ASSAY OF ANTIBIOTIC PRODUCING MICROORGANISMS FROM DIFFERENT SOILS IN THE PREMISES OF FEDERAL UNIVERSITY OYE-EKITI, EKITI STATE. (FUOYE)

BY

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CERTIFICATION

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DEDICATION

I solemnly dedicate this report to God almighty, who has blessed me in all ramifications, impacting me with wisdom, knowledge, understanding and above all strength to achieve this. Also I thank my parents who supported me all through, for their financial and moral support, their love, care and concern.

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ABSTRACT

A total of eight soil samples were collected from different sites within FUOYE environment and were analyzed to determine the presence of antibiotic producing bacteria, fungi and actinomycete Using nutrient agar, sabauraud dextrose agar and glycerol yeast extract agar as culture media. Seven bacteria: *Escherichia coli, Bacillus spp, Pseudomonas spp, Azomonas spp, Gluconobacter spp, Micrococcus spp, and Staphylococcus aureus;* five fungi: *Rhizopus spp, Mucor spp, Rhodotorula spp, Trichoderma spp, Aspergillus spp* and one actinomycete (*Actinomyces spp)* were isolated. These isolates were screened for their antimicrobial potency against selected reference pathogenic organisms: *Staphylococcus aureus, Pseudomonas aerugenosa*, and *Candida albicans. Bacillus spp* isolated was found to inhibit *Staphylococcus aureus* and *Pseudomonas aerugenosa. Actinomyces spp* inhibited all the pathogens at different rate. Fungal isolate, *Rhizopus spp* inhibited *Staphylococcus aureus* while the other fugal isolate did not inhibit any of the reference pathogens.

CHAPTER ONE

1.1 INTRODUCTION

The term soil refers to the outer loose material of the earth. It may be regarded as a three phase's system content (Davies and Williams, 1999). It is composed of solids, liquids and gases, dispersed to a heterogeneous matrix. The bacteria are the most abundant group usually more numerous compared to the other types of microorganisms. (Nester *et al*, 2009). Soil bacteria can be rod (bacilli), coccoid (spherical) or spirally (spirals). Among these, *Bacillus* is more numerous than the others. They are one of the major groups of soil bacteria and are very widely distributed (Davies and Williams, 1999). The number and type of bacteria present in a particular soil would be greatly influenced by geographical location, and other factors like soil type, soil temperature, soil pH, organic matter contents, cultivation, and aeration and moisture contents.

Life on this planet could not be sustained in the absence of microorganisms that inhabit the soil. Benefits of the presence of such microbes in the soil include improved soil structure, disease suppression and degradation of pollutants, nutrient cycling, and retention of nutrient in root zone and increase in biodiversity.

Actinomycetes are best known for their ability to produce antibiotics. Among Actinomycete, Streptomycetes are the most dominant. Over 5000 antibiotics have been identified from the culture of gram positive, gram negative organisms and filamentous fungi, but only a few have been commercially used to treat human, animal and plant diseases. The genus *Streptomycete* is responsible for the formation of more than 60% of known antibiotics (Williams *et al,* 1993). The trend of search for antibiotics in the past and in recent times as a result of drug resistance by microbial species has required combing the earth for various sources of antibiotics including the soil.

Technically defined, antibiotics include a chemically heterogeneous group of small organic molecules of microbial origin that, at low concentrations, are deleterious to the growth or metabolic activities of other microorganisms. Over the past decade, however, genetic and molecular techniques have been applied to demonstrate conclusively that microorganisms synthesize a variety of antibiotics, even under field conditions, in the rhizosphere i.e. that portion of the soil enriched in carbon and energy resources released by plant roots. The fact that Soil is rich in microorganisms capable of antibiotic synthesis is well accepted, but the frequency with which synthesis this occurs at ecologically significant levels in nature has been much less clear (Brun *et al*, 2000). The beneficial activities of these soil inhabitants far outweigh their detrimental effects.

1.2 AIMS AND OBJECTIVES

This research aims at:

Isolating antibiotic producing organisms from soil samples and determining the organisms that are susceptible to these antibiotics.

OBJECTIVES TO ACHIEVING THESE ARE:

i. Obtaining the colony forming units (CFU) per gram of the soil sample
ii. Identification of genera of the isolated organisms.
iii. Identification of antibiotic producers in the above population.
iv. Assaying for the spectrum of antimicrobial activities of the antibiotics isolated

1.3 ORGANISATION OF THE STUDY

This work is organized into four chapters. The chronology is as follows:

CHAPTER ONE: Introduction, aims and objectives of research work, and organization of study.

CHAPTER TWO: Literature review

CHAPTER THREE: Materials and methods

CHAPTER FOUR: Results and Discussion

CHAPTER FIVE: Conclusion and Recommendation

REFERENCES

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ANTIBIOTICS

Antibiotics are a type of antimicrobial used specifically against bacteria and are often used in medical treatment of bacterial infections. They may either kill or inhibit the growth of bacteria. Several antibiotics are effective against a number of fungi, protozoan and some are toxic to humans and animals, even when given in therapeutic dosage. Antibiotics are not effective against viruses such as the common cold or influenza, and may be dangerous when taken inappropriately.

The discovery of antibiotics in the 20^{th} century led to the revolution of medicine and in combination with vaccination, it has led to the near eradication of diseases such as tuberculosis in the developed world. Their effectiveness and easy access led to overuse especially in livestock raising, thereby enabling bacteria to develop resistance.

Sometimes the term antibiotic is used to refer to any substance used against microbes, synonymous to antimicrobial. Some sources distinguish between antibacterial and antibiotic, with antimicrobials used in soaps and cleaners e.t.c., but not as medicine.

Naturally soil is rich in microorganisms capable of antibiotic synthesis but the frequency with which synthesis occurs at ecologically significant levels in has been much less clear.

2.1.1 INDISCRIMINATE USE OF ANTIBIOTICS; A MAJOR SOURCE OF RESISTANCE

According to Nikaido (2011)100,000 tons of antibiotics are produced annually, which are used in agriculture, food, and health. Their use has impacted populations of bacteria, inducing antibiotics resistance. This resistance may be due to genetic changes such as mutation or acquisition of resistance genes through horizontal transfer, which most often occurs in organisms of different taxonomy (Aminov, 2011). Mutations can cause changes at the site of drug action, hindering the action of the antibiotic (Andersson and Hughes, 2011). Most of the resistance genes are in the same cluster as the antibiotic biosynthesis gene (Allen *et al*, 2011). In nature, the main function of antibiotics is to inhibit competitors, which are induced to inactivate these compounds by chemical modification (hydrolysis), and changes in the site of action and membrane permeability(Garza-Ramos *et al,* 2011). A study carried out with *Streptomyces* from urban soil showed that most strains are resistant to multiple antibiotics, suggesting that these genes are frequent in this environment (Nikaido, 2011). Many resistance genes are located on plasmids (plasmid A), which can be passed by conjugation to a susceptible strain; these plasmids are stable and can express the resistance gene (Wright, 2011). The susceptibility to a particular antibiotic can be affected by the physiological state of the bacteria, and the concentration of the antibiotic; this may be observed in biofilms through a mechanism known as persisted formation - small subpopulations of bacteria survive the lethal concentration of antibiotic without any specific resistance mechanisms, although this mechanism does not produce high-level resistance (Sheng *et al*, 2011).

