osondu,PC (2011). Economic assessment of palm oil processing in Owerri Agricultural zone of Imo State. *International Journal of Agriculture andRural Development 14(2)*: 703- 706.

Claudia Spampinato and Dario Leonardi (2013). Candida infections, causes, Targets and Resistance Mechanisms: Traditional and Alternative Antifungal Agents, Biomedical Research International 2013: 204-237.

Clinical and laboratory Standard Institute (2008). Specimen handling and processing Speciality Collection (CLSI.).

Cochran, W.G. (1977). Sampling Techniques, Wiley, New York. 3rd Edition PP 205-212 published in Canada.

Chinnapun, D (2015). Virulent factors involved in pathogenecity of dermatophytes. Walailak *Journal of Science and Technology 12*: 573- 80.

Chitra M., Muga V., Sasikumar Dhanarasu and Awdah Masoud Al-hazimi (2011). Screening of phytochemical and In-vitro

activity of *Euphorbia hirta* L. *Journal of chemical pharmaceutical Research 3(6):* 110-114

Cimanya, RK., Kambu, KL., Tona, Bruyne, T.De, Sanders, A., Totte,T., Pieters, I., Vlietinck, AJ.(2004). Antibacterial and antifungal activities of some extracts and fractions of *Mitracarpus scaber* zucc. (Rubiaceae). *Journal of natural remedies 4(1)*; 17-25.

Crespo, Erchiga, V and Delgado, Florencio V (2002). Malassezia Species in skin diseases.*Curr. Opin Infect.Dis 15*:133-142.

Reddy Prasad, Amirah Izam and Maksudur Rahman Khan (2012). *Jatropha curcas*: Plant of medicinal benefit *Journal of medicinal Plants Research 6(14)*: 2691-2699.

Dabas, PS.(2013).An approach to etiology,diagnosis and management of different types of candidiasis. *Journal of yeast and fungal research 4(6)*:63-74.

Dalis, JS., Kazeem, HM., Kwaga, JKP and Kwanashie CN. (2014). An outbreak of ringworm caused by *Trichophyton verrucosum* in a group of calves in Vom,

Nigeria. *African Journal of Microbiology Research 8(8):* 783-787.

David Ellis, Stephen Davis, Helen Alexiou, Rosemary Handke, Robyn Bartley (2007). Description of Medical Fungi Second Edition. Published by the authors: Mycology unit womens and childrens hospital North Adelaide 5006 Australia PP 1- 156.

David Greenwood, Richard Slack, John Peutherer, Mike Barer (2007)Medical Microbiology: A guide to microbial infections: pathogenesis, immunity, laboratory diagnosis and control.17th

edition. Published by Churchill livingstone Elsevier Newyork.

Deshmukh, SK (2002). Incidence of keratinophilic fungi from selected soils kerals State (India).

Mycopathologia 156(3):177-181.

Deshmukh,S.K. and Verekar,S.A.(2006). The occurrence of dermatophytes and other keratinophilic fungi from the soils of Himachal Pradesh (India). *Czech mycology 58(1-2)*:117-122.

Didier, Pin (2017). Non-dermatophytes dermatoses mimicking dermatophytoses in animals.*Mycopathologia 182*:113-126.

Divya Dheer and Ravi Shankar (2017). Medicinal attributes of 1,2,3- triazoles: current developments. *Journal of Bioorganic Chemistry 71*: 30- 54.

El –Ashmawy WR., El-Hafez E A. and El Saeed H.A. (2015). Clinical study on Dermatophytosis in calves with in vitro Evaluation of Antifungal Activity of Bergamot oil. *Adv. Anim. Vet. Sci. 3(1):* 34-39.

Elangovan Elavarashi, Anupma Jyoti Kindo and Sudha Rangarajan (2017). Enzymatic and non-enzymatic virulence activity of dermatophytes on solid media. *Journal of Clinical DiagnosticReasearch 11(2)*: 23-25.

El-diasty, EM., Ahmed,MA., Okasha,N., Mansour,SF., El-dek,SL., Abd El-Khalek, HM., Youssif,MH..(2013). Antifungal activity of zinc oxide nanoparticles against dermatophytic lesions of cattle.*Romanian JournalBiophys 23(3)*:191-202.

El-Mewafy Abdou, El Mewafy El Ghadban, Omneya Farouk Abou El- leel and Ehab

M.B. Mahdy (2016). Morphological phytochemical and molecular characterization on some *Jatropha* species cultivated in Egypt. *InternationalJournal of Pharmaceutical Science and Scientific Research 3(1)*: 1-13.

El-said, AHM., Sohair, TH. And El-Hadi, AG. (2009). Fungi Associated with hairs of Goat and Sheep in Libya. Mycobiology 37(2): 82-88.

Emenuga VN., and Oyeka CA. (2013). “Epidemiology, Health Effects and Treatment of Cutaneous Mycoses of Goat and Sheep from some Eastern States of

Nigeria. “*American Journal of Infectious Diseasesand Microbiology 1 (16):* 106 - 110.

Fabiana Guillen Moreura, Simora dos Reis, Maria Aparecida Ferreira Costa, Cristina Giatti Marques de Souza,Rosane Msrina Peraita (2005).Production of hydrolytic enzymes by plant pathogenic fungus *Myrothecium verrucaria* in submerged culture.*Brazilian Journal ofMicrobiology 36(1)*:1678-4405.

Falah Al-Ani, F.A.Younes and Odeh Al-Rawashden (2002).Ringworm Infection of

cattle and horses in Jordan. *Acta Veterinaria Bmo 71(1)*:55-60.

Falodun, A (2010). Herbal medicine in Africa-distribution, standardization and prospects *Research Journal of Phytochemistry*, *4(3)*: 154-161.

Farid, M. Toma and Nareen Q. Faqi Abdulla.(2012). Isolation ,Identification and Seasonal Distribution of soil borne fungi in different areas of Erbil Governorate. *Journal of Advanced Laboratory Research in biology3(4)*:246-255.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using a bootstrap. *Evolution 39*:783-791.

Fresco L O (2009).Challenge for food system adaptation today and tomorrow. *Environ.Sci. Policy 12*:378-385.

Gampa Ramakrishnaiah,S.M Mustafa,G.Srihari(2013).Studies on keratinase producing fungi isolated from poultry waste and their enzymatic activity.*Journal of Microbiology Research 3(4)*:148-151.

Ganaie MA., S.Sood, G.Rizvi and T.A Khan (2010) Isolatrin and identification of keratinophilic fungi from different soil samples in Jhansi city (India). *Plant palnology Journal 9:194-197.*

Gebreabiezgi Teklebiohan and Adane Bitew (2015). Profile of dermatophyte and non-dermatophyte fungi in patients suspected of dermatophytosis. *American journal of life Sciences 3(5)*:352-357.

Geeta Singh and Padma Kumar (2013). Phytochemical study and screening for antimicrobial activity of flavonoids of *Euphorbia hirta*. *International journal of*

*applied basic medicinal research 3(2)*:111-116.

Gibbs EP.(2005).Emerging zoonotic epidermics in the interconnected global community.*Vet.Rec.157*:673-679.

Gini, TG and Jothi, CJ (2015). In vitro screening of antibacterial andantifungal activity of *Marsiles quadrifolia* (*Marsileaceae*)Linn. Extract. *American Journal of Phytomedicine and ClinicalTherapeutics 3(4)*: 313 -329.

Gropp,K.,Schild,L.,Schinder,S.,Hube,B., Ziptel,P.E. and Skerka,C.(2009).The yeast

*Candida albicans* evades human complement attack by secretion of aspartic proteases.*Molecular Immunology 47(2)*:405-475.

Gudynaite-Savitch L. and White,T.C(2016).Fungal Biotechnology for industrial enzyme production. Focus on (Hemi) cellulase production strategies, Advances and challenges. In:Schnoll M.,Dattenbock C.(eds).Genes Expression systems in fungi:Advancement and Applications. *Fungal Biology Springer*: 395-439.

Guo,Z. and Xu,X.(2005). “New opporturnity for enzymatic modification of fats and oil with industrial potentials”.*Organic and Biomolecular Chemistry 3(14)*:2615-2619.

Gupta, VK, Singh, J., Kumar, R., and Bhanot, A (2011). Pharmacognostic and preliminary phytochemical study of Occimum gratissium Linn ( Family;lamiaceae). *Asian journal of plant sciences 10*: 365-369.

Gupta Aditya K. and Lyons Danita CA(2015). The rise and fall of oral ketoconazole. Journal of cutaneous medicine and surgery.

Pubmed. https/ doi. Org/ 10.1177/1203475415574970.

Hadi Alwan Mohammed Al-saidy, Ghassan H. jameel and Amaar A. Sultan (2014). Evaluation of Calvatia craniformis mushroom Activity in treatment of Ringworm Disease in cattle. *Journal of Biology, Agriculture and Healthcare 7*:24.

Harborne, JB (1973). Methods of plant analysis.In phytochemical Methods Chapman and Hall, London.

Harborne,J.B.(1998). Phytochemical methods: *A guide to modern technique inplant*

*analysis*. (3rd ed). London: Chapman and Hall.

Hemandez NE, Tereschuk ML, Abdala LR (2000). Antimicrobial activity of flavonoid in medicinal plants from tatidel valle (Tacuman, Argentina). *Journal of Ethanopharmacol, 73(1-2)*: 317-322.

Hoiberg Dale H, ed (2010). Abia Encyclopedia Britannica. I.A.ak Bayers (15th ed). Chicago,Illinois Encyclopedia Britannica Inc. PP.32.

Hope,WM., McEntee.L., Livermore,J., Whalley, S., Johnson,A., Farringtontom,N., Kolamunnage-Dona,R., Schwartz,J., Kennedy,A., Law,D., Birch,M., Rex,JH. (2017). *Aspergillus fumigatus*: New opportunities for treatment of multidrug-resistant fungal disease. *mBio 8(4):*eo1157-17.

Huang Linfang, Chen Shillin and Yang Melhua (2012).*Euphorbia hirta* (Feiyangcao): A review on its ethnopharmacology, phytochemistry and pharmacology. *Journal of Medicinal Plants Research 6(39)*: 5176-5185.

Hube,B.,Stehr,F.,Bossenz,M.,Mazuir,A.,Kretschmar,M.andSchafer,W.(2000).Secreted lipases of Candida albicans:cloning,characterrisation and expression analysis of a new gene family with at least ten members.*Archives of Microbiology174(5)*:362-374.

Hubertine ME Williams, Salmans Ahmed, Junyan Liu, Zhenboxu and Brian M Peters (2020). Vulvovaginal candiasis: A current understanding and burning questions. *Journal of fungi6(1)* :27.

Ibatsam Khokhar, Muhammad Saeem Haider, Sobia Mushtao, Irum Mukhtar (2012).

Isolation and screening of highly cellulolytic filamentous fungi. *Journal of Applied Science EnvironmentManagement 16(3)*: 223-226.

Ibatsam, Khokhar, Irum, Mukhtar and Sobia, Mushtaq (2011). Comparative studies on the amylase and cellulose production of Aspergillus and Penicillium. *Journal of applied Science environmentmanagement 15(4*): 657- 661.

Igor Stojanov, Sandra Jaksic and Prodanov Jasna Z (2007).Presence and importance of saprophyte fungal organisms on dog Skin.*Zbornik MatriceSrpske Za Prirodne Nauke 113(261)*:261-265.

IIondu, EM. And Bosah, BO(2015). Growth inhibition potentials of leaf extracts from four selected Euphorbiaceae against fruit rot. Fungi of African star Apple ( *Chrysophyllum albidum* G Don). *Journal plant pathology and microbiology 6(9)*: 1-6.

Iyeh, I.I., Njoku, O.U., Ekenza, E.C. (2004). Medicinal Evaluation of *xylopia aethiopica* and *Ocimum gratissimum. Journal of medicinal Aromatis Science 26(1):* 44-48.

Jachak S.M. and Saklani A. (2007). Challenges and opportunities in drug discovery from plants.*Journal of current science 92(1)*:1251-1257.

Jaffar A, Al- Tawfig and Ali Boukhamseen (2011). Cerebral Phaeohyphomycosis due to *Rhinocladiella mackenziei* (formerly Ramichloridium mackenziei): case presentation and literature review. *Journal of infection and public health 4(2)*: 96-102.

Jamal,M.Kaled, Hammed,A.Goleh, Abdulla,S.Khalei, Naiyf,S.Alharbi and Ramzi,A.Mothana. (2015). Dermatophyte and Non-dermatophyte fungi in Riyadh City, Saudi Arabia.*Saudi Journal of Biological Sciences 22(5)*:604-209.

