Synthesis of Mushroom-based Copper Nanoparticles and its Characterization

Dissertation submitted in partial fulfillment of the requirement for the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

By

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DECLARATION

I hereby declare that the work presented in this report entitled **"Synthesis of Mushroom-based Copper Nanoparticles and its Characterization"** in partial fulfilment of the requirements for the award of the degree of **Bachelor of Technology** in **Biotechnology** submitted in the Dept. of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat is an authentic record of my own work carried out over a period from January 2023 to May 2023 under the supervision of **Dr. Garlapati Vijay Kumar**, Associate Professor, Department of Biotechnology and Bioinformatics, JUIT.

I also authenticate that I have carried out the above mentioned project work under the proficiency stream Industrial Biotechnology.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

(Jyotika, 191827)

SUPERVISOR'S CERTIFICATE

This is to clarify that the work reported in B. Tech. thesis entitled "**Synthesis of Mushroom-based Copper Nanoparticles and its Characterization"** submitted by Jyotika Gupta (191827) at Jaypee University of Information Technology, Waknaghat, India, is the bonafide record of their original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

(Signature of supervisor)

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(Jyotika Gupta, 191827)

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ABSTRACT

This study is conducted to analyze a greener synthetic method for generating copper oxide nanoparticles using shiitake and oyster mushrooms. Due to its distinctive qualities, including its low cost, simple synthesis methods, high water solubility, and environmentally friendly nature, research and innovation in producing nanoparticles (NPs) from biomaterials have received a lot of interest. The high nutritional value, immune-modulating, antimicrobial (antibacterial, antifungal, and antiviral), antioxidant, and anticancer properties of NPs derived from macrofungi, including different mushroom species like *Agaricus bisporus*, Pleurotus species, Lentinus species, and Ganoderma species. Fungi have a high metal tolerance and bioaccumulation capacity due to their aptitude for intracellular metal uptake and maximal wall binding. Compared to chemical and physical methods, biological processes for synthesizing copper nanoparticles (CuNPs) are more cost-efficient, environmentally benign, and easily scalable.

Keywords: Copper Oxide; Nanoparticles; DPPH Assay; MHA Test; Bioactive effects; Antioxidant Assay.

CHAPTER 1

Introduction

1.1 Introduction

1.1.1 Ethnomycology

Eukaryotic fungi are organisms that may exist in a wide range of typical settings and frequently create decomposer organisms. Of the estimated species of 1.5 million fungi only 70,000 have been named so far. Based on more recent data, high-throughput sequencing techniques have calculated that there are 5.1 million different species of fungi. It is important to remember that these living creatures have the capacity to break down extracellular material and generate specialized enzymes that hydrolyze complex elements into simpler molecules that are then ingested and used as an energy source. The study of fungi's involvement in nanobiotechnology is deemed essential.

Fungi have drawn more attention in studies on the biological production of metallic nanoparticles because of their tolerance and capacity to bioaccumulate metals. The benefits of using fungi in the manufacture of nanoparticles are their ease of scaling up (e.g., using a thin solid substrate fermentation technique). Fungi are great producers of extracellular enzymes and can manufacture vast quantities of enzymes.

Mushrooms belonging to the fungi are widely known for their wonderful taste and amazing health benefits. Packed with high amounts of essential vitamins and minerals, it's a great addition to your diet and adds flavour to a variety of recipes.The mushrooms used in this study shiitake and oyster mushrooms are edible and an excellent source of low-fat, low-calorie protein, carbohydrates, fibre, vitamin D2 (ergocalciferol) and minerals. Considered a healthy food. A variety of bioactive effects, including immunomodulatory, anti tumour, anti viral, anti diabetic, and anti inflammatory, are produced by different polysaccharide fractions that have been extracted.

1.1.2 Medicinal Properties

Beta-D-glucan polysaccharides, which are found in shiitake and oyster mushrooms, are thought to enhance the immune system's response to cancer. With components that encourage the production of white blood cells, the shiitake mushroom, whose medicinal benefits have been well-known for millennia, also functions as a natural anti-inflammatory and immune system booster. Shiitake mushrooms are also excellent sources of vitamins B and D2, which are necessary for the proper operation of the body's digestive, metabolic, and cell-creating processes.

In addition, shiitake and oyster mushrooms may provide the following health benefits:

(i) Heart Health

Since mushrooms like oysters have a savoury flavour and no cholesterol. Consuming oyster mushrooms helped diabetic patients lower their triglyceride and cholesterol levels as well as high blood sugar levels, according to one study. Shiitake mushrooms contain beta-glucan, a soluble fibre type that is beneficial for lowering cholesterol.Shiitake mushrooms contain potassium, which lowers blood pressure as well.

(ii) Support for the Immune System

In addition to enhancing heart health, beta-glucan also improves immune system performance. Oyster mushrooms are a rich source of other antioxidants that can help shield your immune system from oxidative stress and free radical damage so it can fight off ageing.Having a constant food source may minimize a deficiency since the body does not store much copper. For most people, a single serving of cooked shiitake mushrooms contains more copper than they require daily.The production and function of several immune cells, that includes T cells, neutrophils, phagocytes, B lymphocytes, natural killer cells, and antibodies, are supported by copper, which is essential for the immune system.

(iii) Assistance with Cancer

The abundant beta glucan present in both mushrooms may be particularly helpful in squelching and eliminating cancer cells. A study conducted in 2019 that followed more than 36,000 Japanese men between the ages of 40 and 79 found a link between eating mushrooms and a lower risk of prostate cancer. Ergothioneine, an antioxidant found in mushroom variations like king oyster, shiitake , and maitake varieties that might lessen oxidative stress, is the cause of the association, according to researchers.

(iv) Control of Diabetes

Clinical trials have established the ability of another beta-glucan, the SX fraction, to lower blood sugar levels. It aids in the treatment of diabetes by reducing insulin resistance and helping to activate insulin receptors.

(v) Prevention of Neurodegenerative Diseases

Ergothioneine, an amino acid, also acts as a cytoprotectant. In other words, it guards against the harm caused by free radicals and oxidative stress on cells.This is why oysters and mushrooms may be able to protect against neurodegenerative conditions like Alzheimer's, Parkinson's, and dementia.

1.1.3 Adverse Results

Although these both are typically regarded as a healthy food, there isn't much research on how it will affect you over the long term if you take it as a supplement. If you intend to add supplements containing shiitake and oyster mushroom extract to your regimen, always talk to your doctor first.

(i) Allergic reactions

Although shiitake mushrooms aren't regarded as a typical allergen, it is still possible to be allergic to them. Although exceedingly uncommon, it has been demonstrated in one instance that handling shiitake mushrooms can cause asthma through an IgE-mediated reaction. Other instances of allergic responses to mushrooms have been reported. One published case study suggests that severe food responses to cross-reacting mushroom proteins may be caused by earlier sensitization to mould allergens. If you are allergic to mould, eating mushrooms may cause oral allergy syndrome symptoms. Itchiness or swelling of the mouth, face, lip, tongue, or throat may be among these symptoms. Before taking mushrooms, check with your doctor if you have a mould allergy.