Microorganisms growing in a biofilm are associated with chronic and recurrent human infections and are resistant to antimicrobial agents(Hassan *et al,* 2011).The spread of resistant strains is not only linked to antibiotic use, but also to the migration of people, who disperse

resistant strains among people in remote communities where the use of antibiotics is very limited (Allen *et al*, 2011).Due to the difficulty of obtaining new antibiotics, the drug industry has made changes to existing antibiotics; these semi-synthetics are more efficient and less susceptible to inactivation by enzymes that cause resistance. This practice has become the strategy for the current antibiotics used today and is known as the second, third, and fourth generation of antibiotics.

The world's demand for antibacterials (antibiotics) is steadily growing. Since their discovery in the 20th century, antibiotics have substantially reduced the threat of infectious diseases. The use of these "miracle drugs", combined with improvements in sanitation, housing, food, and the advent of mass immunization programs, led to a dramatic drop in deaths from diseases that were once widespread and often fatal. Over the years, antibiotics have saved lives and eased the suffering of millions. By keeping many serious infectious diseases under control, these drugs also contributed to the increase in life expectancy during the latter part of the 20th century.

The increasing resistance of pathogenic organisms, leading to severe forms of infection that are difficult to treat, has further complicated the situation, as in the case of carbapenem-resistant *Klebsiella pneumoniae*, and other microorganisms. Infections caused by resistant bacteria do not respond to treatment, resulting in prolonged illness and greater risk of death. Treatment failures also lead to long periods of infectivity with high rates of resistance, which increase the number of infected people circulating in the community and thus expose the population to the risk of contracting a multidrug-resistant strain.

As bacteria become resistant to first generation antibiotics, treatment has to be changed to second or third generation drugs, which are often much more expensive and sometimes toxic. For example, the drug needed to treat multi-drug resistant *Streptococcus pneumoniae*,

Staphylococcus aureus, *Klebsiella pneumoniae*, and *Mycobacterium tuberculosis*, can cost 100 times more than first generation drugs used to treat non-resistant forms. Most worrisome is that resistance to virtually all antibiotics has increased.

Even though the pharmaceutical industry has intensified efforts to develop new drugs to replace those in use, current trends suggest that some infections will have no effective therapies within the next ten years. The use of antibiotics is the critical factor in the selection of resistance. Paradoxically, underuse through lack of access and inadequate treatment may play a role as important as overuse. For these reasons, proper use is a priority to prevent the emergence and spread of bacterial resistance. Patient-related factors are the main causes of inappropriate use of antibiotics. For example, many patients believe that new and expensive drugs are more effective than older drugs.

In addition to causing unnecessary expenditure, this perception encourages the selection of resistance to these new drugs, as well as to the older drugs in their class. Self-medication with antibiotics is another important factor that contributes to resistance, because patients may not take adequate amounts of the drug. In many developing countries, antibiotics are purchased in single doses and taken only until the patient feels better, which may occur before the bacteria is eliminated.

Physicians can be pressured to prescribe antibiotics to meet patient expectations, even in the absence of appropriate indications, or by manufacturers' influence. Some doctors tend to prescribe antibiotics to cure viral infections, making them ineffective against other infections. In some cultural contexts, antibiotics administered by injection are considered more effective than oral formulations. Hospitals are a critical component of the problem of antimicrobial resistance worldwide. The combination of highly susceptible patients, patients with serious infections, and intense and prolonged use of antibiotics has resulted in highly resistant

nosocomial infections, which are difficult to control, making eradication of the pathogen expensive.

In September 2001, the World Health Organization (WHO) launched the first global strategy to combat the serious problems caused by the emergence and spread of antimicrobial resistance. Known as the WHO Global Strategy for the Containment of Antimicrobial Resistance, the strategy recognizes that antimicrobial resistance is a global problem that must be addressed in all countries. No nation, however effective, can close its border to resistant bacteria, thus proper control is required in all places. Much of the responsibility lies with national governments, with a strategy and particular attention to interventions that involve the introduction of legislation and policies governing the development, licensing, distribution, and sale of antibiotics.

Finding new antibiotics that are effective against bacterial resistance is not impossible, but it is a complex and challenging area of research. It is also an area that has not been the primary focus of the pharmaceutical industry in recent years, because antibiotics generally represent a relatively low return of investment, and the high standards for drug development are also factors that influence this lack of interest.

Despite the expected growth trends for the global market of antibiotics, their long-term success is primarily influenced by two main factors - resistance and generic competition. Antibiotic resistance forces reduction in use. The increase in antibiotic resistance makes curing infections difficult. A major disadvantage is the difficulty of the industry to find new antibiotics - those in use are generally ongoing modifications to produce new forms. Despite the advantages large companies have in the development of new antibiotics: a) well-defined targets, b) mode of research effectively established, c) biomarkers for monitoring, d) sophisticated tools to study dosing, and e) faster approval by regulatory agencies, they have

given priority to other diseases, because the return on investment for antibiotics is low, despite representing a market of $ 45 billion, second only to drugs for cardiovascular problems and central nervous system. Another problem is competition from generics at far lower prices. In some cases the large companies have transferred the responsibility to small businesses to develop new antibiotics, such as daptomycin, developed by Cubist and licensed to Lilly.

2.1.2GENOME AND NEW ANTIBIOTICS

With the availability of genomes from a large number of pathogens, hundreds of genes have been evaluated as targets for new antibiotics. A gene is recognized as essential when the bacterium cannot survive while the gene is inactive, and can become a target when a small molecule can alter its activity (Simmons *et al,* 2011). Genetic analysis has shown that a gene may encode a function that is important in one bacterium but not in another.167 genes have been determined as essential for bacterial growth and are potential targets for new antibiotics (Freiberg and Brötz-Oesterhelt, 2011). GlaxoSmithKline has conducted studies with the antibiotic GKS299423 acting on topoisomerase II, in order to prevent the bacteria from developing resistance (Jones, 2010).

2.2 SOIL

Soil is the mixture of minerals, organic matter, gases, liquids, and countless organisms that together support life on earth. It is a natural body known as pedosphere and performs four important functions; it is a medium for plant growth, it is a modifier of atmosphere of earth, it is a means of water storage, supply and purification; it is a habitat for organisms; all of which in turn, modifies the soil.

2.3 ANTIBIOTIC PRODUCING MICROORGANISMS

In the beginning of 20th century, the idea of growth inhibition of one microorganism present in the vicinity of other one came into existence. Later, it was demonstrated that growth inhibition of the former micro- organism was mediated by secretion of toxic metabolites by the microorganisms. This toxic metabolite was termed as 'antibiotic' and the phenomenon of act of growth inhibition by antibiotics as 'antibiosis. The antibiotics are defined as "the complex chemical substances, the secondary metabolites which are produced by microorganisms and act against other microorganisms". In nature, there is universal distribution of antibiosis among the microorganisms owing to which they are involved in antagonism. Those microorganisms which have capacity to produce more antibiotics can survive for longer time than the others producing antibiotics in less amount. However, antibiotics produced by microorganisms have been very useful for the cure of certain human diseases caused by bacteria, fungi and protozoa. Due to continuous endeavour made in this field, the antibiotics discovered at present are about 5,500. Total world production of antibiotics is more than one million tons per annum. This success has been possible only due to continuous researches made during the last 4 decades

Soil contains myraids of microorganisms, including bacteria, fungi, protozoa, algae, and viruses. The most prevalent are bacteria, including the moldlike actiomycetes, and fungi.