Jain, PK., V.K.Gupta, A.K.Misra, R.Gaur, V.Bajpai and S.I ssar. Current status of *Fusanum* infection in Human and Animal. Asian Journal of animal and vertenary Advances. 2011;6 (3): 201-227.

Jati Batoro and Dian Siswanto (2017_. Ethno Medicina; survey of plants used by local society in Poncokusumo district Malang, East Java Province, Indonesia, *Asian Journal Medical Biological Research 3(2)*: 158-167.

Jawetz, Melnick and Adelberg (2010).Medical Microbiology.25th edition.Published by McGraw-Hill.pp 625-663.

John Didacus Njoku, Gordon Amangabara, Patricia N. Duru (2013). Spatial assessment of urban flood risks in Aba metropolis using geographic information systems techniques. *Global Advanced Research Journal of Environmental Science and Toxicology 2*: 086-092.

Jones KE.,Patel NG.,Levy MA.,Storeyard A.,Balk D.,Gittleman JL and Daszak P,(2008).Global trends in emerging infectious diseases.*Nature 451*:990-993.

Jones PG and Thornton PK (2009).Croppers to livestock keepers: livelihood transitions

to 2050 in Africa due to climate change. *Environ Sci policy.12*:427-437.

Joseph H., Skalski. Theodore J. Kottom and Andrew H. Limper (2015). Pathobiology of Pneumocystis pneumonia: Life cycle, cell wall and cell signal transduction. FEMS Yeast Research 15(6):046.

Juveriyah Kausar, Durai Muthumani, Agaath Hedina, Sivasamy and Vijaya Anand (2016). Review of the phytochemical and pharmacological activities of *Euphorbia hirta* Linn. *Pharmacognosy Journal 8 (4):* 310-313.

Karaye P. Gloria, Dogo G.I. Abraham and Karaye Kuduk (2017). Evaluation of antimycotic activity of crude methanolic extract of *mitrocarpus scaber* on *candida albicans* and *Trichophyton mentagrophytes. Saudi Journal of medical and pharmaceutical Sciences 3(3)*: 217-220.

Karesh WB., Dobson A., Lloyd-Smith JO., Lubroth J., Dixon MA., Bennett M., Aldrich S., Harrington T., Formenty P., Hoh EH., Machalaba CC., Thomas MJ and Heymann DL. (2012). Ecology of zoonoses: natural and unnatural histories. *Lancet 380*:1936-1945.

Karina Q. De Sagun- Bella, MD, Archimedes Lee D. Agahan, MD, Leo DP,. Cubillan, MD., Noel S. Carino, MD, Roslyn De Mesa- Rodriguez, RMT, PhD (2013). Antifungal activities of Voriconazole on local isolates: an in-vitro study. *Philippine Journal of Ophthamology 38*: 29-34.

Keith C.da Cunha, Deanna.A.Sutton, Annette W. Fothegill, Josepa Gene, Josep Cono, Hugo Madrid, Sybren de. Hoog, Pedro W. Crous, Josep Guarro (2013). In-vitro antifungal susceptibility and molecular identity of 99 clinical isolates of the opportunistic fungal genus *Curvularia*. *Diagnostic Microbiology and Infectious Diseases 76*:168-74.

Keyvan, Pakshir, Moosa, Rahim Ghiasi, Kamiar, Zomorodian and Ali, Reza G haravi (2013). Isolation and molecular identification of keratinophilic fungi from public parks soil in Shiraz,Iran. Biomed Research International 2013:619576.

Khandelwal,K.R.,(2006).Pratical pharmacognosy,Techniques and Experimentals.16th Edn.,Nirali Prakashan,Pune,India,ISBN:81-85790-30-2,pages:107.

Kings DA., Peckham C., Wagge JK.,Brounlie J and Woolhouse ME.(2006).Epidemiology,Infectious

diseases: preparing for the future. *Science 313*:1392-13.

Kirby,W.M and Bauer,A.W.(1996). Antibiotic susceptibility testing: a standard single disc method. *American Journal Clinical Pathology 45*:493-494.

Kluezek- Turpeinem B., Maijala P., Tuomela M., Hofrichter M., Hatakka A,(2005). Endoducanase activity of compost dwelling fungus pacilomyces inflatus is stimulated by humir acids and other low molecular mass aromatics. *World Journal of Microbilogy Biotechnology 21(8-9)*: 1603-1609.

Koche DK, Kokate PS, Suradkar SS and Bhadange DG (2012).Prelimanry Phytochemistry and Antibacterial Activity of ethanolic Extract of Ocimum Gratissimum Leaves. *Bioscience Discovery 3(1)*: 20-24.

Korkmaz, H. H.Hur and S. Diyncer. (2004). "Characterization of alkaline keratinase of Bacillus licheniforms strain HKY from poultry waste."*Annals of Microbiology 54*: 201-211.

Kumar A. and Sharma S. (2008). An evaluation of multipurpose of seed crop for

industrial uses (*Jatropha curcas)*: A review *Bios Eng 97*: 201-207.

Kupiec PM., Green AN., Marks KC (2017). Black adherence nodules on the scalp hair shaft. *Cutis 100(1)*: 14; 38; 39.

Kyriakidis, I., Tragiannidis, A., Munchen, S. and Groll, AH. (2017). Clinical hepatoxicity associated with antifungal agents. *Expert Opinion Drug Safety 16(2)*: 149-165.

. Ladipo, M.K., Doherty, V.F. and Kanife U.C. (2010). Phytochemical Screening and antibacterial investigation of the extract of

*Ocimum gratissimum* (Scent Leaf) on selected Enterobacteriaceae. *Production agriculture and technology 6(2):* 75-84.

Lawal-Adebowale, O.A. (2012). Factors influencing small Ruminant Production in selected urban communities of Abeokuta, Ogun State. *Nigerian Journal of Animal production, 39(1):* 48-228.

Lin Y and Tanaka S. (2006).Ethasnol fermentation from biomass resources:Current State and Prospects.*Applied Microbiology 69*:627-642.

Livia ,S.Ramos,Marta H.Branquinha,Andre L.S.Santos(2017).Different classes of hydrolytic enzymes produced by multidrug-resistant yeasts comprising the *Candida haemulonii* complex.*Medical Mycology 55(2)*:228-232.

Luis Henriques Guimaraes, Simone C.Peixoto-Nogueira, Michele Michelin, Ana Carolina S. Rizzatti, Valeria C. Sandrim, Fubiana F.Zanoelo, Ana Carla M.M.Aquino, Altino B-Junior, Maria de Lourdes T.M.Polizeli (2006). Screening of filamentous fungi for production of enzyme of biotechnological interest.*Brazillian Journal of Microbiology 37*:474-480.

Lynd,LR.,Laser,MS.,Bransby,D.,Dale,BE.,Davison,B.,Hamilton,R.,Himmel,M.,Keller,M.,McMillan,JD.,Sheehan,J. and Wyman, CE.(2008).How biotechnology can transform biofuels.*National Biotechnology 26*:169-172.

Silva, T.G. Oliveria Jr, O.F.L. Fernandes, X.S. Passor, C.R. Costa, L.K.H Souza , J.A. Lemos and J.R. Paula (2005). Antifungal activity of *Ocimum gratissimum* towards dermatophytes. Mycoses *48(3)*: 172-175.

Mahendra Pal (2017). Dermatophytosin in an adult cattle due to *Trichophyton*

*verrucosum. Animal Husb.Dairy Vet. Sci. 1*: doi 10.15761/AHDVS. 1000106.

Mahendra Pal, Pratibha Dave and Anil Kumar Manna (2014).Emerging role of *Aspergillus flavus* in human and animal disorders.*Journal ofmycopathological Research:52(2)*:211-216.

Mahesh Mathur, Prakash Acharya, Alina Karki, Nisha KC, Jyoti Shah (2019). Dermoscopic pattern of pityriasis versicolor. *Journals of clinical cosmestic and investigational dermatology 12*:303-309.

Makino, K., Fukushima, S., Maruo, K., Egawa, K., Nishimoto, K and Ihn, H.(2011). Cutaneous hyalohyphomycosis by *Scedosporium apiospermum* in immunocompromised patient.*Mycoses 54*:259-261.

Malek, E., MoosazadehM., Hanafi, P., Abbasi Nejat, Z.,, Amini A. (2013). Isolation of keratinophilic fungi and aerobic Actinomycetes from park soils in Gorgan, North of Iran. *Jundishapur journal of Microbiology 6(10)*: 11250.

Mandels,M.(1975).MicrobialSources of cellulose.Biotechnology.Bioeng.Symp.,5:81-105.

Marcelo Sandoval – Dennis, Deanna A Sutton, Adela Martin – Vicente, Jose F. Cano-Lira, Nathan Wiederhold, Josep Gurro and Josepa Gene (2015). Cladosporium species recovered from clinical samples in the United States. *Journal of clinical Microbiology* 53(9) : 2990 – 3000.

Marcondes, N.R, Taira, C.L, Vandresen, D.C, Svidz-inski, T.I.E, Kadowaki, M.K and Peralta, R.M (2008) New feather-degrading

filmentous fungi. *Microbiological Ecology 56*: 13-13.

Maria Fernanda Reis Gavazzon Dias, Maria Victoria Pinto Quaresma-Santos, Fred Bernardes – Filho, Adriana Gutstein da Fonseca Amorim, Regina Casz Schectman and David Rubem Azulay (2013). Update on therapy for superficial mycoses: review article part 1 An Bras Dermatol 88(5).

Martinez-Herrara J., Siddhuraju P., Francis G, Davila-Ortiz G and Becker K. (2006). Chemical composition toxic/antimetabolic constituents and effects of different treatments on their levels in four

provenances of *Jatropha curcas L.* from Mexico. Food Chem. 96:80-89.

Martins, O.O and Igwemma, A.A 2000.Applied Statistical Techniques for Businrss and Basic Sciences.2nd. Skillmark media Ltd; Owerri, Nigeria. pp 216-304.

Mary M Maslen (2000). Human cases of cattle ringworm due to *Trichophyton verrucosum* in Victoria, Australia. *Australian Journal of Dermatology 41(2)*: 90-94.

Matasyoh LG, JC Matasyoh, FN wachira, MG Kinyua, AW Thairu Muigai and TK Mukiama (2007). Chemical composition

and antimicrobial activity of the essential oil of *Ocimum grastissimum* leaves groeing in Eastern Kenya. *African Journal of Biotechnology 6(6)*: 760-765.

Maurice D Awouafack, Lyndy J McGaw Sebastian Gotifried, Roukayatou Mbouangouere. Pierre Jane, Micheal Spiteller and Jacobus N Eloff (2013). Antimicrobial activity and cytoxicity of the ethanol extract, fraction and eight compounds isolated from *Eriosema robustum* (Fabaceae). *BMC Complementary and Alternative Medicine 13*: 298.

Mbakwem-Aniebo C., Onianwa O. and Okonko I.O. (2012). Effects of *Ocimum Gratissimum* Leaves on common dermatophytes and causative agent of *pityriasis versicolor* in Rivers State, Nigeria. Journal of Microbiology Research 2(4): 108-113.

Mbata,T.I. Duru,M.C. and Onwumelu, H.A.(2009). Antibacterial activity of crude seed extracts of *Buchholzia coriacea* E. on some pathogenic bacteria. *Journal of Dev.Bio and Tissue Engr. 1(1)*:001-005.

McEvoy, JM, Nde, CW, Sherwood, JS, Logue, CM. (2005). An evaluation of sampling

methods for the detection of Echerichia coli and Salmonella on turkey carcasses. *Journal of food protection68*: 34-39.

Mercer, DK. and Stewart,CS(2019).Keratin hydrolysis by dermatophytes.*Medical Mycology 57(1)*:13-22.

Michael Niyi Aransiola, Charele Ehikhase, Joy C. Mmegwa and Idris Olayinka Wahab (2014). Microbiology: A systems approach by Majorie Kelly Cowan and Kathleen Park Talaro

( 2006).Published by Mc Graw Hill.1st Edition Newyork,America. Pp. 131-139.

Miller RS., Farnsworth ML and Malmberg JL,(2013).Diseases at the livestock-wildlife interface: status,challenges and opportunities in the united states.*Preventive veterinary medicine 110(2)*:119-132.

Mitchell,RS.,Kumar,V.,Abbbas,AK.,Fausto,N. (2007).Robbins Basic pathology.8th(edn.).P hiladelphia:Sanders,Pg.122.

Moallaei, H., F.Zaini, M. Pihet, M.Mahmoudi and J. Hsahemi (2006).Isolation of keratinophilic fungi from soils samples of

forests and farm yards. *IranJournal of Public health 35*:62-69.

Modupeola Omotara Samaila and Kabiru Abdullahi (2011).Cutaneous manifestation of Deep mycosis:An experience in a Tropical pathology laboratory.*Indian J Dermatol 56(3):*282-286.

