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# Ecofriendly Synthesis of Silver Nanoparticles by Using *Morchella esculenta*

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## ABSTRACT

In the present study, biological method used for the synthesis of stable and well-characterized AgNPs was discussed by using *Morchella esculenta*. Size and shape are controlled by changing conditions such as substrate concentration, temperature, mixing speed, and exposure time. Specimens of dried mushrooms were used for AgNP production. AgNPs are synthesized using a green line of nanoparticle synthesis. Silver nitrate has been used as a source of Silver and *Morchella esculenta* mold has been used as a fuel for bio-reduction and synthetic nanoparticles. During the synthesis of AgNPs, the first confirmation of the synthesis of nanoparticles was the change in color reaction from yellow to dark red. The formation of Eco-friendly nanoparticles has been bringing a green revolution in treating wastewater and reducing soil contamination. The low cost and abundant resources of raw materials make it possible to synthesize silver nanoparticles green, which is an important factor in reducing pollution. The research on green synthesis of silver nanoparticles and method to prepare it for further use in pollution control is discussed in this article.

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## 1. INTRODUCTION

*"Morchella* is a member of the Morchellaceae family in the Pezizales order of the Pezizomycetes class under the Ascomycota division in the fungi kingdom, which is extensively recognized as a valuable resource for nutritional and medicinal purposes" [1].

"*Morchella esculenta* is an excellently edible mushroom growing in temperate regions. In India, this mushroom is found growing in the forests of Jammu and Kashmir and Himachal Pradesh. This mushroom is locally known as 'Guchhi'. It has been reported to be used in health care and medicinal purposes among traditional hill societies" [2].

*"Morchella esculenta* (L.) Pers. is commonly found in thick coniferous forests, loamy soil rich in humus, naturally grown in hilly altitudes with cold environments. It is found at altitudes of 2500-3500 m in forest habitats" [3].

### 1.1 Nutritional Content

"Edible mushrooms have now been suggested as promising sources of biological functional ingredients and are the subject of the most recent nutrition research, in addition to expanded uses in innovative functional foods" [4]. "Morels are rich in proteins, fibers, necessary vitamins, minerals, and PUFAs. Recently, a trend toward increasing plant-based foods and reducing meat consumption is growing, and alternative meat is becoming popular" [5,6].

*"Morchella esculenta* is a famous mushroom all around the globe due to its nutritional and nutraceutical assessment. It contains various crucial nutrients such as proteins (32.7%), vitamins (Vit B, C, and D), carbohydrates (38%), dietary fibers (17.6%), minerals, trace rudiments (Copper 21 mg/kg, Manganese 22 mg/kg, Cobalt 0.12 mg/kg, Zinc 153 mg/kg, Iron 304 mg/kg, Calcium 2340 mg/kg and Magnesium 1272 mg/kg, dry weight basis)" (*Genccelep et al., 2009*) [7].

"Interestingly, the crude protein content of fermented mycelia of *M. esculenta* was as high as 39.4%, which is much higher than the average protein level of its fruiting body" [8]. "Three major soluble monosaccharides were detected in morels, with glucose (42.3 μg/g) being the highest, next to fructose and galactose" [9]. "The amino acids identified in morels mainly include alanine, I-5-oxo proline, and ornithine. The sweetness mainly comes from soluble carbohydrates like mannitol, glucose, and some free amino acids including the most abundant L-alanine, as well as L-serine and L-threonine" [10].

### 1.2 Bioactive Compounds

The fruiting bodies of *M. esculenta* possess various active constituents, including tocopherols, carotenoids, organic acids, phenolic compounds, and polysaccharides. Polysaccharides are formed by long chains of monosaccharides linked by glycosidic bonds. The health benefits of polysaccharides have been appreciated for a long time. These molecules are the primary bioactive constituents of *M. esculenta* and have been highlighted for their remarkable pharmaceutical activities, including antioxidant [11], anti-inflammatory [12], immunomodulatory [13], and anti-atherogenic activities [14]. "There are various bioactive compounds found in the cultured broth, mycelium, and fruiting bodies such as volatile oils, flavonoids, alkaloids, ascorbic and organic acids, fats, polysaccharides, tocopherols, glycosides, minerals, proteins, carotenoids, terpenoids, lectins, enzymes, phenolics, and folates" [15].