1.1.4 WARNINGS & SPECIAL PRECAUTIONS

- Breast-feeding and pregnancy: There isn't enough reliable information to say whether eating either mushroom is safe during pregnancy or lactation. Avoid being too cautious.
- Diabetes: Blood sugar levels may be lowered by maitake mushrooms. Your diabetic medication schedule might need to be changed by your doctor.
- It is possible to lower blood pressure with mushrooms. The consumption of mushrooms has the potential to further lower the blood pressure of people who already have low blood pressure.
- Hypereosinophilic syndrome is a blood condition: Shiitake mushrooms should not be consumed if you have this condition. It can worsen as a result.

1.1.5 Copper oxide Nanoparticles

Nanoparticles of tens of atoms are a low size regime in which the electrons and holes are entirely confined. These materials have no dimensions. It is discovered that nanoparticles range in size from 23 to 37 nm. The UV-Visible spectrophotometer is used to measure the optical absorption characteristics. It is possible to define quantum confinement as a reduction in the degree of freedom of nanoparticles whose diameter is lower than their Bohr-excitation radius from N to zero. Two types of copper oxide include cupric oxide, also called copper (II) oxide, cuprous oxide, also known as copper (I) oxide.

While cuprous oxide has a cubic structure, cupric oxide is monoclinic. Each of them has a band gap of 1.2-1.7 and 2 eV, respectively, and they are both p-type semiconductors. Solar cells, gas sensors, inks for FET transistors, and antibacterial materials for medicinal and biological purposes are just a few of the possibilities for CuO nanoparticles. For the synthesis of copper oxide nanoparticles, a number of techniques have been reported, including the precipitation method, hydrothermal method, thermal decomposition technique, and sol-gel technique.

Cupric oxide, also known as copper (II) oxide, and cuprous oxide, also known as copper (I) oxide, are two different forms of copper oxide. While cupric oxide is monoclinic, cuprous oxide has a cubic structure. They are both p-type semiconductors with band gaps of 1.2-1.7 and 2 eV, respectively. A few applications for CuO quantum dots include solar cells, gas sensors, FET transistor inks, and antibacterial materials for biological and medical use. For the synthesis of copper oxide nanoparticles, a number of techniques have been documented, including the precipitation approach, the hydrothermal technique, thermal decomposition method, and sol-gel approach.

1.2 Problem Statement

Low research in the field of mushrooms has suppressed the extravagant nature of fungi. It was explicitly stated in a nature article that fungi are more closely linked to people than plants are. Despite the mushrooms' low calorie and bioactive component contents, there aren't many studies on them. Although it can be challenging for researchers to locate fungus that are not harmful, it is known that certain mushrooms have anticancer properties. The green synthesis which is cheaper and good to environment of nanoparticles from mushrooms has received much attention over the years, but it still requires expensive equipment and sophisticated labs to study its effectiveness and characterization, making copper oxide quantum dots a less studied subject than it might otherwise be given its applications in the biomedical, electronic, and other fields.

CHAPTER 2

Literature Review

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1. Literature Survey

2.1.1 Mycology of Lentinula *edodes*

Fig. 1 Picture of shiitake also known as "oakwood mushroom"

The most well-known and significant cultivated mushroom in Japan is *Lentinula edodes*, also known as shiitake. It is one of the most widely grown mushrooms in the world, behind *Agaricus bisporus*. Shiitake mushrooms are found in clusters on the dead wood of deciduous trees, including beech, sweetgum, poplar, hornbeam, chestnut, oak, maple, ironwood, and mulberry. The ideal temperature range for shiitake mycelium growth is between 20° and 26°C. During spawning logs, low temperatures (14–20°C) are preferred.

2.1.2 Mycology of Pleurotus *ostreatus*

Fig. 2 Picture of oyster mushroom also known as "dhingri" in india.

Pleurotus ostreatus, sometimes referred to as the oyster mushroom, oyster fungus, hiratake, or pearl oyster mushroom, is a common edible mushroom. Natural samples come in a variety of colours, including as white, grey, tan, and dark brown. A broad, fan- or oyster-shaped cap with a diameter of 2–30 centimeters (34–1134 inches) covers the mushroom. When young, the margin is smooth and frequently slightly bent or wavy. Although it may be grown on straw and other materials, it is one of the more popularly sought-after wild mushrooms.

P.ostreatus, a carnivorous fungus, feeds on nematodes by paralysing its prey within minutes of contact, causing necrosis, and producing slurry that can be easily ingested as a source of protein. *P. ostreatus* sprouts on the mass of dead and dying wood that is accumulating quickly. By consuming the dead wood and reintroducing essential nutrients and minerals to the environment in a form that can be utilised by other plants and animals, they really help the forest. Lithium bioaccumulates in oyster mushrooms.

2.2 Nutritional and medicinal uses

2.2.1 Nutritional and medicinal uses of Shiitake

The comprehensive nutritional profile of shiitake mushrooms accounts for the majority of their health advantages. Shiitake mushrooms are a great meat alternative since they are low in calories and have amino acids that are similar to those in red meat. This is especially true when paired with other sources of plant-based protein. Shiitake mushrooms offer good amounts of essential nutrients that are hard to get in other food sources, in addition to fibre. Four shiitake mushrooms provide 10% of the recommended selenium intake, 33% of the recommended B5 intake, and 39% of the recommended copper intake for the day.Shiitake mushrooms will also offer a decent dosage of vitamin D, which is extremely difficult to acquire in food sources if they are grown under special UV lights.

To top it off, shiitakes also contain unique substances called lipids, sterols, terpenoids, and polysaccharides that have been examined for their function in boosting the immune system and protecting against cancer. The immune system appears to be strengthened by shiitake mushrooms. Shiitake mushrooms may make drugs that weaken the immune system less effective since they boost the immune system.

Mycophenolate (CellCept), cyclosporine (Neoral, Sandimmune), daclizumab (Zenapax), tacrolimus (FK506, Prograf), sirolimus (Rapamune), prednisone (Deltasone, Orasone) and others are some medications that impair immune system function.

2.2.2 Nutritional and medicinal uses of Oyster Mushroom

Because they include a variety of vitamins, minerals, and antioxidants in addition to the essentials, oyster mushrooms also offer a respectable nutritional value.Niacin: Nearly all human cells need niacin to operate and process other nutrients.

Vitamin D: Since it normally comes from sunlight, vitamin D is difficult to locate in dietary products. However, one of the best food sources of vitamin D is UV-treated mushrooms.

Iron: Iron is needed for red blood cells. Oyster mushrooms are a wonderful meat substitute since they provide 12% of the daily required iron consumption in just one cup.

Additionally, oyster mushrooms include 8% of the daily recommended intake of numerous essential elements, including riboflavin, potassium, vitamin B6, folate, magnesium, and vitamin c. Flavonoids and phenolics, two types of antioxidants that are abundant in oyster mushrooms. Antioxidants are compounds that lessen or stop the body's cells from ageing. Free radicals, which are associated with conditions like cancer, are fought by antioxidants.

