BIOSYNTHESIS OF BIOACTIVE SILVER NANOPARTICLES BY FUNGAL ISOLATE AND ITS APPLICATION AS ANTIBIOTICS AGAINST SELECTED BACTERIAL PATHOGENS

BY

OSAHON, OSARUGUE PRAISE

18010101003

A RESEARCH SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, MOUNTAIN TOP UNIVERSITY, MAKOGI, IBAFO, OGUN STATE, NIGERIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B. Sc.) IN MICROBIOLOGY

SEPTEMBER, 2022

DECLARATION

I hereby declare that the project report was written under the supervision of DR. G. E. ADEBAMI and it is a product of my own research work. Information derived from various sources has been duly acknowledged in the text and the list of references provided. This researched project report has not been presented for the award of any degree.

______________________________ (Signature and Date)

Osahon, Osarugue Praise

CERTIFICATION

This is to certify that this research project titled "**BIOSYNTHESIS OF BIOACTIVE SILVER NANOPARTICLES BY FUNGAL ISOLATE AND ITS APPLICATION AS ANTIBIOTICS AGAINST SELECTED BACTERIAL PATHOGENS**" was carried out by OSAHON, Osarugue Praise with Matriculation number **18010101003**. This report meets the requirements governing the award of Bachelor of Science (B. Sc.) Degree in Microbiology, Department of Biological Sciences of the Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literary presentation.

______________________________ (Signature and Date)

Dr. G. E. Adebami

Supervisor

______________________________ (Signature and Date)

Dr. (Mrs.) C. I. Ayolabi

Ag. Head of Department

DEDICATION

This project is dedicated to God Almighty, my source of strength, and to my parents Mr. and Mrs. E. O. Osahon.

ACKNOWLEDGEMENTS

I sincerely want to appreciate the Almighty God for his help, guidance, direction, faithfulness, and his unwarranted love towards me and my project. I am extremely grateful to Him.

My profound gratitude goes to my supervisor Dr. Adebami G. E. for his support and also for sparing enough time for me to ensure the success of this project. God bless you, sir.

I would also like to appreciate the Head of Department of Biological Sciences, Dr. (Mrs) C. I. Ayolabi, the coordinator of Microbiology Programme, Dr. Fayemi E. O., and all the wonderful lecturers, may God bless you all more abundantly.

I sincerely want to appreciate the laboratory technicians, Mrs. Osagie and Miss. Adegbala for their contributions and support, God bless you both.

I would like to give a big thank you to my wonderful parents, Mr. and Mrs. Osahon, for their word of encouragement and prayers, without them I won't be able to do this on my own.

Finally, I would like to appreciate all my friends for their love and support and also my fellow project mates for their contribution to the success of this project.

TABLE OF CONTENTS

Front Page……..…………………………………………………………………..……........i
Declaration………………………………………………………………………..….... ii
Certification...iii
Dedication...iv
Acknowledgements...v
Table of Content vi
List of Tables...ix
List of Figures...x
List of Plates………………………………………………………………...……xi
Abbreviations………………..…………………………………………………………..xii
List of Appendix…………………………………………………………..……xiii
Abstract……………………………………………………………………………….xiv
CHAPTER ONE: INTRODUCTION 1
1.1 Background of study 1
1.2 Statement of problem... 3
1.3 Justification of the study 3
1.4 Aim of the study... 4
1.5 Objectives of the study... 4
CHAPTER TWO : LITERATURE REVIEW 5
2.1 Fungi 5
2.1.1 Fungi etymology 5
2.1.2 Characteristics of fungi 6
2.1.3 Reproduction of fungi 8
2.1.4 Fungi adaptations 9
2.1.5 Human applications 9
2.2 Nanotechnology 11

2.2.1 Nanoparticles 11
2.2.2 Classification of Nanoparticles 12
2.2.3 Properties of Nanoparticles 15
2.2.4 Fungi mediated biosynthesis of nanoparticles 16
2.2.5 Characterization of nanoparticles 21
2.3 Food pathogens 22
2.3.1 Common examples of food pathogens 23
2.3.2 Sources of food pathogens 25
2.3.3 Some common food borne infections caused by food pathogen 27
2.3.4 Application of silver nanoparticles as an antibiotic remedy against food pathogens 28
CHAPTER THREE: METHODOLOGY 31
3.1 Materials and Equipments 31
3.2 Culture media and reagents 31
3.3 Isolation of fungal species 31
3.3.1 Sampling 31
3.3.2 Serial dilution 31
3.4 Pure culture technique 32
3.5 Preparation of culture media 32
3.5.1 Potato Dextrose Agar 32
3.5.2 Muller Hinton Agar 32
3.5.3 Nutrient Broth 32
3.5.4 Pour plate method 33
3.6 Screening of the fungal isolates 33
3.7 Characterization of the fungal isolates 33
3.7.1 Morphological characterization 33

3.7.2 Biochemical characterization 33
3.8 Fungi biomass preparation 33
3.9 Biosynthesis of AgNPs 34
3.10 Characterization of AgNPs 34
3.10.1 UV- spectrum analysis 34
3.10.2 Fourier transform infrared spectroscopy (FT-IR) 34
3.11 Application of synthesized AgNPs against pathogenic bacteria 34
CHAPTER FOUR: RESULTS AND DISCUSSION 35
4.1 Colony counts 35
4.2 Screening and selection of fungi isolates 35
4.3 Characterization of fungi isolate 38
4.4 Biosynthesis of silver nanoparticles 41
4.4.1 Visual observation 41
4.5 Characterization of silver nanoparticles 41
4.5.1 Ultraviolet spectroscopy 41
4.5.2 FT-IR analysis of *Aspergillus* sp. AgNPs 44
4.6 Antibacterial activity of *Aspergillus* sp. MDS1 biosynthesized silver nanoparticle against selected bacteria pathogens. 47
CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS 49
5.1 Conclusion 49
5.2 Recommendations 49
REFERENCES
APPENDIX A: STATISTICAL ANALYSIS
APPENDIX B: COMPONENTS OF MEDIA

LIST OF TABLES

Table 2.1	Some fungi species and the nanoparticles they synthesize	20
Table 2.2	Some food pathogens and their sources	26
Table 2.3	Some foodborne infections and the causative food pathogens	27
Table 2.4	Recompilation of studies about the antimicrobial effects of AgNPs against food pathogens	30
Table 4.1	Colony counts for soil sample	36
Table 4.2	Antibiotic susceptibility screening result	37
Table 4.3	Morphological characteristics of *Aspergillus* sp. MDS1 on PDA culture plate	39
Table 4.4	FT-IR spectral positions with their corresponding vibration modes for biomass filtrate and AgNPs synthesized by *Aspergillus* sp. MDS1	46
Table 4.5	Antibacterial activity of the biosynthesized silver nanoparticle by *Aspergillus* sp. MDS1 against some selected bacterial pathogens	48

LIST OF FIGURES

Figure 4.1	UV-Visible spectrophotometer of the biosynthesized silver nanoparticles (AgNPs) by *Aspergillus* sp. MDS1 strain at different incubation periods	43
Figure 4.2	Fourier transformation infrared (FT-IR) spectroscopy of the biosynthesized silver nanoparticles (AgNPs) by *Aspergillus* sp. MDS1 strain	45

LIST OF PLATES

Plate 4.1	Morphological characterizations of *Aspergillus* sp. MDS1	40
Plate 4.2	Visual observation of silver nanoparticle synthesis	42

ABBREVIATIONS

AgNPs/ Ag-NPs – Silver nanoparticles

CNT – Carbon nanotubes

DLS – Dynamic light scattering analysis

DNA – Deoxyribonucleic Acid

FT-IR – Fourier transform infrared spectroscopy

MDR – Multidrug resistant

MDS – MTU dumpsite

MHA – Muller hinton agar

MtNPs – Metal nanoparticles

NPs – Nanoparticles

PDA – Potato dextrose agar

PDI – Polydispersity index

RES – Reticuloendothelial system

SEM – Scanning electron microscopy

sp – Specie

SPR – Surface plasmon resonance

TEM – Transmission electron microscopy

UV – Ultraviolet

WHO – World health organization

XRD – X-ray diffraction

LIST OF APPENDIX

Appendix A Statistical analysis 63

Appendix B Components of media 64

ABSTRACT

Nanotechnology is an advanced discipline of science and technology for dealing with material sizes on the nanoscale. Nanoparticles, which have a dimension of less than 100 nm are among those materials. In this study, fungal isolates from dumpsite soil were screened on solid agar for antibiotic production and silver nanoparticle synthesis. Morphological and biochemical characteristics of the selected fungal isolate were investigated. The visual observation, UV spectrum analysis, and Fourier transform infrared spectroscopy (FT-IR) analysis were done. The antibacterial activity of the synthesized silver nanoparticle was investigated against five selected pathogenic bacteria. A total of sixteen (16) morphologically different fungi were isolated. Isolate MDS1 identified as *Aspergillus* sp. showed the highest antibiotic activity and was used for Silver nanoparticle synthesis. Colour change from yellow to brown, and 400 nm surface plasmon resonance (SPR) peak after 72 hours were obtained. FT-IR showed 15 peaks with ten functional groups contributing to the synthesis of *Aspergillus* sp. nanoparticle. The biosynthesized *Aspergillus* sp. MDS1 nanoparticle showed improved inhibitory effect compared to the *Aspergillus* sp. MDS1 extract, silver nitrate solution, and chloramphenicol antibiotics against *Salmonella enterica* (15 mm), *Staphylococcus aureus* (16 mm), and *Enterococcus faecalis* (15 mm) respectively. This study has shown that *Aspergillus* sp. MDS1, isolated from dumpsite soil is a potential nanoparticle producer which can be developed for the industrial production of biotechnologically important products such as antibiotics.

Key words: silver nanoparticle, *Aspergillus* sp., antibiotics, Ultraviolet spectrum, FT-IR spectroscopy.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

One of the primary methods of contemporary medicine for treating infections is antibiotic treatment. From the 1930s to the 1960s, antibiotics experienced their "golden age," giving rise to a large number of antibiotics (Akinyemi, 2020). Antibiotics have been widely utilized in human and veterinary medicine since their discovery in 1929, either for treating conditions or in an effort to avoid bacterial infections (Uddin *et al*., 2021). Unfortunately, due to the emergence of diseases that were resistant to antibiotics, researchers were unable to keep up the pace of antibiotic discovery, and this age came to an end. Predisposing factors for the establishment of antibiotic resistance include continued use of antibiotics, inability to develop or find new antibiotics, and improper use of antibiotics (Dhingra *et al*., 2020). The "resistome" is a dynamic and growing issue, and antibiotic resistance is a long-standing issue. Large-scale antibiotic production during the past three decades has led to a lack of concern about the possibility of bacterial resistance. As a result of chromosomal alterations or the exchange of genetic material via plasmids and transposons, bacteria have developed resistance to antimicrobial treatments (La Rosa *et al*., 2022). As a result of interactions between different organisms and their environment, it is what is anticipated to occur. Since the majority of antimicrobial substances are naturally occurring chemicals, coresident bacteria have developed defenses against them to survive (Munita and Arias, 2016).

Bacterial pathogens face a dilemma with significant morbidity and mortality rates: antibiotic resistance. Gram-positive and gram-negative bacteria with multidrug resistance patterns are challenging to treat and may potentially be resistant to standard medications. There are currently few new antibiotics, few successful prevention strategies, and few effective therapeutics available, necessitating the creation of novel therapeutic choices and alternative antimicrobial medications (Akova, 2016). It is well acknowledged that the emergence of resistance among the most significant bacterial diseases poses a serious threat to human health on a global scale. In addition to emerging in hospitals, multidrug-resistant organisms are increasingly frequently found in community settings, indicating that reservoirs of antibiotic-resistant bacteria exist outside of hospitals (Munita and

Arias, 2016). Antibiotics have transformed infectious diseases from being incurable and fatal to being curable and low-risk. Antibiotic resistance is prevalent, and some bacteria have even developed multidrug resistance (MDR), as a result of the indiscriminate use of antibiotics. Humans' overuse and abuse of antibiotics has resulted in the selective evolution of bacteria resistant to the drugs currently on the market as well as resistant non-pathogenic microbiota, which has spread resistance genes broadly across the environment (Nitschosuch *et al*., 2016). For instance, MDR *M. tuberculosis* is a significant disease that is present in both developing and industrialized countries and has evolved into the pathogen of the 20th century. According to Mühlen and Dersch (2016), the growth of multidrug-resistant bacteria and the resistance of significant bacterial pathogens to conventional antimicrobial medicines are both rising alarmingly, and it is necessary to find ways to tackle this problem (Khameneh *et al*., 2016).

Conventional treatments are less effective for more diseases brought on by resistant microbes, and even last-resort medications are no longer effective (Freire-Moran *et al*., 2011; Bush *et al*., 2011). A new generation of antimicrobials that can prevent the spread of antibiotic resistance and protect the good microbiota are thus urgently needed. Antimicrobial resistance has been addressed in an effort to find novel antimicrobials or chemically alter existing antimicrobial medications. Sadly, there is no guarantee that new antimicrobials will solve the issue (Huh and Kwon, 2011). Nanotechnology is being used more frequently in medicine, and it is not surprising to see these tools being used to combat the threat of antibiotic resistance. There are numerous ways that nanoparticles can be used to treat infections. They can be used with antimicrobials already on the market to improve their physiochemical action against microorganisms that are resistant to medications (Kumar *et al*., 2018). Focusing mostly on fungus-mediated silver nanoparticles (AgNPs), these particles have shown antibacterial action against many infectious and harmful microbes, such as multidrug-resistant bacteria (Siddiqi, 2018; Marambio-Jones *et al*., 2010) and decaying fungi (Simbine *et al*., 2019). AgNPs have a strong antimicrobial effect and may be developed as a new class of antimicrobial agents for the treatment of bacterial infections, including those that are multidrug resistant, according to a study (Loo *et al*., 2018) on the in vitro antimicrobial activity of green synthesized silver nanoparticles against a number of gram-negative foodborne pathogens.

One of the most straightforward and straightforward methods of producing these nanoparticles is by biosynthesis using fungi. Fungi can produce more NPs than bacterial cells because they have a

stronger cell wall binding potential for metal ions and a higher potential to withstand metal concentrations (Karthikeyan and Loganathan 2012).

Fungi are more effective and less expensive than bacteria at producing NPs, and they also have a stronger propensity to accumulate metals. Consequently, fungi have been extensively researched for the synthesis of various NPs, including silver, etc (Langlois *et al*., 2012). Researchers' focus on biosynthesis of silver nanoparticles has recently increased as a means of overcoming the limitations of physical and chemical methods of manufacture (Sardul, 2017). The most effective method for overcoming bacteria' resistance to antibiotics is the use of silver nanoparticles. Since ancient times, it has been recognized as an efficient antibacterial agent (Klasen, 2000). Due of its increased toxicity to a wide range of microbes and lesser toxicity to mammalian cells, silver has advantages over other metals. Additionally, silver ions are known to be efficient against a variety of pathogens that are resistant to antibiotics (Sharma *et al*., 2013).

1.2 Statement of problem

The menace of antibiotics resistance is a global healthcare challenge. Almost all types of microorganisms have become more resilient to antibiotics in recent decades, posing a threat to emerging infectious disease strains or super strains that are both more expensive to treat and more challenging to heal (Rajeshkumar and Malarkodi, 2014). Currently, there is a rapid increase in the emergence of bacterial pathogens due to the misuse and abuse of antibiotics thereby making these pathogens develop antibiotic resistance genes with increasing pathogenicity. If these are not properly addressed, more problems will be created for humans and also affect the economic profits gained in producing these antibiotics. Thus, the development of multidrug-resistant antimicrobial agent is needed as there is a growing concern in multidrug resistant bacteria pathogens (Loo *et al*., 2018).

1.3 Justification of the study

Antibiotic-resistant bacteria are a growing problem and a major source of concern for people. To prevent these bacterial pathogens, fungi-mediated nanoparticles have proven to be a very effective and formidable treatment alternative. In comparison to bacteria, yeast, actinomycetes, and plants, fungi are simple to handle, require simple nutrients, have a high capacity for wall binding, and have the ability to absorb metals intracellularly. This advantage of fungi over other microbes make it preferable for the synthesis of nanoparticles. Fungi mediated nanoparticles have a variety of

applications in pharmaceutical science, health, and in research. Recently a lot of attention has paid to fungi mediated silver nanoparticles considering their importance in the medical and biotechnological field. Fungi mediated Silver nanoparticles possess antimicrobial properties that have been used extensively in the treatment of antibiotic resistant bacterial infections.

1.4 Aim of the study

This research is aimed at investigating the synthesis of silver nanoparticles by fungi isolates and to determine its antibiotics potentials against selected pathogenic bacteria.