Mohamed Salama, Mohamed A. Mahdy, Amina Mohamed, Aref. T. Mohamed, Eseldin. I. keleb, Assad A. Omar and Nagib A. Elimarzugi (2015). Formulation and evaluation of ketoconazole polymeric films for topical application. *Journal of*

*applied pharmaceuticalscience 5(5)*: 028-032.

Molyneux ,RJ., Lee, ST., Gardner, DR., Panter, KE., James, LF (2007). Phytochemicals: the good, the bad and the ugly?.*Phytochemistry 68(22-24)*: 2973-2985.

Moriarty, B., Hay, R., and Morris-Jones, R. (2012).The diagnosis and management of tinea.*Bio Med Journal: 345*:E4380.

Murray PR., Rosenthal RS and Pfaller MA (2005).Superficial and Cutaneous

mycosis.In Medical microbiology 5th edition Philadelphia, USA pp 745-751.

Muthukrishnan, S. and Mukilarasi, K (2016). Industrial important protease screening and optimization from Micro-fungal isolates of Ayyanar falls Forest samples, Rajapalalyam.*World Applied Sciences Journal 34(3)*; 343-347.

Nalu Teixera de Aguiar Peres,Fernando Cristina Albuquerque Maranhao,Antonio Rossi and Nila Maria Martinez-Rossi(2010). Dermatolophytes: host-pathogen interaction and antifungal resistance. *Anais Brasileiros de Dermatologia 85(5)*:657-667.

Nassar, MSM., Hazzah, WA and Bakr, WMK (2019). Evaluation of antibiotic susceptibility test results: how guilty a laboratory could be ?. *Journal of Egyptian Public Health Association*: *94(4)*: 3709.

Naveed,AM., Naeem,R. and Nasiruddin ( 2009). Non-dermatophyte moulds and yeast as causative agents in Onychomycosis.*Journal of PakistanAssociation of Dermatologists 19(2)*:74-78.

Ndako,J.A., Osemwegie,O.O., Spencer, T.H.I., Olopade,B.K., Yunusa,G.A., and Bande,

J(2012). Prevalence of dermatophytes and other associated fungi among school children.*Global Advanced Research Journal ofMedicine Sciences 1(3)*:049-056.

Niema Aquil, Hanar Baybay,Kaoutar Moustaide, Zakia Douhi, Sara Euoudi and Fatima Zahra Mernissi (2018). A prospective study of tinea capitis in children: making the diagnosis easier with a dermascope. *Journal of medical case reports 12*:383.

Nostratabadi,M, Kordbacheeh, P, Kachueri, R, Safara, M, Rezala, S and Afshan, MA, Isolation of keratinophilic funginfrom the

soil of agareater Tunb, Abu-Musa and Sirri Islands in the Persian Gulf, Iran. Current Medical Mycology. 2017; 3(2): 13-19.

Nouf M.AI. Enazi, Amani, S.Awadd, Monerah R.AI-Othman, Nour K.AI-Anazi, Saleh I. Alqasoumi (2018). Isolation, identification and anti-candidial activity of filamentous fungi from Saudi Arabia. *Saudi Pharmaceutical Journal 26(2)*: 253-257.

Nouioua Wafa, Gaamoune Sofiane and Kaabache Mouhamed (2016). The antioxidant and antimicrobial activities of flavonoids and tannins extracted from

*Phlomis* bovei De Noe. *European Journal of Experimental Biology 6(3)*:55-61.

Nwadiaro Patience, Ogbonna Abigail, Wuyep Ponchang, Adekojo Deborah (2015). Keratinolytic activity of c*ladosporium*and *Trichoderms* species isolated from barbers ladfill. *International Journal of Biosciences 6(10)* : 104-115.

Nweze EI. (2011). Dermatophytes in domesticated animals.*Rev. Inst.* Med. *Trop. Sao Paulo 53 (2):* 95-99.

Nweze EL and Eze EE (2009).Justification for the use of *Ocimum gratissimum* in herbal

medicine and its interaction with disc antibiotics.*BMC complementary and Alternative medicine 9(37):* 1-6.

Nickie D. Greer (2003). Voriconazole, the newest triazole antifungal agent proceedings (Baylor University Medical Centre 16(2): 241-248.

Nwinyi OC, Chinedu NS, Ajani OO, Ikpo CO and Ogunniran KO (2009). Antibacterial effects of extracts of *Ocimum gratissimum* and piper guineense on *Escherichia coli* and *Staphylococcus aureus. African Journal of Food Science* 3(3): 077-081.

Nwokocha, A,Blessing, I.O. Agbagwa and B.E. Okoli (2011). Comparative phytochemical screening of *Jatropha* L.species in the Niger Delta. *Researh journal of phytochemistry* 5:107-114.

Nourchene Toukabri, Cyrine Dhieb, Dalenda El Eueh, Mustapha Rouissi, Mourad Mokni and Najia Sadfi- zouaoul (2017). Prevalence, Etiology and Risk factors of Tinea pedis and tinea unguium in Tunisia. *Canadian Journal of infectious diseases and medical microbiology*. Article ID 6835725. 9 pages.

Okwu, DE, (2004): “Phytochemical and vitamin content of indigenous plant in South-eastern Nigeria”. *Journal of sustain agricultural environment 2*:31-36.

Olapeju Aiyelagbe, Bola J. Oguntuase, Babatunde D. Arimah and Bolanle A. Adeniyi (2008). The antimicrobial activity of *Jatropha multifida* extracts and chromatographic fractions against sexually transmitted infections. *Journal of Medical Sciences (Faisalabad) 8(2)*: 143-147.

Oliver, J., Sibley, GE., Beckmann, N., Dobb, KS., Slater, MJ., McEntee, L.,duPre. S., Livermore, J., Bromley, MJ., Wiederhold,

NP., Hope, WW., Kennedy, AJ., Law, D., Birch, M (2016)/ “F901318” represents novel class of antifungal drug that inhibits dihydrorotate dehydrogenase. *Proceedings of the national academy of sciences of United States of America 113(45)*: 12809-12814.

Olubukola O. Babalola (2010). Pectinolytic and cellulolytic enzymes enhance *Fusarium compactum* virulence on tuberclesbinfection of Egyptian broomrape. *International Journal of Microbiology*. Article ID 273264.

Oritiz, AP., Gonzalez, DV, and Bonifaz, A.(2012). Opportunistic filamentous mycoses:Aspergillosis, Mucormycosis,Phaeohyhomycosis and Hyalohyphomycosis. *Journal of Neutsche dermatologische gesellscha ft(JDDG)10*:611-621.

Orji, JO., Nwuzo, AC., Ejikeugwu, PC, Ugba, EN., Moses, IB., Nwakaeze, EA. and Nwankwo, CP. (2015). Antifungal activities of *Occimum gratissium*and *Gongronema latifolium* leaves on Colletotrichum species isolated from spoilt tomatoes .*International journal of*

*pharmaceutical scienceinvention 4(5)*;42-45.

Osama E.Amer, Mohamed A. Mahmoud AbdEl- Rahim M.A. El- Samawaty and Shaban RM Sayed (2011). Non-liquid nitrogen- based- method for isolation of DNA from filamentous fungi. *African Journal of Biotechnology 10(65)*: 14337-14341.

Ouadja, B., Anani, K., Djrei, B., Ameyapoh, Y.O and Karou. D.S. (2018). Evaluation of the phytochemical composition, antimicrobial and antiradical activites of

Mitracarpus scaber (Rubiaceae). *Journal of medicinal plantsResearch 12(28)*:493-499.

Pahare Shikha, and Shukla RV (2014). Occurrence of keratinophilic fungi in coal mines soils, Korba, Chhattisgarh. *Journal of MycopathologiaRes.52(1)*:76-80.

Pahare,S., Kamalesh Shukla and Shukla RV. (2018). Keratinophilic fungi from warm,moist, cattle-house of Bilaspur Central-India.*Journal ofmicrobiology and experimentation 6(2)*:46-48.

Paixao, GC, Sidrim, JJC, Campos, GMM, Brilhante, RSN and Rocha, MEG

(2001).Dermatophyte, and Saprobe Fungi collected from dogs and cats in the city of Fortaleza, Brazil. *Arqyivo Brasilero de Medicina Veteninaria ootecnia 53(5)*: 568-573.

Pandey,A.,Nigam,P.,Socol,CR.,Soccol,VT.,Singh,D and Mohan, R(2000).Advances in microbial amylases.*Biotechnology Application inBiochemistry 31*:135-152.

Pasqualotto, AC.(2010).Aspergillosis: From diagnosis to prevention. Springer London.

Paula Monteiro de Souza,Mona Lisa de Assis Bittencourt,Carolina Canielles

Caprara,Marcela de Freitas,Renata Paula Coppini de Almeida, Damaris Silveira,Yris Maria Fonseca,Edivaldo Ximenes Ferreira,Filho,Adalberto Pesso,Junior and Perola Oliveira Magalhaes(2015).A biotechnology perspective of fungal proteases.*Brazilian Journal of Microbiology46(2)*:331-346.

Pavan, R.and Manjunath, K. (2014). ‘ Qualitative Analysis of indoor and outdoor Airborne Fungi in Cowshed.” *Journal of mycology 2014* :1-8.

Pearce, JW., Giullano, EA. and Moore, CP.(2009). In-vitro susceptibility patterns

of Aspergillus and Fusarium species isolated from equine ulcerative keratomycosis cases in the Midwestern and Southern United States with inclusion of the new antifungal agent voriconazole. *Vet. Ophthamol 12(5)*: 318-324.

Petrikkos, G, Skiada, A., Lortholary, O., Roilides, E., Walsh, JJ and Kontoyiannis DP (2012). Epidemiology and clinical manifestations of mucormycosis. *Clinical infectious Disesases54(1)*; 23-34.

Pietro Nenoff, Constanze Kruger, Gabriela Ginter- Hanselmayerb and Hans-jurgen Tieta (2014). Mycology – an update.Part 1: Dermatomycoses.Causative agents,

epidemiology and pathogenesis. *Journal of the German Society of dermatology 12(3)*:188-210.

Plancastell, C., Ghidini, F., Donotrio, G., Jottini, S., Taddel, S., Cavirani, S. and Cabassi, CS. (2009). Isolation and characterization of a strain Lichtheimia corymbifera 9ex Absidia corymbifera) from a case of bovine abortion. *Reproductive Biology and Endocrinology 30(7)*: 138.

Polshettiwar, SA, Ganjiwale, RO, Wadher, SJ, Yeole, PG. (2007). Spectrophotmetric estimation of total tannins in some

Ayurvedic eye drops. *Indian Journal Of pharmacological Science 69*: 574 -576.

Ponnusamy,P., Saravanan,M., Lurthu, T. Rectha, Ronald BSM., Marickam,R. and Puvarajan,R.(2018), *Curvularia* dermatomycosis in a goat:Tamil Nadu,India. *Journal of entomology and zoology studies 6(3)*:1446-1448.

Poopathi,S.,Murugan,K.,Salvakumari,J.,Mani, C.,Bala,P.,DeBritto,R.I,J,Paanaik,S.,Santhosh Goud,Prabakaran, K(2016).Keratinase a feather Degrading Enzyme useful for mosquito control. *Journal of Cellular Immunology andserum Biology 2(2)*:50-52.

Prasad, Reddy DM, Izaa, Amirah and Khan, Rahman Maksudur MD(2012). Jatropha curcas: plant of medicinal benefits. *Academic Journals 6(14)*: 2691-2699.

Pricilla, Alexander (2016). Phytochemical screening and mineral composition of the leaves of *Occimum grastissium* (scent leaves). *International journal ofapplied sciences and biotechnology 4(2)*: 161-165.

Prohic, A., Jovovic Sadlkovic T., Krupalija Fazhic M., and Kuskunovic- Vlahovljak, S (2016). Malassezia species in healthy skin

and in dermatological conditions. *Int. J. Dermatol. 55(5)*: 494- 504.

Pu Xiong Ming, Yi Li Xia Ti and lenn S. Bumber (2006). Outbreak of *Trichophyton verrucosum* in China transmitted from cows to humans. *Mycopathologia 161*: 225-228.

Puntenney, SB., y Wang and Forsberg NE. (2003). Mycotic infections in Livestock. Recent insights and Studies on etiology diagnostic and prevention of Hamorrhagic Bowel Syndrome, In: “Proc. South-West Nutrition and Management Conference”. Pheonix, University of Arizona.

Department of Animals Science, Triscon PP. 49-63.

Rafai Ogorek, Agnieszka Lejman, Wojciech Pusz, Anna Miluch and Paulina Miodynska (2012). Characteristics and Taxanomy of *Cladosporium* fungi.*Mikologia Lekarska 19(2)*:80-85.

