

**GREEN SYNTHESIS OF SILVER (Ag) AND GOLD (Au) NANOPARTICLES  
USING *Arspergillus carbonarius* ISOLATED FROM PETROLEUM POLLUTED  
SOIL**

**BY**

**Odomero MONDAY (Miss)**

**LSC1605975**

**DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY**

**FACULTY OF LIFE SCIENCE**

**UNIVERSITY OF BENIN**

**BENIN CITY.**

**NOVEMBER, 2022**

**GREEN SYNTHESIS OF SILVER (Ag) AND GOLD (Au) NANOPARTICLES  
USING *Arspergillus carbonarius* ISOLATED FROM PETROLEUM POLLUTED  
SOIL**

**BY**

**Odomero MONDAY (Miss)**

**LSC1605975**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF  
BACHELOR OF SCIENCE DEGREE (B.Sc.) IN SCIENCE LABORATORY  
TECHNOLOGY (BIOLOGICAL SCIENCES TECHNIQUES), FACULTY OF  
LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.**

**NOVEMBER, 2022**

## CERTIFICATION

This is to certify that the project was carried out by Odomero Monday (Miss), Matriculation Number LSC1605975, a student of the Department of Science Laboratory Technology, Faculty of Life sciences, University of Benin, in partial fulfillment of the requirement for the award of a Bachelor of Science degree, B.Sc. (Hons.) in Science Laboratory Technology.

---

**Dr. F.I . Okolafor**  
**(Project Supervisor)**

---

**Date**

---

**Prof. F.I. Obahiagbon**  
**(Project Coordinator)**

---

**Date**

---

**Dr. E.O. Oshomoh**  
**(Head of Department)**

---

**Date**

---

**External Examiner**

---

**Date**

## **DEDICATION**

This project report is firstly dedicated to God for his grace and goodness. Secondly I wish to also dedicate this project to my parents for their support and endless love then to my project being honest and understanding.

## ACKNOWLEDGEMENT

All thanks to God almighty for giving me the grace, wisdom and strength to complete this my project work and also for His provision, protection and unmerited favour throughout the period of my study.

To my supervisor Dr. F.I Okolafor, I want to say thank you for taking out time to ensure that this work was a success and for your unending support, love and care. May the good Lord continue to bless and reward you accordingly.

My warm regards goes to my H.O D; Dr. E.O Oshomo and the entire lecturers and staffs of the Department of Science Laboratory Technology, University of Benin for their services rendered in making me a better person. Thank you all.

Special gratitude to my parents Mr. and Mrs. Monday Aludu for being there always as regarding the spiritual, financial and all round support. May the good Lord continue to grant you many more years.

I say a very big thank you to my sweet and lovely siblings, Obokparo, Eroure, Ajiri and Edafe thank you all for your support and care. I am forever grateful.

To my friends Ejobona Mike and Victor who stood by me in the thick and thin of my project period and my entire five years of study in University of Benin, I want to say I love you all and may God grant you your heart desires.

Lastly, I wish to express my gratitude to my course mates who assisted me at one point or the other. God bless you all.

## TABLE OF CONTENTS

pages Title page	-	-	-	-	-	-	-	-	-	-	i
Certification	-	-	-	-	-	-	-	-	-	-	ii
Dedication	-	-	-	-	-	-	-	-	-	-	iii
Acknowledgement	-	-	-	-	-	-	-	-	-	-	iv
Table of content	-	-	-	-	-	-	-	-	-	-	v
List of tables	-	-	-	-	-	-	-	-	-	-	ix
List of figures	-	-	-	-	-	-	-	-	-	-	x
List of plates	-	-	-	-	-	-	-	-	-	-	xi
Abstract	-	-	-	-	-	-	-	-	-	-	xii
<b>CHAPTER ONE</b>	-	-	-	-	-	-	-	-	-	-	<b>1</b>
1.0 INTRODUCTION	-	-	-	-	-	-	-	-	-	-	1
1.1 Background of Study-	-	-	-	-	-	-	-	-	-	-	1
1.2 Specific Objectives	-	-	-	-	-	-	-	-	-	-	2
<b>CHAPTER TWO</b>	-	-	-	-	-	-	-	-	-	-	<b>3</b>
2.0 LITERATURE REVIEW	-	-	-	-	-	-	-	-	-	-	3
2.1 Green Synthesis of Nanoparticles	-	-	-	-	-	-	-	-	-	-	3
2.1.1 Microbial Mediated Synthesis of Nanoparticles	-	-	-	-	-	-	-	-	-	-	5
2.1.2 Bacteria Mediated Synthesis	-	-	-	-	-	-	-	-	-	-	5
2.1.3 Fungi Mediated Synthesis	-	-	-	-	-	-	-	-	-	-	5
2.1.4 Algae Mediated Synthesis	-	-	-	-	-	-	-	-	-	-	9
2.1.5 Plant Mediated Synthesis	-	-	-	-	-	-	-	-	-	-	9

2.2	Characterization of Nanoparticles	-	-	-	-	-	-	-	12
2.2.1	Morphological Characterization	-	-	-	-	-	-	-	12
2.2.2	Optical Characterization	-	-	-	-	-	-	-	13
2.3	Antimicrobial Activity of Metal Nanoparticles	-	-	-	-	-	-	-	14
2.3.1	Antibacterial Activity	-	-	-	-	-	-	-	14
2.4	Application of Nanoparticles in Bioremediation	-	-	-	-	-	-	-	15
	<b>CHAPTER THREE</b>	-	-	-	-	-	-	-	<b>17</b>
3.0	MATERIALS AND METHODS	-	-	-	-	-	-	-	17
3.1	Materials	-	-	-	-	-	-	-	17
3.2	Cultivation and Molecular Characterization of Fungus Isolate	-	-	-	-	-	-	-	17
3.3	Biosynthesis of Gold, Silver and Gold-Silver Composite Nanoparticles	-	-	-	-	-	-	-	18
3.4	Characterization of the Synthesized Nanoparticles	-	-	-	-	-	-	-	19
3.4.1	UV-VIS Spectra Analysis	-	-	-	-	-	-	-	19
3.4.2	XRD (X-Ray Powdered Diffraction)	-	-	-	-	-	-	-	20
3.4.3	FTIR Analysis	-	-	-	-	-	-	-	20
3.5	Determination of the Antimicrobial Properties of the Synthesized Gold, Silver and Gold-Silver Composite Nanoparticles	-	-	-	-	-	-	-	20
3.6	Determination of the Remediation Properties of the synthesized Gold, Silver and Gold-Silver Composite Nanoparticles in waste Engine Oil cleanup	-	-	-	-	-	-	-	21
	<b>CHAPTER FOUR</b>	-	-	-	-	-	-	-	<b>22</b>
4.0	RESULTS	-	-	-	-	-	-	-	22
4.1	The Result of the Cultivation and Molecular Characterization of <i>Aspergillus carbonarius</i>	-	-	-	-	-	-	-	22
4.1.1	Cultivation and Morphological Characterization	-	-	-	-	-	-	-	22

4.1.2 Molecular characterization	-	-	-	-	-	-	-	-	22
4.2 The Results of the Synthesis of Gold, Silver and Gold-Silver Composite Nanoparticles	-	-	-	-	-	-	-	-	27
4.2.1 Synthesis of Silver (Ag) Nanoparticles	-	-	-	-	-	-	-	-	27
4.2.2 Synthesis of Gold (Au) Nanoparticles	-	-	-	-	-	-	-	-	27
4.2.3 Synthesis of gold and silver composite nanoparticles	-	-	-	-	-	-	-	-	27
4.3 The results of the Characterization of Ag, Au and Ag-Au Composite Nanoparticles	-	-	-	-	-	-	-	-	29
4.3.1 UV-VIS	-	-	-	-	-	-	-	-	29
4.3.2. The Result of the Fourier Transfer Infrared Spectroscopy (FTIR)	-	-	-	-	-	-	-	-	31
4.3.3 The Result of the X-Ray Diffraction (XRD)	-	-	-	-	-	-	-	-	33
4.4 The Result of the Determination of Antimicrobial Activities of Ag, Au and Au-Ag Composite NPs	-	-	-	-	-	-	-	-	35
4.5 The Results of the Determination of the Bioremediation Properties of Ag, Au and Ag-Au Composite NPs in Waste Engine Oil Clean Up	-	-	-	-	-	-	-	-	37
<b>CHAPTER FIVE</b>	-	-	-	-	-	-	-	-	<b>39</b>
5.0 DISCUSSION	-	-	-	-	-	-	-	-	39
CONCLUSION	-	-	-	-	-	-	-	-	42
REFERENCES	-	-	-	-	-	-	-	-	43
APPENDIX	-	-	-	-	-	-	-	-	50

## LIST OF TABLES

Table 4.1 Morphological characteristics of fungus isolates	-	-	-	-	23
Table 4.1 Molecular characteristics of Fungus Isolate	-	-	-	-	24
Table 4.1 Antimicrobial Properties of Ag- Au Composite NPs Au an Ag Nanoparticles	-	-	-	-	36
Table 4.1 Bioremediation Properties of Ag, Au and Ag-Au Composite in waste engine oil Cleanup	-	-	-	-	38

## LIST OF FIGURES

Fig. 2.1:	Schematic representation of the procedure for green synthesis of silver nanoparticles using various biological entities	4
Fig. 2.2.	Synthesis of nanoparticles from plant extracts - - -	11
Fig. 4.1	UV-VIS absorbance spectra of nanoparticles synthesized by <i>Arspergillus carbonarius</i> - - - - -	30
Fig. 4.2	FTIR spectra of nanoparticles Synthesized by <i>Arspergillus carbonarius</i>	32
Fig. 4.3	X-Ray diffraction patterns of Au-Ag composite NPs, Ag and AuNps synthesized by <i>Arspergillus carbonarius</i> - - -	34

## LIST OF PLATES

Plate 4.1: Colour changes indicating the formation of Ag, Au and Au-Ag NPs

28

## ABSTRACT

Nanoparticles are particles of matter ranging from 1-100nm in diameter. In this study, nontoxic, cost-effective and eco-friendly Ag, Au and Ag - Au composite NPs were synthesized using the culture biomass of *Arspergillus carbonarius* and the application of the NPs in the biosynthesized NPs were utilized in the remediation of waste petroleum products. Polymerase chain reaction (PCR) and internal transcribed spacer (ITS/5.85) rDNA techniques were used to characterize *Arspergillus carbonarius*. The bioreduction properties of the Ag<sup>+</sup> and Au<sup>+</sup> NPs were monitored by UV-Vis spectrophotometry. The NPs obtained were characterized by X-Ray diffraction (XRD) and FTIR (Fourier Transfer Spectroscopy) methods. The intensity of the NPs peak revealed hydroxyl (OH) and carbonyl (C=O) groups formed by the capping of the carboxylic acid moieties. The XRD peaks were compared with the Joint committee on Powdered Diffraction Standard (JCPDS) card as a face-centered cubic (FCC) crystal lattice structure. The Morphological structure of the Synthesized NPs revealed pores strands formed on the periplasmic surface of the mycelia of the *A. Carbonarius*. The Ag, Au and AgAu exhibited potential bioremediation activity in waste engine oil cleanup. Thus, the NPs can be utilized as potential agent for remediation of petroleum products.

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background of Study

Nanotechnology is "the study and manipulation of matter at the nanoscale, with diameters of about 1 and 100 nanometers, wherein distinctive phenomena offer revolutionary applicability," according to the National Nanotechnology Initiative (NNI). (Drexler,1986). The initial coupled most generally used concept of nanotechnology was molecular nanotechnology, particularly focused on the particular technical goal of precisely controlling atoms as well as molecules to produce macroscale objects (Drexler,1992). Eventually, a more inclusive definition of nanotechnology was established by the National Nanotechnology Initiative, which defined it as the process of manipulating matter that have at least a single dimension sized among 1 as well as 100 nanometers. The concept changed from a particular technical aim to a study category that covers all kinds of studies as well as innovations associated with quantum mechanics since quantum mechanical impacts are crucial at this quantum-realm scale. The plural terms "nanotechnologies" as well as "nanoscale technologies" are often used to relate to a broad range of studies as well as operations with a common feature of size. According to the definition of nanotechnology based on size, organic chemistry, semiconductor physics, , as well as other fields of research are all covered.