1.5 Objectives of the study

- To isolate, screen and select antibiotic producing fungi from dumpsite soil
- To identify the selected isolates using morphological and biochemical characterizations
- To synthesize silver nanoparticle using the selected fungal strains
- To apply the synthesized silver nanoparticle as an antibiotic against some selected bacterial pathogens

CHAPTER TWO

LITERATURE REVIEW

2.1 Fungi

Any eukaryotic organism, including yeasts, molds, and the more well-known mushrooms, is referred to as a fungus (plural: fungi). The other eukaryotic kingdoms—Plantae, Animalia, Protozoa, and Chromista are not included in this classification of organisms as a kingdom. Instead, these organisms are grouped together as a kingdom. Kingdom A monophyletic class of eukaryotic heterotrophs having chitinous cell walls, fungi (the true fungi) are spore-reproducing and eukaryotic heterotrophs. As heterotrophs (organisms unable to produce their own food), fungi are a kingdom of often multicellular eukaryotic creatures that play significant roles in the nutrient cycling in ecosystems. The most well-known fungus are mushrooms and kitchen molds. The kingdom may contain up to 1.5 million species, of which 80,000 have been recognized and characterized. Even though the total number of fungal species is projected to be above 1.5 million, only about 80 000 to 120 000 have been described so far (Hawksworth, 2001). As a result, fungus would rank among the world's least-used biodiversity resources. Along with terrestrial plants and animals, fungi are eukaryotes with a huge range of body designs and are one of the main evolutionary lineages to occupy land. Fungi include organisms like edible mushrooms, yeasts, black mold, and Penicillium notatum, which makes the antibiotic penicillin. A fungus is presently the biggest (and maybe the oldest) living thing on the surface of the Earth. Furthermore, fungi have symbiotic relationships with both plants and bacteria and can reproduce both sexually and asexually. However, they are also to blame for a few diseases that affect both plants and animals. The term "mycology" refers to the study of fungi.

2.1.1 Fungi etymology

The Latin word fungus, which was employed by Horace and Pliny, served as the primary inspiration for the English word "fungus." This word is a translation of the Greek word sphongos, which refers to the macroscopic characteristics and morphology of molds and mushrooms. German and Dutch are two languages that also make use of this word's root (mould). The word mycology, which refers to the study of fungi, comes from the Greek terms mykes (mushroom) and logos (disclosure) (Hawksworth, 2006).

2.1.2 Characteristics of fungi

Prior to the development of molecular techniques for phylogenetic study, taxonomists believed that fungi belonged to the plant kingdom due to lifestyle similarities. Both fungi and plants have a largely immobile lifestyle and have a common general morphology and growth environment. In the same way that plants do, fungi also frequently grow in the soil and, in the case of mushrooms, generate conspicuous fruit bodies that occasionally resemble plants like mosses. Since they appear to have separated from plants and animals roughly a billion years ago, the fungi are currently regarded as belonging to a different kingdom (around the start of the Neoproterozoic Era) (Bruns, 2006). The physical, metabolic, and genetic characteristics of fungus differ significantly from those of other kingdoms in that some are shared with other living things while others are exclusive to them. The characteristics of fungi include,

- Nutrition: Heterotrophic (lacking photosynthesis), absorption-based rather than ingestion-based nutrition. Because they are absorptive heterotrophs, they first decompose the organic matter of their hosts before ingesting it and producing digestive enzymes to do so. They digest food outside of their cells by secreting hydrolytic enzymes, a process known as extracellular digestion, and they store the energy from this digestion as glycogen. Most are saprobes, meaning they feed on other dead creatures.
- Cell wall: Usually present, often composed of glucans and chitin, glucans and cellulose only rarely (Oomycota). Fungal cells have a thick cell wall, just like plant cells do. The rigid layers of fungal cell walls contain two complex polysaccharides called chitin and glucans. Fungi's cell walls are structurally stable due to chitin (N-acetyl-D-glucosamine), which is also found in the exoskeleton of arthropods like insects. The cell's wall shields it against some predators and desiccation (drying out).
- Cell structure: Fungi are eukaryotes, and as such, have a sophisticated cellular structure. Fungal cells have a membrane-bound nucleus since they are eukaryotes. Like in other eukaryotic cells, the DNA in the nucleus is encased by histone proteins. Some strains of fungi include auxiliary genomic structures like plasmids (DNA loops) in bacteria, however horizontal genetic information transfer between fungi and bacteria is uncommon in fungi. In a typical fungal cell, there is a real nucleus, mitochondria, and a complex web of internal membranes, including the Golgi apparatus and the Endoplasmic reticulum.

- Growth: Grow as microscopic tubes or filaments called hyphae that contain cytoplasm and nuclei, preferring warm, moist conditions. Mycelium is the name for hyphal networks. Because both growth forms have large surface area to volume ratios, the growth of fungi as hyphae on or in solid substrates, or as single cells in watery environments, is adapted for the effective extraction of nutrients. Hyphae are uniquely adapted for growth on solid surfaces as well as for tissue invasion. A thallus, which can be single or multicellular, is the vegetative body of a fungus. Yeasts are single-celled fungus. Threadlike hyphae are produced by multicellular fungus (singular hypha). Dimorphic fungi can transition from a single cell to a multicellular state based on their surroundings. Unicellular fungi include *Candida species* (the causative agents of thrush, a widespread fungal infection) and *Saccharomyces cerevisiae* (baker's yeast).
- Reproduction: Spores can reproduce both sexually and asexually. The following reproductive processes could take place; sexual (involving meiosis and nuclear fusion), parasexual (involving nuclear fusion followed by progressive de-diploidization) and asexual (purely mitotic nuclear division). Environmental factors cause developmental stages that are genetically predetermined and result in the development of specialized structures for sexual or asexual reproduction. By effectively distributing spores or propagules carrying spores, these structures facilitate reproduction.
- Habitat: Fungi can be found in every ecosystem on Earth, despite the fact that they are frequently unnoticeable. They are common throughout terrestrial and freshwater ecosystems, but less so in the ocean. Fungi may grow with or without light and prefer moist, somewhat acidic surroundings. The amount of oxygen they need varies. The majority of fungi are obligatory aerobes, meaning they need oxygen to survive.
- Ecology: Saprotrophs, mutualistic symbionts, parasites, or hyper parasites have crucial ecological functions in the decomposition and recycling of nutrients in the environment. They play a critical role in resource cycling as decomposers, specifically as saprotrophs and symbionts that convert organic matter to inorganic molecules that can then re-enter anabolic metabolic pathways in plants or other organisms. A few are predators that catch prey, while others are internal or exterior parasites.
- Life cycle: Simple or, more usually, complex. The life cycle of fungi can follow many different patterns. Fungi are thought to have a four-stage life cycle that includes hypha, germ,

spore, and mature mycelium for the majority of the indoor molds. Other life cycle patterns, particularly for non-moldy fungus, diverge from this four-stage cycle by having alternative reproductive processes and physiological traits (such as sapstains, wood rots, etc.)

- Propagules: is any substance that aids in the dissemination of an organism to the subsequent stage of its life cycle. Usually, the propagule's shape is distinct from that of the parent organism. Fungi produce propagules in the form of spores. These are often microscopic spores that are produced in large quantities. Motile spores are restricted to particular species.
- Distribution: Fungi may grow or live in a variety of environments, making them cosmopolitan. Fungi are among the most common organisms on Earth and are crucial to both the environment and human health. They are non-motile organisms; however, they cannot move around.

2.1.3 Reproduction of fungi

The complexity of fungi's reproduction reflects the variety of lifestyles and genetic make-up found in this vast kingdom of living things. According to estimates, a third of all fungi use multiple methods of reproduction. For instance, reproduction may take place during two distinct stages of a species' life cycle: the teleomorph (sexual reproduction) and the anamorph (asexual reproduction). The so-called "perfect" fungi can reproduce both sexually and asexually, in contrast to the so-called "imperfect" fungi, which can only reproduce asexually (by mitosis). To reproduce, all fungi require spores. Spores are tiny cells or clusters of cells that split out from their parent fungus and travel mostly through the air or water. Until conditions are ideal for growth, spores can remain dormant for a very long period. This is an adaptation for opportunism; with food source availability being occasionally unexpected, spores may lay dormant until they may colonize a new food source. Spores are created by fungi through both sexual and asexual reproduction (Phadke *et al*., 2013)

2.1.3.1 Asexual reproduction

Asexual reproduction can take place by spore release or mycelial fragmentation, in which the mycelium splits into various parts where each grow independently. In contrast to sexual reproduction, mycelial fragmentation and vegetative spores enable faster spread and preserve clonal populations that are suited to a specific habitat. The class of fungi known as "Fungi imperfecti," or Deuteromycota, includes all species lacking an identifiable sexual cycle (fungi without the sexual or perfect stage). Deuteromycota, also known as Deuteromycetes, conidial fungi, or mitosporic

fungi, is a taxonomic group that is no longer recognized and is now used to refer to any type of fungus that does not have a known sexual life cycle. By fragmenting, budding, or generating spores, fungi can reproduce asexually. During budding, the cell bulges on one side, the nucleus divides during mitosis, and the bud eventually separates from the mother cell, whereas hyphae fragments can form new colonies (Redecker and Raab, 2006).

2.1.3.2 Sexual reproduction

In the course of sexual reproduction, different individuals fuse their hyphae together. Multicellular fungi typically go through three stages in their life cycle, which might vary depending on the species: haploid (where they have one pair of chromosomes), diploid (where they have two sets of chromosomes), and dikaryotic (where they have two sets of chromosomes but keep them separate). Compatible individuals in sexually reproducing fungi can unite by joining their hyphae into a network that is connected to one another; this process, called anastomosis, is necessary for the start of the sexual cycle. Many ascomycetes and basidiomycetes go through a stage known as dikaryosis, in which the nuclei inherited from the two parents do not merge right away following cell fusion but instead stay distinct in the hyphal cells (Taylor *et al*., 2000).

2.1.4 Fungi adaptations

Fungi are exceptionally adaptable. By increasing the surface area of their gills, they have increased their ability to adapt to their environment over time. This is advantageous to the fungus because it can create more spores, which can result in more of them dispersing. The dissemination may help the fungi survive longer. Increasing the thickness of the distributed spore walls is another method of adaptation. The spores can last more effectively on fresh ground when they have more protection. This may be crucial to the young organism's survival during periods of drought. Overall, the fungi have evolved chitin-based cell walls that are thicker and stronger. This enables additional help and predator defense (Onofri *et al*., 2007).

2.1.5 Human applications

Although people frequently associate fungus with pathogenic organisms and food rot, they actually play a crucial role in many aspects of human life. As we've seen, because they participate in the nutrient cycle in ecosystems, fungi have a significant impact on the health of human populations. They also play different roles in the environment. Fungi are useful in the control of dangerous pest

populations since they are animal pathogens. These fungi are highly specialized to the insects they attack and do not infect mammals or plants (Chandler, 2017). Many fungi are already on the market and are now being investigated for use as microbial insecticides. For instance, to curb the recent spread of the emerald ash borer, a beetle that consumes ash trees, scientists are experimenting with the fungus Beauveria bassiana as a potential biological control agent. It has already been made accessible in, Maryland, Illinois, Michigan, Ohio, Indiana, and West Virginia. Additionally, the mycorrhizal relationship between plant roots and fungi is essential for the productive use of agricultural land. Eighty to ninety percent of plants and grasses would not survive without the fungus partner in their root systems.

Supporters of organic agriculture advertise and sell mycorrhizal fungus inoculants as soil additives at gardening supply stores (López-Gómez and Molina-Meyer, 2006). Herbicides, insecticides, creosote, pentachlorophenol, coal tars, and heavy fuels can all be broken down by some fungi, specifically white-rot fungi, into water, carbon dioxide, and the fundamental elements. It has been demonstrated that fungi may biomineralize uranium oxides, indicating that they might be useful in the bioremediation of radioactively hazardous environments (Fomina *et al*., 2007). Some fungus species are consumed as food. Mushrooms play a significant role in human food. Among the delights are morels, shiitake mushrooms, chanterelles, and truffles. Both commercially grown and wild mushrooms can be consumed. *Agaricus bisporus*, which is sold as button mushrooms when little or Portobello mushrooms when larger, is the species that is grown the most frequently in the West. It can be found in many different recipes, including salads and soups.

To ferment sugars, grains to make beer, and fruits to make wine, yeast is obtained from the environment. To manufacture bread and other items containing wheat, such as pizza dough and dumplings, a unicellular fungus called baker's yeast, sometimes known as *Saccharomyces cerevisiae*, is used. Fermentation is another method used to create alcoholic beverages using yeast species from the *Saccharomyces* genus. In order to make Shoyu (soy sauce), sake, and miso, Shoyu koji mold (*Aspergillus oryzae*) is a necessary component (Erdogan *et al*., 2003). *Rhizopus* species are utilized to make tempeh. The commercial value of several secondary metabolites produced by fungus is enormous. To prevent or slow down the growth of bacteria and reduce competition in the natural environment, fungus naturally produce antibiotics. Important antibiotics like penicillin are made from tiny peptides, however, naturally occurring penicillins like penicillin G also belong to this structurally similar class of -lactam antibiotics (produced by *Penicillium chrysogenum*) have a

relatively narrow spectrum of biological activity (Brakhage *et al*., 2004). By chemically altering the natural penicillins and cephalosporins, which are extracted from fungi, a broad variety of additional penicillins can be synthesized. Cyclosporine, an immunosuppressant that lowers the chance of organ rejection after transplantation, as well as steroid hormone precursors and ergot alkaloids, which are used to control bleeding, are among the useful pharmaceuticals extracted from fungus.

For thousands of years, many cultures have used fungi like *Gymnopilus junonius* and *Psilocybe semilanceata*, which contain the chemical psilocybin, to produce psychedelic experiences (Pan *et al*., 2008). Fungi are significant model study organisms because they are basic eukaryotic species. The red bread mold *Neurospora crassa* has been used to make significant strides in modern genetics. Furthermore, numerous significant genes first identified in *S. cerevisiae* served as a starting point for the discovery of related human genes (Karbalaei *et al*., 2020).

2.2 Nanotechnology

Since the last century, nanotechnology has been a well subject of research. Since Nobel Laureate Richard P. Feynman coined the word "nanotechnology" in his well-known 1959 speech "There's Plenty of Room at the Bottom," there have been several advancements in the field of nanotechnology (Feynman, 1960). Nanotechnology produces a wide range of materials at the nanoscale called nanoparticles (NPs) which are particulate materials with a minimum of one dimension of fewer than 100 nanometers (Laurent *et al*., 2008; Khan, 2017). Nanotechnology is the most advanced branch of science and technology for dealing with nanoscale material sizes. Nanotechnology is a method or ability to design, identify, build and use nanoscale structures and systems to regulate and dimension. Nanotechnology, according to an adopted common definition, is a method or technique for designing, identifying, building, and using tiny structures and systems to control shape and dimension. Nanotechnology has attracted a lot of attention in recent years. Nanoparticles are a key component of nanotechnology (Ealia, 2017).

2.2.1 Nanoparticles

Nanoparticles (NPs) which are particulate materials with a minimum of one dimension of fewer than 100 nanometers (Laurent *et al*., 2008; Khan, 2017). Numerous definitions of "nanoparticle" have been provided in literature. It uses the Standard Terminology of ASTM 2456-06. Nanotechnology defines it as "a particle with lengths in two or three dimensions greater than 1 nm

and less than 100 nm that may or may not display a size-related intensive feature". NPs are also known as zero-dimensional nanomaterials, in contrast to one- and two-dimensional nanomaterials, which have one or two dimensions larger than nanoscales (Sajida, 2020).

2.2.2 Classification of Nanoparticles

Inorganic and organic NPs are the two basic types of nanoparticles. The first class includes liposomes, dendrimers, hybrid polymeric NPs, micelles, and compact polymeric NPs. Fullerenes, quantum dots, silica, and gold nanoparticles make up the second category (Cartaxo, 2018). Organic, inorganic, and carbon-based nanoparticles are presently the most common classes of nanoparticles (Ealia, 2017).

2.2.2.1 Organic Nanoparticles:

Also known as, ferritin, micelles, dendrimers and liposomes. These nanoparticles are harmless and environmentally safe. Some, like micelles and liposomes, have no space within them, which are also called Nanocapsules, and are thermally sensitive and react to electromagnetic radiation like heat and light. They are a superb choice for medicine delivery because of their unique characteristics. In addition to their normal properties like size, composition, surface shape, and so on, their drug-carrying capacity, stability, and delivery systems, whether an entrapped drug or an adsorbed drug system, define their field of applications and efficiency. Organic nanoparticles are frequently employed in biomedical fields, such as medication delivery systems because they are effective and may be injected into particular areas of the body which is also known as targeted drug delivery (Ealia, 2017). Some of the examples of organic nanoparticles are briefly explained below:

- **Micelles**: Micelles are amphiphilic compounds, such as polymers or lipids that form nanostructures. They hide their hydrophobic groups inside the structure and expose the hydrophilic groups when exposed to aquatic surroundings. In lipid-rich settings, on the other hand, their structure may organize in the other direction (Cartaxo, 2018).
- **Dendrimers**: A dendrimer is distinguished morphologically by a branching structure formed by one or more cores. The number of generations permitted to grow over these cores can simply influence the size of these NPs. Dendrimers provide challenges in terms of drug absorption and release because their synthesis is time-consuming (Cartaxo, 2018).