"The flavonoid composition in morels also contributes to its beneficial effects on gut health preservation" [16]. "Fat is a small part of mushrooms that may play a role in regulating lipid levels. In fact, polyunsaturated fatty acids can lower serum cholesterol" [17]. "The fatty acid profile is composed of oleic acid, palmitoleic acid, linoleic acid, α-linoleic acid, palmitic acid, stearic acid, and myristic acid" [18]. "Carbohydrates play an important role in providing energy for the formation and growth of morel fruiting bodies" [19,20]. "Three major soluble monosaccharides were detected in morels, with glucose (42.3 μg/g) being the highest, next to fructose and galactose" [19]. "Several organic acids including succinic acid, malic acid, citric acid, and fumaric acid were also found in morels" [19].

"The average yield of crude polysaccharide-protein complexes in *M. esculenta* fruiting bodies was around 3%, compared to 1.3% of deproteinized polysaccharides" [21]. "The total tocopherol content in *M.esculenta* fruiting bodies ranged from 14.8 to 121.3 μg/100 g DW" [22]. "The content of ergosterol peroxide in *M. esculenta* was 13.4 mg/100 g DW, which was higher than those of *Laetiporus sulfureus* and *Boletus badius"* [23].

## 2. MATERIALS AND METHODS

### 2.1 The Main Objectives of the Present Study

a) To synthesize Silver nanoparticles using Fungi as a fuel for its bioreduction and capping.
b) To characterize synthesized nanoparticles using modern analytical techniques i.e., XRD and FTIR.

### 2.2 Processing of Plant Material

The study area of the present work was Thannamndi, District Rajouri which is the Northern part of Jammu and Kashmir with sub-Mediterranean type of climate. An extensive field survey was carried out in different areas and a good number of edible and non-edible mushroom species were collected. *Morchellas* were collected and washed thoroughly under running tap water to remove all adhering soil. The washed *Morchellas* were then shade dry for more than 15 days. The dried leaf materials were then cut into small pieces and used as working material for AgNP synthesis.

### 2.3 Preparation of Plant Extract

The Mushroom fragments were suspended in distilled water and boiled for a few minutes. Allow it to stand at room temperature for a few hours, then heat the solution having mushroom pieces at 40 to $45^0$c for a few minutes; allow it to stand again for 30 minutes, and then finally heat the mixture at $50^0$c for 5 minutes. The *Morchella esculenta* extract was filtered through Whitman's filter paper. Then take a pure sample of extract and allow it to stand at room temperature for 36 hours.

### 2.4 Preparation of Silver Nitrate

Fresh silver nitrate solution was prepared for the synthesis of the AgNPs, by dissolving 1 mM solution in 100 ml of distilled water. The solution prepared was filtered with the help of Whatman paper No1.

### 2.5 Biosynthesis of Nanoparticles

Fresh *Morchella* extract and silver nitrate extract was mixed in different ratios to select the best for the synthesis of AgNPs. Finial 1:9 ratio was selected after the initial exercise. The selected ratio was used for further work.

The extracts of both Fungi and Silver were mixed in the above-said ratio, and the reaction mixture was kept at room temperature for 36 hours. The solution color changes from light yellow to dark brown. The supernatant was discarded and the pellet obtained was redispersed in deionized water. The centrifugation process was repeated two to three times to wash off any absorbed substances on the surface of the silver nanoparticle. After that solution color changed from light yellow to dark brown color. These changes in the color of the solution indicate the presence of nanoparticles. After that nanoparticles are under the con troll condition. Nanoparticles are ready for further process.

### 2.6 Characterization of Nanoparticle

The Preliminary indication of silver particle production by using mushroom extracts is confirmed by the color change from light yellow colors to dark brown colors.

### 2.7 Fourier Transmission Infrared Spectroscopy

FTIR stands for Fourier transform infrared, the preferred method of infrared spectroscopy. When IR radiation is passed through a sample, some radiation is absorbed by the sample, and some passes through (is transmitted). The resulting signal at the detector is a spectrum representing a molecular 'fingerprint' of the sample. The usefulness of infrared spectroscopy arises because different chemical structures (molecules) produce different spectral fingerprints.