It has a wide range of studies as well as applications, spanning modifications of conventional device physics to completely original methods that utilize molecular self-assembly (Belkin et al., 2015), from creating new nanoscale materials to precise atomic-scale regulation of matter. The design, manipulation, and production of nanoparticles are all topics in nanotechnology, which is an important branch of modern research. The physical method, biological method, and chemical method are the three approaches used to make nanoparticles. The biological

technique, that uses biological processes, such those found in plants, to create nanoparticles, is the most common of the several methods of nanoparticles production. The biological technique was used to generate silver and gold nanoparticles utilizing fungi during this research. a variety of nano-scale Nanotechnology has been used to create a variety of materials.

The aim of this study is to synthesize and characterize silver and gold nanoparticles from *Aspergillus carbonarius*, a fungus isolated from petroleum polluted soil.

## **1.2 Specific objectives**

The specific objective of this study were to:

1. cultivate and molecularly characterize *Aspergillus carbonarius*.
2. synthesize silver and gold nanoparticles from *Aspergillus carbonarius*.
3. characterize synthesized silver and gold nanoparticles from *Aspergillus carbonarius*.
4. determine the antimicrobial properties of the silver and gold nanoparticles synthesized from *Aspergillus carbonarius*.
5. determine the bioremediation properties of the synthesized nanoparticles in waste engine oil cleanup.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Green Synthesis of Nanoparticles

Green synthesis, also known as biological synthesis, involves the creation of nanoparticles (NPs) employing biological entities including as bacteria coupled with plants, and is both environmentally friendly and financially effective. The top-down coupled with bottom-up approaches are the two main tenets of nanoparticle synthesis. In top-down approaches, a variety of procedures, including lithographic techniques, are used to produce nanoparticles and materials. In contrast, bottom up approaches use techniques like chemical vapor deposition to create nanoparticles from simpler molecules. A sustainable alternative to traditional nanoparticle synthesis is the bottom-up method of creating nanoparticles from biological resources. (Anupam *et al.*, 2019). that also requires making nanoparticles using biological processes, like those used by plants. There will be fewer toxic, unsustainable products since the adverse byproducts of conventional nanoparticle manufacturing will be reduced. Utilizing organic systems as well as other natural resources will assist in achieving the goal of creating a greener, better functioning economy; nevertheless, industrial support for the implementation of such alternative tactics will be required. Metallic nanoparticles may be created in a more sustainable as well as ecologically benign way by using biological or plant-based components such as bacteria, fungus, algae, as well as plant extracts. One pot or single-step techniques, which use less energy than other methods, can be used in this more environmentally friendly method. Below is a list of different biological methods for synthesizing nanoparticles.

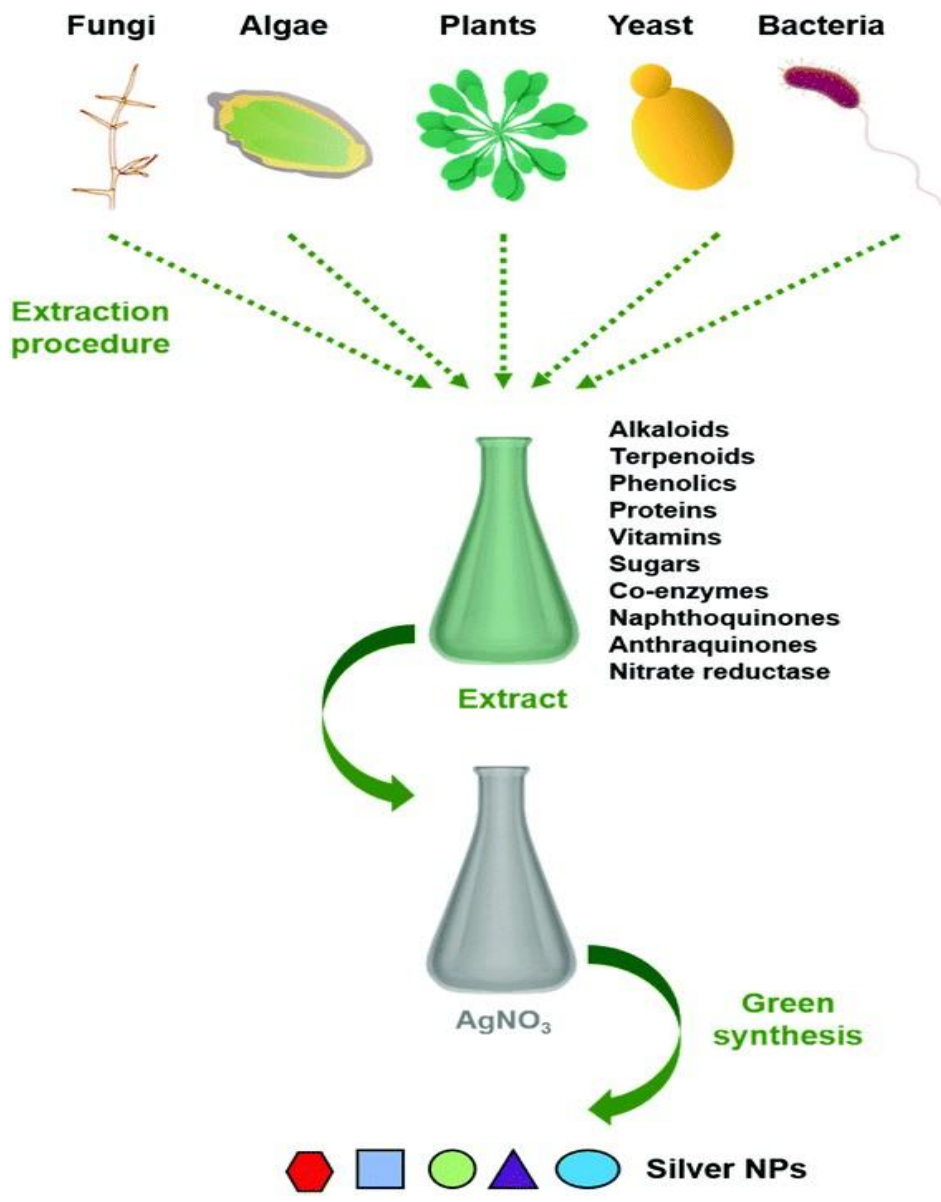


Figure 1: Schematic representation of the procedure for green synthesis of silver nanoparticles using various biological entities.

Source: Anupam *et al.* (2019).

### **2.1.1 Microbial Mediated Synthesis of Nanoparticles**

The biological production of nanoparticles is a bottom-up technique since nanoparticle generation is caused by the decrease of metallic ions through biomolecules released by microorganisms including enzymes, proteins, in addition carbohydrates (Prabhu *et al.*, 2012). Microorganisms can produce external or intracellular nanoparticles, reliant on the category of microbe. Extracellular nanoparticle synthesis, on the other hand, is more favorable since the result may be easily collected without lysis of the cell of the host (Kuppusamy *et al.*, 2014). Owing to its rapid development and ease of cultivation, microbes including as bacteria, fungus, algae, as well as yeasts are widely used in the manufacture of nanoparticles.

### **2.1.2 Bacterial Mediated synthesis**

*Actinobacter sp.*, *Klebsiella pneumoniae*., *Bacillus cereus*, as well as *Pseudomonas spp.* are most often employed microorganisms to produce metallic nanoparticles. (Tollamadugu, K.V. *et al.*, 2011; Iravani, S., 2014; Sunkar, S. *et al.*, 2012). Metallic nanoparticles could be synthesized using bacteria through intracellular or else extracellular methods. The NADH-dependent reductase enzyme, which delivers an electron and *Pseudomonas stutzeri*AG259 bacterium has been utilized to create silver nanoparticles, which oxidize to NAD<sup>+</sup>. The biological decrease of Ag ions to Ag nanoparticles is caused by electron transport (Ahmad *et al.*, 2003). In a related study, Hussein *et al.* (2011) used *Pseudomonas aeruginosa* to decrease Au ions, which resulted in Au nanoparticles are synthesized outside of cells.

### **2.1.3 Fungi Mediated Synthesis**

Myconanotechnology, according to Kashyap (2012), is a discipline in which fungi can be used to synthesize nanomaterials or nanostructures. The use of fungi to synthesize metal nanoparticles including gold and silver has lately been acknowledged as a more efficient and environmentally friendly way for using fungi as nanofactories (Singh *et al.*, 2016).Green

nanotechnology, aims to limit the creation of toxic compounds in general. Meanwhile, compared to other technologies, green nanotechnology requires less energy input; no toxic chemicals are created during synthesis, and they have a high environmental compactibility. As a result, manufactured green nanomaterials and nanostructures can be used in a change of industries (Bartolucci *et al.*, 2020). Dhillon *et al.* (2015), describes numerous sophisticated strategies for improving precision breeding procedures and enabling exact control of the green synthesis process at the nanoscale scale. Specifically, different fungus such as *Aspergillus carbonarius* and *Aspergillus niger* whether intracellularly or extracellularly to create metal nanoparticles, including silver coupled with gold. (Mandal *et al.*, 2006). These fungi have the ability to move organic substances created inside their cell to the exterior of their cell. Fungi as nanofactories are important because they are environmentally benign, affordable, and non-toxic tools that use less energy to synthesize metal nanoparticles than physiochemical approaches. Those that perform extracellular synthesis are of particular attention among the several mechanisms for metal nanoparticles green production, because the material's extracellular position eliminates the requirement for costly and sophisticated downstream processing processes to retrieve intracellular nanoparticles (Singh *et al.*, 2016). When associated to other physiochemical approaches, green production of metal nanoparticles including gold as well as silver utilizing fungi has several advantages. It provides a rapid, cost-effective, clean, non-toxic, as well as environmentally acceptable approach for the synthesis of metal nanoparticles, as well as a greater variety of sizes, shapes, content, as well as physiochemical activities (Shah *et al.*, 2015; Ovais *et al.*, 2018). In general, using fungus in the manufacture of metal nanoparticles has a number of difficulties and requires multiple procedures, such as microbial sample, isolation, culture, and storage. Fungi mediated green synthesis of metals is popular due to its fast development rate, ease of cultivation, as well as capacity to tolerate changes in temperature, pH, and pressure. Among the different

biological sources of metal nanoparticle synthesis, fungi-mediated green synthesis of metal nanoparticles has taken a prominent place (Ali *et al.*, 2020). Several fungi have been identified as possible biofactors for the environmentally friendly and less expensive synthesis of metal nanoparticles comprising metals including copper, silver, as well as gold (Gahlawat *et al.*, 2019; Kato and Suzuki, 2020; Khan *et al.*, 2018). Physiological parameters including reaction temperature, microbiological source, pH, pressure, metal salt concentration, as well as incubation duration influence the creation of various metal nanoparticles. The comprehensive adjustment of these physiological factors is required for the synthesis of nanoparticles with precise size, shape, and chemical contents (Jeyaraj *et al.*, 2019; Singh *et al.*, 2020). Purifying metal Nanoparticles after synthesis is critical before using them in any application. Some of the techniques used to dissociate and enrich the generated metal nanoparticles, as well as to remove reactive bioactive molecules and chemicals, include high-speed centrifugation and repeated washing (Singh *et al.*, 2020). For in cell produced metal nanoparticles, additional purification procedures are required, such as ultrasonification or interaction with a suitable detergent, which releases the metal nanoparticles once the cell wall is broken down. As a result, these additional steps limit the economic benefits of this technique (Soni *et al.*, 2018). The biosynthesis of nanoparticles utilizing fungi has been intensively investigated all over the world, and the synthesis takes place both extracellularly and intracellularly. *Verticillium luteoalbum*, *ricothecium* spp., *Aspergillus oryzae*, and other fungal species have been documented to produce nanoparticles of various shapes and sizes. For instance, Ag NPs with a size variety of 2512 nm were made by exhibiting fungal biomass (*Verticillium*) to an aqueous solution of Ag<sup>+</sup> ions, where the NPs were not hazardous because the biomass (fungal cells) continued to develop. Electrostatic interactions amongst negatively charged mycelia as well as Ag<sup>+</sup> ions caused the NPs to grow on their surface. The fungus's cell wall contains enzymes with carboxylate groups (Mukherjee *et al.*, 2001). When opposed

to their bacterial counterparts, fungi have a few advantages when it comes to the production of metallic nanoparticles. Fungi, for example, secrete enormous amounts of proteins and enzymes per unit of biomass, resulting in bigger amounts of biomass, and hence larger amounts of nanoparticles generated (Jeevanandam *et al.*, 2016). Nevertheless, in way to construct a cost-effective approach for industrial nanoparticle production, a number of limitations in the synthesis of nanoparticles from fungus must be overcome, including the time-consuming process of production as well as the expensive down streaming processing (Narayanan *et al.*, 2010).