2.2.3 Pharmacological Effect of Oyster Mushroom

Research has been done on the varied pharmacological effects and potential health advantages of oyster mushrooms (Pleurotus spp.). The following are some prominent pharmacological effects linked to oyster mushrooms:

- Oyster mushrooms are abundant in bioactive substances such as phenolic compounds, flavonoids, and polysaccharides, all of which have strong antioxidant effects. These substances work to neutralise free radicals and lessen oxidative stress, which is linked to ageing and a number of chronic diseases.

- Effects on the immune system: Polysaccharides in oyster mushrooms, notably -glucans, have an immunomodulatory effect. The body's immune response is strengthened by these substances' capacity to stimulate the activity of immune cells such T cells, natural killer cells, and macrophages.This immune regulation may help to increase resistance to illnesses and infections.

- Anti-inflammatory properties: Research has revealed that oyster mushrooms have anti-inflammatory qualities. Oyster mushrooms contain bioactive substances that can reduce the activity of pro-inflammatory enzymes and reduce the generation of inflammatory chemicals. The management of chronic inflammatory disorders may benefit from this anti-inflammatory activity.

- Oyster mushrooms have antibacterial and antiviral properties that make them effective against a variety of diseases. Numerous studies have proven both their effectiveness against bacteria, including strains that are resistant to antibiotics, and their antiviral potency against particular viruses. These characteristics point to the possibility of oyster mushrooms as all-natural complements or substitutes for traditional antibacterial treatments.

- Oyster mushrooms have been studied for their potential anticancer qualities. Polysaccharides and lectins, two substances present in oyster mushrooms, have shown anticancer effects by preventing the growth of cancer cells, triggering apoptosis (programmed cell death), and altering the immune system's reaction to cancer cells. To completely comprehend and utilise their anticancer potential, more research is necessary.

2.2.4 Pharmacological Effect of Shiitake Mushroom

Numerous studies have been conducted on shiitake mushrooms (Lentinula edodes) to determine its pharmacological effects and potential health advantages. Some prominent pharmacological effects of shiitake mushrooms include the following:

- Shiitake mushrooms feature bioactive ingredients including polysaccharides (especially -glucans) that have immunomodulatory properties. These substances can improve immunological responses, increase cytokine synthesis, and promote immune cell activity. The immune system has been shown to be strengthened by shiitake mushrooms by enhancing the activity of lymphocytes, macrophages, and natural killer cells.

- Shiitake mushrooms are abundant in polyphenols and polysaccharides, two types of antioxidants that help scavenge free radicals and lessen oxidative stress. Shiitake mushrooms may help shield cells from oxidative stress-related cell damage and may lower the risk of chronic disease by neutralizing reactive oxygen species.

- Shiitake mushrooms contain antibacterial qualities and have demonstrated effectiveness against a variety of bacteria and fungi. They also have antiviral capabilities. Additionally, they have antiviral properties against some infections. Lentinan, a polysaccharide found in shiitake mushrooms, and other substances have been investigated for their antiviral properties against influenza viruses.

- Anti-inflammatory properties: Shiitake mushrooms have anti-inflammatory properties. To lessen inflammation, these substances can suppress the synthesis of inflammatory chemicals and alter immunological reactions.

- Shiitake mushrooms have been shown to contain substances, such as eritadenine, that have been proved to decrease cholesterol levels. These substances encourage the body's excretion of cholesterol and inhibit the enzyme responsible for cholesterol production. Shiitake mushroom eating on a regular basis may enhance lipid profiles and lower the risk of cardiovascular illnesses.

2.3 Copper Oxide Nanoparticles

While oxygen is an element of Block P, Period 2, copper belongs to Block D, Period 4. The powdery form of copper oxide nanoparticles is brownish-black in colour. Metal oxide nanoparticles like copper oxide (CuO) have attracted attention due to their antibacterial and biocidal properties and may be utilised in a number of biomedical applications.

One of the most important elements in the synthesis of these nanoparticles is the regulation of particle size, shape, and crystallinity. Diverse synthesis methods have been created to this purpose; among of the more extensively researched ones are the sonochemical approach, the technique of sol-gel, ablation using lasers, the electrochemical method, chemical precipitation, and surfactant-based procedures.

Physical Properties: The table below lists the physical characteristics of copper oxide nanoparticles.

Table 1: Physical properties of copper oxide nanoparticles.

Properties	Metric	Imperial value
Density	6.31 g/cm3	0.227 lb/in3
Molar mass	79.55 g/mol	

Thermal Properties; The table below lists the thermal characteristics of copper oxide nanoparticles.

Table 2: Thermal properties of copper oxide nanoparticles.

Properties	Metric	Imperial value
Melting point	1201°C	2194°F
Boiling point	2000°C	3632°F

One practical method is UV Visible absorption spectroscopy, which is particularly significant since it offers crucial details about the material's optical properties. El Sayed et al. investigated the optical characteristics of polyvinyl alcohol (PVA) and carboxymethyl cellulose (CMC)/CuO nanoparticle-doped thin films. According to reports in the literature, the synthesis temperature may have an impact on the electrical conductivity of CuO NPs. Also the research claims that when the temperature is raised from 300 °C to 700 °C during the synthesis, the removal of H2O vapour from the air causes the electrical conductivity of the CuO NPs to increase from 106 (cm)1 to 105 (cm)1.The magnetic properties of CuO NPs are additionally impacted by their diameters.

Additionally, the magnetic properties of CuO NPs are directly influenced by their form. After obtaining CuO NPs with sizes ranging from 13 nm to 33 nm, the authors of a study on the features of nanoparticles demonstrated a weak ferromagnetic connection. The particle size had very little impact on the process. The most important factor is the nanoparticles' size, which can be changed during synthesis and allows for personalized modelling of their optical, catalytic, electrical, and biological properties. These qualities make them perfect for a range of uses, such as the production of coatings, paints, pharmaceutical stand-ins, beauty products, and other products.

Because of this, controlling size directly as well as other important chemical, physical, and biological characteristics directly or indirectly depends on the applicable synthesis process, tweaking reaction parameters, and bulk material composition. CuO NPs with biological applications and other nanoparticles with green synthesis have been the subject of extensive investigation in recent years.

Because green synthesis is secure for biological systems, better for the environment, and maintains both the chemical and physical characteristics of nanoparticles required for biomedical applications, it is preferred to traditional synthesis.

2.4 Applications

2.4.1 Healthcare facilities

CuO nanoparticles are mostly used as antibacterial agents. Because they have the antibacterial potential to destroy more than 99.9% of Gram-positive and Gram-negative bacteria within two hours of exposure, they are used in hospitals. Studies show that utilizing CuO reduces the incidence of hospital-acquired infections and the costs associated with delivering healthcare in hospitals. One of the most intriguing medical advances is the use of bed linens containing CuO NPs, which reduce microbial adherence and, in turn, microbial infections in hospitals.

Using copper-oxide in medical textiles has a number of advantages, including the following:

- It has potent antibacterial and antifungal properties;
- It acts efficiently against both prone and antibiotic-resistant microorganisms that cause nosocomial infections;
- It prevents the growth of biofilm, or attached communities of microorganisms, on the outer layer of materials covered with CuO NPs;
- It doesn't irritate or sensitize the skin; and
- It is safe for use on people.