Rajah, M., Praveena, G. and William John, S. (2017). Isolation and Identification of fungifrom soil in Loyola college campus, Chennai, India. *International Journal of Current M icrobiology andApplied Sciences 6(2)*: 1789-1795.

Rajrh Mohammad Abu Basma, Zuraini Zakaria, Sasidharan Sreenivasan, Latha Lachimanan (2010). Assessment of *Euphobia hirta* L. Leaf, Flower, stem and Root Extract for their antibacterial and antifungal activity and Brine shrimp lethality.*Molecules* 15: 6008-6018.

Rakshit K. Devapapa, Harinder P.S. Makkar and Klaus Becker (2010). Jatropha toxicity - A Review *Journal of Toxicology and Environmental Health 13(6)* 476-507.

Ramon Perez –Tanoira, Carlos Zarco Olivo, Jose Fortes Alen, Laura Prieto –Perez, Alfonso Cabello, Jose Manuel Ramos

Rinco, Juan Cuadros and Miguel Gorgolas(2018). Tinea nigra Palmaris: a clinical case in a rural Ethiopian hospital. *Revista do Instituto de Medicina Tropical de SaoPaulo 60*:52.

Ravinder,Kaur, Pragyan, Swagatika Panda, Kabir, Sardana and Sahanawaj, Khan.(2015). Mycological Pattern of dermatomycoses in a Tertiary Care Hospital. *Journal of Tropical Medicine 3*:1-5.

Ravinderb Kaur, Pragyan Swagatika Panda and Shahnawaz Khan (2017). Non-dermatophytic molds causing

Onychomycosis:a rising trend in North India. *International Journal of Community Medicine and Public Health4(12)*:4532-4537.

Razirh Sadeti, Amin Baeghi and Reza Abbasi Larki (2015).Iloletion and screening of hypolytc fungi from coastal waters of the southern cespian sci (Noun of Iran). Jundishapur *Journal of Microbiology 8 (4)* e16426.

Reis,P.,Miller,R., Leser, M. and Watzke,H.(2009).Lipase-catalysed reactions at interfaces of two-phase systems and microemulsions.*Applied*

*BiotechnologyBiotechnology 158(3)*:706-721.

Revankar,SG., Patterson, JE., Sutton,DA., Pullen,R. and Rinaldi MG.(2002). Disseminated Phaeohyphomycosis: Review of an emerging mycosis. *Clin.Infec Dis (34)*:467-476.

Rohit Bazaz and David W. Denning (2019). Aspergillosis: causes, types and treatment. *The pharmaceutical Journal 303*; 7927.

Saber, W.A., El-Metwally,MM. and El-Hersh, MS.(2010). Keratinase production and biodegradation of some keratinous wastes

by *Alternaria tenussinum*and *Aspergillus nidulans* .*Research Journal ofmicrobiology 5* : 21-35.

Sadhna Sharma, Bindu Madhar Yenigalla, Suject Kumar Naidu and Premalatha Pidakala (2013). Primary cutaneous aspergillosis due to *Aspergillus tamari* in an immunocompetent host.*Bio-Medical Journal*:Case report.

Saitou, N. and Nei, M.(1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees.*Molecular Biology and Evolution 4*: 406-425.

Sallykutty Thomas (2016). Pharmacognostic and phytochemical constituents of leaves of *Jatropha multifida* Linn and *Jatropha podagrica* Hook. *Journal of pharmacognosy and phytochemistry 5(2)*:243-246.

Salmon, A., Debourgogne, A., Vasbien, M., Clement, L., Plenat, F., Bordigoni, P., and Machouart,.M.(2010). Disseminated *Scopulariopsis brevicailis* infection in an allogenic stem cell recipient: Case report and review of the literature.*Clin. Microbial Infect 16*:508-512.

Sampato, SAP. and Rivitti, EA (2008). Micoses superficial. In: Sampato SAP, Rivitti EA, editors Dematologia 3 Sao Paulo: Artes medicas: 2008 PP. 703-722.

Sandeep Kolwal and Geeta Sumbali (2016). Preferential utilization and colonization of keratin baits by different myco-keratinophiles. *Springerplus 5(1)*: 1204.

Sarangi G., Dash D., Chayani N., Patjoshi SK., Jena S, (2014). Tinea nigra of palm. Arare case repor from Eastern Indisa. *Journal of Medical Microbiology 32*: 86 -8.

Saravanan R., Dhachinamoorthi. D., Senthilkumar K., Srilakshmi M. and Sri Divya T. (2012). Antibacterial activity of *Euphorbia hirta* extracts. *International Journal of Research in Ayurveda and Pharmacy 3(3):* 439-441.

Sasi, Arun, Kani, Manthiri, Panneerselvam, A., Jegadeesh, G., Muthu, K. and Kumar, Ravi M.(2010). Optimizing the conditions of amylase by an Esturian strain of *Aspergillus* Spp. *African journal ofmicrobiology research 4(9)* :581-586.

Schutz, K.,Carle, RJ and Schueber, A. (2006). Taraxacum and view on its phytochemical

and pharmacological profile. *Journal of ethparmacology107(3)*: 313-323.

Scossa F, Benina M, Alseekh S, Zhang Y and Fernie AR (2018). The integration of metabolomics and next generation sequencing data to elucidate the pathways of natural product metabolism in medicinal plants. *Planta Med. 84*: 855- 873.

Seyed Amir Ghiasian (2011). Dermatophytes as a cause of epizoonoses in dairy cattle and humans in Iran: epidermiological and clinical aspects *mycoses 54(4)*: 52-56.

Seyed Amir Yazdanparast, Hussein Dargahi, Saeed Shahrokhi and Roya Horabad Farahani(2012). Isolation and Investigation of keratinophilic fungi in the parks of municipality distinct of Tehran.*Thrita 2(3)*:2-5.

Shakil Ahmed (2007). Air borne fungal spores – A review *Pakistan Journal of Phytopathology 19 (2)* : 179 – 19/1.

Shams-Ghahfarokhi M.,Mosleh-Tehrani F.,Rsnjbar-Bahadori S and Razzaghi-Abyaneh M.(2009).An epidemiological Survey on cattle ringworm in major dairy farms of Mashhad city,Eastern

Iran.*IranJournal of Microbiology 1(3):*31-36.

Sharma Pamita Prashant and Khade Ranjans Ganesh (2019). Qulitrture analysis of aeromycoflors of indoor dairy cittle shed. *International Journal of recent scientific Research 10(4)*: 31720 – 31722.

Shende, R. Wong, SSW, Rapole, S, Beau, R, Ibrahim –Granet O, Monod, M. (2018). Aspergillus fumigatus conidial metalloprotease meplpcleaves host complement proteins. *Journal ofBiological Chemistry 293*: 15538-55.

Shinkafi, S.A. (2013). Antidermatophytic activities, phytochemical screening and chromatographic studies of *Pergularia tomentosa L.*and *mitracarpus scaber* Zucc. (leaves) used in the treatment of Dernatophytoses. *International Research Journal of Microbiology 4(1)*: 29-37.

Shiu Ming Pang, Jonathan Yiyu Pang, Stephanie Frok- Chong and Ailing Yan (2018). Tinea Unguium onychomycosis caused by dermatophytes: a ten year (2005-2014) retrospective study in tertiary hospital in Singapore. *Singapore medical journal 59(10)*: 524- 527.

Shokohi, T., Hedayati, M.T. and Bakhshi,H.(2005).Isolation of fungi and aerobic actinomycetes from surface soil in Sari. *Journal of Kermanshah Universityof medical sciences 8*:25-32.

Shouchuang Wang, Saleh Alseekh, Alisdair R. Fernie, Jie Luo (2019). The structure and function of major plant metabolite modifications. *Molecular Plant 12(7)*:899-919.

Siembieda J., Kock R.,McCracken T and Newman S.(2011).The role of wildlife in trans-boundary.animal diseases. *Anim.Health Res.Rev. 12*:95-111.

Sillma Rampadarath, Daneshwar Puchooa and Mala Ranghoo Sanmukhiya (2014). Antimicrobial, phytochemical and insecticidal properties of Jatropha species and Wild Ricinus Communis L. found in Mauritius.*International Journal of pharmacognosy and phytochemical research 6(4)*:831- 840.

Silva- Navas, Moreno- Risueno, Manzano C, Tellez- Robledo, Navairo Neila, Carrasco V., Pollmann S., Javier Gallego F and del Pozo JC (2016). Flavonols mediate root phototropism and growth through regulation of proliferation to differenciation transition. *Plant cell 28*: 1372- 1387.

Singh Geeta and Kumar Padma (2011).Extraction and antimicrobial activity of alkaloids of *Euphorbia hirta*. *Journal of Pharmacy Research 4(4)*: 1228-1230.

Singh, R., Gupta, N., Goswami, VK. and Gupta, R. (2006). A simple activity staining protocol for lipases and esterases.*Applied Microbiology and Biotechnology 70(6)*:679- 682.

Skiada, A. and Petrikkos, G.(2009). Cutaneous zygomycosis.*Clinical microbia): 679linfections 15(5)*:41-45.

Skiada A, Floeri, CL., Klimko N., Ibrahim A, Roilides E and Petrikkos G (2018). Challenges in the diagnosis and treatment of mucormycosis. *Medical Mycology 56(1)*: 93-101.

Sofowora, A. Medicinal plants and Traditional Medicinal in Africa,2nd Ed. Sunshine House, Ibadan,Nigeria: Spectrum Books Ltd:1993,screening plants for Bioactive Agents:pp.134-156.

Songer J.G and Post K.W (2005).Veterinary Microbiology. Bacteria and Fungal Agents of Animal Disease.Published by Elsevier Saunders.pp 360-369.

Spellberg, B., Edwards, JR and Ibrahim, A.(2005). Novel perspectives on mucormycosis pathophysiology, presentation and management.*Clinical Microbiol. Rev.18*:556-559.

Spinello Antinori, Laura Milazzo, Salvative Sollima, Massimo Gali and Mario Corbellino (2016). Candidiasis in adults: A narrative review. *European Journal of internal medicine 34*: 21- 28.

Stainslaw Tokarzewski, Grazyna Ziolkowska and Aneta Nowakiewicz (2012). Susceptibility testing of Aspergillus niger

strains isolated from poultry to antifungal drugs. Acomparative study of the disk diffusion, broth microdillution ( M38- a) and etest ® methods. 15(1): 125-33.

Subha,Ganguly, Parveez Ahnad Par and Parveen Kumar Parveen (2017). Case study: Investigation of dermatophytosis from the skin scrapings collected from cow.*International Journal of current Microbiology and Applied Sciences 6(3)*:640-643.

Subhash, Gora., Mamata,Gora., Neetu, Jain.,Shamshad, ULHAQ., Meenakshi, Sharma., and Sunita,Mahariya(2017).Isolation and

characterization of keratinophilic fungi and related dermatophytes from various public parks of Jaipur India. *International journal of pharma and bio.sciences 8(2)*:100-106.

Sudarkodi Chandrasekaran, Sundar Pilllai Kumaresan and Murugan Manavalan (2015).Production and Opptimization of protease by filamentous fungus isolated from paddy soil in Thiruvarur District Tamilnadu. *Journal of Applied Biology and Biotechnology 3(6)*: 066-069.

Sujatha, P., Swethalatha, P (2017). Isolation and screening of novel Streptomyces from sediment of bay of Bengal near Srikakulam

coast.*International Journal of Current Research 9*:40-44.

Sundarram, A. and Murthy TPK ( 2014).α-Amylase production and Applications. A review.*Journal of Applied and EnvironmentalMicrobiology 2(4)*:166-175.

Sunitha, VH., Ramesha,A.,Savitha, J and Srinivas, C(2012).Amylase production by endophytic fungi *Cylindrocephalumsp* isolated from medicinal plant *Alpinia calcarata*(Haw) Roscoe.*Brazillian Journal of Microbiology43(3)*:1213-1221.

Suryawanshi, HK and Pandya, ND (2017). Screening, identification of Alkaline

proteases producing fungi from soil of different habitats of Amalner Tahsil (Maharashtra) and their application. *International Journal of Applied Sciences and Biotechnology 5(3)*: 397-402.

Suseela lanka and Tabita Trinkle B (2017). Screening and isolation of lipese producing fungi from marine water obtained from Machilipatnam cosoal Region. *International Journal of Pharamacognosy and phytochemical Research 9 (7):* 928-932.

Sutton, CL., Taylor, ZE., Farona, MB., Handy, ST,(2017). “Antifungal activity of substituted aurone”.*Bioorganic and*

*Medicinal Chemistry Letters 27(4)*: 901-903.