- **Liposomes**: Liposomes are lipid-based vesicles. Two phospholipid layers make up liposome NPs (20–100 nm) (Vahabi, 2017). Unilamellar liposomes, which range in size from 100 to 800 nanometers, are the most common. Amphiphilic materials are used to create these spherical structures, which have a high production cost and content leakage. As main advantages, they are completely biodegradable, compatible, non-toxic, and not immunogenic (Cartaxo, 2018). Liposome NPs, such as Ambisome, have mostly been used as antimicrobials (Vahabi, 2017).

2.2.2.2 Inorganic nanoparticles:

Inorganic nanoparticles are particles that are not made up of carbon. Inorganic NPs (1–100 nm) are made up of several inorganic oxides and have morphological and chemical features such as solubility that vary (Vahabi, 2017). Inorganic NPs, such as metallic NPs, are synthesized by reducing salts via reducing molecules like biopolymers (Vahabi, 2017). Metal oxide-based and metal nanoparticles are generally categorized as inorganic nanoparticles.

- **Metal-based**: Metal-based nanoparticles are those that are produced using either destructive or constructive methods from metals down to nanometric sizes. Almost every metal may be produced as nanoparticles. Cobalt (Co), cadmium (Cd), copper (Cu), gold (Au), zinc (Zn), iron (Fe), and silver (Ag) are a few materials that are frequently utilized to produce nanoparticles. Nanoparticles have unique properties such as size varying from 10 to 100nm, surface properties such as surface volume ratio, pore size, surface charge, crystalline and amorphous structures, shapes such as spherical and cylindrical, color, susceptibility to environmental conditions such as heat, moisture, air, sunlight, and reactivity (Ealia, 2017).

- **Metal oxides based**: Metal oxide-based nanoparticles are produced to alter the properties of the corresponding metal-based nanoparticles. For example, iron nanoparticles (Fe) quickly oxidize to iron oxide (Fe_2O_3) in the presence of oxygen at ambient temperature, improving their reactivity. Because of their improved reactivity and efficiency, metal oxide nanoparticles are commonly produced. Aluminum oxide (Al_2O_3), Iron oxide (Fe_2O_3), Cerium oxide (CeO_2), Magnetite (Fe_3O_4), Titanium oxide (TiO_2), Silicon dioxide (SiO_2), and Zinc oxide (ZnO) are the most typically manufactured (ZnO). When compared to their metal counterparts, these nanoparticles offer outstanding characteristics (Ealia, 2017).

2.2.2.3 Carbon based:

Carbon-based nanoparticles are carbon-only nanoparticles. Fullerenes, carbon nanotubes (CNT), graphene, carbon black, carbon nanofibers, and occasionally activated carbon in Nano size can all be classed under it (Ealia, 2017).

- **Fullerenes**: Fullerenes (C60) are spherical carbon compounds made up of sp2 bonded carbon atoms. Dimensions of the spherical structure, which range from 8.2 nm for a single layer to 4 to 36 nm for multi-layered fullerenes, range from 28 to 1500 carbon atoms (Ealia, 2017). For instance, fullerenes, which are nanomaterials with globular hollow cages, are the building blocks of allotropic carbon forms. They have sparked significant commercial interest in nano-composites for a variety of commercial applications, including efficient gas adsorbents for environmental remediation (Ngoy *et al*., 2014), fillers (Saeed and Khan, 2016, 2014), and in addition to serving as a generally maintained medium for a variety of organic and inorganic enzymes (Mabena *et al*., 2011; khan, 2017).

- **Graphene**: Graphene is a kind of carbon. Graphene is a 2-D planar hexagonal network of honeycomb lattices made up of carbon atoms. The thickness of a graphene sheet is usually approximately 1 nm (Ealia, 2017).

- **Carbon Nano Tubes (CNT)**: Carbon Nano Tubes (CNT) are made by winding a graphene nano foil with a honeycomb lattice of carbon atoms into hollow cylinders. The diameter of a single-layered CNT is only 0.7 nm, while a multilayered CNT has a diameter of 100 nm. The length of a CNT can range from a few micrometers to several millimeters. Ends could be empty or contain a half fullerene molecule (Ealia, 2017).

- **Carbon Nanofiber**: Carbon nanofiber is made from the same graphene Nano foils as CNT, but instead of cylindrical tubes, they are coiled into a cone or cup shape.

- **Carbon black**: A carbon-based amorphous material with a range of diameters from 20 to 70 nanometers with a usually spherical form. The particle contact is so strong that it binds them together in aggregates, forming 500 nm agglomerates (Ealia, 2017).

2.2.3 Properties of Nanoparticles

Nanoparticles have unique features that distinguish them apart from one another. Their differences from one another include their size, shape, polydispersity index, surface, and targeting. To recognize nanoparticles, they must have special properties. Nanoparticles' properties include;

2.2.3.1 Size and polydispersity index

NPs size varies between 1 nm and 100 nm. Particle size influences rolling velocity, particle adhesion, and diffusion. Larger microspheres have been demonstrated to roll quicker than smaller microspheres. When it comes to NP adhesion, the rate of attachment reduces as the size of the NP grows. Because particle adherence to cells can be influenced by a previous step of particle adherence to cells, this fact can have an impact on NP cellular internalization (Cartaxo, 2018). Aside from size, the polydispersity index (PDI) of NP suspensions is important. This index provides information on the sizes of the NPs in a suspension. Essentially, as the PDI value rises, the suspension comprises particles of increasingly varied sizes. Aside from NPs size, NPs PDI is a critical metric since the existence of a polydisperse suspension might generate unanticipated changes in NP behavior (Cartaxo, 2018).

2.2.3.2 Shape

Recent research has revealed that particle form (sphere, ring, disc) plays a significant influence on NP circulation, distribution within the body, cellular uptake, and in vivo behavior. Several research has been conducted to investigate the effect of shape on NP transport across the human body. While spherical particles flow easily, irregularly shaped particles have a significantly higher chance of aligning or tumbling in bifurcations of arteries or filtering organs. To get through the spleen, a spherical particle must have a diameter of less than 200 nm. It can pass through this organ if it is shaped like a disk with a diameter of roughly 7 m and a height of 150 nm (Cartaxo, 2018).

2.2.3.3 Surface properties

To characterize NPs, distinct surface features such as hydrophobicity and surface charge have been employed. Hydrophobicity is extremely important because it effects NP clearance from the body due to RES activity. Nonspecific interactions with proteins are reduced when hydrophobicity is reduced. As a result, macrophages' phagocytosis is reduced (Cartaxo, 2018). The surface electrical potential of NPs, also called the zeta potential, is the potential of a particle or molecule in a given

medium caused by its charge, and it impacts the possibility of particles aggregating. Indeed, the repulsion between NPs becomes stronger as the absolute value of this surface electric potential rises. Positively charged NPs are internally absorbed more nonspecifically than negatively charged or neutral NPs (Cartaxo, 2018).

2.2.3.4 Targeting

In contrast to previous alternative medicines, it is hoped that NPs can target a specific anatomical region after injection, reducing negative effects on healthy tissues. Targeting tactics, both passive and active, are utilized to attain this purpose. The physicochemical/structural parameters of the target site are employed to change the NPs qualities in passive targeting. For instance, to benefit from the greater penetration and retention mechanism seen in tumors with leaky vasculatures, the size of NPs can be reduced. While active targeting, entails attaching molecules to the NPs' surfaces to create actively targeted nanoparticles. Ligands, monoclonal antibodies, tailored antibody fragments, peptides, proteins, nutrients, carbohydrates, and aptamers are all examples of these compounds. The active targeting technique makes use of extremely precise interactions between a molecule found in specific tissues, cells, or organelles of the body and a molecule attached to the NPs' surface (Cartaxo, 2018).

2.2.4 Fungi mediated biosynthesis of nanoparticles

Fungi are single-celled or multicellular organisms found in a wide range of natural habitats and frequently create saprotrophic organisms. Only about 70,000 fungus species have been found out of an estimated 1.5 million on the planet. According to more recent research, there are around 5.1 million fungal species identified using high-throughput sequencing technologies. It is important to note that these organisms can digest extracellular food and release particular enzymes to hydrolyze complicated components into simpler ones that can be ingested and used as energy sources. The relevance of researching the effect of fungi in Nanotechnology is emphasized. Because of their toleration and metal accumulation ability, fungi have got a lot of interest in the research on the microbial biosynthesis of MtNPs (Amin, 2015). Metal nanoparticles like silver, zirconium, gold, silica, iron (magnetite), titanium, and platinum have been produced by the use of using fungal systems, or myco Nano factories (Hany, 2014).

Monodispersed nanoparticles with well-defined dimensions and sizes are mostly produced through fungi-mediated nanoparticle biosynthesis. Fungi are considered good biological agents for creating metal sulfide and metal nanoparticles due to the diversity of their internal enzymes. Fungi, in comparison to bacteria, can produce more nanoparticles giving them more advantage over other microorganisms used (Jaison, 2016). The following are some of the benefits of fungal nanoparticle biosynthesis:

- Fungi have a high capacity for intracellular metal absorption and produce a significant number of enzymes per unit of biomass.
- Reductases are one example of extracellular enzymes produced by fungus that have helped to create metal nanoparticles with a diversity of chemical compositions.
- The ineffective distribution of the metal as a catalyst was also caused by the cultivation of fungus on the surface of inorganic substrates.
- The sizes and forms of nanoparticles produced by different fungus species vary, allowing for a wide range of applications.
- High accumulation: Because of their intrinsic ability to create larger concentrations of proteins that aid in the reduction of metal ions to less toxic forms, fungi that are ordinarily not exposed to high concentrations of hazardous metals can convert into a form that can tolerate high concentrations of metal ions. The stronger the enzyme synthesis, the greater the reduction in metal ions (Dhillon *et al*., 2012).
- High yield: Fungal hyphae grow, branch, and fuse to produce a filamentous explorative mycelium with a high surface area to mass ratio and nutrient transfer capabilities. The hydrated mucilaginous sheath that frequently surrounds hyphae acts as a substrate for geochemical reactions (Gadd, 2007; Gadd and Raven, 2010). The branching network acts as a template for the creation of nanoparticles and nanomaterials (Li *et al*., 2021).
- Easy to culture: Because fungi have basic dietary requirements, they are easy to isolate and subculture. Simple methods for isolating fungus include serial dilutions, plating, and hyphal extraction. Because fungi are totipotent, they can be grown from hyphae or spores and sub-cultured to obtain pure isolates (Saxenaa, 2014).

- Presence of complex proteins that help in nanoparticles synthesis: Fungi produce a lot of extracellular enzymes that accelerate the formation of nanoparticles from heavy metal ions. As a result, fungi may create nanoparticles more quickly than through chemical synthesis (Saxenaa, 2014). Fungi generate substances or biomolecules that function as reducing agents when exposed to metal ions, such as naphthoquinones, anthraquinones, or nitrate reductase (Kim-Hung, 2020). Because fungi and yeast are efficient secretors of extracellular enzymes and because the number of species grows rapidly, cultivating and maintaining them in the laboratory is simple. They can make metal nanoparticles and nanostructures either intracellularly or extracellularly by using a reducing enzyme (Amin, 2015). During intracellular synthesis, metal salts are changed in the mycelia into a less dangerous form that the fungi may use. In extracellular biosynthesis, fungus extracts are employed (Ovais, 2018).

2.2.4.1 Intracellular synthesis of nanoparticles by fungi

The cellular process of microbial cells is exploited to synthesize NPs in the intracellular production of NPs. Ions are carried into microbial cells in the midst of enzymes to create nanoparticles (Hasan, 2015). The microbial cultures are kept in suitable liquid media, and the microbial biomass is rinsed in sterile distilled water before being centrifuged to extract the biomass pellet. After that, the microbial biomass is allowed to react with a metal aqueous solution. The microbial biomass and metals-containing solution are then cultured under the proper incubation conditions until a specified chromatic change is detected. The production of NPs can be seen by the presence of a certain color. The appearance of a whitish-yellow to yellow tint indicates that zinc and manganese NPs are being synthesized. A pale yellow to pinkish color indicates gold NP synthesis, while a pale yellow to brownish color indicates AgNPs synthesis. During this process, positively charged metal ions are held within the negatively charged cell walls of microorganisms. These metal ions are reduced by enzymes bio, generating nanoclusters inside the cell wall that diffuse outside the cell wall into the solution (Koul, 2021). Metal ions are absorbed by microbial cells during the intracellular process, and in the sight of cellular enzymes, metal ions are converted into NPs within the cells (Koul, 2021). Additional procedures, such as ultrasonic therapy or interactions with suitable detergents, are required after intracellular NP formation to release the produced NPs (Kalimuthu *et al.*, 2020) However, supplementary processing steps like as ultrasonication and detergent therapy make extracting the nanoparticles created by intracellular biogenesis difficult (Amin, 2015). The size of

the nanoparticles generated inside the body is smaller than that of extracellularly reduced nanoparticles. The size limit is most likely connected to the nucleation of particles inside the organisms (Hasan, 2015). A step-by-step procedure for the intracellular manufacture of nanoparticles employing *Verticillium* sp. states that trapping, bioreduction, and capping are involved in the production of nanoparticles. Electrostatic interactions occur when metal ions come into contact with the fungal cell surface, trapping the ions. Enzymes located in the cell wall convert metal ions to metal nanoparticles (Hulkoti, 2014).

2.2.4.2 Extracellular synthesis of nanoparticles by fungi

Microbes are cultivated in appropriate conditions during the extracellular production of NPs. In order to create NPs, the microbial enzyme-containing supernatant from centrifuging the microbial cell-containing broth is employed. In a separate vessel, the reductase enzyme-containing supernatant is allowed to react with the metal ions. Metal ions are reduced in cell-free supernatant, resulting in the production of NPs. XRD (X-ray diffraction), TEM (transmission electron microscopy), SEM (scanning electron microscopy), and FT-IR (fluorescence transfer infrared) are used to examine the properties of newly produced NPs (uniformity, morphology, and interaction) (Fourier transform infrared spectroscopy) (Koul, 2021). Metal ions are held beyond the cell membrane and reduced by cellular enzymes to generate NPs in the extracellular process (Koul, 2021). Extracellular biosynthesis is low-cost and simple to process (Maysaa, 2021). This encourages the large-scale manufacturing of NPs to investigate their possible applications. As a result, many investigations on metal NP production have focused on extracellular techniques (Prasad *et al*., 2016). Extracellular nanoparticle synthesis has more uses than intracellular nanoparticle production because it removes superfluous cellular components from the cell (Hasan, 2015).

A vast variety of fungal strains can produce silver nanoparticles (AgNPs) outside of the cell, including *Fusarium oxysporum* (Ahmad *et al*., 2003), *Fusarium semitectum* (Basavaraja *et al*., 2008), *Cladosporium cladosporioides* (Balaji *et al*., 2009), *Aspergillus niger* (Gade *et al*., 2008), *Penicillium brevicompactum* (Shaligram *et al*., 2009), *Aspergillus fumigatus* (Bhainsa and D'Souza, 2006) and *Aspergillus clavatus* (Verma *et al*., 2010) have been previously described (Hany, 2014).