2.4.2 Biomedical Applications

CuO nanoparticles' diverse features, which are significantly impacted by their size, surface characteristics, optical properties, and magnetic attributes, may have a wide range of applications. All of these can be controlled, and consequently can their biological properties, by changing the synthesis procedure. Chemical sensors, antibacterial agents, catalysts for different cross coupling reactions, cancer-fighting formulations, coating materials, etc. are a few examples of potential uses for doping compounds in semiconductors.

Li et al. created a very sensitive and specific approach for the H1N1 flu virus identification in a recent investigation. This method's foundation is the use of CuO NPs to mark antibodies. This approach was developed as an overlapping complex of CuO NP-tagged polyclonal antibodies that can detect and bind antigens that are typical of the H1N1 virus. The technology, an enzymatic chromogenic methodology from the so-called ELISA methods i.e. enzyme linked immunosorbent assay, has shown to be significantly more accurate and speedy than other methods in the sector

2.4.3 The following are some further significant uses for copper oxide nanoparticles:

- As a catalyst to speed up the burning of rocket fuel. It can improve as a catalyst for AP composite propellants, lower pressure index, and greatly boost homogeneous propellant burning rate.
- Its application is advantageous for thermoelectric materials, sensing materials, catalysts, superconducting materials, glass, ceramics, and other disciplines.
- Applications include solar energy conversion, high-tech superconductors, semiconductors, gas sensors, near-infrared tilters, ceramic resistors, magnetic storage, and photoconductive and photothermal devices..
- Because they have better skin penetrating qualities, nanoparticles are used in cosmetic and dermatological goods.

2.5 Toxicity of CuO Nanoparticles

Its application can benefit catalysts, superconducting materials, thermoelectric materials, sensing materials, glass, ceramics, and other fields.Ceramic resistors, magnetic storage, gas sensors, near-infrared tilters, photoconductive and photothermal devices, solar energy conversion, high-tech superconductors, and semiconductors are examples of applications. Nanoparticles are employed in cosmetic and dermatological products because they penetrate the skin better.

CuO nanoparticles have a variety of detrimental effects in vitro and in vivo when tested on human cells and several animal models. In accordance with what happens with poisonous heavy metals, a 1995 study discovered that copper's bioavailability is the most important factor in determining its toxicity.

CuO NPs' toxicity may be affected by changing certain characteristics, such as:

a) size. Small nanoparticles are more hazardous than larger ones

b) Surface charge: A positive charge on a nanoparticle's surface increases its toxicity. This positive charge makes it easier for cells and nanoparticles to connect.

c) Dissolution: The dissolution of copper oxide NPs is temperature and pH dependent, and this has significant effects on their toxicity.

The prolonged exposure of several vertebrate embryos to CuO NPs containing 10 mg Cu/L did not result in a substantial reduction in embryo survival. The ionic form, on the other hand, appeared to be the most harmful, changing all of the studied parameters, including survivability and deformity, particularly when embryos were confronted with 5-10 mg Cu/L.

2.6 Challenges

- Extraction and Purification: Copper oxide nanoparticle extraction and purification from mushrooms can be a challenging process. It entails eliminating contaminants such proteins, polysaccharides, and other biomolecules, then separating the nanoparticles from the mushroom matrix. It can be difficult to develop effective extraction and purification techniques while maintaining the stability and integrity of the nanoparticles.

- Variability in Nanoparticle Properties: Due to intrinsic variances in different mushroom species, growth environments, and extraction processes, copper oxide nanoparticles generated from mushrooms may show variations in size, shape, and composition. For predictable behaviour and reproducible performance, nanoparticle characteristics must be consistent.

- Biocompatibility and Toxicity: It is essential for the safe usage of mushroom copper oxide nanoparticles to comprehend their biocompatibility and potential toxicity. Although it's commonly accepted that mushrooms are safe to eat, turning mushroom extracts into nanoparticles could change how they interact with living things. Thorough toxicity studies, including in vitro and in vivo research, should be performed to determine the potential impact on human well-being and the environment.

- Stability and Agglomeration: Similar to regular copper oxide nanoparticles, mushroom copper oxide nanoparticles can combine, reducing their surface area and changing their physical characteristics. It is necessary to investigate methods to improve stability and avoid agglomeration, such as surface functionalization or stabilization with suitable coatings.

2.7 Limitations

The primary drawback of copper oxide nanoparticles generated from mushrooms is the possibility for variation and inconsistent behaviour in the nanoparticles' make-up and characteristics. Bioactive chemicals are frequently extracted from mushrooms and used as building blocks for nanoparticles in mushroom-based synthesis techniques. However, the makeup of mushrooms can change based on the species, growth circumstances, and time of harvest.

Because of this inherent variety in mushroom content, the characteristics of the copper oxide nanoparticles that are created may vary. Between batches of mushrooms, the concentration of bioactive substances, including those necessary for the production of nanoparticles, may vary, resulting in changes in the size, shape, stability, and other characteristics of the resulting nanoparticles.

This variability in nanoparticle characteristics might make it difficult to reproduce results, scale up processes, and standardize processes in both academic and commercial settings. It can make it more difficult to precisely regulate and improve the nanoparticle properties required for particular applications.

This restriction is being worked around by standardizing extraction procedures, improving mushroom growing conditions, and putting quality control systems in place to guarantee consistency in the composition and characteristics of the resulting copper oxide nanoparticles. To improve the dependability and repeatability of mushroom-based synthesis methods for copper oxide nanoparticles, more study and development is required.

2.8 Objectives

- Synthesis of Mushroom-based copper oxide nanoparticles.
- Characterization of copper oxide nanoparticles.
- Evaluation of biological efficacy of copper oxide nanoparticles (CuO) from mushroom species selected i.e shiitake and oyster mushrooms.
- Comparison study between the two selected mushrooms.

CHAPTER 3

Materials and Methods

3. Materials and Methods

3.1 Procurement of Material

Mushroom species Pleurotus *ostreatus* and *Lentinula edodes* commonly known as oyster mushroom and shiitake mushroom are used in the study which was stored in 4^{o}C. The experiment on the mushroom species started by collection of dried mushrooms 100 grams from the **Vikas Mushroom Farm Solan (H.P.)** India. After the sample procurement the rest experimentation is carried out in Jaypee University of Information Technology.

3.2 Extract Preparation

3.2.1 Material and Apparatus Required

Dried Mushrooms (Oyster and Shiitake), beaker, measuring cylinder, flask, weighing machine, 100 ml distilled water, whatman paper No 4, cotton plug.

3.2.2 Procedure

- Dried mushroom was diced and sliced to powdered form using mortar and pestle. (Fig 3.1) (Fig 3.6)
- 5g of powdered mushroom was dissolved with 100 ml of distilled water. (Fig 3.2.2) (Fig 3.7)
- The solution was heated and stirred for 30 minutes in a magnetic stirrer. (Fig 3.2.3) (Fig 3.8)
- The solution was then kept at room temperature for 10 mins.
- Following that, the fluid was filtered via whatman filter paper. (Fig 3.4) (Fig 3.9)
- After the filtration process the extract is prepared which will be then kept in 4 degree celsius for further experimentation. (Fig 3.5)(Fig 3.10)

Note: Both the mushrooms i.e. shiitake and oyster underwent the same process for the extract preparation.