Suzana Tasic and Natasha Miladinovic Tasic (2007). *Cladosporium* spp: cause of opportunistic mycoses. *Acta Facultatis Medicae Naissenis24(1):15-19.*

Swai ES and Sanka PN (2012). Boune Dermatophytosis caused by *Trychophyton verrucosum*: A case report, *vet world 5(5)*: 297-300.

Swapna S Khatu, Shital Amin Poojary and Nivanjan G. Nagpur (2013). Nodules on the hair: A rare case of mixed piedra.

*International Journal of Trichology 5(4)*: 220 -223.

Swidergall, M and Filler, SG (2017). Oropharyngeal candiasis: Fungal invasion and Epithelial cell Responses. PLoS pathology 13(1): e1006056.

Tabassum Nahida and Hamdani Mariya (2014). Plants used to treat skin diseases. *Pharmacognosy Review 8(15)*:52-60.

Tampieri, MP. (2004). Update on the diagnosis of dermatomycosis. *Parassitologia 46(1-2)*:183-186.

Thes, PM, Soumahoro, I.A., Ackah, JA., Zirihi, GN., and Djaman, AJ.,(2011). Action du savon Misca-mates dans le traitement des teignes. *Phytotherapie: 9(6)* :354-358.

Tomley FM and Shirley M (2009).Livestock infectious diseases and zoonoses.Philos.*Trans R Soc Lond.BBiol Sci.:364(1530):*2637-2642.

Tortorano, AM., Richardson, M., Roilides E., Diepeningen AV., Caira M., Munoz P., Johnson E., Melatiadis J., Pana ZD. Lackner M., Verneji P., Freiberger T., Cornely OA., Arikan- Akdajli S., Dannaoui E., Groll AH., Lagrou K and Chakrabarti A

(2014). ESCMID and ECMM joint guidelines on diagnosis and management of hyalohyphomycosis: *Fusarium* spp. *Scedosporium* spp.and others. *Clinical Microbiology and Infection 20(3)*; 27-46.

Trease,G.E. and Evans,W.C.(1983). Pharmacology (12th ed). Bailliere Tindal:London.

Tzar Mohd Nizam, Rabiatul Adawiyah AG., Binting, Shafika Mohd Saari, Thivyananthini Vijaya Kumar, Marianayati Muhammad, Hartini Satim, Hamidah Yusoff and Jacinta Santhanam (2016). In-vitro antifungal activities against moulds

isolated from Dermatological specimens. *Malaysian Journal of Medical Sciences 23(3)*: 32-39.

UNDP (2008).Human development report 2007/2008:Fighting climate change: human solidarity in a divided word. New York USA.

Valko,M., Rhodes, CJ., Moncol, J., Izakovic, M., Mazar, M,(2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemical biological interactions 160*: 1-40.

Vanbreuseghem, R. (1952). Tecnique biologique pour one isolament des

dermatophytes du sol (biological technique for isolation of dermatophytes from soil). *Annales de la societe belge demedicine tropicale 32*: 173-178.

Vermout,S.,Tabart,J.,Baldo,A.,Mathy,A.,Losson,B. and Mignon,B.(2008). Pathogenesis of dermatophytosis. *Mycopathologia 166*:267-275.

Vyas A.,Pathan,N.,Sharma,R.,Vyas,L.A.(2013).Clinicomycological study of cutaneous mycoses in SawaiManSingh Hospital of

Jaipur,North India.*Annals of Medical Health Science Research 3*:593-597.

Warner, RR.,Schwaitz, JR.,Boissy,V. and Dawson,L.(2001).”Dandruff has an altered stratum corneum ultrastructure that is improved with zinc pyrithione shampoo.”*Journal of American Academy of Dermatology 45(6)*:897-903.

Waseem Ahmad, Shilpa Singh and Sanjay Kumar (2017). Phytochemical screening and antimicrobial study of *Euphorbia hirta* extracts. *Journal of medical plants studies 5(2)*: 183-186.

Wellington, M.,Gigliotti, F (2001). Update on antifungal agents. *Pediatric Infectious DiseaseJournal 20(10)*: 993-995.

Wongsatit Chuakul (2005). Medicinal plants in the Khok Pho District, Pattani Province (Thailand) *The Journal of Phytopharmacy 12(2)*: 23-45.

Woolhouse ME.,Haydon DT and Anita R,(2005).Emerging pathogens:the epidemiology and evolution of species jumps.*Trends Ecol.Evol.20*:238-244.

Wu,S.X., Guo,N.R., Li,X.F., Liao,W.Q., Chen, M., Zhang,Q.Q., Li,C.Y., Li,R.Y.,

Bulmer,G.S., Li,D.M., Xi,L.Y., Lu,S., Liu,B,Zhang,Y.C., Ran, Y.P and Kuan Y.Z (2011).Human pathogenic Fungi in China-Emerging Trends from ongoing National Survey for 1986,1996 and 2006. *Mycopathologia 171*:387-393.

Yan, L., Yang, C., and Tang, J.(2013).Disruption of the intestinal mucosal barrier in *Candida albican* infection. *Microbiological research 168(7)*:389-395.

Zarei Mahdabadi A (2005).A study of dermatophytosis in South West of Iran(Ahvaz). *Mycopathologia;160*: 21-4.

Zarei Mahmoudabadi A (2006).Clinical characteristics and mycology of cutaneous candidiasis in Ahwaz (lran).*Pak.J.Med Sci;22*:43-6.

Zarei Mahmoudabadi A,Yaghoobi R,Sadeghi B (2007).A large outbreak of tinea capitis in a primary school.*J. infect;54*:247-8.

Zarei Mahmoudabadi and Baharea Izadi (2011). Prevalence of cutaneous mycoses among workers. *Turkish .Journal of Medical Science. ; 41(2):*291-294.

Zhang,Y.HP.,Hong J. and Ye,X(2009).Cellulose assy methods.*Molecular Biology 581*:213-231.

**APPENDIX 1: Analysis of variance (anova) to determine the freouency of occurrence of non-dermatophytic molds isolated from air within the market in Abia state**

| Setups | Frequency occurrence |
|---|---|
| Ubakala market | 1.6250 ± 0.3750 |
| Ahia Udele market | 2.7500 ± 0.4910 |
| Lokpanta market | 3.1250 ± 0.6928 |

Mean ± standard error of mean

I accept the null hypothesis that there is no significant different in the frequency occurrence of non- dermatophytic molds isolated from air within the market in Abia State at (P=0.05).

**APPENDIX 2: Analysis of variance ( anova) to determine the frequency occurrence of non- dermatophytic molds isolated from air within the market in Imo state**

| Setup | Frequency occurrence |
|---|---|
| Afor Ogbe market | 4.1250 ± 0.5154 |

| | |
|---|---|
| Ekeubahaeze market | 2.2500 ± 0.6478 |
| Okigwe market | 2.1250 ± 0.7425 |

Mean ± standard error of mean

I accept the null hypothesis that there is no significant difference in frequency occurrence of non- dermatophytic molds isolated from air in Imo State at (P= 0.05).

# APPENDIX 3: Analysis of variance (anova) to determine the frequency occurrence of non-dermatophytic molds isolated from soil within market in Abia state

| Setups | Frequency occurrence |
|---|---|

| | |
|---|---|
| Ubakala market | 6.1667 ± 1.8514 |
| Ahia Udele market | 1.0000 ± 0.6325 |
| Lokpanta market | 3.8333 ± 0.6009 |

Mean ± standard error of mean

I reject the null hypothesis and accept the alternative hypothesis that there is significant difference in frequency occurrence of non-dermatophytic molds from soil in cattle market in Abia State at (P = 0.05).

# APPENDIX 4: Analysis of variance (anova) to determine the frequency occurrence of non- dermatophytic molds isolated from soil within the market in Imo state

| Setups | Frequency Occurrence |
|---|---|

| | |
|---|---|
| AforOgbe market | 4.0000 ± 0.9820 |
| Ekeubahaeze market | 2.8750 ± 0.7181 |
| Okigwe market | 2.6250 ± 0.8852 |

Mean ± standard error of mean

I accept the null hypothesis that there is no significant difference in the frequency occurrence of non- dermatophytic molds from soil in cattle market in Imo State at (P =0.05).

# APPENDIX 5: Analysis of variance ( anova) to determine the mic's of the four selected plant ( leaves) extract against 12

**non—dermatophytic molds isolated from the study**

For MIC (mg/ml)

| Isolates | Mean |
|---|---|
| *Jatropha multifida* | 191.67 ± 8.33$^{c}$ |
| *Occimum gratissium* | 145.83 ± 16.81$^{bc}$ |
| *Euphorbia hirta* | 125.00 ± 20.64$^{b}$ |
| *Mitracarpus scaber* | 50.00 ± 14.10$^{a}$ |

Mean ± standard error of mean

Columns with different superscripts are significantly different at ($P < 0.05$).

## APPENDIX 6: Analysis of variance (anova) to determine mfc of four selected plant

**(leaves) extract against 12 non-dermatophytic molds isolated from the study**

For MFC (mg/ml)

| Isolates | Mean |
|---|---|
| *Jatropha multifida* | 200.00 ± 0.00[b] |
| *Occimum gratissium* | 191.67 ± 8.33[b] |
| *Euphorbia hirta* | 166.67 ± 17.77[b] |
| *Mitracarpus scaber* | 83.33 ± 12.81[a] |

| | |
|---|---|

Mean ± standard error of mean.

Columns with different superscripts are significantly different at ($P < 0.05$)

# APPENDIX 7: Multiple Comparison Analysis On Minimum Inhibitory Concentration (Mic) And Minimum Fungicidal Concentration (Mfc) For *Euphorbia hirta, Occimum gratissimum, Jatropha multifida* AND *Mitracarpus scaber*

```
ONEWAY MIC MFC BY ISOLATES
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=TUKEY LSD ALPHA (0.05).
```

**Oneway**

[DataSet0]

# Descriptives

| | | | | | | 95% Confidence Interval for Mean | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | Mean | Std. Deviation | Std. Error | Lower Bound | Upper Bound | Minimum | Maximum |
| MIC (Mg/ | Jatropha multifida | 12 | 191.6667 | 28.86751 | 8.33333 | 173.32 | 210.0082 | 100.00 | 200.00 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ml) | Ocimum gratissimum | 12 | 145.83333 | 58.225081 | 16.808811 | 108.8389 | 182.8277 | 50.00 | 200.00 |
| | Euphobia hirta | 12 | 125.00000 | 71.50969 | 20.643907 | 79.5649 | 170.4351 | 25.00 | 200.00 |
| | Mitracarpus scaber | 12 | 50.0000 | 48.8504 | 14.1.01990 | 18.9619 | 81.0381 | 25.00 | 200.00 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total | 48 | 128.12500 | 73.594048 | 10.621887 | 106.7566 | 149.4934 | 25.00 | 200.00 |
| MFC (Mg/ml) | Jatropha multifida | 12 | 200.00000 | .000000 | .000000 | 200.00000 | 200.00000 | 200.00000 | 200.00000 |
| | Occimum gratissium | 12 | 191.66667 | 28.867751 | 8.333333 | 173.3251 | 210.0082 | 100.00000 | 200.00000 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Euphobia hirta | 12 | 166.6667 | 61.5457 | 17.766673 | 127.56248 | 205.7710 | 50.00 | 20 0.00 |
| Mitracarpus scaber | 12 | 83.3333 | 44.3812 | 12.811777 | 55.1348 | 111.5318 | 50.00 | 20 0.00 |
| Total | 48 | 160.4167 | 60.9833 | 8.802197 | 142.7089 | 178.1244 | 50.00 | 20 0.00 |

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| MIC (Mg/ml) | Between Groups | 125572.917 | 3 | 41857.639 | 14.282 | .000 |
| | Within Groups | 128958.333 | 44 | 2930.871 | | |
| | Total | 254531.250 | 47 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| MFC (Mg/ml) | Between Groups | 102291.667 | 3 | 34097.222 | 20.693 | .000 |
| | Within Groups | 72500.000 | 44 | 1647.727 | | |
| | Total | 174791.667 | 47 | | | |