### **Fungi Mediated Synthesis of Gold Nanoparticle**

Recently, Kitching *et al.* (2015) examined the biosynthesis of gold nanoparticles from fungus. They are oxidation resistant and scatter well (Daniel and Astruc, 2004). Fungi use a simpler method to synthesize nanoparticles, either intracellularly or extracellularly. The gold ions are trapped as well as reduced by proteins and enzymes on the fungi's surface. They also form largescale aggregates (Sanghi *et al.*, 2011). The characteristics of gold nanoparticles generated from various sources vary. They were tested for their anti-cancer cytotoxic activities (Misra *et al.*, 2011). The reduction of AuCl or AuCl<sub>3</sub> intracellularly and extracellularly follows the same mechanism (Gupta and Vector, 2013). Because AuCl just takes one electron to produce gold nanoparticles, it is reduced in one step, but AuCl<sub>3</sub> requires three electrons and is reduced in three steps. When AuCl<sub>3</sub> is dissolved in water, reactions occur in the mycelia of fungi that include proteins and other materials, resulting in the development of metal nanoparticles (Das *et al.*, 2012). It was discovered that old fungal biomass is less effective than fresh biomass at producing Au nanoparticles. It's probable because fresh fungal biomass secretes more proteins and enzymes than aged fungal biomass.

## **Fungi Mediated Green Synthesis of Silver Nanoparticles**

Silver nanoparticles generated extracellularly or intracellularly by fungus have been the subject of extensive research (Bhainsa and D'Souza, 2006; Ahmad *et al.*, 2003). Metal and fungus have different particle sizes. It has been discovered that by monitoring the concentration, pH, and temperature of the solution, their size may be adjusted. It's also worth noting that intracellular synthesis produces smaller nanoparticles. Although the specific method of intracellular silver nanoparticle creation is unknown, it is certain that they occur on the surface of mycelia in the presence of fungi. According to one theory, metal ions in solution are drawn to the positive charges of fungal mycelia as well as the slightly negative charges on the cell wall induced from carboxylic groups on enzymes or amino groups on proteins. This is accompanied by metal ion elimination as well as the creation of metal nanoparticles. (Sneha *et al.*, 2010).

### **2.1.4 Algae Mediated Synthesis of Nanoparticles**

Current study is focusing on the production of nanoparticles utilizing algae. These are capable of producing various metal in addition metal oxide nanoparticles (Patel *et al.*, 2015). The manufacture of nanoparticles is carried out using a variety of algae species. *Chlorella vulgaris*, for example, was utilized to make gold (Au) nanoparticles. A marine alga (*Sargassum wightii*) was also found to produce gold (Au) and silver (Ag) outside of the cell (Govindaraju *et al.*, 2009).

### **2.1.5 Plant-Mediated Synthesis Nanoparticle**

Plants get the capacity to naturally reduce as well as hyper-accumulate metal ions (Kulkarni *et al.*, 2014; Kale *et al.*, 2013). Because of these unique characteristics, plants have been proposed as an ecologically friendly method for biologically producing metallic nanoparticles however for detoxifying purposes (Khan *et al.* 2013; Kale *et al.*, 2013). Extracts from plant organs like leaves, roots, as well as fruits are first mixed with a metal ion aqueous solution to

produce nanoparticles. The bioreduction of metal ions form nanoparticles involves a variety of substances that function as reducing agents, including sugar, flavonoids, proteins, enzymes, polymers, and organic acids (Iravani, 2011; Makarov et al., 2014; Nadaroglu et al., 2017; Park et al., 2016; Siddiqi et al., 2016). Stable Au, Ag, and Au-Ag alloy nanoparticles were created using leaf extracts from *Jasminum sambac* (Yallappa et al., 2015). It is believed that variations in the content as well as concentration among these active biomolecules among plants, in addition to their relationship between overall with aqueous metal ions, are the causes of the variety in nanoparticle sizes as well as shapes produced (Li et al., 2011). The characteristics of the plant extract, including its composition, metal salt concentration, reaction time, reaction solution pH, as well as temperature, have an impact on the quality, size, as well as morphology of a generated nanoparticles. create stable Au, Ag, and Au-Ag alloy nanoparticles (Yallappa et al., 2015). It is believed that variations in the content and concentration of the these bioactive biomolecules among plants, in addition to their relationship between overall with aqueous metal ions, are the causes of the variety of nanoparticle sizes as well as shapes produced (Li et al., 2011). The characteristics of the plant extract, including its composition, metal salt concentration, reaction time, reaction solution pH, as well as temperature, have an impact on the performance, size, as well as morphology of the generated nanoparticles.

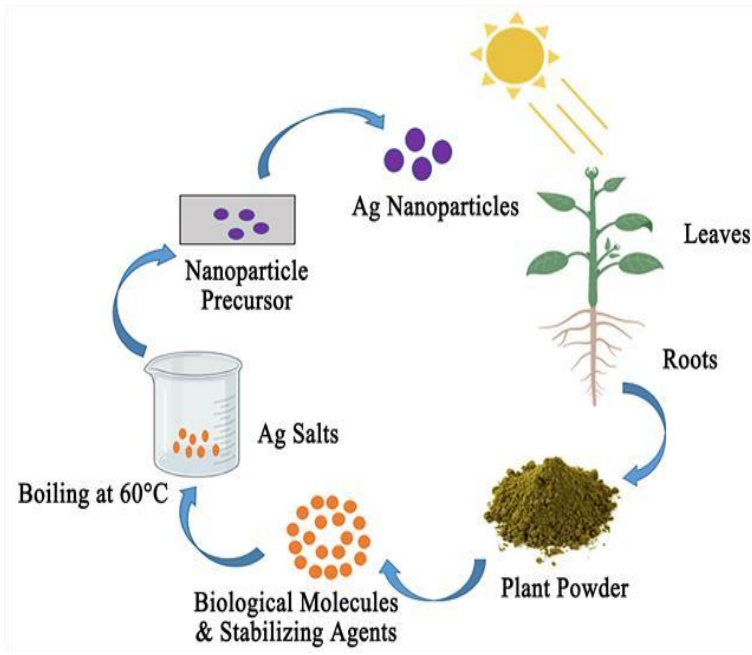


Figure 2.2: Synthesis of nanoparticles from plant extracts.

Source: Khan *et al.* (2022).

## **2.2 Characterization of Nanoparticles**

Many characterization approaches have been employed to investigate the different physicochemical features of NPs. Examples of these methods include X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), infrared (IR), SEM, TEM, Brunauer-Emmett-Teller (BET), as well as particle size analysis.

### **2.2.1 Morphological Characterization**

Because NPs' morphology largely determines their characteristics, this aspect of the study is constantly the main focus. Despite the existence of a number of characterisation techniques for morphological research, polarized optical microscopy (POM), scanning electron microscopy (SEM), and transmission electron microscopy are the most widely used (TEM). The electron scanning principle serves as the foundation for the SEM method, which offers comprehensive information on nanoparticles just at nanoscale level. This method has been used by researchers to examine the shape of its nanomaterials in addition to the dispersion of nanoparticles inside the bulk or matrix, predicated on a thorough review of the literature. This technique demonstrated how SWNTs have been dispersed within the nylon-6 and poly (butylene) terephthalate polymer matrix (PBT). Both Saeed and Khan (2014) and (2016) make reference to this. The same group as well proffered a POM analysis of its materials, which demonstrated the presence of star-like spherulites in the produced materials, the size which its reduced as the SWNT level is been progressively expanded. Similar to TEM, which would be based on the idea of electron transmittance, it could, depending on the magnification, reveal information about only the bulk material. This approach is used to investigate the various morphologies of gold nanoparticles. Khlebtsov and Dykman (2010), 2010a, and 2010b. Important information regarding materials with two or more layers is disclosed by the quadrupolar hollow shell structure of Co<sub>3</sub>O<sub>4</sub> nanoparticles that was discovered by TEM. When employed as the anode in Li-ion batteries, it has been

demonstrated that these NPs are extremely active. The same porous multishell structure results in shorter Li<sup>+</sup> diffusion channel length, good cycle performance, higher rate capacity, and greater specific capacity. There is also ample room to buffer volume expansion (Wang *et al.*, 2013).

### **2.2.2. Optical Characterization**

Photochemists understood how to use this method to comprehend how its photochemical processes function because optical properties are crucial in photocatalytic uses. These explanations are founded on both the well-known Beer-Lambert law and fundamental lighting rules (Swinehart, 1962). These methods could be used to analyze the NPs' absorbance, reflectance, luminescence, as well as phosphorescence characteristics. The best NPs for photo-related uses are recognized to have distinctive colors, especially metallic as well as semiconductor NPs. Throughout order to comprehend the underlying principle for each implementation, it is therefore always beneficial to understand the absorption as well as reflectance principles of these materials. To investigate the optical characteristics of NPs materials, the recognised optical instruments UV-visible (UV- Vis), photoluminescence (PL), as well as the null ellipsometer may be employed. A complete tool for determining optical absorption, transmittance, as well as reflectance in the UV/vis variety is the diffuse reflectance spectrometer (DRS). The first two work well together, but the third (DRS) is a special method primarily for solid samples. The method is rather dependable for figuring out the bandgaps of NPs and other nanomaterials. Materials' bandgap plays a crucial role in defining their photoactivity and conductivity. In carbon nanodot-carbon nitride, a metal-free water-splitting photocatalyst was discovered (C<sub>3</sub>N<sub>4</sub>). Utilizing UV-Vis spectroscopy, a bandgap value of 2.74–2.77 eV was determined, but this material's photoactivity is highly linked with this value (Liu *et al.*, 2015a; Liu *et al.*, 2015b).

## 2.3 Antimicrobial Activity of Metal Nanoparticles

### 2.3.1 Antibacterial Activity

Numerous studies have been done to enhance antimicrobial properties due to the growing microorganism resistance to conventional antibiotics and antiseptics. Owing to in vitro antimicrobial testing, the metallic nanoparticles effectively inhibit a wide variety of bacteria species (Dizaj et al., 2014). Metallic nanoparticles' antibacterial potency is influenced by two elements: (a) the material utilized to create them as well as (b) its particle size. Antimicrobial drug resistance among bacteria has steadily grown over time, presenting a serious threat to public health. For instance, methicillin resistance, penicillin resistance, as well as vancomycin resistance are characteristics of antimicrobial drug resistant microbes (Fair and Tor, 2014). The production of Au nanoparticles is crucial for the generation of effective antibacterial medications due to their non-toxic nature, unique ability to be functionalized, and polyvalent properties (Lima et al., 2013; Tiwari et al., 2011; Zhou et al., 2012). On the contrary hand, the antibacterial activity of gold nanoparticles is not related to the production of reactive oxygen species (Cui et al., 2012). In order to assess the antibacterial capability of Au nanoparticles, researchers attempted to link nanoparticles towards the bacterial membrane without altering the membrane potential, which decreased the ATP level. TRNA binding to the ribosome was similarly hindered by this attachment. The antibacterial activity of zinc oxide (ZnO), copper oxide (CuO), and iron oxide (Fe<sub>2</sub>O<sub>3</sub>) nanoparticles against gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and gram-positive bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) was investigated by Azam et al. (2012) . (*Staphylococcus Aureus* and *Bacillus subtilis*). As a result, the ZnO nanoparticles were shown to have the strongest antibacterial activity. Fe<sub>2</sub>O<sub>3</sub> nanoparticles, on the other hand, have the least effective antibacterial properties. Mahapatra et al. (2008) investigated the antibacterial activity of copper oxide nanoparticles against *Klebsiella pneumoniae*, *Shigella*, as well as *Salmonella*

*paratyphis*. CuO nanoparticles were discovered to have adequate antibacterial action against those microorganisms. Nanoparticles were thought to cross the bacterial cell membrane and disrupt the bacteria's essential enzymes, causing cell death. Green generated nanoparticles, for example, have more antibacterial activity than chemically manufactured or marketable nanoparticles. Due to the plants used in nanoparticle manufacturing [such as *Ocimum sanctum* (Tulsi) and *Azadirachta indica* (neem)] have therapeutic qualities (Ramteke *et al.*, 2013; Verma and Mehata, 2016). When compared to commercial silver nanoparticles, green generated silver nanoparticles displayed an effective in addition broad zone of clearance in contrast to several bacterial strains .

#### **2.4 Applications of Nanoparticles in Bioremediation**

For a number of reasons, different nanomaterials are used in bioremediation. For instance, when a substance is scaled down to the nanoscale, its surface area per unit mass rises; as a consequence, more of the material may come into contact with nearby materials, which might influence its reactivity. Chemical reactions may occur with less activation energy when NMs are present due to their a quantum influence. Surface plasmon resonance is the other phenomenon exhibited by NPs that may be used to identify dangerous chemicals. To clean the environment, a range of metallic and nonmetallic NMs may be used, each with a unique form and size. It may utilize a variety of single metal NPs, bimetallic NPs, carbon base NMs, and other NPs because I they can diffuse or penetrate into a polluted zone where microparticles cannot, and (ii) they have better reactivity to redox-amenable contaminants. With contaminants like carbon tetrachloride, oxide-coated Fe<sub>0</sub> has been found to form weak and outer-sphere complexes (CT). Oxide coating increases reactivity throughout batch experiments as well as field assessments, allowing CT to just be converted into methane, carbon monoxide, or format via electron transfer, as opposed to benzoquinone, bytrichloroethene, as well as other chlorinated aliphatic hydrocarbons, which can be

converted into chemicals with lesser toxicities such as through electron transfer (Nurmi et al., 2005). Additionally to its application in the field, TiO<sub>2</sub> nanotubes may be used in a lab environment to decompose pentachlorophenol by a photoelectrocatalytic reaction (PCP). NPs may immobilize microorganisms that can biodegrade or biorecover certain chemicals. Magnetic nanoparticles (Fe<sub>3</sub>O<sub>4</sub>) was functionalized with ammonium oleate as well as put on the surface of *Pseudomonas delafieldii* to immobilize cells instead of employing micron-sized medium or a permanent surface. By applying an external magnetic field to them, these magnetic Nanoparticle-coated microbial cells was focused at a particular location on the reactor wall, separated from the bulk solution, and used again to cure the very same substrate. When used in a bioreactor at a high biomass concentration, these microorganisms demonstrated that they could desulfurize the organic sulfur of fossil fuel (dibenzothiophene) exactly as well as non-NP- (Shan *et al.*, 2005).

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Materials

*Aspergillus carbonarius* was isolated from petroleum polluted soil and cultured on potatoes dextrose agar (PDA) at ambient temperature. The isolated fungi was identified using morphological and molecular characteristics. The materials used , which includes potato dextrose, Agar, chemical silver nitrate and chemical gold chloride were purchased from Pyrex Nigeria limited, and used as received.

#### 3.2 Cultivation and Molecular Characterization of Fungus Isolate

##### 3.2.1 Cultivation

An innoculating loop was flamed till red hot and allowed to cool. Thereafter, the fruiting body of *Aspergillus carbonarius* was collected. The lid of an already poured potato dextrose agar (PDA) (Appendix 5) petri plate was removed and the Fungus was carefully streaked on the plate. The plate was then covered, labelled appropriately and incubated upright for 5 days at 37°C.

##### 3.2.2 Molecular characterization

###### DNA Extraction

A 3R BashingTMLysis Tube was filled with a fungal cell that weighed 50 to 100mg (wet weight) and had been resuspended in up to 200ul of water, isotonic buffer (like PBS), or up to 200mg of tissue. The tube received 750ul of Lysis solution. The preparation was treated for 5 minutes at maximum speed after being placed in a bead with a 2ml tube holder assembly. Centrifugation at >10,000 g for 1 minute was performed on a 3R BashingBead TMLysis Tube.

A collection tube containing up to 400ul of supernatant were transferred to a Zymo SpinTMIV Spin Filter (orange top) before being centrifuged at 7,000 g for one minute.

The filtrate inside the collecting tube from step 4 was mixed with 1,200ul of fungal DNA binding buffer. After being transferred to a Zymo-spin TM IIC column in a collection tube, 800ul of the mixture from step 5 were centrifuged at 10,000 g for one minute. After discarding the collecting tube's flow-through, do step 6 again. The Zymo-spin TM IIC column received 200ul of DNA Pre-wash buffer before being centrifuged at 10,00 g for one minute. The Zymo-spin TM IIC received 500ul of fungal DNA wash buffer before being centrifuged at 10,000 g for one minute. Transferring the Zymo-spin TM IIC column to a clean 1.5 ml micro centrifuge tube, adding 100 ul (35 ul minimum) of DNA Elution Buffer directly to the matrix of the column, and centrifuging at 10,000 g for 30 seconds to elute the DNA were the next steps. Fungus Isolate Molecular Characterization Using Internal Transcribed Spacer Method.

2.5ul of 10x PCR buffer, 1ul of 25mM MgCl<sub>2</sub>, and 1ul of each forward primer (ITS 1: TCC GTA GGT GAA CCT GCG G) and reverse primer make up the PCR cocktail mix (ITS4 TCCTCCGCTTATTGATATGS) 1 ul DMSO, 2 ul 2.5 m MDNTPs, 0.1 ul 5 u/ul Taq DNA polymerase, and 3 ul 10 ng/ul DNA make up this mixture. Using 13.4 ul of nuclease-free water, the total reaction volume was increased to 25 ul using 36 cycles of denaturation at 94 °C for 30 seconds, annealing at 54 °C for 30 seconds, and elongation at 72 °C for 45 seconds. after which the temperature is maintained at 10 °C indefinitely after a last elongation stage at 72 °C for 7 minutes. On 1.5% agarose electrophoresis gels that had been dyed with Safe View, amplified fragments could be seen. The DNA ladder utilized is a 50 bp ladder from NEB, and the amplicon size is around 650 bp. Sequencing was done using an ABI 3500.

### **3.3 Biosynthesis of Gold, Silver and Gold-Silver Composite Nanoparticles**

#### **3.3.1 Synthesis of Gold Nanoparticles**

In this work, the culture biomass of the fungal isolate was used to reduce the gold chloride ion and create gold nanoparticles extracellularly. The fungal biomass was combined with 100

ml of a 1 mM gold chloride solution (Appendix 2) in a 250 ml Erlenmeyer flask, as well as the mixture was then incubated for 24 hours at room temperature as well as in the dark to create the gold nanoparticles. A flask containing distilled water and the Fungus biomass was used as control. The mixture was then centrifuged for 5 minutes and washed with tris buffer to concentrate the gold nanoparticles, and were then collected for further characterization.

### **3.3.2 Synthesis of Silver Nanoparticles**

In this work, the culture biomass of the fungal isolate was used to reduce the silver nitrate ion and produce silver nanoparticles extracellularly. The 250 ml Erlenmeyer flask was used to combine the fungal biomass with 100 ml of a 1 mM silver nitrate solution (Appendix 3) solution to create the silver nanoparticles, which were then cultured for 24 hours at room temperature as well as the dark. A flask containing distilled water and the Fungus biomass was used as control. The mixture was then centrifuged for 5 minutes and washed with tris buffer to concentrate the silver nanoparticles, and were then collected for further characterization.

### **3.3.3 Synthesis of Composite of Gold and Silver Nanoparticles**

The composite of gold and silver nanoparticles were synthesized by mixing the fungus biomass with 100ml of 1mM silver nitrate and gold chloride solutions in a 250ml Erlenmeyer flask and incubated at ambient temperature in the dark for 24 hours. A flask containing distilled water and the Fungus biomass was used as control. The mixture was then centrifuged for 5 minutes and washed with tris buffer to concentrate the nanoparticles, and were then collected for further characterization.

## **3.4 Characterization of Nanoparticles**

### **3.4.1 UV-vis spectra analysis**

By routinely collecting aliquots (2 ml) of the suspension in a quartz cuvette and examining the UV-vis spectra of the resultant diluents, it was possible to track the bioreduction of  $\text{Ag}^+$

or AuCl<sub>4</sub> in aqueous solution. As a function of bioreduction time at ambient temperature, UV-vis spectroscopy examinations of generated silver or gold nanoparticles were performed with a resolution of 1 nm on Camspec M501 spectrophotometers from 300 to 900 nm.

#### **3.4.2 X-ray Powder Diffraction (XRD) measurement**

Following bioreduction, the hydrosols as well as residual biomass-containing residual solutions were dried at 60 °C, as well as the dried mixture were collected for analysis by an X'Pert Pro x-ray diffractometer (PANalytical BV, Netherlands) operating at a voltage of 40 kV as well as a current of 30 mA with Cu K radiation to determine whether Ag or Au had formed.

#### **3.4.3 Fourier-transform infrared spectroscopy (FTIR) analysis of silver and gold nanoparticles**

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 10 ml after reaction was centrifuged at 4800 rpm for 10 min and the resulting suspension was redispersed in 20 ml sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified suspension was completely dried at 60 °C. Finally, the dried nanoparticles were analysed by FTIR machine (Llantrisant, UK).

#### **3.5 Determination of Antimicrobial Properties of Gold, Silver and Gold-Silver Composite Nanoparticles**

The synthesized nanoparticles were examined for their potential to be used as antimicrobial agents. The tested clinical isolates were *Staphylococcus* sp., *Bacillus* sp. and *Escherichia coli*. The organisms were inoculated into agar plates with 0.5MC farland solution. A cork borer was sterilized and used to bore holes into the plates. A micropipette was used to introduce Gold, silver and the composite of gold and silver nanoparticles into the plates labelled as Au, Ag and Au-Ag, respectively. The plates were covered and incubated at room temperature for

24 hours. The zones of inhibition was then measured to determine the antimicrobial properties of the nanoparticles.

### 3.6 Determination of Remediation Properties of Silver, Gold and Silver-Gold Composite Nanoparticles

The NPs synthesized by the Fungus Isolate were screened tested for their potentials to be used as an agent of remediation of waste engine oil (WEO) using the zone of clearance method reported in our previous study. Two hundred (200) microlitre ( $\mu\text{L}$ ) of WEO was spread to the surface of solid Mineral salt agar (Appendix 7) and winograsky agar. (Appendix 6) Perforated Whatman filter paper number one measuring 0.6 cm was sterilised using the hot air oven. The sterilised filter paper (0.6 cm) was soaked in synthesized Ag NP, Au NP, and Ag-Au NPs for 1 min, thereafter the soaked filter paper was carefully placed on top of the agar plated ceded with WEO. The plates were incubated right-up forfor 24 andto 72 h, thereafter the zones of clearance were measured daily with a meter rule.

## CHAPTER FOUR

### RESULTS

#### **4.1 The Result of the Cultivation and Molecular Characterization of *Aspergillus carbonarius***

##### **4.1.1 Cultivation and Morphological Characterization**

The Growth of the fungus isolate was confirmed on the potatoe dextrose agar plate after an incubation period of 5 days. The fungus revealed characteristics features such as black colour, further microscopic examination revealed that the basal mycelium was white and moderately compact, conidial head were globose with a septate hyphae (Table 4.1).

##### **4.1.2. Molecular characterization**

Further confirmatory test using moleculer method revealed that the fungus isolates matched with NCBI data base with a 99.6% similarity, thereby confirming the fungus to be *Arspergillus carbonarius* (Table 4.2).