BACTERIA: Bacteria are microorganisms that live in the water-filled pore spaces within and between soil aggregates. They include predominantly members of the orders Pseudomonadales and Eubacteriales

ACTINOMYCETES: Predominantly members of the genus Streptomyces; characterized by pleomorphism and filamentous structure

FUNGI: Predominantly members of the zygomecetes (Rhyzopus, Mucor, and Absida) anddeuteromycetes (*Pencillium, Aspergilus, Alternaria, Stemphylium, and Cladosporium*).

2.3.1 ANTIBIOTIC PRODUCING BACTERIA

Antibacterials are a type of antimicrobial used specifically against bacteria and are often used in medical treatment of bacterial infections. They may either kill or inhibit the growth of bacteria. Examples include:

Bacillus

Bacillus species of the family *Bacillaceae* is the largest in the order. The genus contain gram positive, endospore forming, chemotherotrophic rod that are usually motile with peritrichious flagella, it is aerobic and catalase positive. Many species of Bacillus are of considerable importance because they produced the antibiotic (Waites *et al*, 2008). Bacillus species produces many kinds of antibiotics which share full range antimicrobial activity such as bacitracin which is produce by *Bacillus licheniformis* is a mixture of at least 5 polypeptides. These antibiotics consist of 3 separate compounds, bacitracin A, B and C. Bacitracin A is the chief constituent. It is active against many kind of many Gram positive organisms such as *Stapylococci, Streptococci*, anaerobic *cocci, Cornyebacter* and *Clostridia* but not against most other Gram negative gram bacteria (McEvoy, 1993).

Gramicidin which is produced by *Bacillus brevis*, a linear polypeptide antibiotic mixture of gramicidin A, B, C and D. Gramicidin D, a channel forming ionophore that flip-flop slowly across the membrane and surprisingly was found to inhibit phosphate group ATPase species for example *B. thuringiensis* and *B. sphaericus* term a solid protein crystal, the paraporal body next to their endospores during spore formation of *B. thuringiensis* parasporal body contain protein toxin that kill over species of moths by dissolving in the alkaline gut of

caterpillars and destroying the epithelium. The *B.sphaericus* parasporal body contains protein toxic a for mosquito larvae and may be useful in controlling them to make mosquito that carry macana (Waites *et al*, 2008).

2.3.2 ANTIBIOTIC PRODUCING FUNGI

Penicillium

The antibacterial effect of *penicillin* was discovered by Alexander Fleming in 1929. He noted that a fungal colony had grown as a contaminant on an agar plate streaked with the bacterium *Staphylococcus aureus* , and that the bacterial colonies around the fungus were transparent, because their cells were lysing. Fleming had devoted much of his career to finding methods for treating wound infections, and immediately recognised the importance of a fungal metabolite that might be used to control bacteria. The substance was named penicillin, because the fungal contaminant was identified as *Penicillium notatum*. Fleming found that it was effective against many Gram positive bacteria in laboratory conditions, and he even used locally applied, crude preparations of this substance, from culture filtrates, to control eye infections. However, he could not purify this compound because of its instability, and it was not until the period of the Second World War (1939-1945) that two other British scientists, Florey and Chain, working in the USA, managed to produce the antibiotic on an industrial scale for widespread use. All three scientists shared the Nobel Prize for this work, and rightly so - penicillin rapidly became the "wonder drug" which saved literally millions of lives. It is still a "front line" antibiotic, in common use for some bacterial infections although the development of penicillin-resistance in several pathogenic bacteria now limits its effectiveness.

Penicillin has an interesting mode of action: it prevents the cross-linking of small peptide chains in peptidoglycan, the main wall polymer of bacteria. Pre-existing cells are unaffected,

but all newly produced cells grow abnormally, unable to maintain their wall rigidity, and they are susceptible to osmotic lysis. This morphogenetic effect of penicillin can be demonstrated by growing either Gram-positive or Gram-negative bacteria in the presence of sub-lethal concentrations of penicillin. By affecting the cross-linking of the bacterial cell wall, penicillin has caused the bacterium to grow as larger cells with less frequent cell divisions. Penicillin is not a single compound but a group of closely related compounds, all with the same basic ring-like structure (a beta-lactam) derived from two aminoacids (valine and cysteine) via a tripeptide intermediate. The third amino acid of this tripeptide is replaced by an acyl group, and the nature of this acyl group confers specific properties on different types of penicillin. The two natural penicillins obtained from culture filtrates of *Penicillium notatum* or the closely related species *P. chrysogenum* are penicillin G and the more acid-resistant penicillin V. They are active only against Gram-positive bacteria (which have a thick layer of peptidoglycan in the wall) and not against Gram-negative species, including many serious pathogens like *Mycobacterium tuberculosis* (the cause of tuberculosis). Nevertheless, the natural penicillins were extremely valuable for treating wound pathogens such as Staphylococcus in wartime Europe. An expanded role for the penicillins came from the discovery that natural penicillins can be modified chemically by removing the acyl group to leave 6-aminopenicillanic acid and then adding acyl groups that confer new properties. These modern semi-synthetic penicillins such as Ampicillin, Carbenicillin and Oxacillin have various specific properties such as: resistance to stomach acids so that they can be taken orally, a degree of resistance to penicillinase (a penicillin-destroying enzyme produced by some bacteria) extended range of activity against some Gram-negative bacteria. Although the penicillins are still used clinically, their value has been diminished by the widespread development of resistance among target microorganisms and also by some people's allergic reaction to penicillin.

Apart from penicillin, the most important antibiotics from fungi are the cephalosporins (beta-lactams with similar mode of action to penicillin, but with less allergenicity) and griseofulvin (from Penicillium griseofulvum and related species) which is used to treat althlete's foot and related fungal infections of the skin.

2.3.3 ANTIBIOTIC PRODUCING ACTINOMYCETE

The phenomenal success of penicillin led to the search for other antibiotic-producing microorganisms, especially from soil environments. One of the early successes (1943) was the discovery of streptomycin from a soil actinomycete, *Streptomyces griseus*. Actinomycetes are bacteria that produce branching filaments rather like fungal hyphae, but only about 1 micrometre diameter. They also produce large numbers of dry, powdery spores from their aerial hyphae. *Actinomycetes*, especially *Streptomyces* species, have yielded most of the antibiotics used in clinical medicine today.

Actinomycetes are gram-positive, free-living, saprophytic bacteria, widely distributed in soil, water, and colonizing plants (Oskay *et al*, 2004). From the 22,500 biologically active compounds that have obtained from microbes, 45% are produced by actinomycete, 38% by fungi, and 17% by unicellular bacteria (Pandey *et al,* 2011). The species belong to the genus Streptomyces constitute 50% of the total population of soil actinomycetes and are well known for producing a variety of bioactive secondary metabolites including antibiotics, immune modulators, anticancer drugs, antiviral drugs, herbicides, and insecticides. Streptomyces species are gram-positive, aerobic microorganisms with high DNA G+C contents and produce about half of all known antibiotics from microorganisms. In fact, Streptomyces species are the resource of 75% of commercially produced and medically useful antibiotics (Wiley *et al*, 2008).

Although thousands of antibiotics have been isolated from Streptomyces, these represent only a small fraction of the repertoire of bioactive compounds produced. Therefore, isolation of new Streptomyces from natural resources and characterization of their secondary metabolites is a valuable endeavour.

2.2.3.1 Morphological characteristics of *Streptomyces*

Streptomyces is a genus of Gram-positive bacteria that grows in various environments, with a filamentous form similar to fungi. The morphological differentiation of Streptomyces involves the formation of a layer of hyphae that can differentiate into a chain of spores. This process is unique among Gram-positives, requiring a specialized and coordinated metabolism. The most interesting property of *Streptomyces* is the ability to produce bioactive secondary metabolites such as antifungals, antivirals, antitumoral, anti-hypertensives, and mainly antibiotics and immunosuppressives (Omura *et al* , 2011). Another characteristic is of the genus is complex multicellular development, in which their germinating spores form hyphae, with multinuclear aerial mycelium, which forms septa at regular intervals, creating a chain of uninucleated spores. (Ishikawa and Ohnishi, 2011).