## Post Hoc Tests

## Multiple Comparisons

| Dependent Variable | (I) ISOLATES | (J) ISOLATES | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| MIC (Mg/ | Tukey HSD | Jatropha multifida | Occimum gratissium | 45.8333 | 22.101 | .178 | -13.1779 | 104.8446 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ml) D | | Euphobia hirta | 66.6666667* | 22.1.01155 | .02 | 7.655 | 125.677 |
| | | Mitracarpus scaber | 141.6666667* | 22.1.01155 | .00 | 82.65 | 200.677 |
| | Occimum gratissium | Jatropha multifida | -45.83333 | 22.1.01155 | .17 | -104.846 | 13.1779 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Euphobia hirta | 20.83333 | 22.10155 | .78 | -38.17279 | 79.84646 |
| | Mitracarpus scaber | 95.83333* | 22.10155 | .000 | 36.82211 | 154.8446 |
| Euphobia hirta | Jatropha multifida | -66.66667* | 22.10155 | .021 | -125.67779 | -7.65549 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Ocimum gratissium | -20.8333333 | 22.101555 | .78 | -79.84846 | 38.1779 |
| | Mitracarpus scaber | 75.000000* | 22.101555 | .008 | 15.9887 | 134.0113 |
| Mitracarpus scaber | Jatropha multifida | -141.6667* | 22.101555 | .000 | -200.679 | -82.6554 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Occimum gratissium | -95.83333* | 22.101155 | .000 | -154.8446 | -36.8221 |
| | | Euphobia hirta | -75.00000* | 22.101155 | .008 | -134.0113 | -15.9887 |
| LSD | Jatropha multifida | Occimum gratissium | 45.83333* | 22.101155 | .044 | 1.2906 | 90.3761 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Euphobia hirta | 66.6666667* | 22.1.01055 | .00 4 | 22.12 3994 | 11 1.2 09 4 |
| | Mitra carpus scaber | 141.6666667* | 22.1.01055 | .00 0 | 97.12 3994 | 18 6.2 09 4 |
| Occimum gratissium | Jatropha multifida | -45.833333* | 22.1.01055 | .04 4 | -90.37 61 | -1.2 90 6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Euphobia hirta | 20.8333333 | 22.101155 | .351 | -23.7094 | 65.3761 |
| | Mitracarpus scaber | 95.8333333* | 22.101155 | .000 | 51.2906 | 140.3761 |
| Euphobia hirta | Jatropha multifida | -66.666667* | 22.101155 | .004 | -111.2094 | -22.1239 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Occimum gratissium | -20.8333333 | 22.101155 | .35 | -65.3761 | 23.7094 |
| | | Mitracarpus scaber | 75.000000* | 22.101155 | .001 | 30.4573 | 119.5427 |
| | Mitracarpus scaber | Jatropha multifida | -141.6667* | 22.101155 | .000 | -186.2094 | -97.1239 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Occimum gratissium | -95.8333333* | 22.101555 | .00 | -140.37761 | -51.2906 |
| | | | Euphobia hirta | -75.000000* | 22.101555 | .001 | -119.5427 | -30.4573 |
| MFC (Mg/ | Tuke HS | Jatropha multifida | Occimum gratissium | 8.33333 | 16.5.57170 | .955 | -35.9132 | 52.5799 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ml) D | | Euphobia hirta | 33.3333333 | 16.5717070 | .19 | -10.919932 | 77.579999 |
| | | Mitracarpus scaber | 116.666667* | 16.5717070 | .000 | 72.420131 | 160.912 |
| | Occimum gratissium | Jatropha multifida | -8.333333 | 16.5717070 | .958 | -52.579932 | 35.91332 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Euphobia hirta | 25.000000 | 16.571770 | .441 | -19.2465 | 69.2465 |
| | Mitracarpus scaber | 108.33333* | 16.571770 | .000 | 64.0868 | 152.5799 |
| Euphobia hirta | Jatropha multifida | -33.333333 | 16.571770 | .199 | -77.5799 | 10.9132 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Occimum gratissium | -25.000000 | 16.571700 | .441 | -69.2465 | 19.24 65 |
| | Mitracarpus scaber | 83.333333* | 16.571700 | .000 | 39.0868 | 127.5799 |
| Mitracarpus scaber | Jatropha multifida | -116.6667* | 16.571700 | .000 | -160.913 2 | -72.4201 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Occimum gratissium | -108.33333* | 16.57170 | .000 | -152.57799 | -64.08668 |
| | | Euphobia hirta | -83.33333* | 16.57170 | .000 | -127.57799 | -39.08668 |
| LSD | Jatropha multifida | Occimum gratissium | 8.33333 | 16.57170 | .618 | -25.06447 | 41.73114 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Euphobia hirta | 33.33333333 | 16.571700 | .05 | -.0647 | 66.7314 |
| | Mitracarpus scaber | 116.66667* | 16.571700 | .00 | 83.260 | 150.0647 |
| Occimum gratissium | Jatropha multifida | -8.33333 | 16.571700 | .618 | -41.7314 | 25.0647 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Euphobia hirta | 25.000000 | 16.571770 | .139 | -8.39811 | 58.39811 |
| | Mitracarpus scaber | 108.3333* | 16.571770 | .000 | 74.93531 | 141.734 |
| Euphobia hirta | Jatropha multifida | -33.33333 | 16.571770 | .050 | -66.73314 | .0647 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Ocimum gratissium | -25.000000 | 16.571770 | .139 | -58.3981 | 8.398 |
| | Mitracarpus scaber | 83.333333* | 16.571770 | .000 | 49.9353 | 116.731 |
| Mitracarpus scaber | Jatropha multifida | -116.6667* | 16.571770 | .000 | -150.064 | -83.267 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Occimum gratissium | -108.33333* | 16.57170 | .000 | -141.73134 | -74.93532 |
| | Euphobia hirta | -83.33333* | 16.57170 | .000 | -116.73134 | -49.93532 |

*. The mean difference is significant at the 0.05 level.

## Homogeneous Subsets

**MIC (Mg/ml)**

| | ISOLATES | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|---|
| | | | 1 | 2 | 3 |
| Tukey HSD[a] | Mitracarpus scaber | 12 | 50.0000 | | |
| | Euphobia hirta | 12 | | 125.0000 | |
| | Occimum gratissimum | 12 | | 145.8333 | 145.8333 |

| | | | | | |
|---|---|---|---|---|---|
| | Jatropha multifida | 12 | | | 191.6667 |
| | Sig. | | 1.000 | .782 | .178 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000.

**MFC (Mg/ml)**

| | ISOLATES | N | Subset for alpha = 0.05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD[a] | Mitracarpus scaber | 12 | 83.3333 | |
| | Euphobia hirta | 12 | | 166.6667 |
| | Occimum gratissimum | 12 | | 191.6667 |

| | | | | |
|---|---|---|---|---|
| | Jatropha multifida | 12 | | 200.0000 |
| | Sig. | | 1.000 | .199 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000.

# APPENDIX 8

**ABIA STATE GOVERNMENT OF NIGERIA**

**MINISTRY OF AGRICULTURE**

P.M.B. 7224, UMUAHIA

*Telegrams: Moa*

*Tel:*

*Your Ref:* .....

*Dept:* Veterinary Services

*Our Ref:* DVS./OI/RCH/01/18

*Date.* 4th June, 2018

**TO: SURVEILLANCE/MEAT INSPECTION OFFICERS INCHARGE ;**
**ALL ABIA CONTROL POSTS AND ABATTOIRS.**

The bearer Mrs Chioma Nwofo is a student of department of Applied Microbiology and Brewing of the Nnamdi Azikiwe University.

She is conducting a research on Dermatophytosis and other cutaneous diseases on cattle.

Please give her every help she needs.

Thanks.

Yours

**Dr. Nwokedi Onu**
Director of veterinary Services

**APPENDIX 9**

GOVERNMENT OF IMO STATE OF NIGERIA

# MINISTRY OF AGRICULTURE, ENVIRONMENT AND NATURAL RESOURCES

STATE SECRETARIAT COMPLEX, BLOCK 2, Port Harcourt Road, PMB 1165, Owerri, Imo State.

*Your Ref:*.....

*Our Ref:* NAR/Vet/XX

*Date:* 13th March, 2017

Veterinary Services Department

1.To All Vet. Staff
2. Imo Cattle MarketOkigwe.
3.All Concerned,

**RE: PERMISSION TO SCREEN CATTLE FOR SKIN INFECTIONS**

Please, the bearer Nwofor, Chioma N. is currently doing her PH.D at Nnamdi Azikiwe Univerity, Awka. She wants to carry out a research on fungal infections in cattle. This is purely an academic assignment and does not carry any ethical implications.

Please, give her your maximum co-operation, especially access to your cattle.

Thanks for your appropriate response.

**Dr. C. O. Nwagu**
HOD, Vet. Services

**APPENDIX 10: Frequency of occurrence of non- dermatophytic molds genera isolated from Abia and Imo states Nigeria**

| GENUS | TOTAL OCCURRENCE | % OCCURRENCE |
|---|---|---|
| *Aspergillus* Species | 338 | 43.6 |
| *Fusarium* Species | 269 | 34.7 |
| *Penicillum* Species | 23 | 3.0 |

| | | |
|---|---|---|
| *Cladosporium* Species | 38 | 4.9 |
| *Pestalotiopsis* Species | 1 | 0.1 |
| *Talaromyces* Species | 1 | 0.1 |
| *Absidia* Species | 100 | 12.9 |
| ~~*Curvularia* Species~~ | ~~5~~ | ~~0.6~~ |
| **~~Total~~** | **~~775~~** | |

**APPENDIX 11: Frequency of occurrence of non – dermatophytic molds from cattle skin in Abia state and Imo statesNigeria.**

| Isolates | Abia State | Imo State | Total (%) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| *Aspergilluscitrinum* | 18 | 5 | 23(30) |
| *Aspergillusfumigatus* | 21 | 7 | 28(3.6) |
| *Aspergillusterreus* | 8 | 13 | 21(2.7) |
| *Aspergilluswelwitschiae* | 67 | 38 | 105(13.5) |
| *Aspergillusflavus* | 50 | 26 | 76(10.0) |
| Aspergillus *aculeatus* | 33 | 36 | 69(9.0) |
| Aspergillus *sydowii* | 16 | 23 | 39(5.0) |

| | | | |
|---|---|---|---|
| *Talaromyceskendrinckii* | 0 | 1 | 1(0.1) |
| *Curvulariakusanol* | 0 | 5 | 5(0.6) |
| *Cladosporium tenuissium* | 20 | 18 | 38(4.9) |
| *Pestalotiopsismicrospora* | 0 | 1 | 1(0.1) |
| *Fusarium solani* | 10 | 15 | 25(3.2) |
| *Fusarium lichenicola* | 87 | 52 | 139(17.9) |
| *Fusarium succisae* | 56 | 34 | 90(12.0) |

| | | | |
|---|---|---|---|
| *Fusarium oxysporum* | 0 | 15 | 15(2.0) |
| *Absidia corymbifera* | 63 | 37 | 100(12.9) |
| **Total** | **449** | **326** | **775** |

**APPENDIX 12: Frequency of occurrence of non – dermatophytic molds from cattle skin in Abia state.**

| **Isolates** | **Ubakala/ Lokpanta/** | **Ahia Udele/** | **Total(%)** |
|---|---|---|---|

| | Abia Central | Abia South | | |
|---|---|---|---|---|
| **Abia South** | | | | |
| *Aspergillus citrinum* | 9 | 7 | 2 | 18(4.0) |
| *Aspergillus fumigatus* | 11 | 7 | 3 | 21(4.7) |
| *Aspergillus terreus* | 4 | 4 | 0 | 8(2.0) |
| *Aspergillus welwitschiae* | 13 | 21 | 33 | 67(15.0) |
| *Aspergillus flavus* | 20 | 7 | 23 | 50(11.1) |
| *Aspergillus aculeatus* | 11 | 14 | 8 | 33(7.3) |

| | | | | | |
|---|---|---|---|---|---|
| *Aspergillus sydowii* | 7 | 0 | 9 | 16(3.6) | |
| *Fusarium solani* | 4 | 6 | 0 | 10(2.2) | |
| *Cladosporrum tensussium* | 5 | 9 | 6 | 20(4.5) | |
| *Fusarium lichenicola* | 26 | 29 | 32 | 87(19.4) | |
| *Fusarium succisae* | 5 | 27 | 24 | 56(12.5) | |
| *Absidia corymbifera* | 19 | 20 | 24 | 63(14.0) | |
| **Total** | **134** | **151** | **164** | **449** | |

**APPENDIX 13: Frequency of occurrence of non – dermatophytic molds from cattle skin in Imo state.**

| **Isolates** | **Afor Ogbe** | **Eke Ubahaze** | **Okigwe** | **Total (%)** |
|---|---|---|---|---|
| *Pencillum Citrinum* | 5 | 0 | 0 | 5(1.5) |
| *Aspergillus fumigatus* | 0 | 3 | 4 | 7(2.1) |
| *Aspergillus terreus* | 10 | 3 | 0 | 13(4.0) |

*Aspergillus welwitschiae* 12 16 10 38(12.0)

*Aspergillus flavus* 16 0 10 26(8.0)

*Aspergillus aculeatus* 13 6 17 36(11.0)

*Aspergillus sydowii* 4 19 0 23(7.1)

*Talaromyces kendrinkii* 1 0 0 1(0.3)

*Curvulara kusanol* 5 0 0 5(1.5)

*Cladosporrum tenuissium* 10 0 8 18(5.5)