Fig 3.1

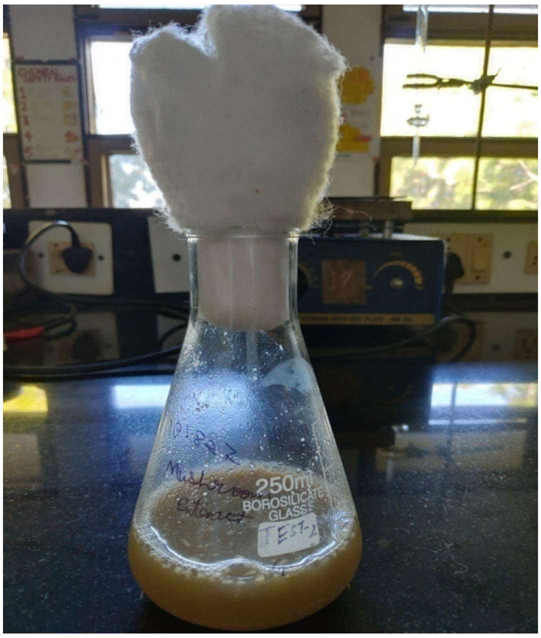

Fig 3.2

Fig 3.3

Fig 3.4

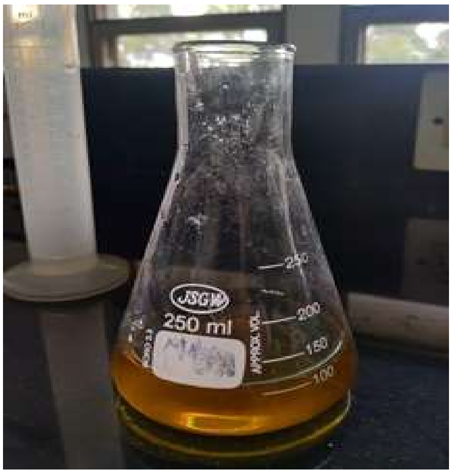

Fig 3.5

Fig 3.6

Fig 3.7

Fig 3.8

Fig.3.9

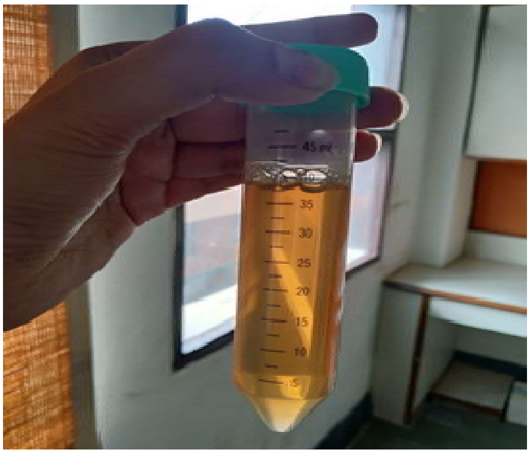

Fig 3.10

Fig.3 Setup of Mushroom Extraction Preparations

3.3 Biosynthesis of CuNPs

- The green synthesis of CuNPs began with the addition of 90 ml of 0.1 M cupric sulphate solution to the reaction mixture. ($CuSO_4.5H_2O$). (Fig 4.1) (Fig 4.3)
- Then the mixture was added to 10 ml of aqueous extract of pleurotus mushroom.
- After this process the solution was kept on a rotatory shaker for 24 hr. (Fig 4.2)
- Note: Same process was done for the other species of mushroom . i.e. Shiitake mushroom.(Fig 4.2)

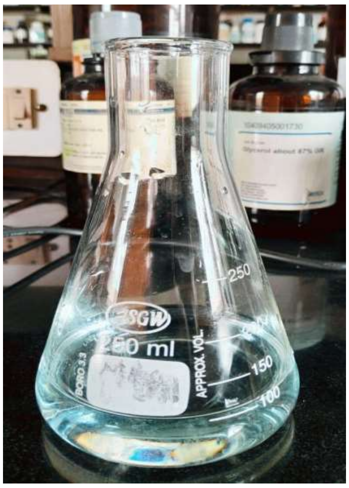

Fig 4.1

Cupric sulphate solution

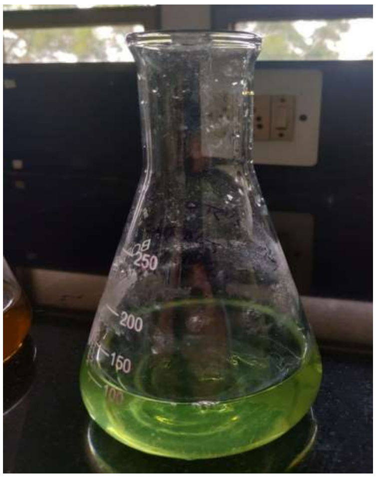

Fig 4.2

Green color of Cu_2O NPs in **oyster extract**

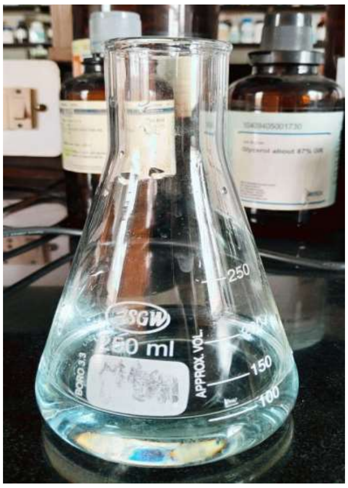

Fig 4.3

Cupric sulphate solution

Fig 4.4

Green color of Cu_2O NPs in Shiitake extract

Fig.4 Setup for biosynthesis of CuNPs

3.4 Antimicrobial Assay

MHA Preparation

- For preparation of 250ml MHA Agar 5.5 gm of available MHA broth was taken.
- In a conical flask add 50 ml of distilled water.
- Add 1.5% to 50 ml volume i.e 3.75g of Agar Agar.
- Again add 200 ml of distilled water to make up the volume 250 ml.
- Autoclave the conical flask with the MHA Agar for 40 min.
- After 40 min take out the conical flask from the autoclave keep it in UV for 20 min.

Pouring

- Take the sterilised plates and put them in U.V. along with the autoclaved MHA Agar.
- After the 20 min turn off the U.V. .
- Pour the plates with the agar formed slowly before the Agar gets solidified.
- Remove bubbles from the molten agar surface by swiftly (and cautiously) moving a Bunsen burner flame over the agar.
- Incubate the plates for 24 hrs.

Streaking

- Streak the whole surface of the Mueller Hinton Agar plate with the swab.
- Repeat the streaking method two more times, turning the plate 60 degrees each time to achieve even inoculum dispersion.
- To allow the surface inoculum to be adsorbed, leave the plate for 5 minutes.