### 2.8 XRD Analysis

XRD stands for X-Ray diffraction. It is a process often used in these fields. It is actually a technique for examining the chemical make- up of unidentified solids. Let us say for instance, they found something that cannot be classified right away; the XRD process will then be used to check the make- up of the said item. For XRD to work there must be a sample of the materials first. The powdered sample will then be placed in a holder. It will be illuminated with X-rays of a given wavelength. The intensity of the reflected radiation will then be recorded with the use of a goniometer. From there, the data collected will be analyzed using different computing methods. The intensity will be measured using various D spacing. The result will also be analyzed to find immediately the matches of the item that was given the XRD for analysis. You also have to realize that the greater the tolerance, the more results will be recorded.

X-ray diffraction techniques are used for the identification of crystalline phases of various materials and the quantitative phase analysis subsequent to the identification. X-ray diffraction techniques are superior in elucidating the three-dimensional atomic structure of crystalline solids. The properties and functions of materials largely depend on the crystal structures. X-ray diffraction techniques have, therefore, been widely used as an indispensable means in materials research, development, and production.

"The Bragg equation, $n\lambda = 2d\sin\theta$ is one of the keystones in understanding X-ray diffraction. In this equation, n is an integer, λ is the characteristic wavelength of the X-rays impinging on the crystallized sample, d is the interplanar spacing between rows of atoms, and θ is the angle of the X-ray beam with respect to these planes. When this equation is satisfied, X-rays scattered by the atoms in the plane of a periodic structure are in phase and diffraction occurs in the direction defined by the angle θ. In the simplest instance, an X-ray diffraction experiment consists of a set of diffracted intensities and the angles at which they are observed. This diffraction pattern can be thought of as a chemical fingerprint, and chemical identification can be performed by comparing this diffraction pattern to a database of known patterns" [24].

Here is a typical powder X-ray diffraction or XRD pattern, in this case, of a cement sample.

## 3. RESULTS AND DISCUSSION

### 3.1 Synthesis and Characterization of AGNPS

In the present work, AgNPs were synthesized using the green route of nanoparticle synthesis. Silver nitrate was used as a silver source and *Morchella esculenta* a fungus was used as fuel for the bio-reduction and capping of nanoparticles.

Results obtained from a series of laboratory studies that attempted to synthesize and characterize green synthesized silver nanoparticles showed the successful synthesis of nanoparticles. During experiment, the extract of whole fungi was reacted with the extract of silver nitrate and allowed to remain at room temperature for 36 hrs. The precipitates formed in the reaction were then collected by centrifuging the reaction mixture. The collected pellets were washed with the help of distilled water thrice followed by drying the pellets in an oven for 10 hours at $60^0$ C. Finally synthesized nanoparticles were homogenized using mortar and pestle and then collected in air-tight bottles for further use.

### 3.2 Preliminary Observation

During the synthesis of AgNPs, the preliminary confirmation for the synthesis of nanoparticles was the color change of the reaction mixture from yellow to dark red-brown. Further, confirmation of ZnO synthesis was carried out by using the UV-Vis technique and obtained results showed peaks in the region at 405 to 430 nm, which could be attributed to the AgNPs nanoparticles. The synthesized nanoparticle was then subjected to conformational details about the shape, size, morphology, and functional group attached if any which may be responsible for the reduction and capping of synthesized nanoparticles by, XRD and FTIR.

## 4. CONCLUSION

Habitually *Morchella esculenta* is used as medicine in poles apart countries like Japan, China, and Malaysia for the healing of several diseases. It has a variety of pharmacological properties like anti-inflammatory, antioxidant, immunostimulant, antitumor, anticancer, and antimicrobial properties due to the attendance of poles apart bioactive compounds. Therefore, in the present study metal based nano synthesis was attempted. The collected *Morchella esculenta* were screened for the potent Silver Nanoparticle. The objective set for the present study was successfully achieved during this research. So, it may recommend here to use the leaf extract-mediated nanoparticles for antimicrobial activity, moreover, nanoparticles may be also used for some drug-targeting studies.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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