When a spore finds favourable conditions of temperature, nutrients, and moisture, the germtube is formed and the hypha develops. The aerial hypha follows, and a stage set initiates the organization of various processes such as growth and cell cycle. Esporogenic cell may contain 50 or more copies of the chromosome; the order, position, and segregation of chromosomes during sporulation is linear, which involves at least two systems (ParAB and FtsK), which lead to differentiation and septation of apical cells into chains of spores. Several other genes that are essential for the sporulation of aerial hyphae have been reported in *S. coelicolor*, for example, the genes *whiG, whiH, whiI, whiA, whiB*, and *whiD.* The explanation

for the presence of spores in *Streptomyces* is probably that these fragments appeared mycelia under selective pressure, which might involve the need to survive outside of plants and invertebrates, or in extreme environments.

The ability of the spores to survive in these hostile environments must have been increased due to the pigment and aroma present in the spores in some species, (Chater and Chandra, 2011) which stimulates cell development and secondary metabolite production (Chi *et al*, 2011). Another important point is the tip of the hypha, which is considered to be the most important region where membrane proteins and lipids may be secreted, especially in the apical area of growth. In some *Streptomyces*, secondary metabolism and differentiation can be related. (ou *et al*, 2011, li *et al,* 2011). Phylogenetically, *Streptomyces* are a part of Actinobacteria, a group of Gram-positives whose genetic material (DNA) is GC-rich (70%) when compared with other bacteria such as *Escherichia coli* (50%). The great importance given to *Streptomyces* is partly because these are among the most numerous and most versatile soil microorganisms, given their large metabolite production rate and their biotransformation processes, their capability of degrading lignocellulose and chitin, and their fundamental role in biological cycles of organic matter. (Bentley, 2011). Two species of *Streptomyces* have been particularly well studied: *S. griseus*, the first *Streptomyces* to be used for industrial production of an antibiotic - streptomycin, and *S. coelicolor*, the most widely used in genetic studies. Various strains have been sequenced and their genomes have been mapped.

Table 1.0: Antibiotics, organisms that produce them and their modes of action.

ANTIBIOTIC	SOURCE ORGANISM	ACTIVITY SITE OR MODE OF ACTION
Penicillin	*Penicillium chrysogenium*	Gram-positive bacteria Wall synthesis
Cephalosporin	*Cephalosporium acremonium*	Broad spectrum Wall synthesis
Bacitracin	*Penicillium griseolfulvum*	Dermatophytic fungi Microtubules
Polymyxin B	*Bacillus polymyxa*	Gram-negative bacteria Cell membrane
Amphotericin B	*Streptomyce s nodosus*	Fungi Cell membrane
Erythromycin	*Streptomyces erythreus*	Gram-positive bacteria Protein synthesis
Neomycin	*Streptomyces fradiaes*	Broad spectrum Protein synthesis
Streptomycin	*Streptomyces griseus*	Gram-negative bacteria Protein synthesis
Tetracycline	*Streptomyces rimosus*	Broad spectrum Protein synthesis
Vancomycin	*Streptomyces orientalis*	Gram-positive bacteria Protein synthesis
Gentamycin	*Micromonospora purpurea*	Broad spectrum Protein synthesis
Rifamycin	*Streptomyces mediterranei*	Tuberculosis Protein synthesis

Source:Archive.bio.ed.ac.uk/jdeacon/microbes/penicill.htm, 24th March, 2015.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was carried out in Federal University Oye Ekiti. Soil samples were collected in faculty of science, faculty of social science, daycare, and the administrative building. The inhabitants of these areas include mainly students, staffs, and farmers that have their farms situated within the school premises.

3.2 Identification of sampling points

A total of eight(8) sampling points were selected for the study. A detailed table is given below:

Table 2.0: Sampling point, sample type, physical properties and pH of each sample type.

Sample stations	Sampling point code	Type of sample	Colour	Texture	pH	Moisture Content(g)
Faculty of Science	A	Clay soil	Reddish brown	Grainy	7.10	9.61
School Auditorium	B	Clay soil	Reddish brown	Grainy	7.00	9.63
Administrative Building	C	Loamy soil	Blackish brown	Grainy and rough	9.81	43.0
Faculty of Science	D	Loamy soil	Blackish brown	Grainy and rough	9.79	43.3
Faculty of Social Science	E	Sandy soil	Light brown	Smooth	3.27	62.5
Faculty of Science	F	Sandy soil	Light brown	Smooth	3.20	46.3
Dumping sites of Faculty of Science and Daycare Centre	G(F/Sc)		Dark brown	Smooth and grainy	7.71	19.9
	H(D/C)		Light brown	Smooth and grainy	7.69	23.7

3.3 Principle: Microorganisms are omnipresent and always exist in a competitive environment. During their metabolism, they produce many types of secondary metabolites which inhibit the growth of the surrounding strains. In such a competitive environment, most microorganisms produce antibiotics to maintain their predominance.

3.4 Materials:

The following materials were used Sterile Petri plates, Sterile test tubes and test tube rack, spatula, sterile distilled water, foil paper, cotton wool, syringes(5ml and 2ml), digital weighing balance, paper tape, inoculating loop, Bunsen burner, grease free slide, cover slip, microscope, filter paper, autoclave, sterile hand gloves, micropipette, sterile nose mask, sterile hand trowel.

3.4.1 Chemical reagents used

Glycerol, Ethanol (70%).

3.4.2 Media used

Nutrient agar, Glycerol yeast extract agar, Saboraud dextrose agar and McConkey agar.

3.5 Collection of soil samples

3.5.1 Soil samples

From respective locations, soil samples were collected from a depth of 0-10cm using a sterile trowel. About 50g of each soil samples collected from each site were placed in sterile labelled plastic bottles and transported immediately to the laboratory for further tests.

3.5.2 Procedure for sample preparation

After collecting the samples, analysis was carried out immediately. A setup for serial dilution was done in which each sample was serially diluted to a dilution factor of five (10^{-5}). Before

serial dilution, the test tubes were sterilized after which 9ml of distilled water were added to each tube (six tubes altogether labelled stock, $10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and\ 10^{-5}$ respectively) in a sterile environment. Serial dilution was performed for the eight soil samples collected.

1g of each soil sample was inoculated into the tube labelled stock , 1ml of the stock solution was then inoculated into the 10^{-1}dilution factor and mixed thoroughly to homogenize the solution, after which 1ml of each aliquots was inoculated from the latter into the 10^{-2} and this process is carried out till the 10^{-5} dilution factor for the eight soil samples.

After the serial dilution, 1ml from each of 10^{-3} and 10^{-5} dilution factor were inoculated into six plates each (two each for bacteria, fungi and actinomycete culture).

Table 3.0: Sample preparation

Sample site	Sample code	Plate poured	Total
School Auditorium(clay)	**A**	**6**	**12**
Administrative Building(clay)	**B**	**6**	**12**
Faculty of Science (loamy)	**C**	**6**	**12**
Faculty of social Science (loamy)	**D**	**6**	**12**
Faculty of Social Science(sandy)	**E**	**6**	**12**
Faculty of Social Science(sandy)	**F**	**6**	**12**
Dumping site of Faculty of science	**G**	**6**	**12**
Dumping site of Daycare centre	**H**	**6**	**12**
TOTAL		**48**	**96**

3.6 Media preparation and sterilization

3.6.1 Nutrient agar

Using the manufacturer's specifications (28g per 1000ml of distilled water), nutrient agar was prepared and sterilized in the autoclave for the purpose of obtaining and isolating a pure culture.