2.2.4.3 Biosynthesis of nanoparticles by different fungi species

Different fungi species have been used for fungi biosynthesis. Fungal biomass was reported in the production of metal, metal oxide, and other diversity of nanoparticles which can be seen in the table below;

Table 2.1: Some fungi species and the nanoparticles they synthesize

Fungi Species	Nanoparticles Synthesized
Verticillium luteoalbum, Fusarium oxysporum, *Aspergillus oryzae*, *Trichothecium* sp., *Collitotrichum* sp., *Penicillium* sp.	Gold (Au) nanoparticles
Fusarium oxysporum, *Aspergillus fumigatus*, *Phanerochaete chrysosporium, Aspergillus flavus*, *Aspergillus niger*, *Fusarium semitectum*, *Fusarium solani*, *Penicillium brecompactum*, *Alternata*, *Trichoderma viride*, *Rhizopus nigricans*.	Silver (Ag) nanoparticles
Aspergillus flavus	TiO_2 nanoparticles
Aspergillus terreus	Zinc oxide (ZnO) nanoparticle
Fusarium oxysporum	Barium titanate ($BaTiO_3$) and Bismuth oxide (Bi_2O_3)
Fusarium oxysporum, Cariolus versicolor	Silica, magnetite, zirconia, titanium, cadmium selenide, and cadmium sulfide nanoparticles.
F. oxysporum	CdTe nanoparticles

2.2.5 Characterization of nanoparticles

Nanoparticles can be characterized using the following methods;

- **UV–visible spectral analysis**: Using a UV-Spectrophotometer to measure the absorbance (optical density/light absorbance) of Nanoparticles at different concentrations between the wavelengths of 200 and 800 nanometers. Research is done by (Al juraifani and Ghazwani, 2015) on the biosynthesis of AgNPs from *A. niger*, *F. oxysporum*, and *A. solani* used UV-spectroscopy to record the production of AgNPs by reduction of $AgNO_3$ by fungi. The results showed strong surface plasmon resonance focused at 445,435,440 nm for *A. solani*, *A. niger*, and *F. oxysporum* respectively. Absorption band range from 435 – 445 nm.
- **X-ray diffraction analysis (XRD)**: The determination of the structural properties of nanoparticles, such as the crystallinity and the chemical composition of synthesized nanoparticles. For instance, after the biosynthesis of silver nanoparticles from the endophytic fungus *Aspergillus clavatus*, Verma *et al*. (2010) observed the XRD analysis of AgNPs and noticed that the XRD peaks at 38.06^0 and 44.1392^0 indicating that the precipitate is made up of pure crystalline silver and corresponds to the diffraction facets of Ag.
- **Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM):** for the observation and confirmation of the size, shape, and morphology of Nanoparticles. Vago *et al*. 2015 after the biosynthesis of gold (Au) nanoparticles observed the size and shape of nanoparticles using TEM and noticed that they appeared Spherical, while in some cases triangular shapes were seen also. The size was also observed which was between 10 and 20nm. The average size of NPs was ≈12 nm.
- **Fourier transform infrared spectroscopy (FT-IR)**: Performed to identify the functional groups present on Nanoparticles. In a study carried out by Hulkoti *et al*. (2014) in the synthesis of silver (Ag) nanoparticles from *Aspergillus fumigatus*, he observed the nanoparticles produced using FT-IR techniques that amino acid groups were binding to them which accounted for the stability of NPs and the proteins present acted as stabilizing and capping agent.
- **Dynamic light scattering (DLS) analysis**: Estimating Particle size using Zetasizer (S90, Malvern Instruments UK) and for observing nanoparticle size at an extremely low level. For

instance, in characterizing the structure of silver nanoparticles from white-rot fungi, (Chan and Don, 2013) measured the average size of AgNPs in the sample solution using DLS and noticed that the fungi have the ability to synthesize smaller diameter nanoparticles in the radius of 50±10 to 65±10 nm.

- **Zeta potential analysis**: Evaluating the Surface area characterization, including, the stability and surface charge of colloidal Nanoparticles using a Zetasizer Nanomachine. During research carried out by Zomorodian *et al*. (2016), on the biosynthesis of silver (Ag) nanoparticles using *Aspergillus* species. He observed that *A. fumigatus* produced smaller Ag nanoparticles (mean=49.00) with higher monodispersity (SD=19.64) in comparison with other species.

2.3 Food pathogens

Food pathogens are microorganisms, primarily bacteria and viruses, that infect food and, when consumed, cause food-borne diseases. Biological agents that can result in an outbreak of a foodborne illness include foodborne pathogens (such as viruses, bacteria, and parasites) (Bintsis, 2017). Consuming food tainted with food germs frequently results in foodborne illness, also known as food poisoning. Numerous diseases caused by foodborne microorganisms have a negative impact on both human health and the economy (Bintsis, 2017). In the past, many food-borne diseases have been identified. These conventional pathogens are now more common. Food-borne infections are still being discovered, and they are responsible for millions of illnesses and chronic difficulties in people. People's health is increasingly at risk due to the prevalence of bacteria that can cause food poisoning and a variety of clinical symptoms worldwide. It has been noted that new diseases are emerging, including *Listeria monocytogenes*, *E. coli* 0157:H7, *Campylobacter species*, *Yersinia enterocolitica*, and *Salmonella enteritidis* (Chukwu *et al*., 2016). Foodborne illnesses are most frequently brought on by bacteria, which come in a range of sizes, varieties, and characteristics. Some hazardous bacteria, such as *Clostridium botulinum*, *Clostridium perfringens*, *Bacillus subtilis*, and *Bacillus cereus*, are spore-forming and therefore very heat-resistant (Bacon, 2003). Some are able to produce toxins that can withstand heat (e.g. *Staphylococcus aureus*, *Clostridium botulinum*). The majority of infections are mesophilic, with ideal growth temperatures between 20 and 45 °C. However, some foodborne pathogens (psychrotrophs), such *Listeria monocytogenes* and *Yersinia enterocolitica*, can grow in the refrigerator or at temperatures lower than 10 °C (Bacon, 2003).

2.3.1 Common examples of food pathogens

2.3.1.1 *Campylobacter*

Members of the family *Campylobacteriaceae* include *Campylobacter jejuni* and other species of *Campylobacter*. It is among the most typical causes of diarrheal disease. Every year in the US, C. jejuni is to blame for 76 fatalities, 8,500 hospitalizations, and around 850,000 illnesses (Scallan, 2011). The World Health Organization (WHO) predicts that 1% of Western Europe's population will contract *Campylobacter* each year (Humphrey, 2007). It is the second-most prevalent bacterial cause of diarrhea in the US. The majority of instances of campylobacteriosis, a disease brought on by the *Campylobacter* bacterium, are linked to consuming raw or undercooked poultry and pork or from these foods' cross-contamination with other foods. Although freezing helps to minimize the amount of *Campylobacter* bacteria on raw meat, it won't entirely eradicate them, thus it's crucial to properly cook meals. The most common age groups for campylobacteriosis are newborns and young children.

2.3.1.2 *Clostridium botulinum*

The spore-forming bacteria *Clostridium* sp. are members of the *Bacillaceae* family. They comprise obligatory anaerobic or aerotolerant spore-forming rods that do not generate spores in the presence of air and are typically Gram-positive, at least in the early stages of growth (Bintsis, 2017). They release a toxin that can result in botulism, a condition that can make it impossible for the breathing muscles to move air in and out of the lungs and is potentially fatal.

2.3.1.3 *Clostridium perfringens*

The *Bacillaceae* family member *Clostridium perfringens*, formerly known as *Clostridium welchii*, is a significant contributor to foodborne illness (Bintsis, 2017). The bacterium *Clostridium perfringens*, also referred to as *C. perfringens*, is widespread in our environment. Under optimal circumstances, it can multiply quite quickly. The most vulnerable are infants, young children, and elderly people.

2.3.1.4 *E. coli* O157:H6

E. coli, also referred to as *Escherichia coli*, is a vast genus of bacteria. Although the majority of *E. coli* strains are not harmful, some of them can be quite sickening. One strain, *E. Coli* O157:H7

(STEC), is frequently linked to outbreaks of food poisoning because its symptoms can be very serious. About 73,000 cases of foodborne sickness are brought on by this bacterium, which has the ability to produce a deadly toxin, each year in the United States.

2.3.1.5 *Listeria monocytogenes*

One of the biggest causes of food-borne illness fatalities, particularly in pregnant women, new mothers, the elderly, and people with impaired immune systems, is *Listeria monocytogenes*. Pregnant women who contract infections run the risk of miscarriages, stillbirths, and birth abnormalities that can be fatal to the developing fetus (Buchanan, 2017). Listeriosis is a dangerous infection that mostly affects those who are at a high risk for food poisoning and is brought on by eating food contaminated with *Listeria monocytogenes* bacteria. Most other bacteria cannot grow in refrigerator conditions, but *Listeria* can.

2.3.1.6 *Salmonella*

One of the most frequent causes of enteric illnesses (food poisoning) worldwide, this genus of *Enterobactericiae* has pathogenic traits. Every year, 1.4 million cases of foodborne illness are caused by it. They were given their names in honor of the researcher Dr. Daniel Salmon, who discovered the first bacteria, *Salmonella choleraesuis*, in a pig's intestine (Bacon, 2003). Salmonellosis is an infection brought on by a type of bacteria known as *Salmonella*. It is one of the most frequent bacterial causes of diarrhea and the main factor in hospitalizations and fatalities linked to foodborne illnesses. Pregnant women, elderly individuals, smaller children, and anyone with compromised immune systems are particularly susceptible to *salmonella* infection. *Salmonella* can easily spread if you don't maintain good hygiene and use the right cooking techniques because, the bacterium can dwell in the intestinal tract of both humans and other animals.

2.3.1.7 *Staphylococcus aureus*

Healthy persons and animals with clear nasal passages frequently have *Staphylococcus aureus* (staph) on their skin, throats, and noses. Therefore, unless it is transferred to food products, where it can grow and produce toxic toxins that cause vomiting soon after ingestion, it typically doesn't cause illness. Symptoms of staphylococci include nausea, cramping in the stomach, vomiting, or diarrhea. Cooking can kill staphylococcal germs, but their toxins are heat-resistant and cannot.

Staph infections can affect everyone, but certain people are more vulnerable than others, including those with chronic illnesses like diabetes, cancer, vascular disease, eczema, and lung disease.

2.3.1.8 *Shigella*

Shigella are facultative anaerobic, non-spore-forming, non-motile Gram-negative rods. *S. sonnei* appears to be more tolerant of lower temperatures than the other serogroups, yet they can develop at a range of 6 to 48 °C. Although growth has been documented between pH 4.8 and 9.3, optimal growth happens between pH 6.0 and 8.0. It is thought to be the cause of 448,000 cases of diarrhea per year. *Shigella* can easily spread from one person to another and from infected people to food due to poor hygiene.

2.3.2 Sources of food pathogens

Food pathogens can be gotten from different sources. It occurs when individuals consume food or beverages that have been contaminated with pathogens, chemicals, or poisons. It can also be as a result of cross contamination from food handlers and cooking/processing equipment. Some food pathogens and their sources can be seen in the table below;

Table 2.2: Some food pathogens and their sources

Food Pathogens	Sources
Campylobacter	Meat, raw and undercooked poultry unpasteurized (raw) milk raw, untreated water and contaminated produce or contaminated water.
Clostridium botulinum	Improperly prepared home-canned foods Honey should not be fed to children less than 12 months old.
Clostridium perfringens	Food that has been abused in terms of time and/or temperature, improper cooking, chicken, meat, gravies, and food that has been left on steam tables or at room temperature for an extended amount of time.
E. coli O157:H6	Raw or undercooked ground beef, unpasteurized juices, raw milk, and ciders or drinking unpasteurized beverages or dairy products, raw fruits, vegetables (such as lettuce, other leafy greens, and sprouts) and contaminated food, especially soft cheeses made from raw milk, Contaminated water, including drinking untreated water and swimming in contaminated water, animals and their environment, particularly sheep, cows, goats and Feces of infected people.
Listeria monocytogenes	Unpasteurized dairy products, including soft cheeses, smoked fish, sliced deli meats, hot dogs, deli-prepared and pate salads (egg, ham, seafood, and chicken salads). Found in refrigerated raw sprouts, dairy products, raw and undercooked meat, poultry, seafood, raw fruits and vegetables.
Salmonella	Undercooked chicken and meat, raw or undercooked eggs, fresh fruits and vegetables (such as sprouts and melons), and unpasteurized dairy products. Environments and animals: Especially birds (baby chicks), reptiles (snakes, turtles, lizards), amphibians (frogs), and pet food and treats. It can also spread through contact with sick animals or food handlers who have not cleansed their hands after using the restroom or through contact with contaminated animals.
Staphylococcus aureus	Cooked foods high in protein that are held too long at room temperature, unpasteurized dairy products and salty foods such as ham and other sliced meats. Foods that are made or come in contact with hands and require no additional cooking are at highest risk, including: Salads, such as egg, ham, tuna, macaroni, chicken, and potato. Bakery products, such as cream-filled pastries, cream pies and sandwiches. If they don't wash their hands before handling food, people who have the bacteria *Staphylococcus aureus* (Staph), which is frequently present on the skin, can contaminate it. Foods that are not cooked after handling, such as puddings, sliced meats, pastries, and sandwiches, are especially risky if contaminated with Staph.
Shigella	Salads, unclean water, and any food handled by someone who is infected with the bacterium. Consumption of contaminated food or drink or contact with an infected individual. *Shigella* outbreaks in food are most frequently brought on by ill food handlers.

2.3.3 Some common food borne infections caused by food pathogen

Consuming contaminated foods or beverages that are infected with food germs results in foodborne disease. Foods can be contaminated by a wide range of pathogens or disease-causing bacteria, leading to a wide range of foodborne illnesses. The majority of foodborne illnesses are bacterial, viral, and parasitic infections. Other illnesses are food poisonings brought on by dangerous poisons or substances.

Table 2.3: Some food borne infections and the causative food pathogens

Food Borne Infections	Causative Pathogen
Botulism	*Clostridium botulinum.*
Perfringens Poisoning	*Clostridium perfringens* (*welchii*)
Staphylococcal Food Poisoning	*Staphylococcus aureus*
Salmonellosis	*Salmonella* sp. (*S. enteritidis*, *S. choleraesuis*, S. *typhimurium*, *S. infantis* and those causing fevers are *S. typhi*, *S. paratyphi*).
Listeriosis	*Listeria monocytogenes*
Diarrheal illness	*Camphylobacter jejuni*

2.3.4 Application of silver nanoparticles as an antibiotic remedy against food pathogens

One of the most significant areas of current research, nanotechnology has quickly produced the most promising medical applications (Sardul *et al*., 2017). New procedures and products can now be developed with the help of nanotechnology. It can be used for a variety of things, including advancing human and animal health, producing more enduring consumer goods, and boosting industrial and agricultural output (Tharayil *et al*., 2021). Because of this, nanoparticles have been employed for a variety of purposes, most notably in the development of antibiotics to combat bacterial resistance (Santos *et al*., 2021). In order to accelerate the pace of disease symptom identification and provide quick therapies, nanotechnology is employed in medication delivery systems and nutrient release systems (Nano encapsulation). Additionally, it can be used to produce nanoscale sensors to detect bacterial, viral, or chemical contamination of crops in addition to fertilizers and other nanoscale additives (Zorraquín-Pea *et al*., 2020).

According to Zorraquín-Pea *et al*. (2020), microbial food deterioration is a significant global issue that can shorten food's shelf life while raising the risk of foodborne illnesses. Given the waste of ruined goods and the effects of foodborne diseases on the public health, microbial contamination of foods is one of the major issues facing the food business (Carbone *et al*., 2016). One of the things that puts people's health in peril is food-borne illness, especially for those who are at risk of social exclusion and in developing nations.

The development of nanotechnology in this field is a result of the ongoing hunt for potent antimicrobials for use in the food industry. The nanomaterial with the best antibacterial action is silver nanoparticles (Ag-NPs), which has a lot of potential for use in food processing and packaging (Zorraquín-Pea *et al*., 2020). For the treatment of burns and persistent wounds, as well as for having antibacterial characteristics, silver and its compounds are well-known (Shakibaie *et al*., 1998). When compared to bulk silver metal, AgNPs have a higher bactericidal activity due to their large surface area to volume ratio (Cho *et al*., 2005; Panyala *et al*., 2008). Since ancient times, silver has been employed in the study of medicine. The primary use of this precious metal was as a safe and efficient disinfectant and antibacterial agent (Chan *et al*., 2012). However, it has been utilized as an alternate treatment for a number of illnesses since the 1990s (Fung and Bowen, 1996). Due to the rise of germs that are resistant to antibiotics as a result of antibiotic misuse, silver has recently gained popularity (Madhumathi *et al*., 2010). One of the most important products in the globe for

issues with public healthcare is antibiotic resistance. Nearly all types of microorganisms have become more resilient to antibiotics in recent decades, posing a threat to emerging super strains of infectious diseases that are both more expensive to treat and more difficult to heal. Pathogens with known drug resistance characteristics are on the rise, and preventing their spread and impact on human health is a major problem (Rajeshkumar and Malarkodi, 2014). It has been discovered that these silver nanoparticles work well against both aerobic and anaerobic microbes (Sandhu *et al.*, 2017). Researchers have researched the antibacterial properties of silver, silver ions, and silver nanoparticles in order to determine how they work against a variety of bacteria (Pal *et al.*, 2007). Morones *et al*. (2005) evaluated the bactericidal effect of AgNPs, and identified three main mechanisms of action of the nanoparticles which are:

- AgNPs between 1 and 10 nm in size are bound to the cell membrane's surface and severely impair its capacity for permeability and respiration.
- AgNPs can enter bacterial cells and harm them, possibly by interacting with sulfur- and phosphorus-containing substances like DNA.
- Silver nanoparticles (AgNPs) emit silver ions, which have the potential to be very reactive and may react with the negatively charged cell membrane, adding to their ability to kill bacteria.