Table 4.1: Morphological Characterization of Fungus Isolate

SAMPLE CODE	DESCRIPTION ON PLATE	PROBABLE ISOLATE
C	<p>The fungus isolate revealed characteristics features on plate such as black colour.</p> <p>Further microscopic examination revealed that the basal mycelium was white and moderately compact, conidial head were globose with a septate hyphae.</p>	<i>Aspergillus sp.</i>

Table 4.2: Gene Bank confirmation Profile of the 16S rRNA sequence output of the fungus

(Sample C) isolated from petroleum polluted soil

Sample	Closets match on NCBI database	% similarity	Sequence (FASTA format)	Accession number
C	<i>Aspergillus carbonarius</i> NBRC 4030	99.6	>TTTCCGTAGGTGAACCTGCGGAAGGATCAT TACCGAGTGCTGGGTCCTTCGGGGCCCAAC CTCCACCCGTGCTTACCGTACCCTGTTGCT TCGGCGGGCCCGCCTTCGGGCGGCCCGGG CCTGCCCCCGGGACCGCGCCCGCCGGAGAC CCCAATGGAACACTGTCTGAAAGCGTGCAG TCTGAGTTGATTGATACCAATCAGTAAAAC TTCAACAATGGATCTCTTGGTTCCGGCATCG ATGAAGAACGCAGCGAAATGCGATAACTAAT GTGAATTGCAGAATTCAGTGAATCATCGAGTC TTTGAACGCACATTGCGCCCCCTGGTATTCCGG GGGGCATGCCTGTCCGAGCGTCATTTCTCCCT CCAGCCCCGCTGGTTGTTGGGCCGCGCCCCC GGGGGCGGGCCTCGAGAGAAACGGCGGCACCG TCCGGTCCTCGAGCGTATGGGGCTCTGTCACCC GCTCTATGGGCCCGGCCGGGGCTTGCCTCGACC CCCAATCTTCTCAGATTGACCTCGGATCAGGTAG GGATACCCGCTGAACTTAAGCATATCAATAAGCG GAGGAAAAGAAACCAACCGGGATTGCCTCAGTAA CGGCGAGTGAAGCGGCAAGAGCTCAAATTTGAAA GCTGGCCCCCTCCGGGGTCCGCGTTGTAATTTGCAG AGGATGCTTTGGGTGCGGCCCGTCTAAGTGCCC TGGAACGGGCCGTCAGAGAGGGTGAGAATCCCGT CTTGGGCGGGGTGTCCGTGCCCCGTGTAAGCTCCT TCGACGAGTCGAGTTGTTTGGGAATGCAGCTCTAA ATGGGTGGTAAATTTTCATCTAAAGCTAAATACTGG	LC573577.1

CCGGAGACCGATAGCGCACAAAGTAGAGTGATCGA  
AAGATGAAAAGCACTTTGAAAAGAGAGTTAAACAG  
CACGTGAAATTGTTGAAAGGGAAGCGCTTGCGACCA  
GACTCGCCCTCGGGGTTTCAGCCGGCACTCGTGCCGGT  
GTACTTCCCCGGGGGCGGGCCAGCGTCAGCTTGGGCG  
GCCGGTCAAAGGCCTCCGGAATGTAGTGCCCCCGGG  
GCACCTTATAGCCGGAGGTGCAATGCGGCCAGCCTGG  
GCTGAGGAACGCGCTTCGGCACGGACGCTGGCATAAT  
GGTCGCAA

---

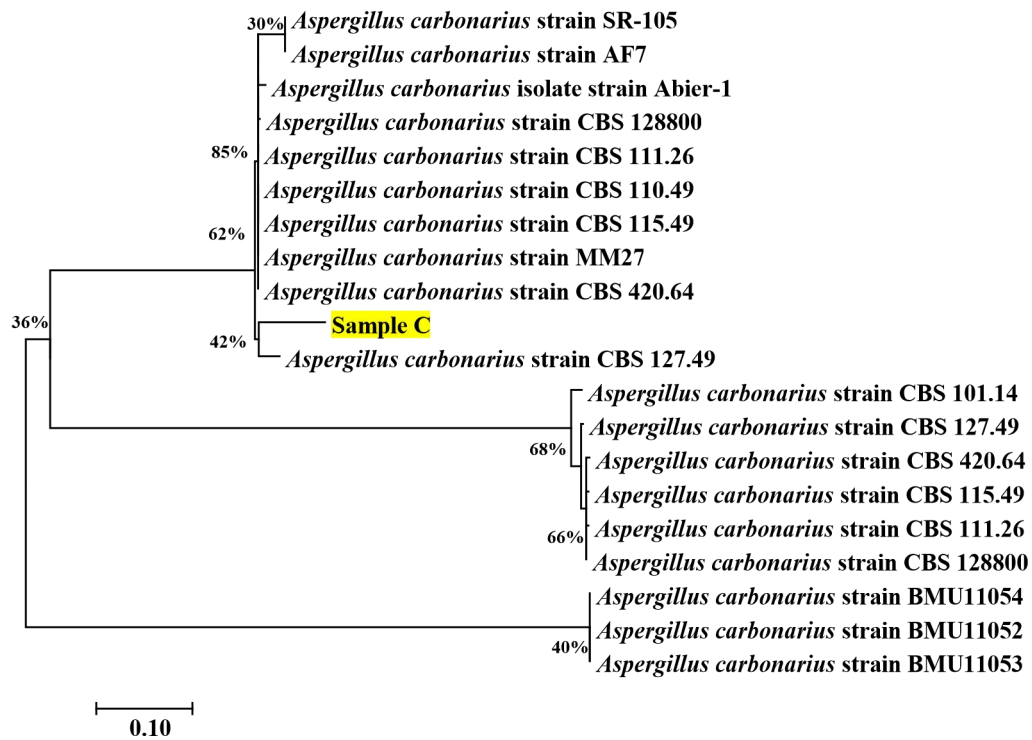


Figure 4.1: Maximum likelihood phylogenetic tree of *Aspergillus carbonarius* isolated (Sample B) from petroleum-polluted soil compared with *A. carbonarius* from Gene Bank database

## **4.2 The Results of the Synthesis of Gold, Silver and Gold-Silver Composite Nanoparticles**

### **4.2.1 Synthesis of Silver (Ag) Nanoparticles**

The synthesis of silver nanoparticles was confirmed by a change in colour (figure 4.1) from colourless to brown after the introduction of the fungus biomass to silver nitrate ( $\text{AgNO}_3$ ) solution, indicating the formation of silver nanoparticles.

### **4.2.2 Synthesis of Gold (Au) Nanoparticles**

The synthesis of gold nanoparticles was confirmed by a change in colour from colour from pale yellow to light purple after the introduction of the fungus biomass to gold chloride ( $\text{AuCl}_3$ ) solution, indicating the formation of gold nanoparticles.

### **4.2.3 Synthesis of gold and silver composite nanoparticles**

The synthesis of the gold and silver composite was confirmed by a change in colour from yellow to brown after the introduction of the fungal biomass into  $\text{AuCl}_3$  and  $\text{AgNO}_3$  solution, indicating the formation of Au-Ag composite nanoparticles.

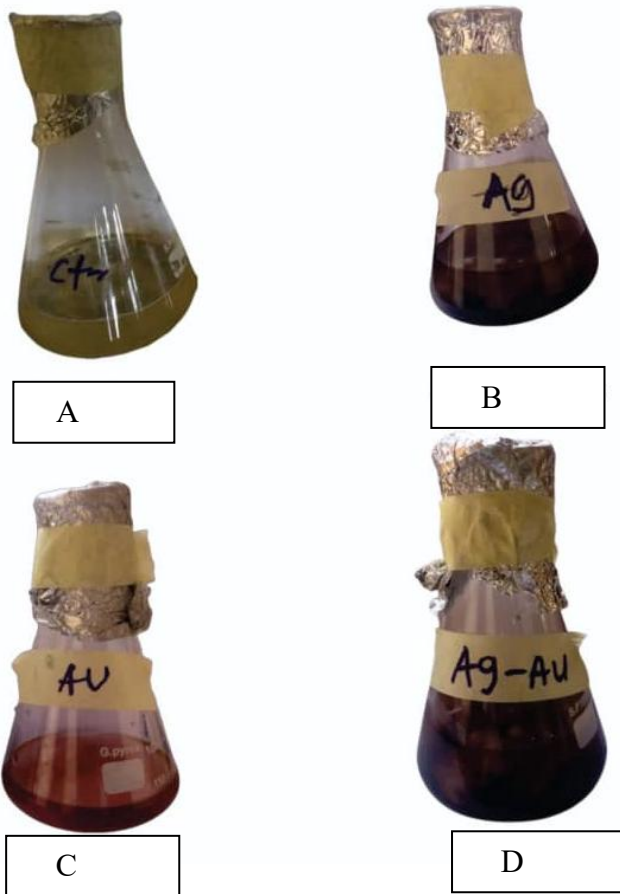


Plate 4.1: Biosynthesis of nanoparticle A:(control), B: (AgNPs), C: (AuNPs) and D: (Ag-Au composite NPs)

### **4.3 The results of the Characterization of Ag, Au and Ag-Au Composite Nanoparticles**

#### **4.3.1 UV-VIS**

The result of the ultraviolet-visible (UV-VIS) spectra analysis carried out to determine the optical density of the Au, Ag and Ag-Au Composite nanoparticles synthesized by *Arspergillus carbonarius* revealed a clear absorption peak at 300-390 nm (Figure 4.2).

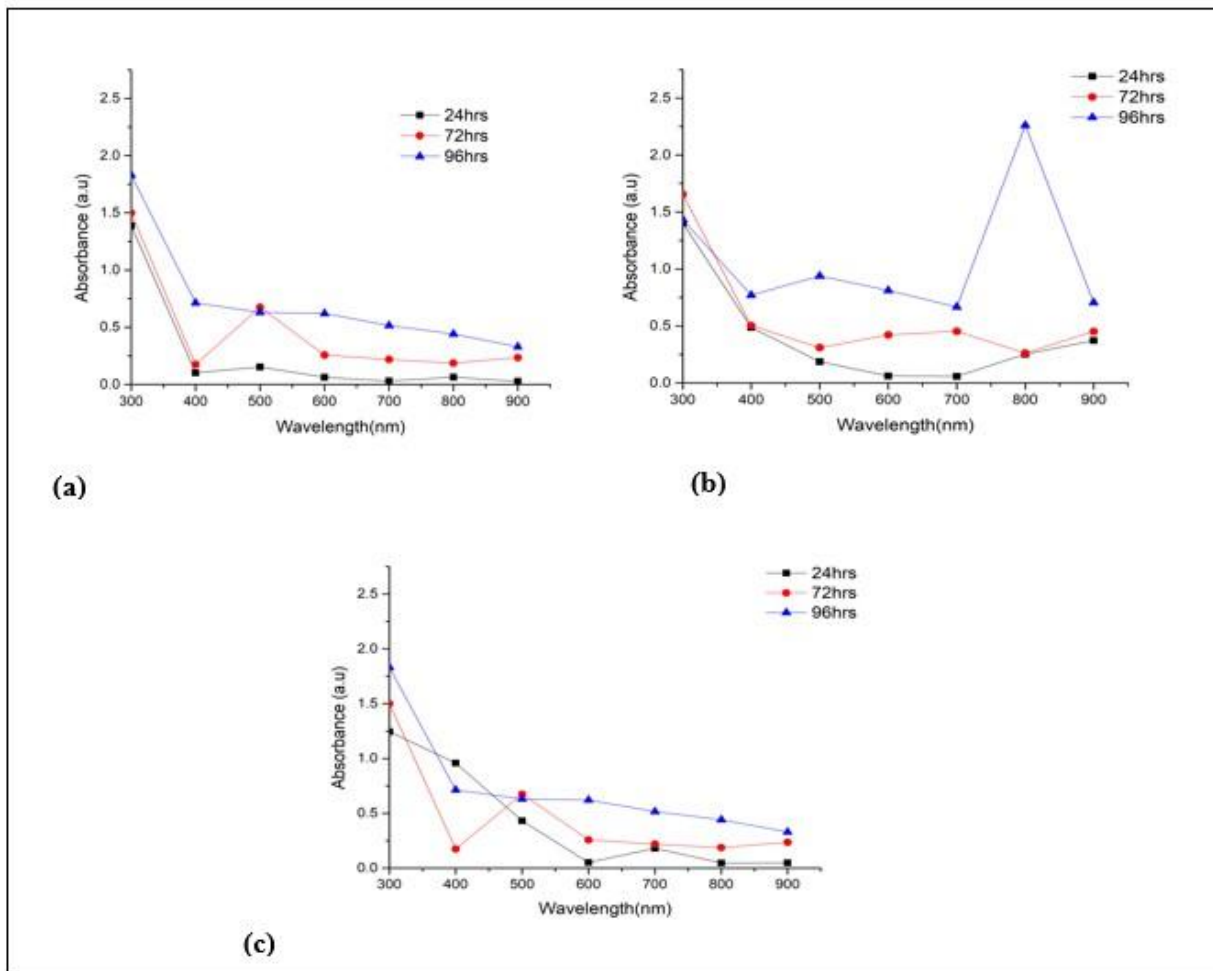


Figure 4.2: UV-VIS Absorbance spectra of nanoparticles synthesized by *Aspergillus carbonarius* (a) Ag NP (b) Au NP, and (c) Ag-Au composite NP

#### **4.3.2. The Result of the Fourier Transfer Infrared Spectroscopy (FTIR)**

The result of the FTIR measurements carried out in order to identify the presence of various functional groups in the nanoparticles synthesized by *Arspergillus Carbonarius* revealed bands at wavelength of 1639-3294 $\text{cm}^{-1}$  for Ag NP, 1641-3302 $\text{cm}^{-1}$  for AuNp and 16423267 $\text{cm}^{-1}$  for Ag-Au Composite nanoparticles.