3.6.2 Sabouraud dextrose agar

SDA was prepared using the manufacturer's specification (65g in 1000ml of distilled water) and sterilized.

3.6.3 Glycerol yeast extract agar

Under asceptic conditions, glycerol yeast extract was composed using the yeast extract medium and glycerol. The required specification for glycerol yeast extract agar include yeast extract (23g per 1000ml) and glycerol (5ml per 1000ml).

3.6.4 McConkey agar

Under aseptic conditions, McConkey agar was prepared using the manufacturer's specification (48.5g in 1000ml of distilled water) after which it was sterilized in an autoclave.

3.7 Microbiological analysis

The bacterial isolates were identified and characterized using standard biochemical tests (Cheesebrough, 2006). The tests employed include gram stain, motility, colonial morphological characteristics, catalase, methyl red, Voges-Proskaeur, urease reduction, starch hydrolysis. The fungal isolates were identified according to Oyeleke and Okunsami (2008) based on the colour of aerial hyphae, conidial arrangement as well as morphology. The same biochemical tests for bacterial isolates were carried out to identify and characterize actinomycete isolates.

From the dilution of the samples, 1ml aliquot was transferred aseptically into the respective plates and about 15ml of freshly prepared nutrient agar medium was pour plated with the aliquot and allowed to solidify at room temperature. The inoculated plates were incubated at 37°C for 24 hours. The same process was carried out for actinomycete using glycerol yeast extract agar and incubated for 72hours at the same temperature.

The fungal plates were pour plated using a treated sabouraud dextrose agar medium. The plates were then incubated at 25°C for 72 hours.

3.7.1 Enumeration of mixed colonies of microbes from soil samples

After speculate time for the growth of bacteria, fungi and actinomycete, a mixed colony of isolates were observed after which the respective plates were further examined morphologically and individual colonies were subcultured for further tests.

3.7.2 Pure culturing

After morphological observation, the mixed colonies were further subcultured to get pure strains of bacteria, fungal and actinomycete isolates by inoculating each individual colony into a fresh agar respectively in a sterile environment.

Distinct colonies of the bacterial isolates growing on the nutrient agar medium was inoculated into a freshly prepared nutrient agar medium and McConkey agar medium respectively. Also distinct colonies of fungal isolates from the sabouraud dextrose medium were also inoculated into a freshly prepared sabouraud dextrose medium.

Cultural characteristics of the discrete bacterial colonies such as colours, pigmentation and opacity were observed. This was followed by characterization of the isolates that included microscope examination for cell morphology, gram reaction and biochemical tests on 24 hours old pure cultures as well as fungal and actinomycete isolates.

3.7.3 Preservation of isolated microorganisms

Agar was prepared by pouring10ml of molten agar into clean, sterile McCartney bottles and sterilized with the autoclave at 121°C for 15 minutes. The McCartney bottles were allowed to cool and solidify in a slant/sloppy position. After cooling to 40°C, the McCartney bottles were inoculated with each colony, with a sterilized inoculating loop. The streaking method was used to introduce the inoculums on the agar in the McCartney bottles. The inoculum was inoculated aseptically. The culture samples were incubated for 24 hours at a temperature of 37°C. The same process was carried out to prepare slant for preserving the fungi and actinomycete using the sabouraud dextrose and glycerol yeast extract medium respectively. Fungi cultures were incubated at a temperature of 25°C for 72hours while actinomycete were incubated at a temperature of 37°C for 72 hours. After growth was observed on the slants of fungi, bacteria and actinomycete, it was preserved in the refrigerator at a temperature of 4°C.

3.8 Identification of isolated Bacteria and Actinomycete isolate

Several methods were employed in the identification of isolated microorganisms. Below are the various methods:

3.8.1 Gram staining

This is the most important and frequently used technique in bacteriology. It helps in the differentiation of bacteria into two main groups namely gram positive and gram negative bacteria. The 24 hour isolate was smeared on a clean grease free slide. It was prepared by placing a drop of sterile water in the middle of the slide. Then the inoculating loop was sterilized by flaming and then left to cool. The bacteria was then picked with the already sterile loop and placed on the drop of water on the slide and left to emulsify. The smear was then heat-fixed by passing through the flame thrice. The inoculating loop was then flamed to make it sterile again. The same process was performed for the other bacterial isolates.

After heat fixing, a drop of crystal violet was placed on it and left to stand for 60 seconds after which it was washed away with water. Then a drop of iodine was placed on it and it was left to stand for 60 seconds, it was then washed away with water, after which a drop of ethanol was placed on it and left to stand for 30 seconds, it was then washed away with water. Then a drop of safranin was placed on the smear and left to stand for 60 seconds. The stained slides were then left to air dry.

The prepared slides were then examined by placing a drop of oil immersion on it and covering it with a cover slip after which it was viewed under the oil immersion lens (×100 objective lens).

3.8.2 Staining for Bacteria spores

Spores are produced per bacterial cells. Certain bacteria produce endospores; members of the genera are *Bacillus*, *Clostridium* e.t.c.

The test was carried out by heat fixing the bacterial smear and passing slide through the flame three times. The slides were then flooded with malachite green stain and heated over a beaker of boiling water for ten minutes but avoided from drying by adding more stain occasionally. They were then washed under tap water and flooded with safranin for 20 seconds, and then they were washed away with tap water and air dried. They were examined under the oil immersion lens.

3.9 Biochemical tests for Bacterial and Actinomycete isolates

3.9.1 Motility test

This is a test used for identifying motile microbes that possess flagella. A drop of oil immersion was placed around the edge of the depression of the slide, and a loopful of the culture was transferred to the centre of the clean dry coverslip placed on a flat white slab. The cavity slide was then inverted over the cover slip. Quickly and carefully, the slide was inverted and the culture drop appears hanging. The culture was examined immediately for motility under the microscope by reducing the light to reduce heating effect. The low power objective was used to focus the edge of the drop after which, the high power objective was used to get a fine view.

3.9.2 Sugar fermentation test

This test determines the ability of a bacterium to ferment the sugar glucose as well as its ability to convert end products (pyruvic acid) into gaseous byproducts.

Nutrient broth containing glucose, inositol, arabinose, xylose, lactose, maltose, mannitol and sorbitol were prepared in test tubes, with inverted durham tubes sterilized in the autoclave. After sterilization and cooling, the unknown cultures were inoculated into the indicator – sugar-broth, and one tube was left uninoculated to serve as control. The test tubes were incubated at 35°C for 2-5 days observed for acid and gas production and the results recorded.

3.9.3 Starch hydrolysis

Most bacteria possess amylase (enzyme), which can hydrolyze complex molecules of starch to sugars. The starch-agar plates were inoculated with a single streak of the respective unknown culture and it was incubated at 35°C for 48 hours. The plate was then flooded with

iodine solution and was observed. The presence of clear zones around the colony indicates a positive result.

3.9.4 Methyl-red test (MR test)

Methyl red is an indicator of low pH (red below pH of 4.4), used to show the mixed acid fermentation ability of bacteria. Test tubes of MR-VP broth were prepared: the test tubes were inoculated with inoculum of the unknown bacterial culture, while one test tube was left inoculated to serve as control. The test tubes were incubated at 35°C for 48 after which methyl red was added, if the colour remains red, it indicates a positive result while if it changes to yellow it indicates a negative result. This process was carried out for each bacterial isolate.