An overview of the effectiveness of silver nanoparticles to inhibit the growth of different microorganisms is shown in Table 2.4;

Table 2.4: Recompilation of studies about the antimicrobial effects of Ag-NPs against foodborne pathogens.

Ag-NPs concentration	Food pathogens	Main result	Reference
0–400µg Ag/mL	*Proteus vulgaris* and *Shigella sonnei, Staphylococcus aureus, Bacillus megaterium.*	The smaller size of Ag-NPs produced a greater growth inhibition. For both sizes the MIC values for the bacteria were between 75–400 ug/mL.	(Khurana *et al*., 2014)
197 µg Ag/mL	*Campylobacter jejuni* (collection strain and isolates of patients and food chain)	The concentrations between 9.85 and 39.4 µg/mL were bactericidal after 24 h of incubation. In addition, the lower concentrations (1.23 and 4.92 µg/mL) significantly inhibited the growth of the collection strain.	(Silvan *et al.,* 2018)
2.37, 4.75, 9.5 and 19 µg Ag/mL	*Escherichia coli* and *Salmonella typhimurium*	The concentration of 4.75 µg/mL Ag-NPs completely inhibited the growth of the two bacteria and the concentration of 9.5 µg/mL was sufficient to kill them.	(Chandhru *et al*., 2019)
0–100 µg Ag/mL	*Escherichia coli* O157:H7, *Vibrio parahaemolyticu*, *Pseudomonas aeruginosa, Salmonella typhimurium, Listeria monocytogenes* and *Staphylococcus aureus.*	Ag-NPs exerted a strong antimicrobial activity against all the pathogens tested. MIC of *V. parahaemolyticus* and *S. aureus* were 6.25 µg/ mL and 50 µg/mL, respectively, and MBCs of *V. parahaemolyticus* and *S. aureus* were 12.5 µg/mL and 100 µg/mL, respectively.	(Du *et al*., 2019)
0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 20.0 and 30.0 µg Ag/mL	*Escherichia coli Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*	The nanoparticles produced a total inhibition of *E. coli* growth at the concentration of 7.5 µg/mL. On the contrary, a concentration of >30 µg/mL is required for the complete inhibition of *S. aureus* and the resistant strain.	(Navarro *et al.,* 2019)
0–400 µg Ag/mL .	*Proteus vulgaris* and *Shigella sonnei, Staphylococcus aureus,* and *Bacillus megaterium*	The smaller size of Ag-NPs produced a greater growth inhibition. For both sizes the MIC values for the bacteria were between 75–400 ug/mL	(Khurana *et al*., 2014)

CHAPTER THREE

METHODOLOGY

3.1 Materials and Equipments

The following materials and equipment were used for the experiment: sterile distilled water, antibiotic capsule, Erlenmeyer flask, filter paper, NaCl, Whatman filter paper, 10 mM $AgNO_3$ solution, petri dish, deionized water, plastic sieve, inoculating loop, cover slips, glass slide, cotton wool, aluminum foil, conical flask, dropper, spatula incubator, weighing balance, light microscope, Bunsen burner, hot air oven, autoclave, weighing balance, water bath, centrifuge, UV- visible spectrophotometer, and Fourier transform infrared spectrophotometer (Khabat *et al*., 2011).

3.2 Culture media and reagents

The media used during the experiment were: potato dextrose agar (PDA), Muller Hinton Agar and Nutrient broth. Reagent used during the experiment include lacto-phenol blue stain.

3.3 Isolation of fungal species

3.3.1 Sampling

Soil sample was obtained from dumpsites located within the school premises. The soil sample was collected from a depth of 5-10 cm using sterile spatula and stored in sterile plastic vials. The bottles were labelled properly and transported to the laboratory for examination and analysis (Eddy *et al*., 2006). The samples were stored at 27 ^{0}C when not in use (Nwuche *et al*., 2011).

3.3.2 Serial dilution

Serial dilution was performed for isolation of soil fungi (Berkhade, 2018). One gram of soil sample was weighed and placed in a sterile test tube with 9ml distilled water. The sterile test tubes were taken and labelled per dilutions ranging from 10^{-1} to 10^{-6}. 1g of the respective soil sample was weighed and added to the first dilution blank of 9.0 mL of distilled water. To the second dilution blank (10^{-2}), 1.0 mL of the first dilution blank (10^{-1}) was added. The tubes were shaken, and repeated dilutions were performed all the way to the last tube (10^{-6}). 0.1 mL of each diluents was inoculated aseptically using pour plate method in a disposable petri dish with potato dextrose agar (PDA).

3.4 Pure culture technique

Fungi colonies obtained were sub-cultured using stabbing method on a solidified potato dextrose agar plate and incubated at 28 ^{0}C for 72 hrs

3.5 Preparation of culture media

Potato dextrose agar (PDA), Muller Hinton Agar (MHA), and Nutrient broth were used for fungal culturing in this study. The preparations of these media were done as follows:

3.5.1 Potato Dextrose Agar

For the isolation and culture of fungi, potato dextrose agar (PDA) was employed. PDA is a common general-purpose media widely used in microbiological research to differentiate the morphological features, pigmentation and identify fungi cultures (Griffith *et al*., 2007). 39 g of commercially available Potato Dextrose Agar were weighed, dissolved in 1 liter of sterile distilled water, and thoroughly mixed while adhering to aseptic procedures and conditions. Chloramphenicol antibiotic was added to prevent bacteria growth. The mixture was boiled to dissolve the culture medium completely. The agar was then sterilized by autoclaving at 121^0 C for 15 minutes. The medium was allowed to cool to 45^0 C before pouring into the sterile petri dishes.

3.5.2 Muller Hinton Agar

Muller Hinton Agar (MHA) was used to test the antibiotic susceptibility of the fungi isolates. Maintaining aseptic methods and conditions, 36 g of commercially prepared Muller Hinton Agar was measured and dissolved in 1 litre of sterile distilled water in a conical flask and mixed thoroughly. The mixture was boiled to dissolve the culture medium completely. The agar was then sterilized by autoclaving at 121^0 C for 15 minutes. The medium was allowed to cool to 45^0 C before pouring into the sterile petri dishes.

3.5.3 Nutrient Broth

Nutrient broth was used for the isolation, cultivation and to maintain the stock of the fungi isolates. Maintaining aseptic methods and conditions, 13 g of commercially prepared nutrient broth was measured and dissolved in 1 litre of sterile distilled water in a conical flask and mixed thoroughly. The medium was then sterilized by autoclaving at 121^0 C for 15 minutes. The medium was allowed to cool to 45^0 C.

3.5.4 Pour plate method

Pour plate method was used to culture the fungal colonies on Potato Dextrose Agar. 0.1 mL from the serially diluted samples (10^{-4} and 10^{-6}) was poured into the sterile petri plates. After few minutes the molten Potato Dextrose agar was poured gently into the respective petri dishes then the petri dishes were mixed thoroughly by rotating gently prior to agar solidification (Pereira *et al.*, 2010). After solidification, the plates were incubated at room temperature for 72 hrs.

3.6 Screening of the fungal isolates

Screening of fungal isolates was done on Muller Hinton Agar using pathogenic *E. coli*. Sub-cultured fungi colonies were further inoculated into nutrient broths and incubated at 28^0 C for 72 hours. Fungi cultures were tested against pathogenic *E. coli* to determine the antibiotic susceptibility of the fungi isolates using cup borer method. Wells were first created on the solidified Muller Hinton Agar medium, then the pathogenic *E. coli* was streaked carefully on the surface of the medium before 100 micro litre of each fungi isolate were pipetted into the wells. All this was done under aseptic procedures. Plates were incubated at 37^0 C for 24 hours.

3.7 Characterization of the fungal isolates

After screening, the selected positive isolates were inoculated on Potato Dextrose Agar and incubated at the room temperature for 72 hrs.

3.7.1 Morphological characterization

The morphological characteristics of the selected isolates grown on PDA was observed and studied for their growth patterns, nature of spores and pigmentation (Berkhade, 2018).

3.7.2 Biochemical characterization

A portion of the fungal isolates was picked using a sterile inoculating needle and mounted on a glass slide with a drop of lactophenol blue on it and covered with cover slips. The preparation was examined under a light microscope using x10 magnification lens. The cell morphology was recorded with respect to spore chain morphology and mycelium structure (Berkhade, 2018).

3.8 Fungi biomass preparation

The fungi biomass was prepared by inoculating the selected fungi isolates in 250 mL nutrient broth each for 48 hrs in a rotary orbital shaker at 120 rpm at room temperature (Gajbhiye *et al.*, 2009).

3.9 Biosynthesis of AgNPs

For the biosynthesis of silver nanoparticles, 250 mL $AgNO_3$ solution was mixed with 250 mL of cell biomass of the fungi in an Erlenmeyer flask and incubated in a dark condition for 24 hrs at room temperature until a color change was observed (Magdi *et al*., 2014)

3.10 Characterization of AgNPs

3.10.1 UV- spectrum analysis

The UV spectrum analysis was used to follow up the reaction process and measure the absorbance of the silver nanoparticle. A blank was measured before the biosynthesized silver nanoparticle solution was poured gently into a cuvette and placed inside the UV spectrophotometer. A blank was then measured before running each wavelength of the sample (Sardul *et al*., 2017). The absorbance of the silver nanoparticle was measured at different time intervals (24 hrs, 48 hrs and 72 hrs respectively).

3.10.2 Fourier transform infrared spectroscopy (FT-IR)

FT-IR was used to identify the types of chemical bonds and determine the chemical functional groups present in the silver nanoparticle. The dried and powdered silver nanoparticle was placed in the FT-IR, then the FT-IR spectrum was recorded (Vahabi *et al*., 2011)

3.11 Application of synthesized AgNPs against pathogenic bacteria

The silver nanoparticle synthesized using the selected fungal strains were tested for antimicrobial activity using Agar-well-diffusion method against the selected pathogenic bacteria (*Salmonella enterica*, *Proteus* sp., *Enterococcus faecalis*, *E. coli* and *Staphylococcus aureus*). The slant cultures of bacteria were sub cultured on nutrient agar plate. Each strain was streaked homogeneously onto the individual plates using sterile inoculating loop. Hollow wells of 5 mm diameter were dug on Muller Hinton agar. 100 µm of Ag-NPs, fungal strain, silver nitrate solution and chloramphenicol, and streptomycin antibiotics were pipetted into the wells on the agar plates and incubated at 37 °C for 24 hrs. After 24 hours of incubation, the zone of inhibitions was measured (Rajeshkumar and Malarkodi, 2014).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Colony counts

Sixteen (16) morphologically different fungal species were isolated from the dumpsite located within the school premises. Table 4.1 shows the colony count in each of the serial dilution plated for 10^{-4} and 10^{-6} respectively after a 72 hrs incubation period. The colony counts for the first sampling ranged from 60 to 6 cfu/mL, second sampling range from 42 to 4 cfu/mL and third sampling range from 40 to 1 cfu/mL colony respectively. The persistence of fungi in this environment is due to the increase in refuse in the dumpsite. It was found that fungi species dominated dumpsites and this is in agreement with findings reported by Sangale *et al*. (2019).

4.2 Screening and selection of fungi isolates

Table 4.2 shows the results of the screening of fungi isolates for antibiotic susceptibility against pathogenic *E. coli* on solid agar. Positive isolates were measured based on the zone of inhibition. Eleven fungi isolates had positive result with zone of inhibition ranging from 15mm to 29mm and five fungi isolates with negative results. Isolates MDS2, MDS9, MDS10, MDS11 and MDS14 showed no zone of inhibition while Isolate MDS1 had the highest activity and was further used for the biosynthesis of nanoparticle. The observed results showed similar results with that of Li *et al*. (2012) which fell on six different fungi sp. in the antifungal screening of various fungi species. Sethi *et al*. (2013) reported similar findings in the antibiotic screening of fungi species isolated from soil samples, where the fungal *Rhizopus stolonifer* showed high inhibitory activity against the selected pathogens.

Table 4.1: Colony counts for soil sample

Sampling time	Sampling location	Colony forming units/Serial dilution	
		At 10^{-4} (cfu/mL)	At 10^{-6} (cfu/mL)
First Sampling	MTU dumpsite	$60x10^4$	$6x10^6$
Second Sampling	MTU dumpsite	$42x10^4$	$4x10^6$
Third sampling	MTU dumpsite	$40 x10^4$	$1x10^6$

Table 4.2: Antibiotic susceptibility screening result

Fungi isolates	Zone of inhibition (mm)
MDS 1	29
MDS 2	n.z
MDS 3	15
MDS 4	26
MDS 5	24
MDS 6	25
MDS 7	23
MDS 8	18
MDS 9	n.z
MDS 10	n.z
MDS 11	n.z
MDS 12	28
MDS 13	24
MDS 14	n.z
MDS 15	18
MDS 16	23

Key: n.z – no zone of inhibition

4.3 Characterization of fungi isolate

Table 4.3 and Plate 4.1 shows the morphological identification of the selected isolate MDS1 on PDA medium. The isolate is a growing mould which matures within 3 to 5 days. Growth begins as fluffy white forming a cottony texture and on aging forms black and light yellowish spores at the center with mycelia present at the margin. Mycelia formed were white with heavy sporulation yielding black and yellowish colonies. The reverse is typically whitish forming a hollow base. The morphology was recorded based on their front view, back view and nature of spores. It also illustrates the microscopic image of isolate MDS1, following staining with lacto phenol blue. The microscopic appearance showed non-septate and hyaline hyphae. Sporangiospores appeared medium in length with a round to spherical sporangium present at the top. Abundance of spores were present. Based on the results of morphological and biochemical characterizations, the probable identity of the isolate was concluded to be *Aspergillus* sp. Afzal *et al*. (2013) recorded similar results when carrying out the morphological identification of *Aspergillus* species. Numerous *Aspergillus* species have been used in the synthesis of silver nanoparticles like *A. amigatus* (Duran *et al*., 2005), *A. terreus* (Lofty *et al*., 2021), *A. niger* (Princy *et al*., 2011), *A. flavus* (Ninganagouda, *et al*., 2013), *A. fumigatus* (Zomorodian *et al*., 2016), and *A. oryzae* (Daniels, 2015). Li *et al*. (2012) evaluated the synthesis of silver nanoparticle using the fungal *A. terreus*. Other fungal species that have been employed in the synthesis of nanoparticles, include *Verticilium* sp. (Mukherjee *et al*., 2001), *Fusarium oxysporum* (Verma *et al*., 2011), *Trichoderma asperellum* (Mukherjee *et al*., 2008), and *Phoma glomerata* (Birla *et al*., 2009).

Table 4.3: Morphological characteristics of *Aspergillus* sp. MDS1 on PDA culture plate

Isolate	Front view	Back view	Nature of spores	Probable identity
MDS1	White with black spores at the center Patches of light yellow spores	Hollow at the base	Black/light whitish spores	*Aspergillus* sp.

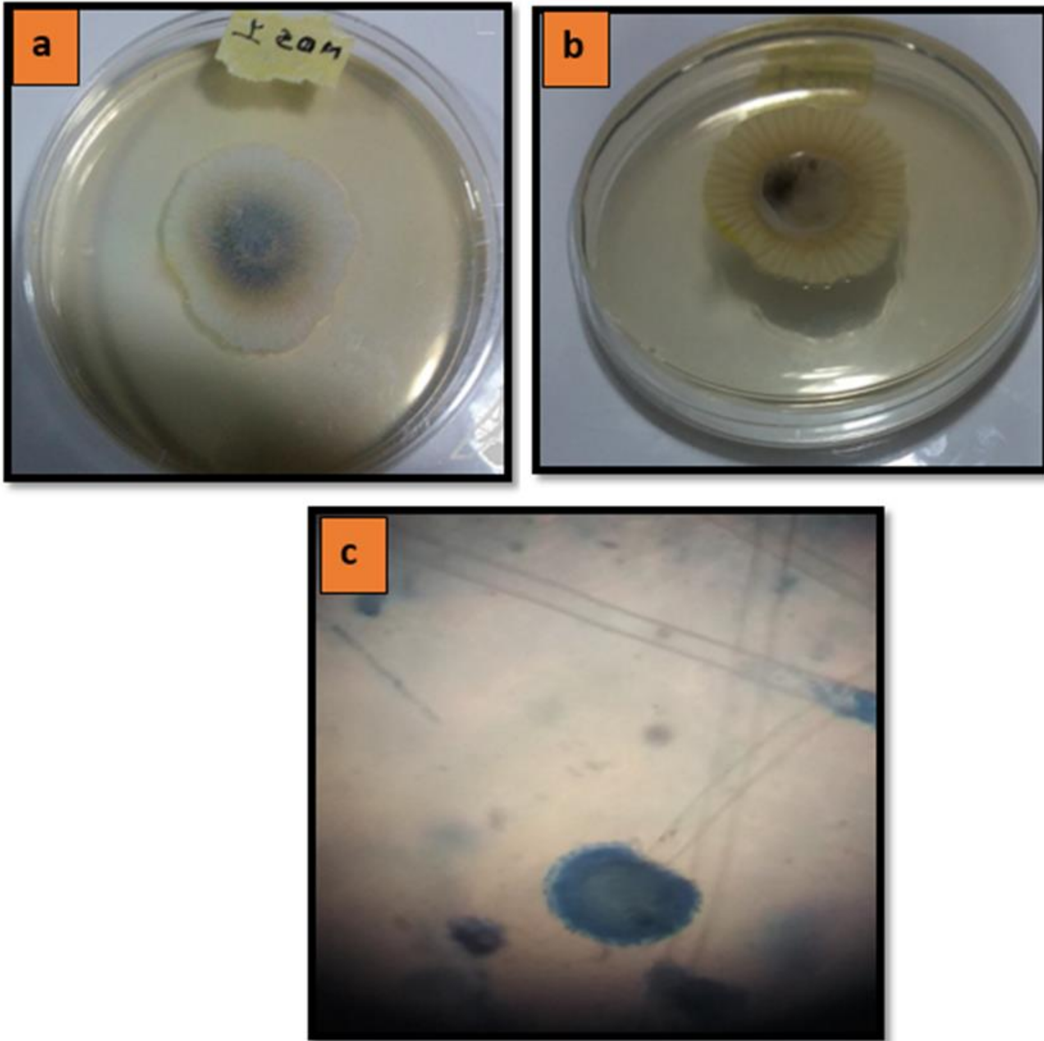

Plate 4.1: Morphological characterizations of *Aspergillus* sp. MDSI (a) Front view on PDA plate (b) Back view on PDA plate (c) Microscopic appearance at 400x magnification

4.4 Biosynthesis of silver nanoparticles

4.4.1 Visual observation

Plate 4.2 shows the result of broth after 72 hrs of incubation, $AgNO_3$ solution and colour change of fungi biomass after biosynthesis. At the end of incubation period, a yellowish to brownish colour change was observed indicating the biosynthesis of silver nanoparticles. The colour change varied at different hours of agitation. Yellowish brown colour was observed after 24 hrs of agitation which seems lighter compared to brownish colour obtained after 72 hrs of agitation. Farrag *et al*. (2020) obtained similar results of brownish colour after 72 hrs of incubation which indicated the synthesis of the silver nanoparticle by the *Aspergillus niger* strain used. This is primarily due to the surface of plasmon resonance of deposited silver nanoparticles. The colour of the silver nanoparticles was due to bio reduction of Ag+ to Ag- leading to the subsequent formation of silver nanoparticle (Vahabi *et al*., 2011; Link and El-sayed, 2003). Al-Zubaidi *et al*. (2019) obtained similar results using the fungal isolate *A. niger* after 48 hrs of incubation confirming the synthesis of the silver nanoparticle.

4.5 Characterization of silver nanoparticles

4.5.1 Ultraviolet spectroscopy

Figure 4.1 illustrates the UV spectroscopy of fungal mediated silver nanoparticle after 24, 48, and 72 hours. At 24 hrs no absorption peak was observed, a declined reading was observed from 200 to 800 nm. At 48 hrs an absorption peak was observed between 300nm and 400 nm. At 72 hrs a slight absorption peak was observed at 400 nm wavelength range indicating the reduction of silver nitrate in the culture broth. Mohmed *et al*. (2017) evaluated the synthesis of silver nanoparticle by *Aspergillus* sp. and obtained similar result during the UV spectrum analysis showing plasmon resonance at 400 nm. The UV spectra clearly demonstrate the increase in silver nitrate solution intensity with time, which indicates the production of more silver nanoparticles in the solution (Devi *et al*., 2014). At 24 hrs the UV spectrum analysis showed no peak, it declined due to the slow production of the nanoparticle. Compared to the result of the UV analysis recorded after 48 hrs a peak could be observed indicating the gradual formation of the silver nanoparticle (Verma *et al*., 2010). A slight peak was observed at 400 nm for 72 hrs UV spectrum analysis. Similar findings were reported by Rajeshkumar and Malarkodi (2014) where they both observed the UV spectrum of the synthesized silver nanoparticle at different time intervals noticing a peak at 400 nm after 48 hrs. Similar observation was reported by Vigneshwaran *et al*. (2007) on biosynthesis of nanoparticles by *Aspergillus flavus*.

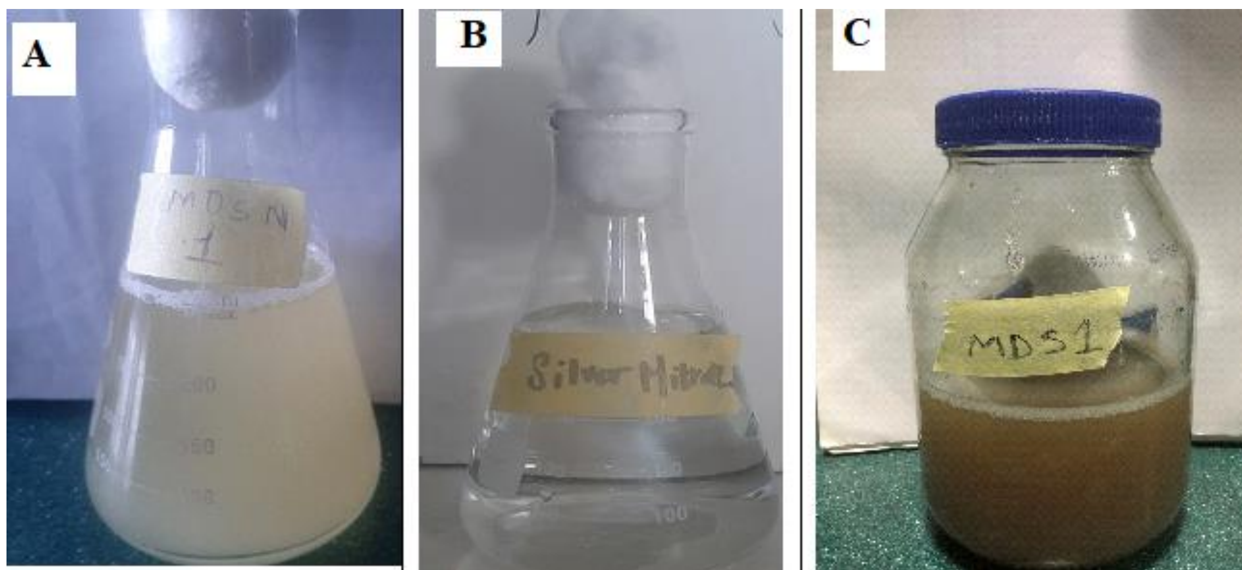

Plate 4.2: Visual observation of silver nanoparticle synthesis. (a) *Aspergillus* sp. MDS1 biomass (b) silver nitrate solution (c) brown colour indicating silver nanoparticle synthesis

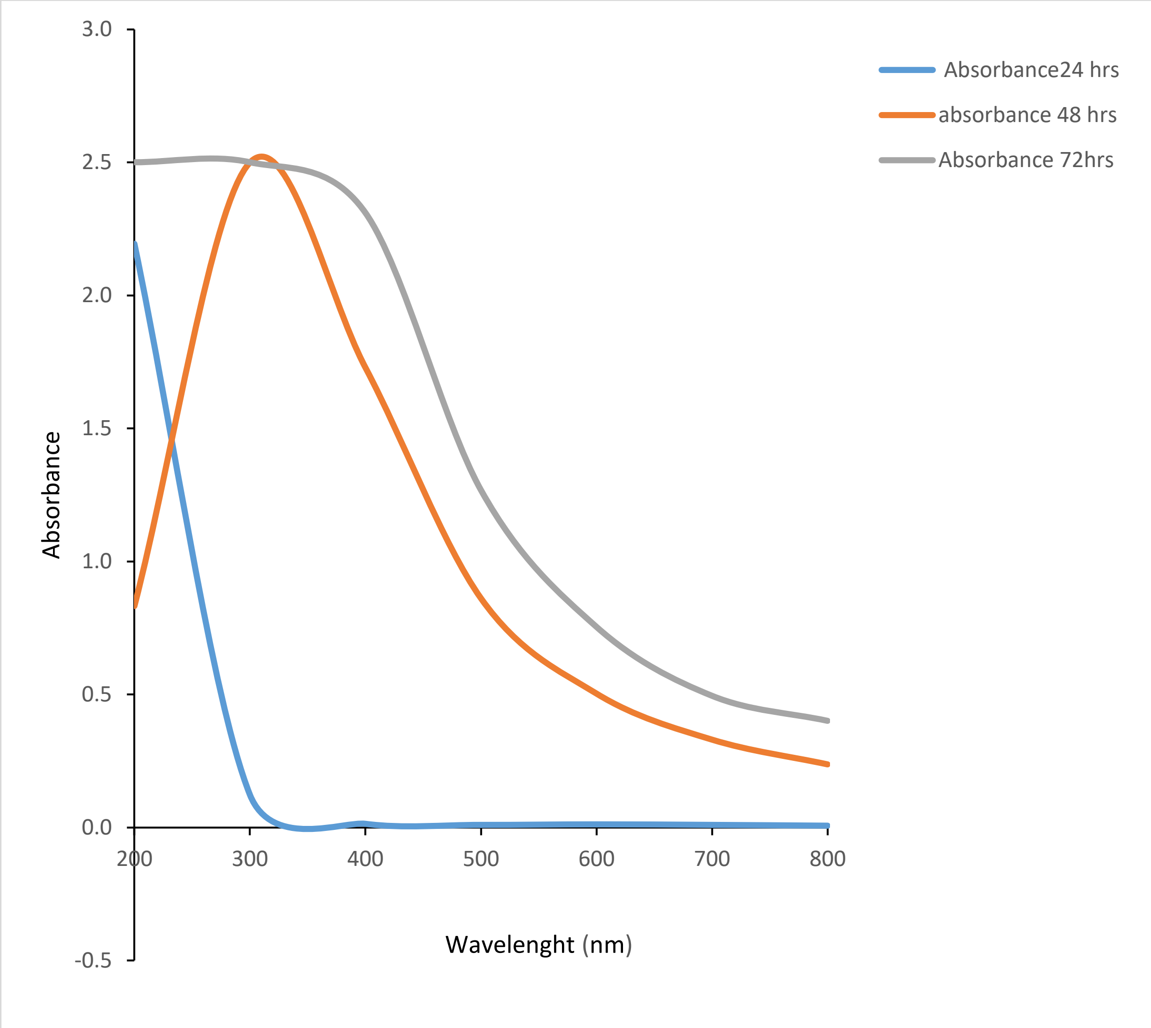

Figure 4.1: UV-Visible spectrophotometer of the biosynthesized silver nanoparticles (AgNPs) by *Aspergillus* sp. MDS1 strain at different incubation periods.

4.5.2 FT-IR analysis of *Aspergillus* sp. AgNPs

The Fourier transformation infrared spectroscopy (FT-IR) spectrum of the greenly synthesized *Aspergillus* sp.-AgNPs is shown in Figure 4.2 and Table 4.4 Fifteen (15) peaks were present between 3762 cm^{-1} to 360 cm^{-1}. The FT-IR spectrum showed a broad peak at 3438 cm^{-1} which corresponds to the broad intermolecular bonding of the alcoholic OH band. The peak at 2926.29 cm^{-1} indicates an alkane C-H stretching vibration. The peak at 1728.45 cm^{-1} indicate the strong aldehyde C=O stretching. The peak at 1637.57 cm^{-1} show the medium C=C stretching of the alkene group. Furthermore, the peak at 1514.38 cm^{-1} indicates a strong nitro compound group from the N-O stretching. The peak at 1381.79 cm^{-1} indicates medium aldehyde C-H bending. The peak at 1329.00 cm^{-1} shows the presence of alcohol group indicating the medium O-H bending. The peak at 1256.29 cm^{-1} indicates the strong aromatic ester bond of the C-0 stretching. The presence of a strong sulfoxide group with S=O stretching is indicated in the 1039.46 cm^{-1} peak and a peak at 605.00 cm^{-1} indicates the presence of a halo compound with C-I stretching. Chan and Don (2013) reported similar results using the fungal *Pycnoporus sanguineus* and *Schizophyllum commune* and recorded the same wavelength indicating the chemical group C – H and bonding class alkanes. Awad *et al*. (2022) recorded similar FT-IR analysis results during the synthesis of silver nanoparticle by *Aspergillus niger* strain. The obtained FT-IR results confirmed the presence of various bioactive molecules such as aldehyde, alcohol, alkenes, carboxylate, sulfoxide, and amino acids that have been reported previously as a potential reducing agent for the biosynthesis of metal and metal oxide NPs (Salem *et al*., 2021). These result were in agreement with other reports found that the proteins play key role in the formation of silver nanoparticles and act as capping and stability agents in the synthesis of AgNPs (Jaidev *et al*., 2010).

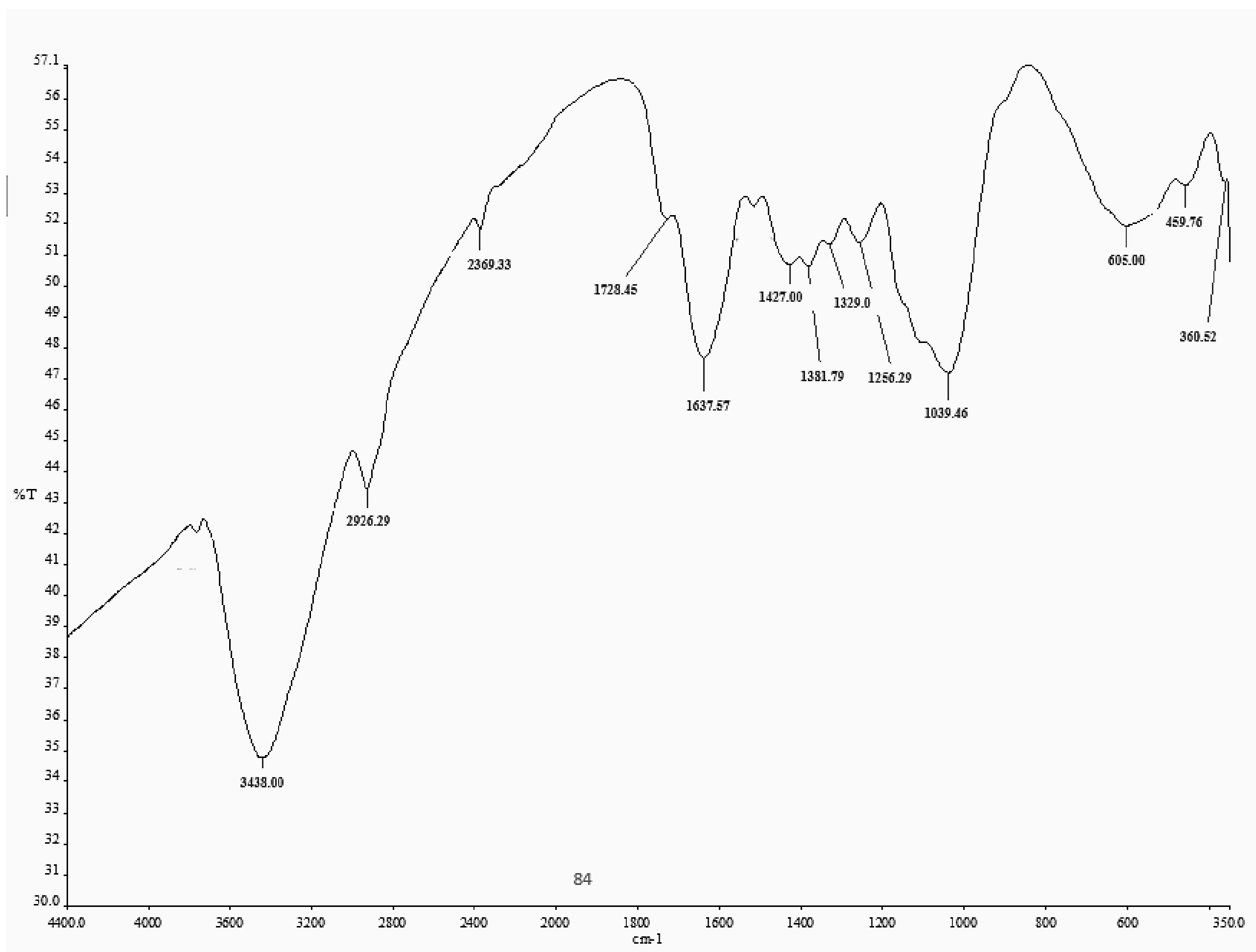

Figure 4.2: Fourier transformation infrared (FT-IR) spectroscopy of the biosynthesized silver nanoparticles (AgNPs) by *Aspergillus* sp. MDS1 strain.

Table 4.4: FT-IR spectral positions with their corresponding vibration modes for biomass filtrate and AgNPs synthesized by *Aspergillus* sp. MDS1

Absorption frequency (cm^{-1})	Chemical groups	Compound class
3762.00	-	-
3438.00	O-H stretching	Alcohol
2926.29	C-H stretching	Alkane
2369.33	-	-
1728.45	C=O stretching	Aldehyde
1637.57	C=C stretching	Alkene
1514.38	N-O stretching	Nitro compound
1427.00	-	-
1381.79	C-H bending	Aldehyde
1329.00	C-H bending	Alcohol
1256.29	C-O stretching	Aromatic ester
1039.46	S=O stretching	Sulfoxide
605.00	C-I stretching	Halo compound
459.76	-	-
360.52	-	-

4.6 Antibacterial activity of *Aspergillus* sp. MDS1 biosynthesized silver nanoparticle against selected bacteria pathogens.

Table 4.5 shows the antibacterial activity of *Aspergillus* sp. MDS1 mediated silver nanoparticle compared with silver nitrate solution, *Aspergillus* sp. MDS1 and chloramphenicol against pathogen bacteria such as *Salmonella enterica*, *Proteus* sp., *Enterococcus faecalis*, *E. coli* and *Staphylococcus aureus*. The silver nanoparticle exhibited strong inhibition against *Staphylococcus aureus* having a zone of 16 mm compared to the silver nitrate solution having a zone of 13mm. a zone of 12 mm was recorded for *Aspergillus* sp. MDS1 and no inhibition activity by chloramphenicol. *Aspergillus* sp. MDS1 mediated silver nanoparticle showed no inhibition activity against *Proteus* sp. and *E. coli*. The current study proved that the biologically silver nanoparticles seem to present potential and effective bactericidal covering material (Rajeshkumar and Malarkodi, 2014).

Several new studies on the antimicrobial activity of nanoscale materials, including the antibacterial characteristics of silver and its NPs, are accessible as interest in the use of NPs for biological purposes rises (Rai *et al*., 2009). Ahluwalia *et al*. (2014) evaluated the synthesis of silver nanoparticle by *Trichoderma harzianum* and tested it against *Staphylococcus aureus* and *Klebsiella pneumoniae* in vitro.

Similar to this study, Dhanaraj *et al*. (2018) conducted a research by testing the activity of the silver nanoparticles produced by *Aspergillus niger* against *Escherichia coli* and *Salmonella typhimurium*. The silver nanoparticle completely inhibited the growth of the two bacteria. Sagar and Ashok (2018) also tested the antibacterial activity of the silver nanoparticle synthesized by *Aspergillus* sp. against selected gram negative bacteria (*Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) and observed that the silver nanoparticle possessed antibiotic potential against *Escherichia coli* (10 mm), *Staphylococcus aureus* (9 mm) and *Pseudomonas aeruginosa* (9 mm) respectively.

Table 4.5: Antibacterial activity of the biosynthesized silver nanoparticle by *Aspergillus* sp. MDS1 against some selected bacterial pathogens

Bacterial pathogens	Silver nanoparticle	Silver nitrate solution	*Aspergillus* sp. MDS1 extract	Chloramphenicol
Proteus sp.	n.z	n.z	n.z	n.z
Staphylococcus aureus	16 mm	13 mm	12 mm	n.z
Enterococcus faecalis	15 mm	12 mm	14 mm	n.z
Salmonella enterica	15 mm	n.z	14 mm	n.z
E. coli	n.z	n.z	n.z	n.z

Key: n.z – no zone of inhibition

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

In conclusion, soil samples from dumpsite were collected, cultured, and screened for nanoparticle producing fungi. Out of sixteen (16) isolates screened, isolate MDS1 showed the highest inhibitory potential on solid agar and was selected for further studies. The results of the morphological and biochemical characterizations of the isolate showed the probable identity to be *Aspergillus* sp. The isolate was able to reduce $AgNO_3$ for nanoparticle biosynthesis. The synthesized nanoparticle was characterized using UV-Visible Spectrophotometer and Fourier transform infrared spectroscopy (FT-IR) which confirmed the synthesis of nanoparticle. The biosynthesized *Aspergillus* sp. nanoparticles showed improved inhibitory activities against the five selected pathogenic bacteria including *S. enterica*, *S. aureus*, *E. coli*, *E. faecalis*, and *Proteus* sp. compared to the *Aspergillus* sp. MDS1 extract, silver nitrate solution and synthetic chloramphenicol antibiotics. Thus, this study revealed that the biosynthesized silver nanoparticle by *Aspergillus* sp. is a good antimicrobial agent that can be commercially developed as antibiotics against common pathogenic bacteria.

5.2 RECOMMENDATIONS

According to the study's findings, the following recommendations was made:

- Nanoparticle produced by *Aspergillus* sp. MDS1, can be used as antibacterial to inhibit the growth of pathogenic bacterial.
- Strain improvement should be done on *Aspergillus* sp. MDS1 to improve the yield of nanoparticle production.

REFERENCES

Ahluwalia, V., Kumar J., Sisodia R., Shakil N. A. and Walia S. (2014). Green synthesis of silver nanoparticles by Trichoderma harzianum and their bio-efficacy evaluation against *Staphylococcus aureus* and *Klebsiella pneumonia*. *Industrial Crops and Products*, 55, 202-206.

Ahmad, A., Mukherjee P., Senapati S., Mandal D., Khan M.I., Kumar R. and Sastry M. (2003). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum. Colloids Surfaces. B: Biointerfaces,* 28(4), 313-318.

Akinyemi, O. M. (2020). Antibiotic Resistance: An Investigation on effectiveness of antibiotics treatment on bacterial growth. *Open Access Library Journal*, *7*(05), 1-4

Akova, M. (2016). Epidemiology of antimicrobial resistance in bloodstream infections. *Virulence*, 7(3), 252-266.

Al juraifani, A.A and Ghazwani A.A. (2015). Biosynthesis of silver nanoparticles by *Aspergillus niger, Fusarium oxysporum,* and*, Alternaria solani*. African Journal of Biotechnology, 14(26), 2-5.

Al-Zubaidi, S., Al-Ayafi A. and Abdelkader H. (2019). Biosynthesis, characterization and antifungal activity of silver nanoparticles by *Aspergillus niger* isolate. *Journal of Nanotechnology Research*, *1*(1), 23-36.

Amin, M.B., Namvar F., Moniri M., Paridah M.D., Tahir A. and Mohamad R. (2015). Nanoparticles Biosynthesized by Fungi and Yeast: A Review of Their Preparation, Properties, and Medical Applications. *Molecules,* 20, 1-9.

Awad, M. A., Eid A. M., Elsheikh T. M., Al-Faifi Z. E., Saad N., Sultan M. H. and Fouda A. (2022). Mycosynthesis, Characterization, and Mosquitocidal Activity of Silver Nanoparticles Fabricated by Aspergillus niger Strain. *Journal of Fungi*, *8*(4), 396.

Bacon, R. T., and Sofos J. N. (2003). Characteristics of biological hazards in foods. *Food safety handbook*, 10, 157-95.

Balaji, D.S., Basavaraja S., Deshpande R., Mahesh D.B., Prabhakar B.K. and Venkataraman A. (2009). Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus*. Colloids Surfaces. B: Biointerfaces,* 68(1), 88-92.

Baldauf, S. L and Palmer J. D (1993). "Animals and fungi are each other's closest relatives: congruent evidence from multiple proteins". *Proceedings of the National Academy of Sciences of the United States of America*, 90 (24), 2-5.

Barkhade, T. (2018). Extracellular biosynthesis of silver nanoparticles using fungus *Penicillium* species. *International Journal of research-Granthaalayah,* 6(1), 277-283.

Basavaraja, S., Balaji S.D., Lagashetty A., Rajasab A.H. and Venkataraman A. (2008). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum. Material Research Bulletin.,* 43(5), 1164-1170.

Bhainsa, K.C and D'Souza S.F. (2006). Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus. Colloids Surfaces. B: Biointerfaces*, 47(2), 160-164.

Bintsis, T. (2017). Foodborne pathogens. *AIMS microbiology*, *3*(3), 529- 530.

Birla, S. S., Tiwari V. V., Gade A. K., Ingle A. P., Yadav A. P. and Rai M. K. (2009). Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Letters in Applied Microbiology*, 48(2), 173-179.

Brakhage A. A., Spröte P., Al-Abdallah Q., Gehrke A., Plattner H. and Tüncher A. (2004). "Regulation of Penicillin Biosynthesis in Filamentous Fungi". Molecular Biotechnology of Fungal beta-Lactam Antibiotics and Related Peptide Synthetases. *Advances in Biochemical Engineering/Biotechnology*, 88, 45–90.

Bruns, T. (October 2006). "Evolutionary biology: a kingdom revised". *Nature*, 443 (7113), 758–761.

Buchanan, R. L., Gorris L. G., Hayman M. M., Jackson T. C. and Whiting R. C. (2017). A review of Listeria monocytogenes: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food control*, *75*, 1-13.

Bush, K., Courvalin P., Dantas G., Davies J., Eisenstein B., Huovinen P. and Zgurskaya H. I. (2011). Tackling antibiotic resistance. *Nature Reviews Microbiology*, 9(12), 894-896.

Carbone, M., Donia D. T., Sabbatella G. and Antiochia R. (2016). Silver nanoparticles in polymeric matrices for fresh food packaging. *Journal of King Saud University – Science*, 28(4), 273-279.

Cartaxo, A.L. (2018). Nanoparticles types and properties understanding these promising devices in the biomedical area. *International. Journal of Nanomedicine,* 1(8), 1-4.

Chan, S. and Don M. (2012). Characterization of Ag nanoparticles produced by white-rot fungi and it's in vitro antimicrobial activities. *The International Arabic Journal of Antimicrobial Agents*, *2*(3), 1-8.

Chan, Y.S and Don M.M. (2013). Biosynthesis and structural characterization of Ag nanoparticles from white-rot fungi. *Materials Science and Engineering,* 33, 1-7.

Chandler, D. (2017). "Basic and Applied Research on Entomopathogenic Fungi". Microbial Control of Insect and Mite Pests, 5, 69–89.

Chellat, M. F., Raguž L. and Ried R. (2016). Targeting antibiotic resistance. *Angewandte Chemie International Edition*, 55(23), 6600-6626.

Cho, K. H., Park J. E., Osaka T. and Park S. G. (2005). The study of antimicrobial activity and preservative effects of nanosilver ingredient. *Electrochimica Acta*, *51*(5), 956-960.

Daniels, G. (2015). Biosynthesis of silver nanoparticles by *Aspergillus oryzae* (MTCC No. 1846) and its characterizations. *Nanoscience and Nanotechnology*, *5*(1), 14-21.

Devi, L. S. and Joshi S. R. (2014). Evaluation of the antimicrobial potency of silver nanoparticles biosynthesized by using an endophytic fungus, *Cryptosporiopsis ericae* PS4. *Journal of Microbiology*, 52(8), 667-674.

Dhanaraj, S., Bharathiraja P., Dhandapani R., Subbaiya R. and Kathireshan A. K. (2018). Biosynthesis and characterization of silver nanoparticles from Aspergillus niger and its antibacterial activity. *Research Journal of Pharmacy and Technology*, 11(12), 5282-5286.

Dhillon, G.S., Brar S.K., Kaur S. and Verma M. (2012). Green approach for nanoparticle biosynthesis by fungi: current trends and applications. *Critical Reviews in Biotechnology*, 32(1), 49-73.

Dhingra, S., Rahman A. A., Peile E., Rahman M., Sartelli M., Hassali M. A. and Haque M. (2020). Microbial resistance movements: an overview of global public health threats posed by antimicrobial resistance, and how best to counter. *Frontiers in Public Health*, 8, 3-22.

Du, J., Hu Z., Yu Z., Li H., Pan J., Zhao D. and Bai Y. (2019). Antibacterial activity of a novel *Forsythia suspensa* fruit mediated green silver nanoparticles against food-borne pathogens and mechanisms investigation. *Material Science and Engineering*, 102, 247–253.

Durán, N., Marcato P. D., Alves O. L., De Souza G. I. and Esposito E. (2005). Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. *Journal of nanobiotechnology*, 3(1), 1-7.

Ealia, S. A. M. and Saravanakumar M. P. (2017). A review on the classification, characterization, synthesis of nanoparticles and their application. *IOP conference series: materials science and engineering*, 263(3), 1-15.

Eddy, N. O., Odoemelem S. A. and Mbaba A. (2006). Elemental composition of soil in some dumpsites. *Electronic Journal of Environmental, Agriculture and Food Chemistry*, 5(3), 1349-1363.

Erdogan A., Gurses M. and Sert S. (2003). "Isolation of moulds capable of producing mycotoxins from blue mouldy Tulum cheeses produced in Turkey". *International Journal of Food Microbiology*, 85 (1–2), 83–85

Farrag, M. M., Mostafa A. M., Mohamed M. E. and Huseein A. M. (2020). Green biosynthesis of silver nanoparticles by *Aspergillus niger* and its antiamoebic effect against *Allovahlkampfia spelaea* trophozoite and cyst. *Experimental Parasitology*, *219*, 108031.

Feynman, R. (1960). Nanotechnology. *Caltech division of engineering and applied science*, *23*, 22-36.

Fomina M., Charnock J. M., Hillier S., Alvarez R. and Gadd G. M. (2007). "Fungal transformations of uranium oxides". *Environmental Microbiology*, 9 (7), 1696–710.

Freire-Moran, L., Aronsson B., Manz C., Gyssens I. C., So A. D., Monnet D. L. and ECDC-EMA working group. (2011). Critical shortage of new antibiotics in development against multidrug-resistant bacteria—Time to react is now. *Drug resistance updates*, 14(2), 118-124.

Fung, M. C. and Bowen D. L. (1996). Silver products for medical indications: risk-benefit assessment. *Journal of toxicology: Clinical toxicology*, *34*(1), 119-126.

Gadd, G. M. (2007). Geomycology: biogeochemical transformations of rocks, minerals, metals, and radionuclides by fungi, bio weathering, and bioremediation. *Mycology Research*, 111, 3-49

Gadd, G. M. and Raven J.A. (2010). Geomicrobiology of eukaryotic microorganisms. *Geomicrobiology. Journal*, 27, 491-519.

Gade, A.K., Bonde P., Ingle A.P., Marcato P.D., Durán N. and Rai M.K. (2008). Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. *Journal of Biobased Material and Bioenergy,* 2(3), 243-247.

Gajbhiye, M., Kesharwani J., Ingle A., Gade A. and Rai M. (2009). Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. *Nanomedicine: Nanotechnology, Biology and Medicine*, 5(4), 382-386.

Griffith, G. W., Easton G. L., Detheridge A., Roderick K., Edwards A., Worgan H. J. and Perkins W. T. (2007). Copper deficiency in potato dextrose agar causes reduced pigmentation in cultures of various fungi. *FEMS microbiology letters*, 276(2), 165-171.

Hany, M.M., Mohammed H.E. and Marwa M.A (2014). Biosynthesis of Silver Nanoparticles Using Fungi and Biological Evaluation of Mycosynthesized Silver Nanoparticles. *The Egyptian Society of Experimental Biology,* 10(1), 1-3.

Hasan, S. (2015). A Review on Nanoparticles: Their Synthesis and Types. *Research Journal of Recent Sciences*, 4, 1-3.

Huh, A. J. and Kwon Y. J. (2011). "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotic's resistant era. *Journal of controlled release*, 156(2), 128-145.

Hulkoti, N.I. and Taranath T.C. (2014). Biosynthesis of nanoparticles using microbes—A review. *Elsevier*, 121, 475-482

Humphrey T., O'Brien S. and Madsen M. (2007) Campylobacters as zoonotic pathogens: A food production perspective. *International Journal of Food Microbiology*, 117, 237–257.

Jaidev, L. R. and Narasimha G. (2010). Fungal mediated biosynthesis of silver nanoparticles, characterization and antimicrobial activity. *Colloids and surfaces B: Biointerfaces*, *81*(2), 430-433.

Jaison, J., Yen S. C. and Michael K. D. (2016). Biosynthesis of Metal and Metal Oxide Nanoparticles. *Chemical Bioengineering Reviews,* 3(2), 55-67.

Kalimuthu, K., Cha B. S., Kim S. and Park K. S. (2020). Eco-friendly synthesis and biomedical applications of gold nanoparticles: A review. *Microchemical Journal*, *152*, 1-38.

Karbalaei, M., Rezaee S. A. and Farsiani H. (2020). Pichia pastoris: A highly successful expression system for optimal synthesis of heterologous proteins. *Journal of cellular physiology*, *235*(9), 5867-5881.

Karthikeyan, B. and Loganathan, B. (2012). Strategic green synthesis and characterization of Au/Pt/Ag trimetallic nanocomposites. *Materials Letters*, 85, 53-56.

Khabat, V., Mansoori G. A. and Karimi S. (2011). Biosynthesis of silver nanoparticles by fungus Trichoderma Reesei. *Insciences journal*, 1(1), 65-79.

Khameneh, B., Diab R., Ghazvini K. and Bazzaz, S. F. (2016). Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them. *Microbial Pathogenesis*, 95, 32-42.

Khan, I., Khalid S. and Idrees K. (2017). Nanoparticles: Properties, applications, and toxicities. *Arabian Journal of Chemistry,* 30, 2-24.

Khurana, C., Vala A. K., Andhariya N., Pandey O. P. and Chudasama B. (2014). Antibacterial activity of silver: The role of hydrodynamic particle size at nanoscale. *Journal of Biomedical Material Research*, 102, 3361–3368.

Kim-Hung, H., Xuan-Hung P., Jaehi K., Sang H. L., Hyejin C., Won-Yeop R. and Bong-Hyun J. (2020). Synthesis, Properties, and Biological Applications of Metallic Alloy Nanoparticles. *International journal of molecular sciences,* 21, 8-29.

Klasen, H. J. (2003). A historical review of the use of silver in the treatment of burns. Part I early uses. *Burns*, 30, 1–9

Koul, B., Poonia A.K., Yadav D. and Jin J.O. (2021). Microbe-Mediated Biosynthesis of Nanoparticles: Applications and Future Prospects. *Biomolecules*, 11, 20-22.

Kumar, M., Curtis A. and Hoskins C. (2018). Application of nanoparticle technologies in the combat against anti-microbial resistance. *Pharmaceutics*, 10(1), 11-17.

La Rosa, R., Johansen H. K. and Molin S. (2022). Persistent Bacterial Infections, Antibiotic Treatment Failure, and Microbial Adaptive Evolution. *Antibiotics 2022*, 11, 3-8.

Langlois, C., Li Z. L., Yuan J., Alloyeau D., Nelayah J., Bochicchio D. and Ricolleau, C. (2012). Transition from core–shell to Janus chemical configuration for bimetallic nanoparticles. *Nanoscale*, 4(11), 3381-3388.

Laurent, S., Forge D., Port M., Roch A., Robic C., Vander E.l. and Muller R.N, (2008). Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Chemistry Reviews,* 108(6), 2064-2110.

Li, G., He D., Qian Y., Guan B., Gao S., Cui Y. and Wang L. (2011). Fungus-mediated green synthesis of silver nanoparticles using *Aspergillus terreus*. *International journal of molecular sciences*, *13*(1), 466-476.

Li, Q., Liu F., Li M., Chen C. Gadd G. M. (2021). Nanoparticle and Nano mineral production by fungi. *Fungal Biology Reviews,* 30(40), 2-14.

Link, S. and El-Sayed M. A. (2003). Optical properties and ultrafast dynamics of metallic nanocrystals. *Annual review of physical chemistry*, 54(1), 331-366.

Loo, Y. Y., Rukayadi Y., Nor-Khaizura A. R., Kuan C. H., Chieng B. W., Nishibuchi M. and Radu S. (2018). In vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. *Frontiers in microbiology*, 9, 1-7.

López-Gómez J. and Molina-Meyer M. (2006). "The competitive exclusion principle versus biodiversity through competitive segregation and further adaptation to spatial heterogeneities". *Theoretical Population Biology*, 69 (1), 94–109.

Lotfy, W. A., Alkersh B. M., Sabry S. A. and Ghozlan H. A. (2021). Biosynthesis of silver nanoparticles by *Aspergillus terreus*: characterization, optimization, and biological activities. *Frontiers in bioengineering and biotechnology*,9, 265-268.

Mabena, L.F., Sinha R. S., Mhlanga S. D. and Coville N. J. (2011). Nitrogen-doped carbon nanotubes as a metal catalyst support. *Applied Nanoscience,* 1, 67–77.

Madhumathi, K., Kumar S., Abhilash S., Sreeja V., Tamura H., Manzoor K. and Jayakumar R. (2010). Development of novel chitin/nanosilver composite scaffolds for wound dressing applications. *Journal of Materials Science: Materials in Medicine*, *21*(2), 807-813.

Magdi, H. M., Mourad M. H. and El-Aziz M. A. (2014). Biosynthesis of silver nanoparticles using fungi and biological evaluation of mycosynthesized silver nanoparticles. *Egyptian journal of experimental biology*, *10*(1), 1-12.

Marambio-Jones, C. and Hoek E. (2010). A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *Journal of nanoparticle research*, *12*(5), 1531-1551.

Maysaa, T. A., Walid I. K. and Adbulsalam K. A. (2021). Biosynthesis of metal nanoparticles using microorganisms and its medicinal applications. *Novel Research in Microbiology Journal,* 5(1), 1-14.

Mohmed, A. A., Saad E., Fouda A., Elgamal M. S., and Salem S. S. (2017). Extracellular biosynthesis of silver nanoparticles using *Aspergillus* sp. and evaluation of their antibacterial and cytotoxicity. *Journal of Applied Life Sciences International*, *11*(2), 1-12.

Morones, J. R., Elechiguerra J. L., Camacho A., Holt K., Kouri J. B., Ramírez J. T. and Yacaman M. J. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology*, *16*(10), 2346-2353.

Mühlen, S. and Dersch P. (2015). Anti-virulence strategies to target bacterial infections. *How to overcome the antibiotic crisis*, 398, 147-183.

Mukherjee, P., Ahmad A., Mandal D., Senapati S., Sainkar S.R., Khan M.I. and Kumar R. (2001). Fungus-mediated synthesis of silver nanoparticles and their immobilization in

the mycelial matrix: a novel biological approach to nanoparticle synthesis. *Nanotechnology Letters*,1, 515–519.

Mukherjee, P., Roy M., Mandal B. P., Dey G. K., Mukherjee P. K., Ghatak, J. and Kale, S. P. (2008). Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*. *Nanotechnology*, 19(7), 75-103.

Munita, J. M. and Arias C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology spectrum*, *4*(2), 4-2.

Navarro Gallón, S. M., Alpaslan E., Wang M., Larese-Casanova P., Londoño M. E., Atehortúa L., Pavón J.J. and Webster T.J. (2019). Characterization and study of the antibacterial mechanisms of silver nanoparticles prepared with microalgal exopolysaccharides. *Materials science and engineering*, 99, 685–695.

Ngoy, J. M., Wagner N., Riboldi L. and Bolland O. (2014). A CO2 capture technology using multi-walled carbon nanotubes with polyaspartamide surfactant. *Energy Procedia,* 63, 2230–2248

Ninganagouda, S., Rathod V., Jyoti H., Singh D., Prema K., and Haq M. U. (2013). Extracellular biosynthesis of silver nanoparticles using *Aspergillus flavus* and their antimicrobial activity against gram negative MDR strains. *International Journal of Pharmaceutical and Biological Science*, *4*(2), 222-229.

Nitsch-Osuch, A., Gyrczuk E., Wardyn A., Życinska K. and Brydak L. (2016). Antibiotic prescription practices among children with influenza. *Advances in Experimental Medicine and Biology*, 905, 25–31.

Nwuche, C. O. and Ogbonna J. C. (2011). Isolation of lipase producing fungi from palm oil mill effluent (POME) dump sites at Nsukka. *Brazilian Archives of Biology and Technology*, *54*, 113-116.

Onofri, S., Selbmann L., De Hoog G. S., Grube M., Barreca D., Ruisi S. and Zucconi L. (2007). Evolution and adaptation of fungi at boundaries of life. *Advances in Space Research*, 40(11), 1657-1664.

Ovais, M., Ali T. K., Muhammad A., Irshad A., Susheel K. N. and Sudip M. (2018). Biosynthesis of Metal Nanoparticles via Microbial Enzymes: A Mechanistic Approach. *International journal of molecular sciences,* 19, 4-11.

Pal, S., Tak Y. K. and Song J. M. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. *Applied Environmental Microbiol*ogy, 73, 1712–1720.

Pan A., Lorenzotti S. and Zoncada A. (2008). "Registered and investigational drugs for the treatment of methicillin-resistant Staphylococcus aureus infection". *Recent Patents on Anti-Infective Drug Discovery*, 3 (1), 10–33.

Panyala, N. R., Peña-Méndez E. M. and Havel J. (2008). Silver or silver nanoparticles: a hazardous threat to the environment and human health? *Journal of applied biomedicine*, 6(3), 118-129.

Pereira, V. J., Fernandes D., Carvalho G., Benoliel M. J., San Romão M. V. and Crespo M. B. (2010). Assessment of the presence and dynamics of fungi in drinking water sources using cultural and molecular methods. *Water Research*, 44(17), 4850-4859.

Phadke, S. S., Feretzaki M. and Heitman J. (2013). Unisexual reproduction enhances fungal competitiveness by promoting habitat exploration via hyphal growth and sporulation. *Eukaryotic cell*, 12(8), 1155-1159.

Prasad, R., Pandey R. and Barman I. (2016). Engineering tailored nanoparticles with microbes: quo Vadis? *Nanomedicine and Nanobiotechnology,* 8(2), 316-330.

Princy, S., Karthik S. and Malini R. (2011). Biosynthesis of Silver Nanoparticles by *Aspergillus niger*. *Asian Journal of Research in Chemistry*, *4*(1), 31-34.

Rai, M., Yadav, A. and Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology advances*, 27(1), 76-83.

Rajeshkumar, S. and Malarkodi C. (2014). In vitro antibacterial activity and mechanism of silver nanoparticles against foodborne pathogens. *Bioinorganic chemistry and applications*, vol 2014, 1-10.

Redecker, D. and Raab P. (2006). Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi). Recent developments and new gene markers. *Mycologia*, 98(6), 885-895.

Sagar, G. and Ashok, B. (2012). Green synthesis of silver nanoparticles using Aspergillus niger and its efficacy against human pathogens. *European Journal of Experimental Biology*, *2*(5), 1654-1658.

Saeed, K and Khan I. (2014). Preparation and properties of single-walled carbon nanotubes/poly (butylene terephthalate) nanocomposites. *Iranian Polymer Journal,* 23, 53–58.

Saeed, K and Khan I. (2016). Preparation and characterization of single-walled carbon nanotube/nylon 6, 6 nanocomposites. *Instrumentation Science and Technology,* 44, 435–444.

Sajida, M and Justyna P.W. (2020). Nanoparticles: Synthesis, characteristics, and applications in analytical and other sciences. *Microchemical Journal,* 154, 3-7.

Salem, S. S. and Fouda, A. (2021). Green synthesis of metallic nanoparticles and their prospective biotechnological applications: an overview. *Biological Trace Element Research*, *199*(1), 344-370.

Sangale, M. K., Shahnawaz M. and Ade A. B. (2019). Potential of fungi isolated from the dumping sites mangrove rhizosphere soil to degrade polythene. *Scientific reports*, *9*(1), 1-11.

Santos, T. S., Silva T. M., Cardoso J. C., Albuquerque-Júnior L. D., Zielinska A., Souto E. B. and Mendonça, M. D. (2021). Biosynthesis of silver nanoparticles mediated by entomopathogenic fungi: Antimicrobial resistance, nanopesticides, and toxicity. *Antibiotics*, 10(7), 1-13.

Sardul, S. S., Harshita S. and Shyamji S. (2017). Biosynthesis of silver nanoparticles by endophytic fungi: Its mechanism, characterization techniques and antimicrobial potential. *African Journal of Biotechnology*, 16(14), 683-698.

Saxenaa, J., Madan M. S., Sarika G. and Abhijeet S. (2014). Emerging role of fungi in nanoparticle synthesis and their applications. *World journal of pharmacy and pharmaceutical sciences,* 3, 9-14.

Scallan, E., Hoekstra R. M., Angulo F. J., Tauxe R. V., Widdowson M. A., Roy S. L. and Griffin P. M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerging infectious diseases*, 17(1), 7-15.

Sethi, S., Kumar R. and Gupta S. (2013). Antibiotic production by microbes isolated from soil. *International Journal of Pharmaceutical Sciences and Research*, *4*(8), 2967-2968.

Shakibaie, M. R., Dhakephalker P. K., Kapadnis B. P., Salajaghe G. A. and Chopade B. A. (1998). Plasmid mediated silver and antibiotic resistance in Acinetobacter baumannii BL54. *Iranian Journal of Medical Science*, 23(12), 30-36.

Shaligram, N. S., Bule M., Bhambure R., Singhal R. S., Singh S. K., Szakacs G. and Pandey A. (2009). Biosynthesis of silver nanoparticles using an aqueous extract from the compactin-producing fungal strain. *Process Biochemistry,* 44(8), 939-943.

Sharma, S., Kumar S., Bulchandini B., Taneja S. and Banyal S. (2013). Green synthesis of silver nanoparticles and their antimicrobial activity against gram positive and gram-negative bacteria. *International Journal of Biotechnology and Bioengineering Research*, 4(7), 711-714.

Siddiqi, K. S., Husen A. and Rao R. A. (2018). A review on biosynthesis of silver nanoparticles and their biocidal properties. *Journal of nanobiotechnology*, 16(1), 1-28.

Silvan, J. M., Zorraquin-Pena I., Gonzalez de Llano D., Moreno-Arribas M. V. and Martinez-Rodriguez A.J. (2018). Antibacterial activity of glutathione-stabilized silver nanoparticles against *Campylobacter* multidrug-resistant strains. Frontiers in Microbiology, 9, 458-459.

Simbine, E. O., Rodrigues D. C., Lapa-Guimaraes J., Kamimura E. S., Corassin C. H. and Oliveira F. D. (2019). Application of silver nanoparticles in food packages: a review. *Food Science and Technology*, 39, 793-802.

Taylor, J. W., Jacobson D. J., Kroken S., Kasuga T., Geiser D. M., Hibbett D. S. and Fisher M. C. (2000). Phylogenetic species recognition and species concepts in fungi. *Fungal genetics and biology*, 31(1), 21-32.

Tharayil, A., Rajakumari R., Kumar A., Choudhary M. D., Palit P. and Thomas S. (2021). New insights into application of nanoparticles in the diagnosis and screening of novel coronavirus (SARS-CoV-2). *Emergent Materials*, 4(1), 101-117.

Uddin, T. M., Chakraborty A. J., Khusro A., Zidan R. M., Mitra S., Emran T. B. and Koirala N. (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. *Journal of Infection and Public Health*, 14(12), 1750-1766.

Vago, G. S., Safran G., Bela R. H. and Lagzi P. (2015). One-step green synthesis of gold nanoparticles by mesophilic filamentous fungi. *Chemical physics letters,* 645, 2-10.

Vahabi, K. and Sedigheh K. D. (2017). Biosynthesis of Nanoparticles by Fungi: Large-Scale Production. *Springer,* 12, 1-14.

Verma, V. C., Kharwar R. N. and Gange A. C. (2010). Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus *Aspergillus clavatus. Nanomedicine (Lond),* 5(1), 33-40.

Vigneshwaran, N., Ashtaputre N. M., Varadarajan P. V., Nachane R. P., Paralikar K. M. and Balasubramanya R. H. (2007). "Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*," *Materials Letters*, 61(6), 1413–1418.

Zomorodian, K., Seyedmohammad P., Arman S., Pouyan M., Keyvan P., Mohammad J. R. and Ali A. M. (2016). Biosynthesis and Characterization of Silver Nanoparticles by *Aspergillus* Species. *BioMedical Research International*, 2016(8), 1-4.

Zorraquín-Peña, I., Cueva C., Bartolomé B. and Moreno-Arribas M. V. (2020). Silver nanoparticles against foodborne bacteria. Effects at intestinal level and health limitations. *Microorganisms*, *8*(1), 2-25.

APPENDIX A: STATISTICAL ANALYSIS

TABLE A: UV spectrum of fungi mediated silver nanoparticle

Wavelength (nm)	Absorbance 24 hrs	Absorbance 48 hrs	Absorbance 72 hrs
200	2.194	0.833	2.500
300	0.123	2.500	2.500
400	0.014	1.729	2.310
500	0.010	0.862	1.268
600	0.012	0.504	0.754
700	0.010	0.331	0.495
800	0.007	0.238	0.401

APPENDIX B: COMPONENTS OF MEDIA

Potato Dextrose Agar

Infused potato	200 g
Dextrose	20 g
Agar powder	20 g
Distilled water	1 litre

Nutrient Broth

Peptones	10 g
Beef extract	1 g
Yeast extract	2g
Sodium chloride	5g
Distilled water	1 litre

Mueller Hinton Agar

Beef extract	2.0 g
Acid hydrolysate of casein	17.5 g
Starch	1.5 g
Agar	17.0 g
Distilled water	1 litre