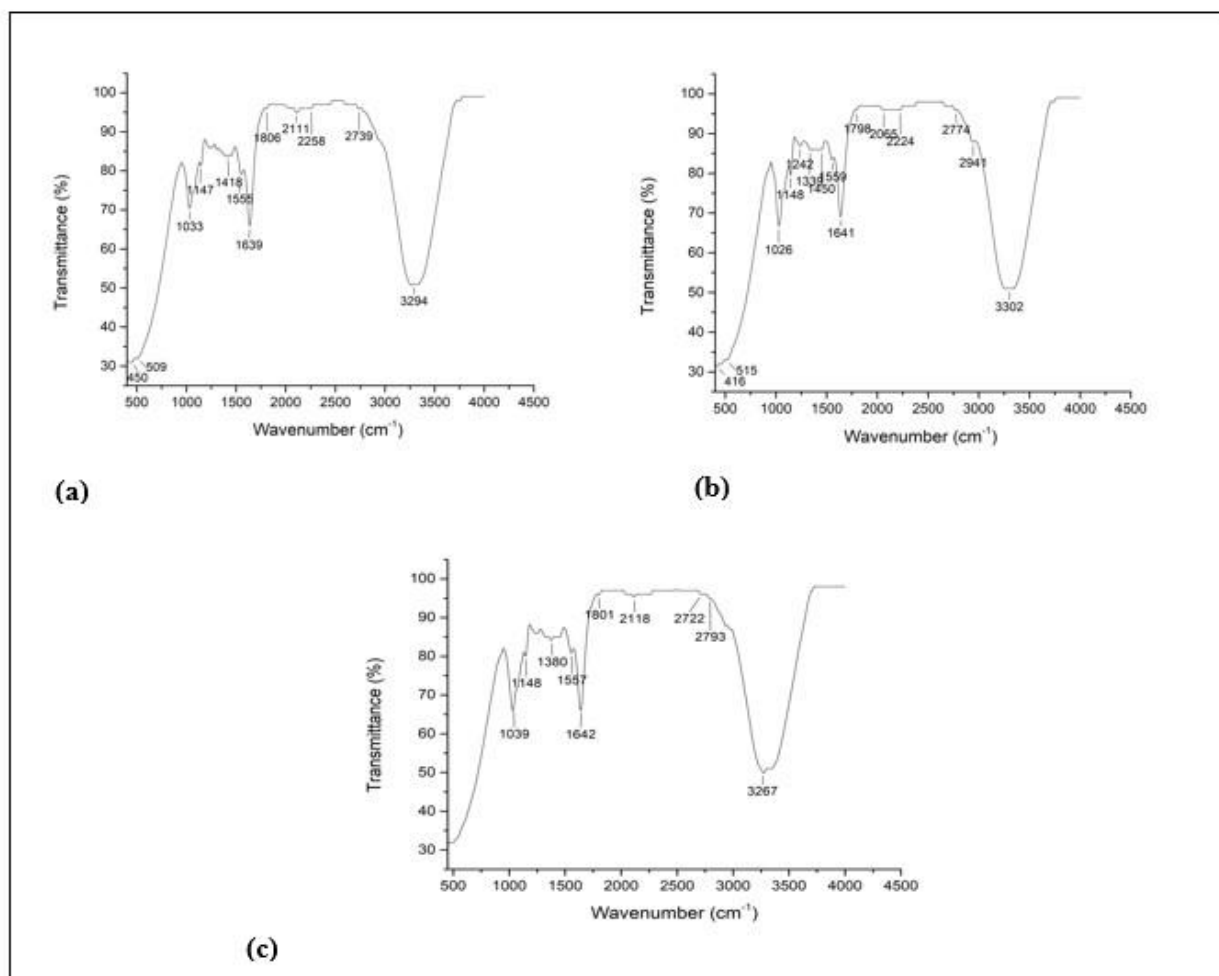


Figure 4.3: FTIR spectra of nanoparticles synthesized by *Aspergillus carbonarius* (a) Ag NP (b) Au NP & (c) Ag-Au composite NP

### 4.3.3 The Result of the X-Ray Diffraction (XRD)

The x-diffraction analysis carried out to determine the crystalline nature of the NPs synthesized by *Arspergillus Carbonarius* revealed the following peaks at  $2\theta$  with the corresponding miller indices (hkl) in parentheses, 38.1(111), 44.5(200), 64.5(220) and 77.5(311) for AuNPs, 38.5(111) for AgNPs and 38.1(111), 44.5(200), 64.5(220) and 77.5(311) for Ag and none for Au in the Au-Ag composite nanoparticles. The peaks were compared with the standard JCPDS (Joint committee on powder diffraction standards) card series to determine their crystallinity and they corresponded with the JCPDS numbers 00004-0783 for AgNPs, and 00-004-0784 for AuNPs (Figure 4.4).

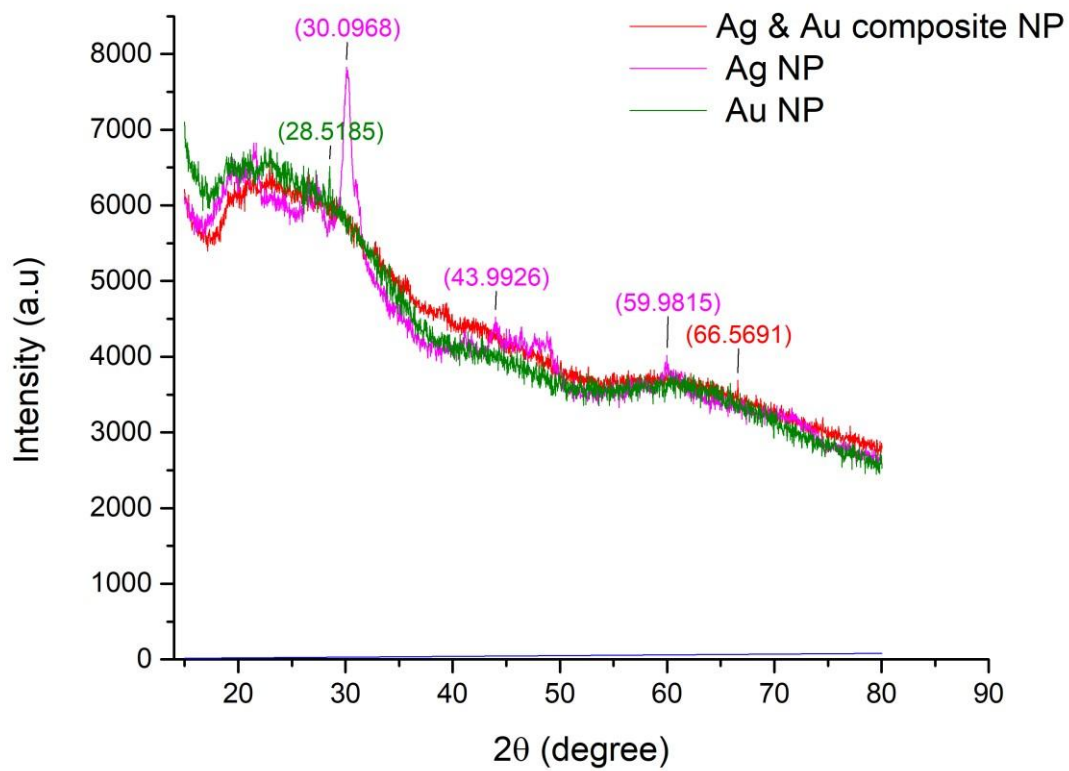


Figure 4.4: X-ray diffraction patterns of Ag-Au composite NP, Ag and Au NP's synthesized by *Aspergillus carbonarius*

#### **4.4 The Result of the Determination of Antimicrobial Activities of Ag, Au and Au- Ag Composite NPs**

The result for the determination of the Antimicrobial Properties of the synthesized nanoparticles against various clinical isolates (*Staphylococcus* sp., *Klebsiella* sp., *Bacillus* sp. and *Escherichia coli*) revealed that gold nanoparticles had an inhibition zone of 13mm against *Staphylococcus* sp. and none against the other tested strains. No zone of inhibition was noticed for both Ag and Ag-Au composite nanoparticles.

Table 4.3: Antimicrobial properties of NP's synthesized by *Aspergillus carbonarius*

<b>Nanoparticles</b>	<b><i>Staphylococcus sp.</i></b>	<b><i>Klebsiella sp.</i></b>	<b><i>Bacillus sp.</i></b>	<b><i>Escherichia coli</i></b>
<b>Inhibition Zone Diameter (mm)</b>				
Ag-NP	0	0	0	0
Au-NP	13	0	0	0
Ag-Au composite NPs	0	0	0	0

#### **4.5 The Results of the Determination of the Bioremediation Properties of Ag, Au and Ag-Au Composite NPs in Waste Engine Oil Clean Up**

The result for the determination of the remediation properties of the synthesized NPs in waste engine oil Cleanup revealed that the Au, Ag & Au-Ag composite nanoparticles had considerable remediation properties evidenced by zones of clearance of 15 – 15mm mineral salt agar plate and 5-16mm on winograsky agar plate.

**Table 4.4: Application of NP's synthesized by *Arspergillus Carbonarius* in waste engine oil clean up**

<b>Medium</b>	<b>Ag NPs</b>	<b>Au NPs</b>	<b>Ag-Au NPs</b>	<b>composite</b>
<b>Zone of clearance in agar plate (mm)</b>				
Mineral salt	0	15	15	
Winograsky 1	5	0	16	

## CHAPTER FIVE

### DISCUSSION OF RESULTS

Morphological characterization, which entails physical and microscopic analysis revealed the fungus isolate to be a strain of *Arspergillus sp.* Further confirmation was carried out molecularly using PCR and ITS methods confirmed the species to be *A. Carbonarium*. Using the Tamura-Nei model and the Maximum Likelihood approach, the evolutionary history was inferred (Tamura and Nei, 1993). The tree (-4217.5791) with the highest log probability is displayed. By automatically applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances calculated using the Maximum Composite Likelihood (MCL) technique, and then choosing the topology with the best log likelihood value, the initial tree(s) for the heuristic search were created. With branch lengths expressed as the number of substitutions per site, the tree is scaled. Next to each internal node in the tree is the percentage of places where at least 1 unambiguous base is present in at least 1 sequence for each downstream clade. The analysis involved 20 nucleotide sequences. There were a total of 1334 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.*, 2018).

The change in colour from colourless to brown observed during the synthesis of AgNPs was due to the excitation of the surface plasmon resonance at different properties of the AgNPs, (Maddinedi *et al.*, 2017). The change in colour from light yellow to light purple observed during the synthesis of AuNPs are characteristics of the surface plasmon resonance (SPR) of different sizes of AuNPs in the solution (Germain *et al.*, 2003). The change in colour from pale yellow to brown observed during the synthesis of the Ag-Au Composite nanoparticles was due to the surface plasmon resonance of different sizes of Au and Ag nanoparticles. The color intensity of the biomass and AgCl<sub>3</sub> and AuNO<sub>3</sub> medium was sustained even after 24, 72 and 96 hours of incubation, indicating that the synthesized nanoparticles were well dispersed

in the solution, no obvious aggregation was noticed. The above reactions were monitored by ultraviolet visible Spectroscopy. The intensity of the SPR band increased from 24 hours to 96 hours. The Ag and Au-Ag nanoparticles were stable at 24 to 96 hours while the AuNPs showed stability at 24 to 72 hours and instability at 96 hours. These results were consistent with the reports of Verma *et al.* (2010).