3.9.5 Nitrate reduction test

This test demonstrates the ability of a bacterium to produce the enzyme nitratase, capable of converting nitrate to nitrite. Seven test tubes of nitrate peptone water were prepared in which the unknown bacteria culture were inoculated and this was repeated for other unknown culture. One was left to serve as control. The test tubes were incubated for 3 days at 35°C respectively. Two tablets of nitrate tablets were then added to the sample. A deep red colour indicates positive result.

3.9.6 Catalase test

The enzyme catalase acts as a catalyst in the breakdown of hydrogen peroxide and water. Catalase test was used to know the specie of a particular bacterium that relates with the hydrogen peroxide to produce oxygen and water. A Drop of 3% hydrogen peroxide was applied on a loopful of the colony of the organisms that was 24 hours culture, which was placed on a slide. The presence of catalase is shown by the formation of gas bubbles.

3.9.7 Urease production test

This test demonstrates the ability of a bacterium to produce the enzyme urease, capable of hydrolyzing urea. Phenol red indicator is added (fuchsia above pH 8.4) to show rise in pH due to accumulation of ammonia.

In this test, four slants of urea agar medium were prepared for each isolate with two serving as the control basal medium that lacks urea. Two of the slants were inoculated with the unknown bacteria culture, while the two controls were inoculated with the same unknown bacteria culture media. All slants were incubated at 35°C for 5 days and were examined daily for colour change.

3.9.8 Voges Proskauer test

This test is used to show bacterial production of acetoin, also known as 2, 3-butanediol. MR-VP broth was prepared in test tubes and organisms were inoculated in each tube and incubated at a temperature of 35°C for 48 hours after which they were observed. A Cheryl red colour indicated a positive result while negative results were yellow-brown colour.

3.10 Staining for fungal hyphae

Lactophenol cotton blue is the popular stain used in staining fungal hyphae. This stains the cytoplasm light blue and it is used in mounting fungal specimens.

A drop of lactophenol cotton blue was placed on a clean grease free slide using a sterile needle. Thereafter a small piece of mycelium free of medium was picked and transferred to the stain on the slide and then covered carefully with a cover slip to avoid bubbles. The slides were then observed under the low power and the high power objectives of the microscope and observation were recorded.

3.11 Assay of bacterial, fungal and actinomycete isolated from soil sample

After identifying the microorganisms isolated from the soil, they were then inoculated against the selected pathogenic organisms. The pathogens used were: *Pseudomonas auregenosa, Staphylococcus aureus* and *Candida albicans.*

3.11.1 Agar well method of antibiotic assay

Nutrient agar was prepared, sterilized and poured into petriplates under aseptic conditions in a laminar air flow. The petriplates were then left to solidify. Then the wells were made at selected areas in the petriplates. Then pathogenic organisms were swabbed on separate Petri plates with a sterile swab. The petriplates were left for a while. The isolates were inoculated in broth for 72 hours, 0.5 micro litres of each isolate in broth was taken using a micropipette and loaded into the wells. Then the petriplates were incubated overnight without inverting at optimum temperature of 37°C for 24 hours. Strains showing the more inhibition were selected and checked for the increasing concentrations of the antibiotic with effect to the increasing concentrations of the sodium nitrate. Agar well method was also carried out for fungal and actinomycete isolates.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.2 Bacteria and Fungi

The results of the colony forming units and spore forming units of the bacteria and fungi isolates are shown in table 4.0, while plates 1 and 2 show the growth of bacteria generally and as pure culture.

Table 5 shows the morphological description, biochemical test results as well as the probable bacteria isolates while plate 3 shows the zone of inhibition of *Bacillus* isolate against *Staphylococcus aureus.* Table 6.0 shows zones of inhibition of bacteria isolates while Figure 1 also compares the zones of inhibition of suspected bacterial antibiotic producers.

Table 7.0 shows macroscopy and microscopy of fungal isolates while table 8.0 shows the zones of inhibition of fungal isolates against test organisms.

Table 4.0: The result of colony forming unit and spore forming unit obtained from the experiment carried out for isolation of bacteria and fungi isolates from clay, loamy and sandy soils respectively.

Sites	Shape (bacteria)	Color (bacteria)	Texture (bacteria)	Optical characterization (bacteria)	Elevation. (bacteria)	Bacteria Cfu/ml	Fungi Sfu/ml
School auditorium	**Round**	**White**	**Rough**	**Opaque**	**Flat**	**2.5×10^4**	**1.0×10^4**
Administrative building	**Circular**	**Golden yellow**	**smooth**	**Opaque**	**Flat**	**3.0×10^4**	**1.2×10^4**
Faculty of science	**Irregular**	**Pale**	**smooth**	**Transparent**	**Convex**	**3.5×10^4**	**1.0×10^4**
Faculty of social science	**Round**	**Bluish green**	**Rough**	**Transparent**	**Flat**	**4.0×10^4**	**8×10^4**
Faculty of social science	**Irregular**	**Light yellow**	**Rough**	**Opaque**	**Elevated**	**2.5×10^4**	**9×10^4**
Faculty of science	**Circular**	**Yellow**	**Rough**	**Transparent**	**Raised**	**1.0×10^4**	**1.0×10^4**
Dumping site of faculty of science	**Irregular**	**Yellow**	**Rough**	**Transparent**	**Raised**	**3.5×10^4**	**1.2×10^4**
Dumping site of Daycare centre	**Round**	**Whitish**	**Glossy**	**Transparent**	**Raised**	**2.6×10^4**	**1.3×10^4**

Plate 1: Growth of bacterial culture after 24 hours of incubation.

Plate 2: Pure culture streaking of bacterial colonies.

Table 5.0: Morphological description, Biochemical tests and identification of probable bacteria isolates.

SITES	SA-clay	AB- clay	FS-loamy	FSS-loamy		FSS-sandy	FS-sandy		FSD-dumping site		DCD- dumping site	
Pigmentation												
Nutrient agar	Yellowish green	Pink	White	White	Yellow	White	Bluish green	Yellowish green	Golden yellow	White	Yellow	Golden yellow
McConkey Agar				Deep pink						Deep pink		
Elevation	Flat	Raised	Raised	Raised	Raised	Raised	Flat	Flat	Flat	Raised	Raised	Flat
Surface Texture	Slimy	Slimy	Rough	Glossy	Rough	Rough	Rough	Slimy	Smooth	Glossy	Rough	Smooth
Optical	Transparent	Opaque	Transparent	transparent	Transparent	Transparent	Transparent	Transparent	Opaque	transparent	transparent	Opaque
Gram stain Reaction	–	–	+	–	+	+	+	–	+	–	+	+
Cell shape	Ovoid	Rod	Rod	Rod	Cocci	Rod	Rod	Ovoid	Cocci	Rod	Rod	Cocci
Endospore (spore stain)	–	–	+	–	–	+	+	–	+	–	+	+
Sugar utilization												
Glucose	A	A	AG	AG	AG	AG	A	A	A	AG	AG	A
Inositol	-	-	-	+		-		-		+	-	
Arabinose	-	A	A			A	–	-			A	
Xylose	-	-	A			A		-			A	
Lactose	-	-	AG			AG	–	-	A		AG	A
Maltose	-	-	A	Mostly +	A	A	–	-		Mostly +	A	
Mannitol	-	-	-	+		-	+	-		+	-	
Sorbitol	-	-	A	+		A	–	-		+	A	
Motility	+	–			–	–	+	+	+			+
Nitrate reduction	–	–	+	+	–	+	–	–	+	+	+	+
Starch hydrolysis	–	–	+	+	+	+	+	–	+	+	+	+
Catalase	+	+	+	–	+	+	+	+	+	–	+	+