*Pestalotiopsis microsporum* 1 0 0 1(0.3)

| | | | | | |
|---|---|---|---|---|---|
| *Fusarium solani* | 9 | 6 | 0 | 15(4.6) | |
| *Fusarium lichenicola* | 16 | 16 | 20 | 52(16.0) | |
| *Fusarium succisae* | 14 | 8 | 12 | 34(10.4) | |
| *Fusarium oxysporum* | 10 | 0 | 5 | 15(4.6) | |
| ~~*Absidia corymbifera*~~ | ~~14~~ | ~~7~~ | 16 | 7(11.3) | |
| **Total** | **140** | **84** | **102** | **326** | |

**APPENDIX 14: Frequency of occurrence of non – dermatophytic molds from cattle skin with lesion only in Abia state.**

| **Isolates** | **Ubakala Market** | **Ahia Udele Market** | **Lokpa Market** | **Total (%)** |
|---|---|---|---|---|
| *Penicillum citrinum* | 0 | 3 | 0 | 3(5.0) |

*Aspergillus fumigatus* 0 1

0 1(2.0)

*Aspergillus welwitschiae* 0

7 6 13(21.3)

*Aspergillus aculeatus* 1 2

0 3(5.0)

*Aspergillus flavus* 0 1

2 3(5.0)

*Aspergillus sydowii* 0 0

2 2(3.3)

*Cladosporrum tenuissinum* 1

2 1 4(7.0)

*Fusarium lichenicola* 2 2

4 8(13.1)

| | | | | | |
|---|---|---|---|---|---|
| *Fusarium succisae* | 0 | 7 | 5 | 12(20.0) | |
| ~~*Absidia corymbifera*~~ | ~~1~~ | ~~7~~ | ~~4~~ | ~~12(20.0)~~ | |
| **Total** | **5** | **32** | **24** | **61** | |

**APPENDIX 15: Frequency of occurrence of non dermatophytic molds from cattle skin with lesions only in Imo state, Nigeria.**

| Isolates | AforOgbe Ekeubahaeze | Okigwe Market | Total (%) Market | Market |
|---|---|---|---|---|
| *Aspergillus welwitschiae* | 5 | 4 | 11 | 20(28.2) |
| *Aspergillus aculeatus* | 7 | 4 | 0 | 11(15.5) |
| *Aspergillus flavus* | 0 | 0 | 1 | 1(1.4) |

| | | | | |
|---|---|---|---|---|
| *Aspergilllus sydowii* | 2 | 4 | 0 | 6(8.5) |
| *Talaromyces kendrinckii* | 1 | 0 | 0 | 1(1.4) |
| *Cladosporium tenuissinum* | 4 | 0 | 0 | 4(5.6) |
| *Pestalotiopsis microsopora* | 1 | 0 | 0 | `1(1.4) |
| *Fusarium lichenicola* | 6 | 3 | 1 | 10(14.1) |
| *Fusarium succisae* | 0 | 1 | 6 | 7(9.9) |
| *Absidia corymbifera* | 4 | 2 | 4 | 10(14.1) |

| | | | |
|---|---|---|---|
| **Total** | **30** | **18** | **23** **71** |

# APPENDIX 16: Frequency of occurrence of non dermatophytic molds isolated from air within the market in Abia state

| Isolates | Ubakala Market | Ahia Udele Market | Lokpanta Market | Total (%) |
|---|---|---|---|---|
| *Penicillumm citrinum* | 2 | 3 | 5 | 10 (16.7) |
| *Aspergillus welwitschiae* | 3 | 3 | 6 | 12 (20) |
| *Aspergillus flavus* | 2 | 2 | 4 | 8 (13.3) |
| *Aspergillus aculeatus* | 2 | 2 | 2 | 6 (10) |
| *Aspergillus sydowil* | 0 | 2 | 0 | 2 (3.3 |

| | | | | | |
|---|---|---|---|---|---|
| *Clasdosporium tenussimum* | 0 | 2 | 2 | 4 (6.7) | |
| *Fusarrium lichenicola* | 2 | 2 | 2 | 6 (10.0) | |
| *Absidia corymbifera* | 2 | 6 | 4 | 12(20.0) | |
| **Total** | **13** | **22** | **25** | **60** | |

**APPENDIX 17: Frequency of occurrence of non dermatophytic molds isolated from air samples in Imo state.**

| Isolates | Afor Ekeubehaeze Market | Ogbe Okigwe Market | Total Market | (%) |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| *Penicillum citrinum* | 3 | 2 | 0 | 5(7.4) |
| *Aspergillus welwitschiae* | 5 | 3 | 4 | 12(17.6 |
| *Aspergillus flavus* | 5 | 3 | 0 | 8 (11.8) |
| *Aspergillus aculeatus* | 4 | 1 | 3 | 8 (11.8) |
| *Clasdosporium tenussimum* | 1 | 0 | 1 | 2 (3.0) |
| *Fusarium lichenicola* | 5 | 5 | 4 | 14 (20.6) |
| *Fusarium oxysporum* | 5 | 0 | 0 | 5 (7.4) |

| | | | | |
|---|---|---|---|---|
| *Absidia corymbifera* | 5 | 4 | 5 | 14 (20.6) |
| **Total** | **33** | **18** | **17** | **68** |

**APPENDIX 18: Frequency of occurrence of non dermatophytic molds from soil in cattle market in Abia, Nigeria**

| **Isolates** | **Ubakala** | **Ahia** | **Udele** | **Lokpanta** | **Total** |
|---|---|---|---|---|---|
| | | **Market** | **Market** | | |
| **Market** | **(%)** | | | | |
| *Aspergillus flavus* | 6 | 0 | 6 | | 12(18.2) |

| | | | | |
|---|---|---|---|---|
| *Aspergillus welwitschiae* | | 6 | 4 | 3 |
| 13 (20.0) | | | | |
| *Aspergillus sydowil* | | 0 | 0 | 2 |
| 2 (3.0) | | | | |
| *Fusarium Succisae* | | 3 | 0 | 3 |
| 6 (9.1) | | | | |
| *Fusarium lichenicola* | | 9 | 1 | 4 |
| 14 (21.2) | | | | |
| ~~*Absidia corymbifera*~~ | | ~~13~~ | ~~1~~ | ~~5~~ |
| 19 (29.0) | | | | |
| **Total** | **37** | **6** | **23** | |
| **66** | | | | |

# APPENDIX 19: Frequency of occurrence of non dermatophytic molds from soil in cattle market in Imo state, Nigeria

| Isolates | Afor Ekeubehaeze Market | Ogbe Okigwe Market | Total Market (%) |
|---|---|---|---|
| *Aspergillus flavus* | 5 | 5 | 6 16(21.1) |
| *Aspergillus welwitschiae* | 8 | 4 | 2 14 (18.4) |
| *Aspergillus sydowil* | 0 | 2 | 0 2 (3.0) |
| *Fusarium succisae* | 4 | 3 | 3 10 (13.2) |
| *Fusarium lichenicola* | 6 | 5 | 5 16 (21.1) |

| | | | | |
|---|---|---|---|---|
| *Fusarium Solani* | 2 | 0 | 0 | 2 (3.0) |
| *Cladosporium tenussimum* | 1 | 0 | 0 | 1(1.3) |
| *Absidia corymbifera* | 6 | 4 | 5 | 15 (20.0) |
| **Total** | **32** | **23** | **21** | **76** |

**APPENDIX 20: Frequency of occurrence of keratinophilic non dermatophytic molds from soil within cattle market in Abia state using hair bait technique**

| Isolates | Ubakala Market | Ahia Market | Udele Lokpanta Market | Total (%) |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| *Penicillum citrinum* | 0 | 0 | 2 | 2 (6.0) |
| *Aspergillus flavus* | 3 | 2 | 4 | 9(26.0) |
| *Aspergillus aculeatus* | 0 | 0 | 2 | 2 (6.0) |
| *Aspergillus welwitschiae* | 4 | 3 | 0 | 7 (20) |
| *Absidia corymbifera* | 3 | 3 | 3 | 9 (26.0) |
| *Fusarrium lichenicola* | 2 | 2 | 2 | 6 (17.1) |
| **Total** | **12** | **10** | **13** | **35** |

# APPENDIX 21: Frequency of occurrence of keratinophilic non dermatophytic molds

**from samples from within cattle markets in Imo state using hair bait technique**

| **Isolates** | **Afor Ogbe Ekeubehaeze Market** | **Okigwe Market** | **Total (%)** |
|---|---|---|---|
| *Penicillumm citrinum* | 0 | 0 | 1 1(2.2) |
| *Aspergillus flavus* | 3 | 2 | 2 7(16.0) |
| *Aspergillus aculeatus* | 2 | 0 | 2 4 (9.0) |
| *Aspergillus fumigatus* | 0 | 0 | 2 2 (4.4) |

| | | | | | |
|---|---|---|---|---|---|
| *Aspergillus welwitschiae* | 2 | 4 | 3 | 9 (20.0) | |
| *Cladosporium tenussimum* | 0 | 0 | 1 | 1 (2.2) | |
| *Absidia corymbifera* | 3 | 4 | 0 | 7 (16.0) | |
| *Fusarrium lichenicola* | 2 | 3 | 2 | 7 (16.0) | |
| *Fusarium Succisae* | 2 | 1 | 2 | 5 (11.1) | |
| *Aspergillus terreus* | 0 | 0 | 2 | 2(4.4) | |
| **Total** | **14** | **14** | **17** | **45** | |

# APPENDIX 22: Some laboratory

ing

PCR MACHINE

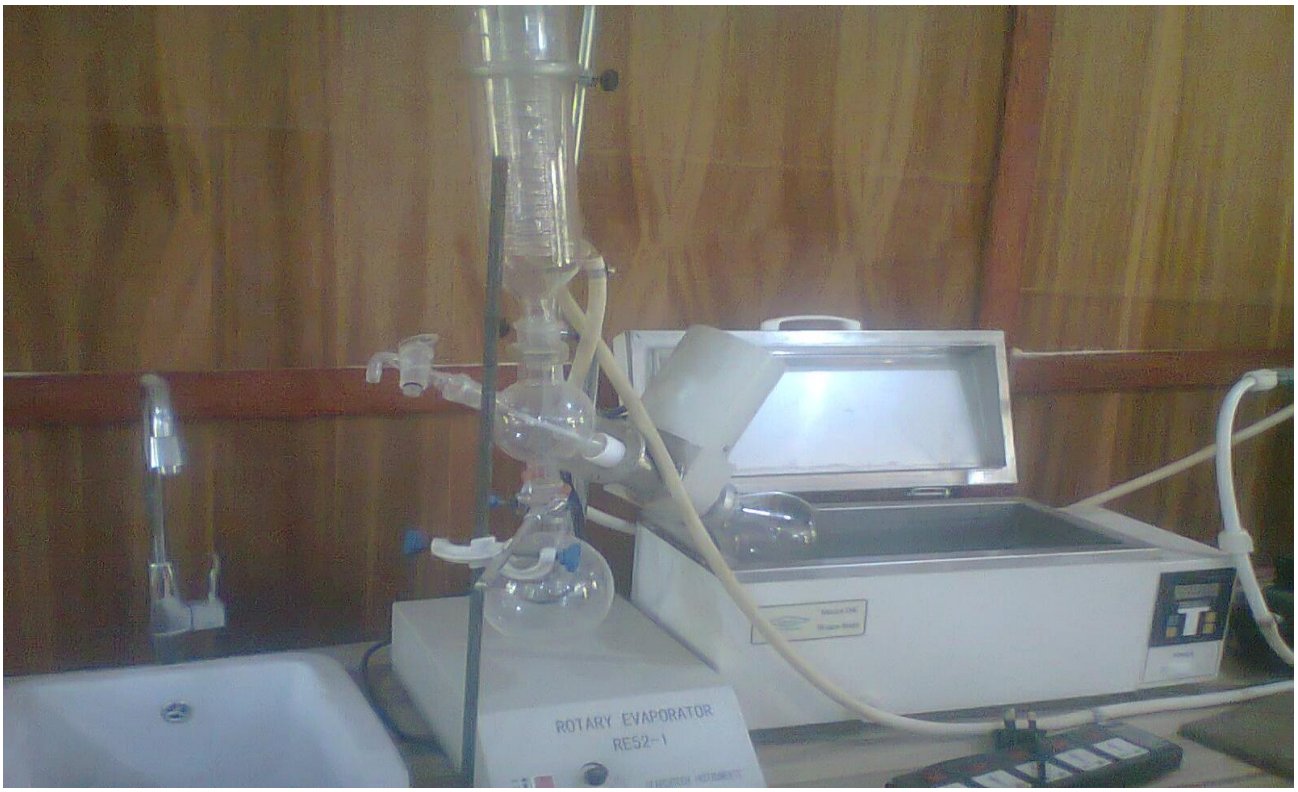

NANODROP MACHINE

ELECTROPHORESIS

CENTRIFUGE MACHINE

# HEATING MANTLE

SOXHLET EXRACTOR



ROTARY EVAPORATOR

# APPENDIX 23: Hair bait technique plate (a), agar plate exposed at strategic point within the market (b), some lesion elicited on albino mice used for pathogenecity test (c &d)


B

D

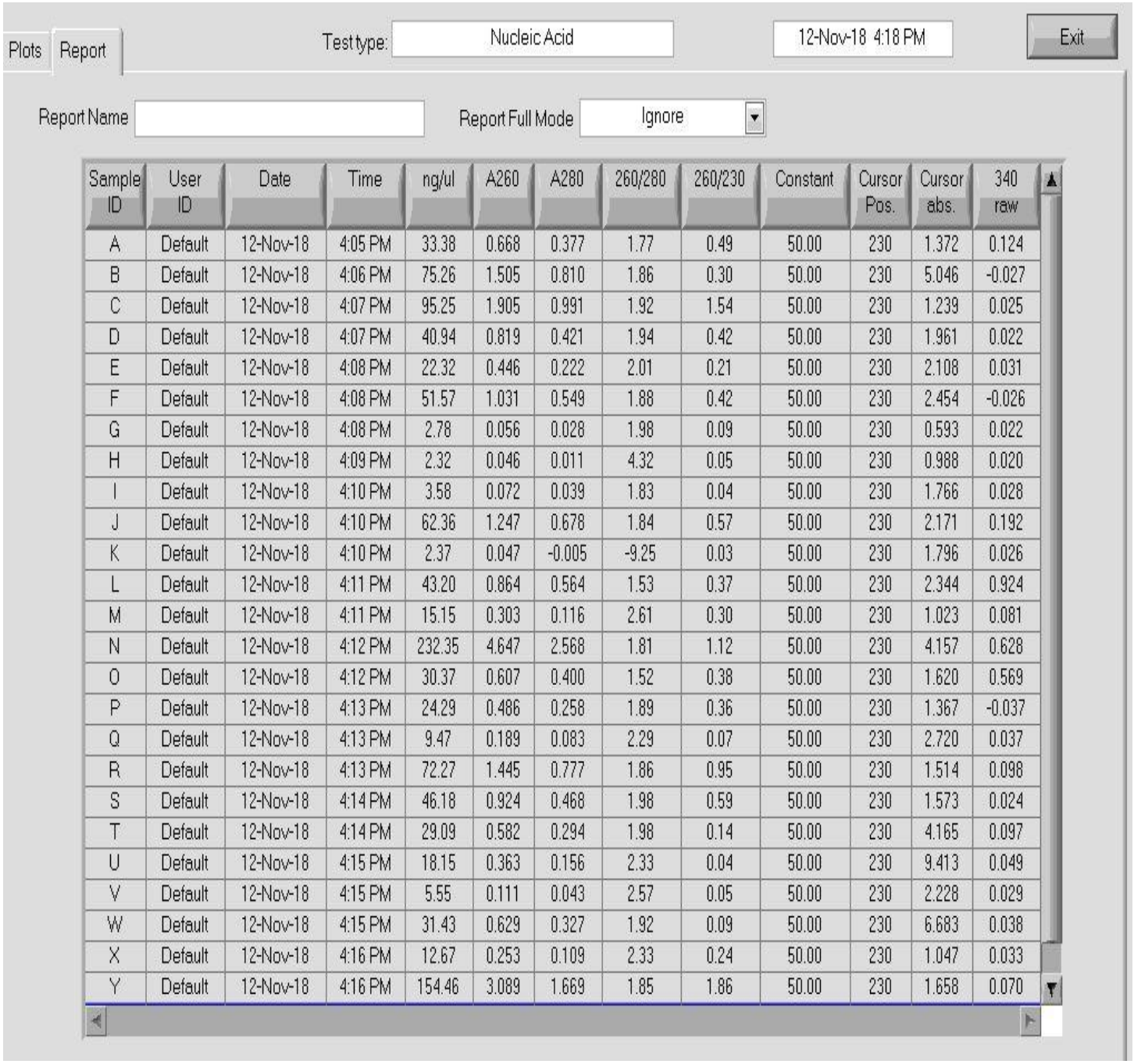
**APPENDIX 24: Some local plants used in this study; *Euphorbia hirta* (A), *Occimum gratissimum* (B), *Jatropha multifida* (C)**

A

B

C

# APPENDIX 25: Result from nano drop 1000 spectrophotometer




| Sample ID | User ID | Date | Time | ng/ul | A260 | A280 | 260/280 | 260/230 | Constant | Cursor Pos. | Cursor abs. | 340 raw |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Default | 12-Nov-18 | 4:05 PM | 33.38 | 0.668 | 0.377 | 1.77 | 0.49 | 50.00 | 230 | 1.372 | 0.124 |
| B | Default | 12-Nov-18 | 4:06 PM | 75.26 | 1.505 | 0.810 | 1.86 | 0.30 | 50.00 | 230 | 5.046 | -0.027 |
| C | Default | 12-Nov-18 | 4:07 PM | 95.25 | 1.905 | 0.991 | 1.92 | 1.54 | 50.00 | 230 | 1.239 | 0.025 |
| D | Default | 12-Nov-18 | 4:07 PM | 40.94 | 0.819 | 0.421 | 1.94 | 0.42 | 50.00 | 230 | 1.961 | 0.022 |
| E | Default | 12-Nov-18 | 4:08 PM | 22.32 | 0.446 | 0.222 | 2.01 | 0.21 | 50.00 | 230 | 2.108 | 0.031 |
| F | Default | 12-Nov-18 | 4:08 PM | 51.57 | 1.031 | 0.549 | 1.88 | 0.42 | 50.00 | 230 | 2.454 | -0.026 |
| G | Default | 12-Nov-18 | 4:08 PM | 2.78 | 0.056 | 0.028 | 1.98 | 0.09 | 50.00 | 230 | 0.593 | 0.022 |
| H | Default | 12-Nov-18 | 4:09 PM | 2.32 | 0.046 | 0.011 | 4.32 | 0.05 | 50.00 | 230 | 0.988 | 0.020 |
| I | Default | 12-Nov-18 | 4:10 PM | 3.58 | 0.072 | 0.039 | 1.83 | 0.04 | 50.00 | 230 | 1.766 | 0.028 |
| J | Default | 12-Nov-18 | 4:10 PM | 62.36 | 1.247 | 0.678 | 1.84 | 0.57 | 50.00 | 230 | 2.171 | 0.192 |
| K | Default | 12-Nov-18 | 4:10 PM | 2.37 | 0.047 | -0.005 | -9.25 | 0.03 | 50.00 | 230 | 1.796 | 0.026 |
| L | Default | 12-Nov-18 | 4:11 PM | 43.20 | 0.864 | 0.564 | 1.53 | 0.37 | 50.00 | 230 | 2.344 | 0.924 |
| M | Default | 12-Nov-18 | 4:11 PM | 15.15 | 0.303 | 0.116 | 2.61 | 0.30 | 50.00 | 230 | 1.023 | 0.081 |
| N | Default | 12-Nov-18 | 4:12 PM | 232.35 | 4.647 | 2.568 | 1.81 | 1.12 | 50.00 | 230 | 4.157 | 0.628 |
| O | Default | 12-Nov-18 | 4:12 PM | 30.37 | 0.607 | 0.400 | 1.52 | 0.38 | 50.00 | 230 | 1.620 | 0.569 |
| P | Default | 12-Nov-18 | 4:13 PM | 24.29 | 0.486 | 0.258 | 1.89 | 0.36 | 50.00 | 230 | 1.367 | -0.037 |
| Q | Default | 12-Nov-18 | 4:13 PM | 9.47 | 0.189 | 0.083 | 2.29 | 0.07 | 50.00 | 230 | 2.720 | 0.037 |
| R | Default | 12-Nov-18 | 4:13 PM | 72.27 | 1.445 | 0.777 | 1.86 | 0.95 | 50.00 | 230 | 1.514 | 0.098 |
| S | Default | 12-Nov-18 | 4:14 PM | 46.18 | 0.924 | 0.468 | 1.98 | 0.59 | 50.00 | 230 | 1.573 | 0.024 |
| T | Default | 12-Nov-18 | 4:14 PM | 29.09 | 0.582 | 0.294 | 1.98 | 0.14 | 50.00 | 230 | 4.165 | 0.097 |
| U | Default | 12-Nov-18 | 4:15 PM | 18.15 | 0.363 | 0.156 | 2.33 | 0.04 | 50.00 | 230 | 9.413 | 0.049 |
| V | Default | 12-Nov-18 | 4:15 PM | 5.55 | 0.111 | 0.043 | 2.57 | 0.05 | 50.00 | 230 | 2.228 | 0.029 |
| W | Default | 12-Nov-18 | 4:15 PM | 31.43 | 0.629 | 0.327 | 1.92 | 0.09 | 50.00 | 230 | 6.683 | 0.038 |
| X | Default | 12-Nov-18 | 4:16 PM | 12.67 | 0.253 | 0.109 | 2.33 | 0.24 | 50.00 | 230 | 1.047 | 0.033 |
| Y | Default | 12-Nov-18 | 4:16 PM | 154.46 | 3.089 | 1.669 | 1.85 | 1.86 | 50.00 | 230 | 1.658 | 0.070 |

**APPENDIX 26: Equipments and glassware**:

These items of equipment and glassware used:

**Microscope**: It was used to observe the microscopic view of the fungal isolates

**Cell disruptor Machine** (Distruptor Genie): Used to break the cell membrane during DNA extraction used for PCR analysis.

**Centrifuge** (Sorvall MC 12V): Used to separate DNA from other cell debris.

**Hot air oven** (gallenkamp England):It was

used for sterilization of all equipment made of glassware at 160℃ for 1hr.

**Incubator** (gallenkamp England and Uniscope-SM9082,Surgifield medicals England): It was used for incubating culture media used at 27°C.

**Autoclave** (gallenkamp England): It was used for sterilization of all culture media used at 121℃ for 15mins.

**Transilluminator** (PrepOne™ Sapphire blue light): It is used to visualize ethidium bromide-stained DNA in gels. Always wear protective eyewear when observing DNA on a transilluminator to prevent damage to the eyes from UV light.P

**Weighing balance** (MB- 2610 Made in China): It was used for the purpose of weighing appropriate media and plant extract.

**Eppendorf pipette** (Uniscope, Labnet, Oxford research pipette): Used during centrifuging and PCR analysis.

**Microwave** (Panasonic NN-SM332W): It is used to dissolve agarose powder with Tris-borate-EDTA (TBE).

**PCR Machine** (GeneAmp PCR system 9700): It is used for amplification of DNA.

**Spectrophotometer** (Nanodrop 1000): It is used to test DNA purification and quantification.

**Glasswares** (Pyrex, England): Conical flasks, Measuring Cylinder, Test tubes were the glasswares used.

**Agarose Voltameter** ( model EPS-300,11v, size 1-34 well,CBS Scientific company Inc): It is used to convey electric current to the agarose gel.

**Surgical syringe** (Visconject ADV$^{TM}$): It is used to dispense fluids.

**Soxhlet extractor**: It is used to extract of active ingredients from the local plant leaves.

**Rotary evaporator**: It was used to dry the extracts.

**Nanodrop scale**: To test the level of purity of DNA extracts.

**Sterilization of Material**

Asceptic standard was ensured in this study. The material used were sterilized by standard laboratory methods as described by (Chesbrough, 2010).

Glass wares were washed with detergent under running tap and sterilized with hot air oven at 160ºC for 1 hour. Glass rod spreader (hockey stick) was dipped intermittently in absolute alcohol and brought over a burning flame to burn off while inoculation wire loop was sterilized intermittently by flaming to red hot over a bunsen flame and warming gently in the air to cool.

Culture media were sterilized by autoclaving at 121ºC for 15 minutes at 15psi pressure unit. Hands were washed intermittently in running

tap water. Sterile disposable hand gloves and nose masks were worn and replaced intermittently to avoid contamination.

**Preparation of Media**

The media used for this study were prepared according to the manufacturer's instruction on the product labels.

**Sabouraud Dextrose Agar**

60g of the powder was dissolved in 1 litre of deionised water and allowed to stand for 10 minutes for complete dissolution. It was swirled to homogenize and sterilized by autoclaving at 121ºC for 15 minutes. It was allowed to cool to about 40ºC. Plates were prepared, containing 20mg of chloramphenicol to inhibit bacteria growth. It was then poured

20ml into sterile disposable petri dishes and allowed to solidify at room temperature. They were then stored in the refrigerator until required for use.