The FTIR bands at 1639-3294 cm<sup>-1</sup> for Ag, 1641-3302 cm<sup>-1</sup> for Au and 1642-3267 cm<sup>-1</sup> for Au-Ag composite nanoparticles respectively, corresponds to O-H stretching vibration indicating the presence of hydroxyl groups and C=O stretching indicating the presence of carbonyl functional groups formed as a result of the capping of the carboxylic acid moieties. This result is consistent with the findings of Lin *et al.* (2005).

The X-Ray Diffraction (XRD) analysis showed one distinct Bragg's reflections corresponding to the (111) Orientation of the face-centered cubic (FCC) silver. The data obtained was matched well with the JCPDS file No.00-004-0783 indicating the crystalline nature of silver nanoparticles. The XRD spectrum of gold nanoparticles also showed four clear diffraction peaks at planes corresponding to FCC Au and was matched with the JCPDS file No. 00-0040784 also indicating the crystalline nature of AuNPs. This is consistent with the findings of Elbeshehy *et al.* (2015). The XRD spectrum of Au-Ag composite NPs showed four planes corresponding to FCC Ag and was matched with JCPDS file No. 00-004-0784 for AgNPs, and none for AuNPs. The absence of AuNPs in the Au-Ag composite may be due to an increase in the concentration of gold chloride (AuCl<sub>3</sub>) solution used for the synthesis of the NPs. The best concentration to synthesize nanoparticles is 1 mM of AuCl<sub>3</sub> solution, an increase in concentration (2mM-10mM) may become unfavorable thereby preventing the formation of the AuNPs. This is consistent with the findings of Mishra *et al.* (2011).

In respect to the antimicrobial properties of the synthesized nanoparticles, AuNPs exhibited antimicrobial activity against *Staphylococcus* sp., whereas no zone of inhibition was recorded against the other tested organisms. Ag and Au-Ag composite nanoparticles did not exhibit any antimicrobial activity (Table 2). Thus, the Ag, Au and Au-Ag composite nanoparticles synthesized by *Arspergillus carbonarius* cannot be considered as excellent broad - spectrum antimicrobial agents. This result is in contrast to the findings of Jaidev and Narasimha (2010).

In respect to the bioremediation properties of the nanoparticles in waste engine oil Cleanup, Au and Au-Ag composite nanoparticles exhibited a very high bioremediation activity in waste engine oil cleanup with broad clearance zones in both mineral salt and winograsky agar plates (Table 3). Thus the Ag, Au and Au-Ag composite nanoparticles can be considered as potential bioremediation agents. This is consistent with the findings of Vanalaker *et al.* (2018).

## CONCLUSION

The culture biomass of *Arspergillus carbonarius* was successfully explored for the synthesis of Au, Ag and Au-Ag composite NPs. The mycosynthesis protocols employed in this study were found to be simple, rapid, cost effective and environmentally benign and thus, this extracellular method of biosynthesis has a huge potential to become developed into simple bioprocess system for sustainable production of NPs in larger amount.

## BIBLIOGRAPHY

- Ahmad, A., Mukherjee, P., Senapat, S, Mandal, D., Khan, M.I., Kumar, R. and Sastry, M.(2003). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum* .*Colloids and Surfaces Biointerface* **28**:313–318.
- Ahmad, A., Senapati, S., Khan,M.I. ,Kumar, R., Ramani, R., Srinivas, V. and Sastry. M. (2003). Intra-cellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, *Rhodococcus* species. *Nanotechnology* **14**(7) : 824–828.
- Ali, M., Ahmed, T., Wu, W., Hossain, A., Hafeez, R. Islam. and Masum M.(2020). Advancements in plant and microbe-based synthesis of metallic nanoparticles and their antimicrobial activity against plant pathogens. *Nanomaterials* **10**(6): 1146
- Anupam, R., Onur, B., Sudip, S., Amit, K. M. and Deniz, M .Y. (2019). Green synthesis of silver nanoparticles: biomolecular nanoparticles organizations targeting antimicrobial activity. *RSC Advances* **17**: 122
- Azam, A., Ahmed, A.S. and Oves, M. (2012). Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. *International Journal of Nanomedicine*. **7**:6003–9.
- Bartolucci, C., Antonacci, A., Arduini, F., Moscone, D., Fraceto, L. and Campos, E. (2020). Green nanomaterials fostering agrifood sustainability. *Trends in Analytical chemistry* (125) 115840.
- Belkin, A., Hunker, A. and Bezryadin, A. (2015). "Self-Assembled Wiggling Nano-Structures and the Principle of Maximum Entropy Production". *scientific Reports*: **5** :8323
- Bhainsa, K.C. and D'Souza, S.F.(2006). Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. *Colloids and Surface Biointerfaces* **47**:160–164.
- Cui, Y., Zhao, Y., and Tian, Y.(2012). The molecular mechanism of action of bactericidal gold nanoparticles on *Escherichia coli*. *Biomaterials* **33**:2327–33.
- Daniel, M.C. and Astruc, D.(2004). Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chemical Reviews* **104**:293–346.
- Das, S.K., Liang, J., Schmidt, M, Laffir, F. and Marsili, E.(2012). Biomineralization mechanism of gold by zygomycete fungi *Rhizopous oryzae* *ACS NANO* **6**:6165–6173

- Dhillon, N. and Mukhopadhyay, S.(2015). Nanotechnology and allelopathy: synergism in action. *Journal of crop and weed* **11**(2): 187–91.
- Dizaj, S.M., Lotfipour, F., Barzegar-Jalali, M.(2014). Antimicrobial activity of the metals and metal oxide nanoparticles. *Materials Science and Engineering* **44**: 278–84.
- Drexler, K. E. (1986). *Engines of creation: The coming era of nanotechnology*. (1st edition).Anchor press, New York,283p.
- Drexler, K.E. (1992). *Nanosystems: molecular machinery, manufacturing and computation* .(5th edition).John Wiley & Sons. New york, 556p.
- Elbeshehy,K.F.E., Elazzazy, A.M. and Aggelis G. (2015).
- Emery,A.A., Saal, J.A., Kirklin, S., Hegde, V.I. and Wolverson C.(2016). High-throughput computational screening of perovskites for thermochemical water splitting applications. *Chemistry of Materials* **28** (16): 5621-5634
- Fair, R.J., Tor, Y.(2014). Antibiotics and bacterial resistance in the 21st century. Perspectives in *Medicinal Chemistry* **6**:145-153
- Gahlawat, G. and Choudhury A.R.(2019). A review on the biosynthesis of metal and metal salt nanoparticles by microbes. *RSC Advances* **16**(23): 12944–67.
- Gross, J., Sayle, A., Karow, A.R., Bakowsky, U. and Garidel, P.(2016). Nanoparticle tracking analysis of particle size and concentration detection in suspensions of polymer and protein samples: Influence of experimental and data evaluation parameters *European Journal of Pharmaceutics and Biopharmaceutics* **104**: 30-41,
- Gupta, S. and Bector, S. Biosynthesis of extracellular and intracellular gold nanoparticles by *Aspergillus fumigatus* and *A. flavus*. *Antonie Van Leeuwenhoek* **103**:1113–1123.
- Hulkoti, N.I. and Taranath T.(2014). Biosynthesis of nanoparticles using microbes—a review. *Colloids and surface interfaces*. 121: 474–83.
- Husseiny, M. I., El-Aziz , M. A.,Badr ,Y. and Mahmoud, M. A. (2007). Biosynthesis of gold nanopar-ticles using *psuedomonas aerigosa* Spectrochimica Acta. Part A: *molecular spectroscopy* **67**(3-4): 1003–1006.
- Iqbal, N., Khan, I., Yamani, Z.H and Qurashi, A.(2016). Sonochemical assisted solvothermal synthesis of gallium oxynitride nanosheets and their solar-driven photoelectrochemical water-splitting applications *Scientific Reports* **6**(1): 1-11.
- Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green chemistry* **13**(10): 2638–2650.

- Iravani, S. (2014). Bacteria in nanoparticle synthesis: Current status and future prospects. *International school of research notices* 35-93.
- Jaidev.L.R and Narasimha.G. (2010).Fungal mediated biosynthesis of silver nanoparticles, characterization and antimicrobial activity. *Colloids and surfaces Biointerfaces* **81**(2): 430-433
- Jain, N., Bhargava, A., Majumdar, S., Tarafdar, J.and Panwar., J.(2011). Extracellular biosynthesis and characterization of silver nanoparticles using *Aspergillus flavus* NJP08: a mechanism perspective. *Nanoscale*. **3**(2): 635–41
- Jeevanandam, J., Chan, Y. S. and Danquah, M. K. (2016). Biosynthesis of Metal and Metal Oxide Nanopar-ticles. *Chemical and Biochemical review* **5**(2): 55–67.
- Jeyaraj. M., Gurunathan, S., Qasim, M., Kang, M-H. and Kim J-H.(2019). A comprehensive review on the synthesis, characterization, and biomedical application of platinum nanoparticles. *Nanomaterials* **9**(12): 1719.
- Kale, A., Bao, Y., Zhou, Z., Prevelige, P. E. and Gupta, A. (2013). Prevelige, P.E.; Gupta, A. Directed self-assembly of CdS quantum dots on bacteriophage P22 coat protein templates. *Nanotechnology* **24**(4): 216-222.
- Kato, Y. and Suzuki, M.(2020). Synthesis of metal nanoparticles by microorganisms. *Crystals*. **10**(7): 589.
- Khan, I., Ali, S., Mansha, M. and Qurashi A.(2017). Sonochemical assisted hydrothermal synthesis of pseudo-flower shaped Bismuth vanadate (BiVO<sub>4</sub>) and their solar-driven water splitting application. *Ultrasonics Sonochemistry* **36**: 386-392,
- Khan, N. , Ali, S. , Latif, S. and Mehmood, A. (2022) .Biological Synthesis of Nanoparticles and Their Applications in Sustainable Agriculture Production. *Natural Science* **14**:226-234.
- Khan, S. A. and Ahmad, A. (2013). Fungus mediated synthesis of biomedically important cerium oxide nanoparticles. *Materials research bulletin* **48**(10): 4134–4138.
- Khan, T., Abbas, S., Fariq. A. and Yasmin A.(2018). Microbes: Nature’s cell factories of nanoparticles synthesis. In: Prasad R, Jha AK, Prasad K (Editors) *Exploring the Realms of Nature for Nanosynthesis*. Cham: Springer; p. 25–50.
- Khlebtsov, L.A. and Dykman. (2010). Optical properties and biomedical applications of plasmonic nanoparticles *Journal of Quantitative Spectroscopy and Radiative Transfer* **111** (1), 1-35.

- Khlebtsov, N and Dykman L. (2011). Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies *Chemical Society Review* **40**(3) 1647-1671.
- Kitching, M., Ramani, M. and Marsili, E.(2015). Fungal biosynthesis of gold nanoparticles: mechanism and scale up. *Microbial Biotechnology* **8**:904–917.
- Kulkarni, N. and Muddapur, U. (2014). Biosynthesis of metal nanoparticles: A review. *Journal of Nanotechnology*
- Kuppusamy, P., Yusoff, M. M., Maniam, G. P. and Govindan, N. (2016). Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications—An updated report. *Saudi pharmaceutical journal* **24**(4): 473–484.
- Lima. E., Guerra. R., Lara, V. and Guzmán, A.(2013). Gold nanoparticles as efficient antimicrobial agents for *Escherichia coli* and *Salmonella typhi*. *Chemistry Central Journal* **7**(11): 102-112
- Liu, D., Li, C., Zhou, F., Zhang, T., Zhang, H., Li, x., Duan, G., Cai, W. and Li, Y.(2015). Rapid synthesis of monodisperse Au nanospheres through a laser irradiation -induced shape conversion, self-assembly and their electromagnetic coupling SERS enhancement. *Scientific Reports* **5**(1): 7686,
- Liu, J., Liu, Y., Liu, L., Han, Y., Zhang, X., Huang, H., Lifshitz, Y., Lee, S .T., Zhong, J. and Kang.Z.(2015).Metal-free efficient photocatalyst for stable visible water splitting via a two-electron pathway. *Science* **80**(347) 970-974.
- Mahapatra, O., Bhagat, M., Gopalakrishnan, C. and Arunachalam K.D.(2008). Ultrafine dispersed CuO nanoparticles and their antibacterial activity. *Journal of Experimental Nanoscience* **3**:185–93
- Makarov, V. V., Love, A. J., Sinitsyna, O. V., Makarova, S. S., Yaminsky, I. V., Taliansky, M. E. and Kalinina, N. O. (2014). “Green” nanotechnologies: Synthesis of metal nanoparticles using plants. *Acts naturae* **6**(1 (20): 35–44.
- Mandal, D., Bolander, M.E., Mukhopadhyay, D., Sarkar, G. and Mukherjee P.(2006). The use of microorganisms for the formation of metal nanoparticles and their application. *Applied microbiology and biotechnology* **69**(5): 485–92.
- Mansha, M., Khan, I., Ullah, N., and Qurashi A.(2016). Synthesis, characterization and visible-light-driven photoelectrochemical hydrogen evolution reaction of carbazole-

- containing conjugated polymers. *International Journal of Hydrogen Energy* **42**(16) 10952-10961.
- Mishra, A., Tripathy, S.K., Wahab, R. , Being, S.H., Hwang,I. and Yang, Y.B. (2011).Microbial synthesis of gold nanoparticles using the fungus *Penicillium brevicompactum* and their cytotoxic effects against mouse mayo blast cancer C2C12 cells. *Applied Microbiology and Biotechnology* **92**: 617–630
- Mishra, A., Tripathy, S.K., Wahab, R., Jeong, S.H., Hwang, I., Yang, Y.B., Kim, Y.S., Shin, H.S. and Yun, SII. Microbial synthesis of gold nanoparticles using the fungus *Penicillium brevicompactum* and their cytotoxic effects against mouse mayo blast cancer C2C12 cells. *Applied Microbiology and Biotechnology* **92**:617–630.
- Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S., Sainkar, S. R., Khan, M. I., Parishcha, R., Ajayku-mar, P. V., Alam, M., Kumar, R. and Sastry, M. (2001). Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis. *Nano letters* **1**(10): 515–519.
- Nadaroglu, H., Gungor, A.A., Ince, S. and Babagil, A. (2017). Green synthesis and characterisation of platinum nanoparticles using quail egg yolk.*spectrochimica Acta part a \_molecular and Biomolecular spectros\_ copy* **172**: 43–7.
- Narayanan, K. B. and Sakthivel, N. (2010). Biological synthesis of metal nanoparticles by microbes. *Advances in colloids and interface science* **156**(1-2): 1–13.
- Ovais, M., Khalil, AT., Ayaz, M.,Ahmad, I., Nethi, S.K. and Mukherjee S.(2018). Biosynthesis of metal nanoparticles via microbial enzymes: a mechanistic approach. *International journal of molecular sciences* **19**(12):4100
- Park, S., Sung, H. K., & Kim, Y. (2016). Green Synthesis of Metal Nanoparticles Using Sprout Plants: Pros and Cons. *Journal of nanoscience and nanotechnology* **16**(5): 4444–4449.
- Prabhu, S. and Poulouse, E. K. (2012). Silver nanoparticles: Mechanism of anti-microbial action, synthesis, medica lapplications, and toxicity effects. *International Nano letters* **2**(1): 1–10.
- Ramteke, C., Chakrabarti, T., Sarangi B.K and Pandey, R. (2013).Synthesis of silver nanoparticles from the aqueous extract of leaves of *Ocimum sanctum* for enhanced antibacterial activity. *Journal of chemistry* **2013** :1–8.
- Recent advances in synthesis of Cu<sub>2</sub>FeSnS<sub>4</sub> materials for solar cell applications: A review.

- Saeed, K. and Khan, I. (2014). Preparation and properties of single-walled carbon nanotubes/poly(butylene terephthalate) nanocomposites. *Iranian Polymer Journal* **23**(1): 53-5.
- Saeed, K. and Khan, I. (2016). Preparation and characterization of single-walled carbon nanotube/nylon 6,6 nanocomposites. *Instrumentation Science and Technology* **44**(4) 435-444.
- Sanghi, R. Verma, P. and Puri, S. (2011). Enzymatic formation of gold nanoparticles using *Phanerochaete chrysosporium*. *Advances in Chemical Engineering Science* **1**:154–162.
- Shah, M., Fawcett D., Sharma, S., Tripathy, S.K. and Poinern G.E.J. (2015). Green synthesis of metallic nanoparticles via biological entities. *Materials* **8**(11): 7278–308.
- Siddiqi, K. S. And Husen, A. (2016). Fabrication of metal and metal oxide nanoparticles by algae and their toxic effects. *Nano-scale research letter* **11**(1): 363.
- Sikora, A., Shard, A.g. and Minelli, C. (2016). Size and  $\zeta$ -potential measurement of silica nanoparticles in serum using tunable resistive pulse sensing. *Langmuir* **32**: 2216-2224.
- Silver nanoparticles synthesis mediated by new isolates of *Bacillus* spp., nanoparticle characterization and their activity against Bean Yellow Mosaic Virus.
- Singh, A., Gautam, P.K., Verma, A., Singh, V., Shivapriya, P.M. and Shivalkar S. (2020). Green synthesis of metallic nanoparticles as effective alternatives to treat antibiotics resistant bacterial infections: a review. *Biotechnology Report* **25**: 427
- Singh, P., Kim, Y-J., Zhang, D. and Yang, D-C. (2016). Biological synthesis of nanoparticles from plants and microorganisms. *Trends in biotechnology* **38**(7): 588–99
- Singh, P., Kim, Y-J., Zhang, D. and Yang, D-C. (2016). Biological synthesis of nanoparticles from plants and microorganisms. *Trends in biotechnology* **38**(7): 588–99
- Sneha, K., Sathishkumar, M., Mao, J., Kwak, I.S. and Yun, Y.S. (2010). *Corynebacterium glutamicum*-mediated crystallization of silver ions through sorption and reduction processes. *Chemical Engineering Journal* **162**:989–996.
- Son, M., Mehta, P, Soni, A and Goswami G.K. (2018). Green nanoparticles: Synthesis and applications. *IOSRJ Biotechnology and Biochemistry*. **4**: 78–83.
- Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy *Elsevier* **61** (4): 761-765

- Sunkar, S. and Nachiyar, C. V. (2012). Biogenesis of antibacterial silver nanoparticles using the endo-phytic bacterium *Bacillus cereaus* isolated from *Garcinia xanthochymu*. *Asian pacific journal of Tropical Biomedicine* **12**(12):953–959.
- Swinehart D.F.(1962).The Beer-Lambert law. *Journal of Chemical Education* **39**: 333
- Tiwari, P.M., Vig, K., Dennis, V.A. and Singh, S.R.(2011). Functionalized gold nanoparticles and their biomedical applications. *Nanomaterials* **1**:31–63.
- Tollamadugu, K. V., Prasad, T., Kambala, V. S., & Naidu, R. (2011). A critical review on bio-genic silver nanoparticles and their antimicrobial
- Ullah, H., Khan, I., Yamani, Z.H. and Qurashi, A.(2017).Sonochemical-driven ultrafast facile synthesis of SnO<sub>2</sub> nanoparticles: growth mechanism structural electrical and hydrogen gas sensing properties. *Ultrasonics Sonochemistry* **34**: 484-490.
- Verma, A., and Mehata, M.S.(2016) .Controllable synthesis of silver nanoparticles using neem leaves and their antimicrobial activity. *Journal of Radiation Research and Applied Sciences* **9**:109–15.
- Verma, V.C.; Kharwar, R.N. and Gange, A.C.(2010). Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus *Aspergillus clavatus*. *Nanomedicine* **5**: 33–40.
- Wang, J., Yang, N., Tang, H., Dong, Z., Jin, Q. Yang, M., Kisailus, D., Zhao, H., Tang, Z.and Wang,D.(2013). Accurate control of multishelled Co<sub>3</sub>O<sub>4</sub> hollow microspheres as high-performance anode materials in lithium-ion batteries *Angewandte Chemie International Education* **125**(25): 6567-6570.
- Yallappa, S., Manjanna, J. and Dhananjaya, B. L. (2015). Phytosynthesis of stable Au, Ag and Au–Ag alloy nanoparticles using *J. sambac* leaves extract, and their enhanced antimicrobial activity in presence of organic antimicrobials. *Spectrochemical Acta.part A:Spectrochimica Acta. Part A*: 137: 236–243
- Zhongyu, L., Jianming W, and Ru,X. and Yong Y.(2005).
- Zhou, Y., Kong, Y. and Kundu, S. Antibacterial activities of gold and silver nanoparticles against *Escherichia coli* and *bacillus Calmette-Guérin*. *Journal of Nanobiotechnology* **10**(19): 147-155

## APPENDIX

### Appendix 1: Biomass Preparation

To prepare fungus biomass for biosynthesis, *Aspergillus carbonarius* was grown in potato infusion medium in a 250ml Erlenmeyer flask. The flask was inoculated with spores and incubated at 37°C on a rotary shaker for 7 days. The biomass was harvested by centrifuging the mixture to yield the supernatant cell free extract at the rate of 2000rpm. The biomass was washed with 50mM Tris HCL buffer at pH 7.0 to reduce the activity of potatoe and the biomass was decanted through filter paper (whatman filter paper No.1).

### Appendix 2: Preparation of 1mM of Gold Chloride

The molar mass of Gold Chloride (303.33) was divided by 1000 and the result was 0.3g. 0.3g of gold chloride was measured and dispensed into a beaker. A known volume of distilled water was dispensed into the beaker and stirred thoroughly to dissolve the gold chloride completely. Distilled water was added to make up the volume to 1000 and the mixture was dispensed into a winchester bottle. Molar mass of Gold Chloride=303.33 To prepare 1Mm of Gold Chloride:

$$303.33/1000 = 0.3g$$

### Appendix 3: Preparation of 1mM silver Nitrate

The molar mass of silver nitrate (169.87) was divided by 1000 and the result was 0.17g. 0.17g of silver nitrate was measured and dispensed into a beaker. A known volume of distilled water was dispensed into the beaker and stirred thoroughly to dissolve the silver nitrate completely. Distilled water was added to make up the volume to 1000 and the mixture was dispensed into a winchester bottle.

Molar mass of silver nitrate=169.87

To prepare 1mM of silver nitrate:

$$169.87/1000=0.17\text{g}$$

#### **Appendix 4: Preparation of Potatoe Dextrose infusion**

The working space was disinfected with dettol. 200g of Potatoe was sliced and boiled in 1000ml of water for 30 minutes. The mixture was filtered with cheese cloth and the effluent was dispensed into 7 conical flask. 12g of dextrose agar was introduced into the conical flask. HCL was then dispensed into flasks to reduce pH. The flask was shaken vigorously to mix the content thoroughly and autoclaved at 121°C for 15 minutes.

#### **Appendix 5: Preparation of Potatoe Dextrose Agar**

The working space was disinfected with dettol. 40.6g of agar was weighed using a digital weigh balance. Thereafter, the agar was dispensed into 1000ml of distilled water. The medium was shaken vigorously and sterilized by autoclaving at 121°C for 15 minutes. The pH of the Medium was then adjusted to 3.5 by adding sterile tartaric acid. The medium was then dispensed into petri dishes and covered appropriately.

#### **Appendix 6: Preparation of Winograsky Agar**

The working space was disinfected with dettol. 10g of agar was weighed using a digital weigh balance and dispensed into a conical flask. 500ml of Winograsky solution was dispensed into a beaker. The pH of the Medium was adjusted to 7.0 by introducing potassium hydroxide.

The winograsky solution was then introduced into the conical flask containing the agar and shaken vigorously, sealed and allowed to settle. The medium was then sterilized in an autoclave at 121°C for 15 minutes.

#### **Appendix 7: preparation of mineral salt agar**

Preparation was done by weighing 13.9g into 500ml distilled water in a conical flask. The conical flask was then covered with foil paper and autoclaved for 15minutes at 121°C. The conical flask was then allowed to cool after sterilization of the agar and the agar was poured into petri dishes and allow to solidify.