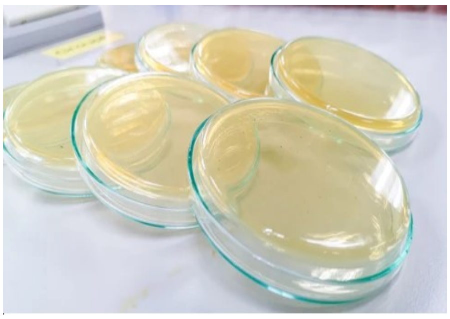

Fig.5 Streaked plates with MHA

3.5 Antioxidant Assay

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method for measuring antioxidants is quick, simple, and cost-effective, and it uses free radicals to evaluate a substance's capacity to either produce hydrogen or scavenge free radicals.

DPPH Preparation and Method

- Prepare 0.3 mM DPPH solution i.e. 4.3mg of DPPH
- Dissolve the amount in 80% ethanol.
- Wrap the conical flask with aluminium foil and keep it in a dark place overnight.(Fig 6.1)
- For test sample prepare series of solution of 1 ml, 2ml, 3ml, 4ml of the nanoparticles soluble extract in 2.5 ml of Dpph solution (Fig 6.2)
- Mix well and In a dark room, incubate at room temperature for 30 minutes.
- Take OD at 517 nm.

Fig 6.1

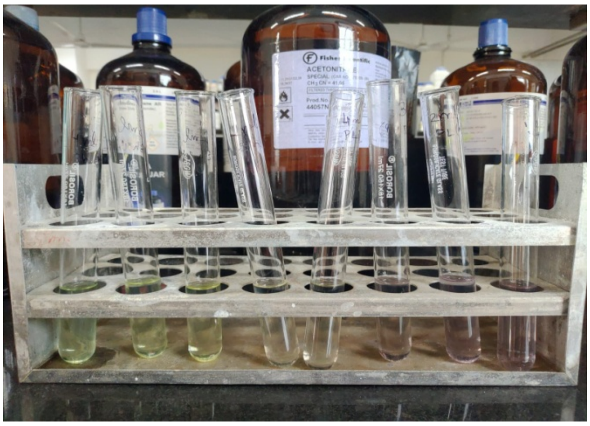

Fig 6.2

Fig.6 Setup for DPPH Assay

3.6 UV-Vis Spectroscopy

UV-Vis spectroscopy, commonly referred to as ultraviolet-visible spectroscopy, is a method for analyzing light absorption or transmission in the ultraviolet (UV) and visible (Vis) parts of the electromagnetic spectrum. It reveals important details about the electronic structure and characteristics of molecules, ions, and materials. UV-Vis spectroscopy works on the basis of transmitting a beam of UV or visible light through a sample and measuring the amount of light absorbed by the sample at different wavelengths.

Prepare the dilutions 2ml, 3ml, 1ml (Fig 7) of nanoparticles extract for both the mushrooms by taking the total volume of 10ml dissolved in distilled water. A UV-vis spectrophotometer will be used to measure the UV-vis absorption between 200 and 700 nm at 25 °C.

Fig 7

Fig. 7 Setup for UV-Vis spectrophotometric analysis

CHAPTER 4

Results and Discussion

4.1 Nanoparticle extraction

The extract obtained will be treated with the certain Metal Oxide and will be centrifuged at 12000 rpm and then left at room temperature for 3 days to notice the colour change. The resultant colour change will result in the presence of the nanoparticles in the extract used of the mushroom species. As the solution turns green, it results in the presence of copper oxide nanoparticles.

Pleurotus nanoparticles extract | Shiitake nanoparticles extract

Fig. 8 Pleurotus and Shiitake nanoparticles extracts

4.2 Qualitative tests

4.2.1 Antimicrobial Activity (MHA Test)

The antimicrobial activity of the extract will be checked by preparing the stock cultures of Salmonella *typhi*, Staphylococcus *aureus,* Proteus *mirabilis,* and Providencia *alcalifaciens.* This will further help in the accurate analysis while doing the medical efficacy tests.

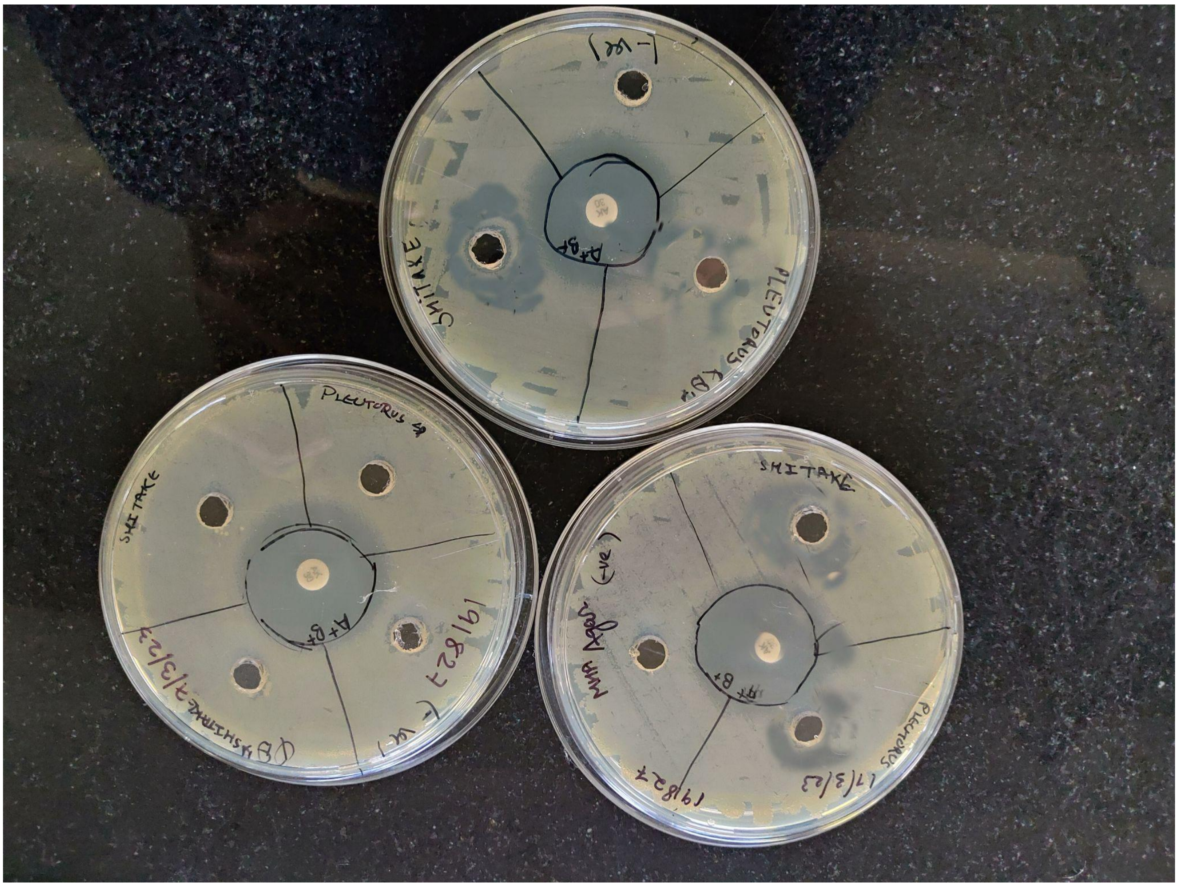

Fig. 9 MHA test results

4.2.2 Antioxidant Test using DPPH

As it can be observed the solution turns yellow and the OD taken at 517 nm gives following results:

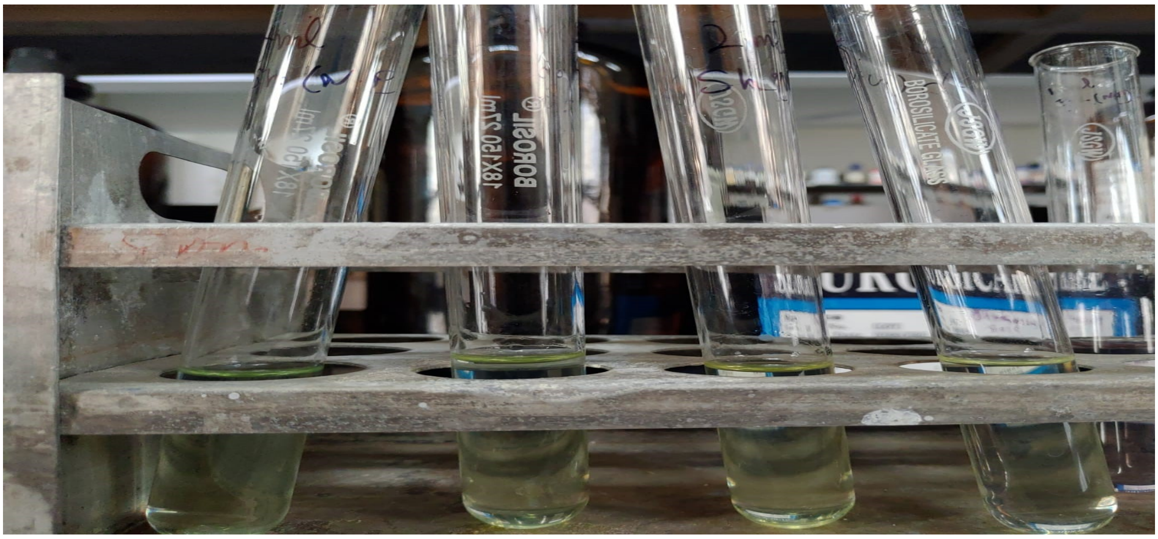

Fig. 10 Shiitake Antioxidant test results

Fig. 11 Oyster Antioxidant test results

Table 3 Pleurotus O.D. readings

S.no	**Sample Name**	**OD- 517 nm (Abs)**
1.	Pl_1ml	0.184
2.	Pl_2ml	0.161
3.	Pl_3ml	0.140
4.	Pl_4ml	0.123

Scavenging effect (%) = 1 - (Absorbance Sample)/(Absorbance control) $\times$ 100

Scavenging effect (%) = 1- (0.152/0.214) $\times$ 100 =1-0.710 *100 = 28.9%

Table 4 Shiitake O.D. readings

S.no	**Sample Name**	**OD- 517 nm (Abs)**
1.	Shi_1ml	0.107
2.	Shi_2ml	0.123
3.	Shi_3ml	0.137
4.	Shi_4ml	0.139

Scavenging effect (%) = 1- (0.509/0.214) $\times$ 100 =1-0.236 *100 = 76.4%

4.3 Characterization using UV-vis Spectroscopy of Copper oxide Nanoparticles

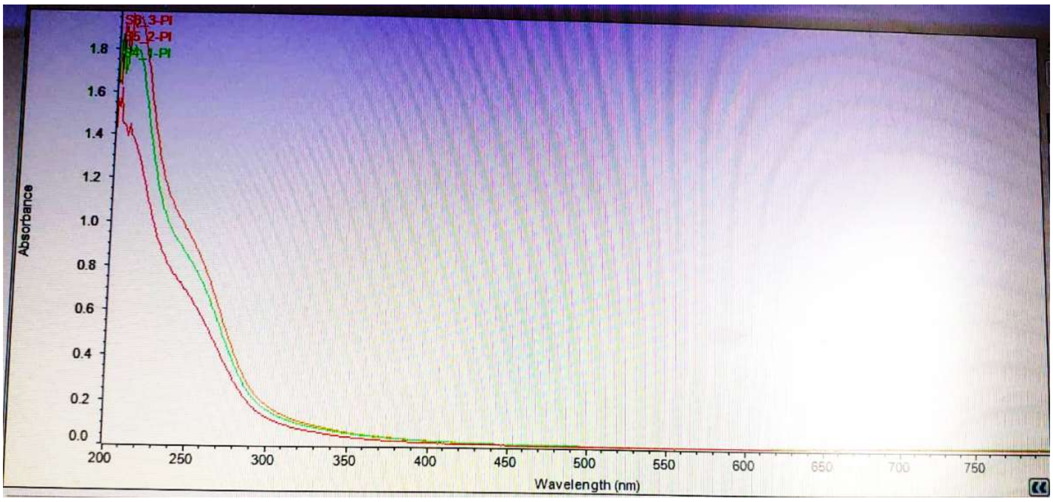

Fig. 12 UV-vis Spectroscopy for Pleutorus nanoparticles diluted was peak at 220 nm

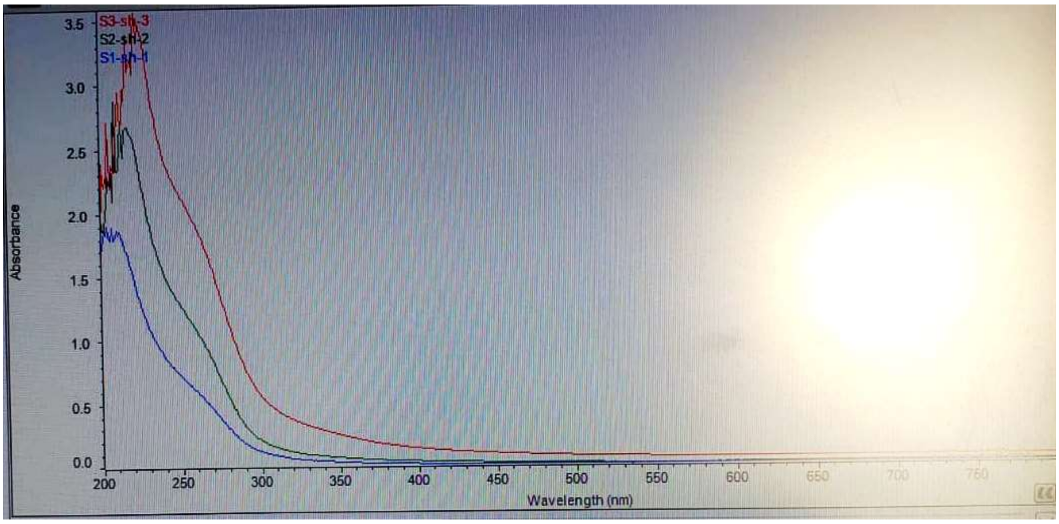

Fig. 13 UV-vis Spectroscopy for shiitake nanoparticles diluted was peak at 230 nm.