Methyl red test	–	–	–	+		–	–	–		+	–	
Voges proskauer	–	–	–	–	–	–	–	–		–	–	
Urease Production	–	–	–	–	+	–		–	–	–	–	–
PROBABLE ORGANISMS	*Azomona sagilis*	*Gluconob acteroxyd ans*	*Bacillus spp*	*Esch erich ia coli*	*Micr ococ cu s spp*	*Bacillu s spp*	*Pse dom ona sspp*	*Az om on asa gili s*	*Stap hylo cocc us aure us*	*Esch erich ia coli*	*Bacill us spp*	*Staphyl ococcu s aureus*

KEYS

A/G =Acid and gas production
A/- =acid production only
-/G =Gas production only
-/- =no production of acid and gas
+ = Positive reaction
- =Negative reaction
W= weakly positive
FS= Faculty of Science
FSS= Faculty of Social Science
AB= Administrative Building
SA= School Auditorium
FSD= Faculty of Science dumping site
DCD= Day-care Centre dumping site

Table 6.0: Zones of inhibition of bacteria isolates.

PROBABLE ORGANISMS	ZONES OF INHIBITION AGAINST CLINICAL ISOLATES(mm)		
	Pseudomonas aerugonosa	*Staphylococcus aureus*	*Candida albicans*
Azomonas spp	0	0	0
Gluconobacter spp	0	0	0
Bacillus spp	11	12	0
Escherichia coli	0	0	0
Micrococcus spp	0	0	0
Bacillus spp	13	15	0
Pseudomonas spp	0	0	0
Azomonas spp	0	0	0
Staphylococcus aureus	0	0	0
Escherichia coli	0	0	0
Bacillus spp	12	14	0
Staphylococcus aureus	0	0	0

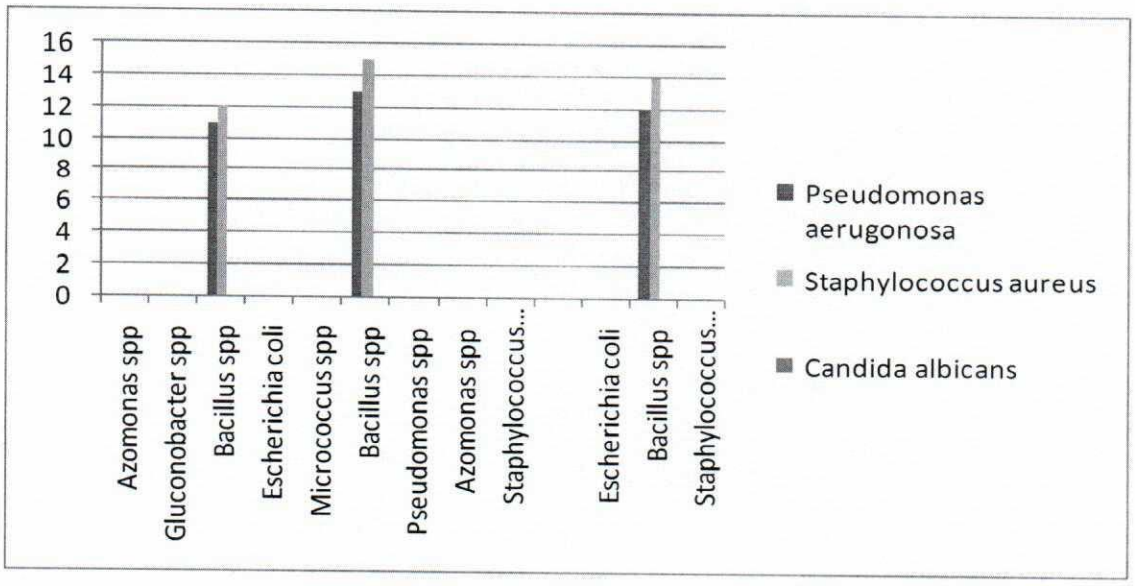

Figure 1: Zones of inhibition (mm) of bacterial isolates against *Staphylococcus aureus*, *Pseudomonas aerugenosa* and *Candida albicans* showing some form of antibiosis.

Plate 3: Zone of inhibition of growth of *Bacillus spp* against *Staphylococcus aureus*

4.2.1 Fungi

Table 7.0: The macroscopy and microscopy of the fungal isolates.

SITES	CULTURAL MORPHOLOGY	MICROSCOPIC OBSERVATION	SUSPECTED ORGANISMS
SA(clay)	Cotton like mycelia growing after 24 hours turning dirty with development of black spores on mycelium. Reverse is pale yellow.	Round, black sporangia are filled with endospores. Unbranched sporangia arising from the stolon.	*Rhizopus spp*
AB(clay)	Green mycelia growth	Septate hyphae with short branched conidiophores, flasked shaped phialides, single celled conidia.	*Trichoderma spp*
AB(sandy)	White cottony aerial mycelium which was white at first but later became yellow	Aseptate mycelium, branched sporangiophores. Globose sporangium present at the tip of the sporangiophore.	*Mucor spp*
	Mucoid like changing colour from orange to pinkish red	Small, oval budding yeast cells	*Rhodotorula spp*
FS(sandy)	White cottony aerial mycelium which was white at first but later became yellow	Aseptate mycelium, branched sporangiophores. Globose sporangium present at the tip of the sporangiophore	*Mucor spp*
FSS(loamy)	Folded velvety colonies in shades of white and green becoming dark with age	Loosely columnar heads, small vesicle, hemispherical and fertile over the upper half, conidiophores are smooth and hyaline	*Aspergillus spp*
FS(loamy)	White cottony aerial mycelium which was white at first but later became yellow	Aseptate mycelium, branched sporangiophores. Globose sporangium present at the tip of the sporangiophore	*Mucor spp*
	Mucoid like changing colour from orange to pinkish red	Small, oval budding yeast cells	*Rhodotorula spp*

FSD(humus)	Green mycelia growth	Septate hyphae with short branched conidiophores, flasked shaped phialides, single celled conidia.	*Trichoderma spp*
FSD(Humus)	Mucoid like changing colour from orange to pinkish red	Small, oval budding yeast cells	*Rhodotorula spp*
DCD(humus)	White cottony aerial mycelium which was white at first but later became yellow	Aseptate mycelium, branched sporangiophores. Globose sporangium present at the tip of the sporangiophore.	*Mucor spp*
	Green mycelia growth	Septate hyphae with short branched conidiophores, flasked shaped phialides, single celled conidia.	*Trichoderma spp*

KEYS
FS=Faculty of Science
FSS=Faculty of Social Science
AB=Administrative Building
SA=School Auditorium
FSD=Faculty of Science Dumping site
DCD=Day-care Centre Dumping site

Table 8.0: Zones of inhibition by fungal isolates against test organisms.

		Zones of inhibition		
Sites	Probable Organisms	*Pseudomonas aerugenosa*	*Staphylococcus aureus*	*Candida albicans*
SA(clay)	*Rhizopus spp*	0	5mm	0
AB(Clay)	*Trichoderma spp*	0	0	0
AB(sandy)	*Mucor spp*	0	0	0
	Rhodotorula spp	0	0	0
FS(sandy)	*Mucor spp*	0	0	0
FSS(loamy)	*Aspergillus spp*	0	0	0
FS(loamy)	*Mucor spp*	0	0	0
	Rhodotorula spp	0	0	0
FSD(humus)	*Trichoderma spp*	0	0	0
FSD(Humus)	*Rhodotorula spp*	0	0	0
DCD(humus)	*Mucor spp*	0	0	0
	Trichoderma spp	0	0	0

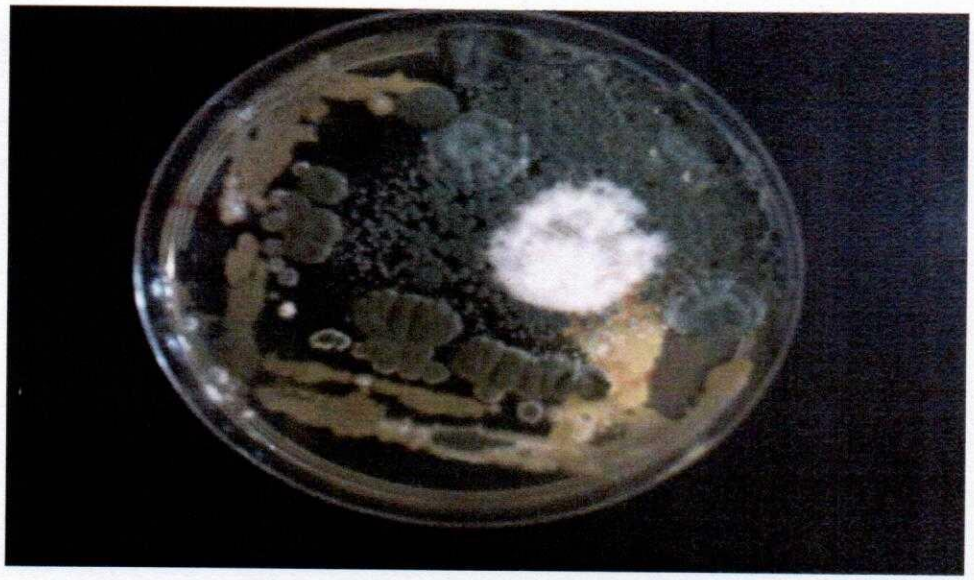

Plate 4: Growth of fungi *Aspergillus spp* on Sabouraud dextrose agar.

Plate 5: Growth of *Rhizopus spp* on sabouraud dextrose agar plate

4.2.2 Actinomycete

Table 9.0: Biochemical test of suspected Actinomycete isolated from soil sample

Gram stain	+
Cell Shape	**Rod**
Endospore (spore stain)	-
Sugar utilization	
Glucose	**A**
Inositol	**A**
Arabinose	-
Xylose	-
Lactose	-
Maltose	**A**
Mannitol	-
Sorbitol	-
Motility	-
Nitrate reduction	-
Motility	-
Starch hydrolysis	-
Catalase	-
Methyl red test	+
Vogesproskauer	-
Urease reduction	-
PROBABLE ORGANISM	*Actinomyces spp*

NB: Actinomycete isolate was isolated from humus soil

Table 10.0: Effect of Actinomycete isolate, *Actinomyces spp* against reference pathogens

Isolate	*Pseudomonas aeregunosa*	*Staphylococcus aureus*	*Candida albicans*
Actinomyces spp	**20mm**	**20mm**	**18mm**

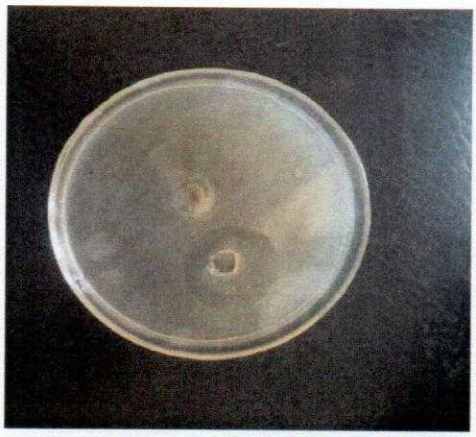

Plate 6: Zone of inhibition of actinomycete against *Staphylococcus aureus*

Plate 7: Plate showing zone of inhibition of actinomycete against *Pseudomonas spp*

4.3 DISCUSSION

Microorganisms that showed the potential of producing antibiotics that were isolated from soil samples taken from FUOYE campus are *Bacillus spp, Rhizopus spp* and *Actinomyces spp*. The repeated occurrence of *Bacillus spp* observed in the contaminated soil may not be surprising as these organisms are indigenous to soil environment and are known to persist in such environment (Atlas and Bartha, 1998). The result conforms to many reviews of literature that *Bacillus spp* are known to produce antibiotics. Spore forming bacteria and other members of the Bacillus genus possess genes for the catabolism of diverse carbon source and antibiotic synthesis (Prescott *et al*, 2008). In a previous research by Muaz and Shahida (Muaz and Shahida, 2010), it was discovered that *Bacillus spp* produces the bacitracin and subtilin.The other bacterial isolates includes *Azomonas spp, Gluconobacter spp, Escherichia coli, Micrococcus spp, Pseudomonas spp ,Staphylococcus aureus* and these showed no antibiotic effect against test organisms . The presence of *Pseudomonas spp* may be as a result of high load of different waste materials on the soil.

The Actinomycete isolated is of the genus *Actinomyces spp.* It exhibited inhibitory effect against the three test organisms used; *Pseudomonas aerugenosa, Staphylococcus aureus* and *Candida albicans* which indicates that it has the potential of producing antibiotics against these organisms. Due to certain conditions such as texture of the soil and other prevailing environmental activities where the sample was collected; there was difficulty in isolating a variety of Actinomycete isolate.

The fungal isolates include *Aspergillus spp, Trichoderma spp, Mucor spp, Rhodotorulla spp,* and *Rhizopus spp*. The result of antibiotic testing on fungal isolates indicates that *Rhizopus spp* has the potential of producing antibiotics against *Staphylococcus aureus,* while the other fungal isolates did not exhibit antibiotic properties. Most of the fungal isolates were soil

inhabiting microorganisms as seen in many other literatures (Atlas *et al*, 1998). All these findings were in conformation with Adesemoye *et al* (2006) and Ogbonna and Igbenijie, (2006). However among these effects, there are other beneficial effects of these fungal isolates present in the soil.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The result indicated that a relatively high number of microorganisms can be isolated from the soil. It also showed that very few bacterial and fungal species are capable of producing antibiotics. According to Williams *et al.*, (1993), Actinomycete is responsible for about 60% of antibiotics produced, as seen in the result above, *Actinomyces spp* has a very high potential of producing antibiotics, and this is in conformation with the research as stated in the results.

Also, the extracellular substance produced by *Bacillus spp, Rhizopus spp or Actinomyces spp* may equally become potent and exhibit marked effect as antibiotics if harnessed and purified as those of the commercially available antibiotics.

The discovery of antibiotics is one of the most useful discoveries in the field of science and particularly in the field of microbiology. This intense search for antibiotics has paid off over the past few decades because more of them have been discovered, though surprisingly only a relatively small number have been applicable to chemotherapy, because finding a new antimicrobial substance is only a first step in drug development.

5.2 RECOMMENDATION

More in-depth studies needs to be done to both screen for more antibiotic producing organisms as well as extracting and purifying suspected antibiotics from these suspected microorganisms.

Also, it is recommended that more studies should be done on how to isolate these microorganisms from other sources apart from soil.

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