These both results are in the close context with the previous research [7]

CHAPTER 5

Conclusions and Future Scope

5.1 Conclusion

- Mushrooms have direct effects on cancer cells, such as apoptosis and cytotoxicity, in accordance with the literature study and other discoveries, making them suitable candidates for consideration as anti-cancer medication development.The employment of various biological organisms instead of hazardous chemicals to reduce and stabilize nanoparticles has aroused the curiosity of many in the field of nanotechnology. Biologically active compounds from fungus and yeast are effective scaffolding for this aim among the different potential bio resources. Since there are numerous species of yeast and fungi and because they are efficient extracellular enzyme secretors, it is simple to grow and maintain them in the laboratory. They can create metal nanoparticles and nanostructures either intracellularly or extracellularly by using reducing enzymes.

- Fungi have the ability to create metal nanoparticles and nanostructures through the process of biomimetic mineralization and the usage of reducing enzymes either intracellularly or extracellularly.

- To sum up, copper oxide nanoparticles have distinct qualities that make them desirable for a variety of applications. They do, however, have restrictions that must be taken into account and dealt with in order for them to be used safely and effectively. Although copper oxide nanoparticles' behaviour can be tailored thanks to its size- and shape-dependent characteristics, scalability and repeatability issues can arise. Their performance and dispersion may be impacted by stability and agglomeration problems. A careful assessment and management of any worries regarding their possible toxicity and interaction with biological systems is also necessary. It is also important to examine the costs and environmental effects of using copper oxide nanoparticles.

- In order to overcome these obstacles and maximize the advantages of copper oxide nanoparticles in a variety of applications, research and development efforts are still being made.

- According to studies, nanoparticles made from mushrooms contain antioxidant, antibacterial, anti-inflammatory, and anticancer effects. Their potential for medicine delivery, biosensing, bioimaging, and environmental cleanup has been studied. These nanoparticles are well suited for biological applications due to their biocompatibility and low toxicity.

- A further benefit of mushroom-based nanoparticle synthesis is that it is environmentally friendly and sustainable. Utilising natural resources and ecologically friendly synthesis techniques lessens the need for chemical agents and the negative effects of traditional nanoparticle synthesis on the environment.

- Despite the encouraging results, there are still difficulties to be solved. Further study is needed in the areas of standardizing synthesis procedures, enhancing the characteristics of nanoparticles, and comprehending how they interact with biological systems. To make their practical application on a broader scale possible, it is also necessary to address scale-up production and cost-effectiveness.

- Overall, there is a lot of promise for creative and long-lasting solutions in many disciplines thanks to research on nanoparticles made from mushrooms. Continued research and development in this field will probably reveal new possibilities and progress in nanotechnology and its applications.

5.2 Future Scope

We may anticipate more fungal research being taken into account in the future. due to the fact that many species are still undiscovered.

Improved synthesis methods: Researchers will likely concentrate on improving the processes used to create copper oxide nanoparticles based on mushrooms. To gain exact control over the size, shape, and composition of the nanoparticles, this involves optimising variables including temperature, pH, and concentration. To increase effectiveness and scalability, cutting-edge methods like green chemistry and biotechnology approaches may be investigated.

- Customised characteristics: Copper oxide nanoparticles generated from mushrooms will be manufactured with tailored qualities to fit certain application needs. The properties of the nanoparticles can be fine-tuned for improved performance in areas including catalysis, energy storage, sensors, and biological applications. This is done by altering the composition, surface functionalization, or doping of the nanoparticles.
- Applications in biomedicine: Copper oxide nanoparticles derived from mushrooms may be used more frequently in this sector. They can be investigated as medicines, imaging agents, or targeted drug delivery systems because of their biocompatibility, low toxicity, and special qualities. Studying their interactions with biological systems, comprehending their modes of action, and investigating their potential in fields like cancer treatment, antibiotic therapy, and regenerative medicine will probably be the main topics of research in the future.
- Environmental remediation: Copper oxide nanoparticles derived from mushrooms may be very important. They are excellent candidates for use in soil remediation, air filtration, and water purification due to their intrinsic qualities like antibacterial and catalytic activity. The efficiency of these techniques in eliminating pollutants, heavy metals, and organic contaminants from various environmental matrices will be the focus of future research.

- Sustainable and scalable production: Production methods that are scalable and sustainable will be developed with a particular focus on copper oxide nanoparticles made from mushrooms. In order to accomplish large-scale manufacturing with the least possible ecological impact, this includes researching methods like fermentation, bioreactors, and mycelium-mediated synthesis. Practical applications in numerous industries will be made possible by affordable and environmentally friendly synthesis techniques.

Overall, copper oxide nanoparticles made from mushrooms have a bright future ahead of them, with improvements anticipated in synthesis processes, customised characteristics, biomedical uses, environmental remediation, and sustainable manufacturing approaches. The innovation and application of these nanoparticles in various domains will be driven by ongoing research and collaboration between scientists, engineers, and industry stakeholders, having a positive impact on technology, healthcare, and environmental sustainability.

References

[1] He, Y., Zhang, L., & Wang, H. (2019). The biological activities of the antitumor drug Grifola frondosa polysaccharide. *Progress in Molecular Biology and Translational Science.* doi:10.1016/bs.pmbts.2019.02.010

[2] Bhat, Ravishankar, et al. "Photo-Irradiated Biosynthesis of Silver Nanoparticles Using Edible Mushroom Pleurotus florida and Their Antibacterial Activity Studies." vol. Volume 2011, 2011, p. 7, doi:10.1155/2011/650979.

[3] Boobalan T., et al. "Mushroom-Derived Carbon Dots for Toxic Metal Ion Detection and as Antibacterial and Anticancer Agents." 2020, pubs.acs.org.

[4] Vinci, John c., et al. "Spectroscopic Characteristics of Carbon Dots (C-Dots) Derived from Carbon Fibers and Conversion to Sulfur-Bridged C-Dots Nanosheets." *Society for Applied Spectroscopy*, vol. Volume 69, Number 9, no. 2015, 2015, p. 9. *https://core.ac.uk/*, https://core.ac.uk/download/pdf/216222229.pdf.

[5] Pacquiao, Melvin R., et al. "Highly fluorescent carbon dots from enokitake mushroom as multi-faceted optical nanomaterials for Cr6+ and VOC detection and imaging applications." *Applied Surface Science*, vol. 453, 30 September 2018, pp. 192-203. *science direct*, https://doi.org/10.1016/j.apsusc.2018.04.199.

[6] Wu, Jian Yong, et al. "10.3390/foods10010095." 2021, frondosa (Maitake) https://doi.org/ 10.3390/foods10010095.

[7] Azam, Nayab, et al. "Carbon Quantum Dots for Biomedical Applications: Review and Analysis." 2021

[8] Rajeshwari Sivaraj, Pattanathu K.S.M. Rahman, P. Rajiv, S. Narendhran, R. Venckatesh, 2014, Biosynthesis and characterization of Acalypha indica mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy