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# Exploring the Classification, Properties, and Biological Green Synthesis of Nanoparticles: A Comprehensive Review

Nandan Singh Karki<sup>1</sup>, Tanuja Bisht<sup>2</sup>, Manoj Pal<sup>3</sup>, Bipin Chandra Pathak<sup>4</sup> and Manisha Bisht<sup>1</sup>\* <sup>1</sup>Department of Chemistry, L.S.M. Govt. P.G. College, Pithoragarh (Uttarakhand), India. <sup>2</sup>Department of Chemistry, Indira Priyadarshini Govt Girls College, Haldwani (Uttarakhand), India. <sup>3</sup>Department of Microbiology, Graphic Era (Deemed to be University), Dehradun (Uttarakhand), India. <sup>4</sup>Department of Zoology, L.S.M. Govt. P.G. College, Pithoragarh (Uttarakhand), India.

(Corresponding author: Manisha Bisht\*) (Received: 25 March 2023; Revised: 18 April 2023; Accepted: 26 April 2023; Published: 20 May 2023) (Published by Research Trend)

ABSTRACT: Green synthesis of nanoparticles using microorganism such as bacteria, fungi, and yeast has gained significant attention in recent years due to its potential as an eco-friendly and cost-effective alternative to traditional chemical synthesis methods. The synthesis of nanoparticles using plant extracts and microorganisms has been extensively explored as plants are a potential source of many reducing and capping agents. It is evident that microorganisms have enormous potential specially to synthesize metallic and metal oxide nanoparticles of various size and shapes. Therefore, the possibilities of different biological sources such as bacteria, fungi, and yeast in green synthesis were discussed and highlighted. However, some challenges are still exist such as the optimization of microbial growth, and the scalability of the synthesis process. Recently, many progressive efforts are being used to tackle these problems as discussed in this review article. This review article mainly emphasized on the classification, properties, and microorganism based green synthesis approaches of nanoparticles.

Keywords: Green synthesis, nanotechnology, nanoparticles, and nanoparticles applications.

## **INTRODUCTION**

Nanoparticles are the smaller size particles having the diameter in nanoscale range, usually from 1nm to 100 nm and exhibit distinct physical and chemical properties in comparison with the majority of the same chemical composition (Roduner, 2006a; Varghese and Sakho 2019). The term "nanometer" was coined in 1914 by Richard Adolf Zsigmondy. "Nanotechnology" was first used by Richard Feynman in his speech during the American Physical Society annual meeting in 1959, which is considered the first academic discussion on nanotechnology (Santamaria, 2012). In this meeting, he delivered a speech titled "There's Plenty of Room at the Bottom", later a concept was proposed. "Why can't we write the entire 24 volumes of encyclopaedia Britannica on the head of a pin". The thought behind it was to develop smaller machines down to molecular level (Bayda et al., 2019). Nanoparticles have more surface area than regular metals. This makes them act differently because of their size, the way they interact with surfaces, and the way their atoms behave. These unique properties aren't found in regular metals. Nanoparticles have been employed in in many chemical reactions and widely popularized being their ability to enhance the rate of reaction by adsorbing the reactant molecules on their own surface that eventually lead decreasing the activation energy barrier (Singh et al., 2019).

Roduner (2006a); Varghese and Sakho (2019) have noted that nanoparticles, with diameters typically

ranging from 1nm to 100 nm, are the smaller size particles that demonstrate distinct physical and chemical properties compared to the majority of particles with the same chemical composition. In 1914, Zsigmondy coined the Richard Adolf term "nanometer". Santamaria (2012) states that Richard Feynman first used the term "nanotechnology" in his speech at the American Physical Society annual meeting in 1959, marking the initial academic discussion on the subject. During this meeting, Bayda et al. (2019) recall that Feynman delivered a speech titled "There's Plenty of Room at the Bottom" and proposed the concept of developing smaller machines at the molecular level. The idea behind it was to write the entire 24 volumes of the encyclopaedia Britannica on the head of a pin. Regular metals lack the unique properties exhibited by nanoparticles, as highlighted by Singh et al. (2019). They note that nanoparticles possess a larger surface area, resulting in distinct behaviors in terms of interactions with surfaces and atomic behaviour. Due to their ability to adsorb reactant molecules on their surface, nanoparticles have found extensive applications in various chemical reactions, leading to enhanced reaction rates and a reduction in the activation energy barrier.

Pearce (2012) emphasizes that nanotechnology, a rapidly expanding field, involves interdisciplinary research and development across disciplines such as physics, chemistry, biology, and medicine. The primary objective of this field, as stated by Pearce, is the design,

manufacturing, and study of materials with diameters smaller than 100 nm. Furthermore, in Fig. 1, various

common areas of application within the nanotechnology field were illustrated.

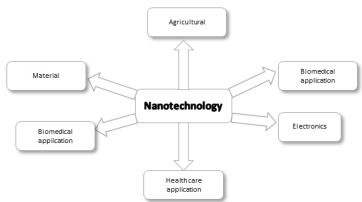


Fig. 1. Application of nanotechnology in different fields.

In their study, Iravani *et al.* (2014) provided a categorization of different methods used for synthesizing nanoparticles. These methods encompass both chemical and physical approaches, as well as environmentally friendly techniques commonly known as green methods. Green methods have gained recognition for their eco-friendly nature, their ability to avoid the need for expensive equipment, their ease of preparation, and their cost-effectiveness.

Within the realm of green methods, various biological sources are utilized, including bacteria, fungi, and different parts of plants such as roots, fruits, flowers, seeds, stems, and pollen grains, to produce nanoparticles. These methods are characterized by their environmentally safe nature, short production times, and lower costs compared to other techniques. Specifically, the synthesis of metal nanoparticles from plant extracts is considered a relatively simpler process compared to using fungal or bacterial cultures, which necessitate sterilized conditions and specialized skills for preservation. Nevertheless, after so much work on the green synthesis of nanoparticles, many research gaps exist, such as;

• The absence of standardized protocols for synthesizing nanoparticles using plants and microorganisms. The methodologies employed in various studies often differ significantly, making it challenging to compare results and establish reliable trends.

• Although significant progress has been made in synthesizing nanoparticles using biological entities, the precise mechanisms by which plants and microorganisms mediate the synthesis are not fully understood. Investigating the exact biochemical and genetic pathways involved in nanoparticle formation could provide valuable insights and lead to more efficient and controlled synthesis techniques.

• While green synthesis methods are generally considered eco-friendly, a comprehensive assessment of the toxicity and biocompatibility of the synthesized nanoparticles is lacking. It is essential to evaluate the impact of these nanoparticles on human health and the environment to ensure their safe application.

• Many studies have successfully demonstrated the green synthesis of nanoparticles at the laboratory scale, but translating these methods to industrial-scale

production remains a challenge. Research focusing on scaling up the production process while maintaining the desired properties of nanoparticles is needed.

Addressing these research gaps would not only advance our understanding of the biological green synthesis of nanoparticles but also pave the way for their wider adoption in sustainable nanotechnology and diverse industrial sectors. This review primarily focuses on the classification, properties, and different methods of synthesizing nanoparticles, including physical, chemical, and biological approaches for future applications.

The focus of this review is primarily on the classification, properties, and diverse methods of synthesizing nanoparticles. This encompasses physical, chemical, and biological approaches, thus providing a comprehensive overview of the field.

## CLASSIFICATION OF NANOPARTICLES

Ijaz *et al.* (2020) highlight that nanoparticles can be classified in various ways, taking into account their morphology, dimensionality, agglomeration, and the materials utilized for their synthesis. Additionally, based on whether a single material or multiple materials are employed in their synthesis, nanoparticles can be categorized as either simple or composite, including core/shell particles. Simple nanoparticles are composed of a single material, whereas composite and core/shell particles are comprised of two or more materials. Specifically, core/shell nanoparticles typically consist of an inner material (core) surrounded by an outer layer material (shell). Kumar and Kumbhat (2016) further note that these nanoparticles can fall into either the organic or inorganic category.

Jeevanandam *et al.* (2018) explain that carbon-based nanomaterials, synthesized solely using carbon precursors, exhibit diverse forms, including spheres, hollow tubes, carbon nanofibers, fullerenes, and graphene. Inorganic-based nanomaterials, as outlined by Jeevanandam *et al.* (2018) are constructed from metals and metal oxides such as silver, gold, iron, titanium dioxide, zinc oxide, manganese dioxide, and vanadium oxide. Moving on to organic-based nanomaterials, Dresselhaus and Terrones (2013) clarify that these are formed through non-covalent interactions of organic materials other than carbon, such as

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liposomes and micelles. Additionally, composite-based nanomaterials, as discussed by Gokarna *et al.* (2014); Li *et al.* (2012), refer to multiphase nanoparticles or nanostructured materials capable of binding with other nanoparticles or bulk materials.

Gleiter (2000) initially proposed a classification of nanomaterials based on their crystalline form and chemical composition; however, this approach faced limited acceptance as it did not account for the dimensionality of nanomaterials. Tiwari *et al.* (2012); Wu *et al.* (2020) highlight the shortcomings of this classification. Presently, nanoparticles are commonly categorized based on their dimensionality into four types, as outlined by Jeevanandam *et al.* (2018).

According to Jeevanandam et al. (2018), zerodimensional nanoparticles/materials possess all three dimensions within the nanoscale, examples being fullerenes and atomic clusters. On the other hand, onenanoparticles/materials dimensional have two dimensions within the nanoscale and one dimension larger than the nanoscale, such as nanotubes and nanofibers. Two-dimensional nanoparticles/materials are characterized by having only one dimension within the nanoscale and the remaining two dimensions outside the nanoscale, such as nanolayers and nanofilms. Lastly, three-dimensional nanoparticles/materials lack any of the three dimensions within the nanoscale but are actually aggregates of 0D, 1D, or 2D nanomaterials. This category includes nanocomposites and box-shaped graphene.

### PROPERTIES OF NANOPARTICLES

Mannix et al. (2015) note that nanomaterials exhibit various properties that differ from their bulk counterparts. Specifically, at the nanoscale, electronic properties demonstrate variations compared to their bulk forms. For instance, the 2D network of boron behaves as a good 2D metal, whereas its bulk form does not exhibit metallic behavior. Singh et al. (2020) highlight that mechanical properties of substances are enhanced at the nanoscale in comparison to their bulk counterparts, owing to minimized crystal defects. This improvement in mechanical properties contributes to the superior performance of nanomaterials on the nanoscale. Furthermore, Alivisatos (1996) emphasizes that optical properties of nanomaterials, such as quantum dots or nanospheres, display variations based on their shape and size. These optical properties, intrinsic to the nanoscale dimensions, further contribute to the unique characteristics and applications of nanomaterials.

Tomar *et al.* (2020) highlight that nanomaterials possess unique properties based on their shape, size, and morphology. One such property is their high surface area, which makes them excellent candidates for heterogeneous catalysts in various chemical processes. Geoffrion and Guisbiers (2020) emphasize that nanomaterials exhibit enhanced quantum behaviour compared to their bulk counterparts, making them effective semiconductors. This quantum behaviour contributes to their superior performance in electronic

applications. Roduner (2006b) notes that magnetic behaviour at the nanoscale can be altered, leading to nonmagnetic elements becoming magnetic and vice versa. This phenomenon showcases the intriguing magnetic properties that can be achieved in nanomaterials. Wu et al. (2020) highlight that mechanical properties such as elasticity, tensile strength, and hardness, which are absent in their bulk counterparts, emerge in nanomaterials. This further enhancement in mechanical properties contributes to the unique characteristics and applications of nanomaterials.

Liu et al. (2022) emphasize that nonmaterial's, particularly nano-scale metals, exhibit excellent catalytic ability due to their higher surface area, which also makes them effective adsorbents in various heterogeneous catalysis processes. This catalytic ability is of great importance in numerous industrial applications. Makvandi et al. (2020) discuss the remarkable antimicrobial properties exhibited by certain nanomaterials, including antibacterial, antiviral, and antifungal properties. These properties make nanomaterials valuable tools in combating pathogennanomaterials related diseases. Furthermore, demonstrate excellent thermal and electrical conductivity compared to their bulk counterparts. This enhanced thermal and electrical conductivity contributes to their utility in various fields and applications.

# METHODS FOR THE SYNTHESIS OF NANOPARTICLES

In general there are two types of approaches for the synthesis of nanoparticles they are named as top-down approach and bottom- up approach (Fig. 2).

Zhuang et al. (2016) discuss the top-down approaches utilized in the synthesis of nanoparticles, which involve breaking down bulk materials to construct nano-scale particles. This approach encompasses various methods such as mechanical milling, etching, sputtering, and electro-explosion. Cook and Clemons (2022) introduce the bottom-up approach as the second type of method for synthesizing nanoparticles. This approach involves the aggregation of smaller atoms and molecules from gas or liquid to generate particles within the nano range. Examples of bottom-up approaches include selfaggregation of polymer molecules, chemical and electrochemical nonstructural precipitation, sol-gel method, chemical vapor deposition (CVD), and bioassisted synthesis. Both top-down and bottom-up approaches have their own advantages and disadvantages in relation to each other. Generally, bottom-up approaches are considered superior to topdown approaches in certain respects. Top-down methods can be expensive to implement, and obtaining ideal surface and edges can be challenging due to the formation of hollow structures and irregularities in nanoparticles. On the other hand, bottom-up approaches offer excellent nanoparticle synthesis capabilities.

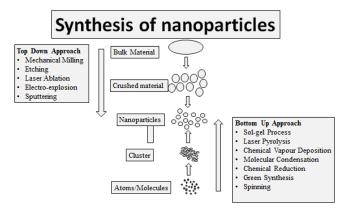


Fig. 2. Top-down and bottom-up approaches for the synthesis of nanomaterials.

Furthermore, that top-down approaches such as the mechanical friction method, which utilizes a ball mill to convert bulk materials into nanoparticles, are costand However, effective. systematic, simple. contamination can occur due to the presence of balls, posing a potential drawback in this method. In summary, the discussion within the review article focuses on the differentiation between top-down and bottom-up approaches for nanoparticle synthesis. Various methods are explored under each approach, and their advantages and disadvantages are highlighted in relation to one another.

Green synthesis of nanoparticles. Nadaroglu et al. (2017a); Nadaroglu et al. (2017b); Shah et al. (2015) emphasize the limitations of nanoparticle synthesis via physical and chemical means, which can be costly, hazardous, and require the use of toxic reagents. Consequently, there has been a shift in focus towards biological methods that are more environmentally friendly and do not rely on such reagents. In recent years, these biological methods have demonstrated the ability to produce nanoparticles with diverse shapes, sizes, and properties, achieving high yields. The synthesis of nanoparticles can be achieved in a single using bio-organisms such as step bacteria, actinobacteria, yeasts, algae, and plant materials. These bio-organisms generate various compounds, including proteins, enzymes, phenolic compounds, amines, alkaloids, and pigments, which facilitate the reduction process and ultimately synthesize nanoparticles. The advantage of employing biological or green synthesis methods is that the reducing agents and stabilizing agents required for nanoparticle synthesis are naturally present in the used bio-organisms. This eliminates the need for externally added reducing agents and stabilizing agents, reducing the potential for hazardous and toxic effects on the environment. Fig. 3 illustrates the synthesis of nanoparticles using biological methods. Gao et al. (2014) highlight the suitability of bacteria for nanoparticle synthesis due to their rapid production, low culturing cost, and favorable growth environment. Bacteria possess the ability to reduce the toxic effects of metals and can generate nanoparticles through both in-situ and ex-situ methods. Utilizing reducing agents such as proteins and enzymes derived from bacteria,

metal ions are reduced to metal atoms, which subsequently aggregate to form metal nanoparticles through biochemical processes.

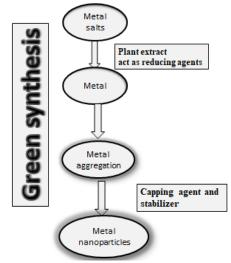


Fig. 3. Biological method for the synthesis of nanoparticles.

Biosynthesis of nanoparticles by using plant extract. Iravani (2011) highlights the use of plant materials and plant extracts as an excellent biological or green method for nanoparticle synthesis. This method is known for its non-hazardous, eco-friendly nature, and cost-effectiveness. It is particularly effective in the synthesis of nanoparticles of noble metals such as Pt, Au, Pd, Ag, and Os as well as bimetallic alloys and metals. Akhtar et al. (2013) discuss the utilization of various biomolecules, including amino acids, citric acid, proteins, reducing sugars, phenols, and alcohols, as both reducing agents and capping agents in the synthesis of nanoparticles. These biomolecules play essential roles in the reduction process and provide stability to the resulting nanoparticles. The review article presents the route and mechanism for the synthesis of metal nanoparticles using various bio metabolites, as illustrated in Fig. 4. This diagram elucidates the stepwise process and interactions involved in the synthesis of metal nanoparticles through the utilization of bio metabolites.

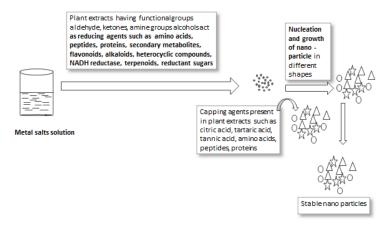


Fig. 4. Synthesis of metal nanoparticles by using bio metabolites.

Ankamwar et al. (2005); Chandran et al. (2006) have conducted studies on the synthesis of Au nanoparticles, utilizing plant extracts obtained from Tamarindus indica, Aloe barbadensis, and Emblica officinalis leaves. Their research demonstrates the significant role of these plant extracts in the preparation of Au nanoparticles. Shankar et al. (2004) successfully synthesized Au nanotriangles by utilizing extracts obtained from lemon grass leaves to treat AuCl- ions. Additionally, they prepared Ag nanoparticles by employing fruit extracts derived from Emblica officinalis. In a separate study, Maensiri et al. (2008) observed the synthesis of In2O3 nanoparticles, which were obtained using Aloe vera plant extract. Furthermore, Shankar et al., (2004) conducted research demonstrating the synthesis of Ag, Au, and bimetallic Ag-Au nanoparticles. They achieved this synthesis by utilizing the leaf broth of Azadirachta indica. A review article published by Kumari et al. (2021), has deliberately discussed the synthesis of green nanoparticles and highlighted the importance of different sources; plants, and microorganisms (bacteria, and fungi) in green synthesis of nanoparticles. The role of fruit; Pithecellobium dulce (pulichinthakaya) peel in synthesis of silver nanoparticles underscored by Yaku et al. (2019) and simultaneously they have used silver nanoparticles in determination of Hg+2and Fe+3 metal ions.

Biosynthesis or green synthesis of nanoparticles by using microorganism. Microorganism like algae,

fungi, prokaryotic bacteria, yeast and actinomycetes can be used to prepare the nanoparticles. All these microorganisms generate protein, enzymes, alkaloids, phenolic compounds and amines which prepare the nanoparticles by reduction process. By using these microorganisms, metal and metal oxide nanoparticles are synthesized (Ag, Au, TiO<sub>2</sub> etc). During biosynthesis or green synthesis microorganisms capture the metal ions from their source and reduce them into metal atoms with the help of reducing agents like enzymes obtained from cellular activities. Recently, Nag et al. (2022), has isolated a cyanobaterium; Chlorogloeopsis fritschii BK (MN968818) from mangrove environment and used it for the synthesis of zinc oxide nanoparticles followed by demonstration of antimicrobial properties. Synthesis of nanoparticles by using microorganisms is divided into two broad categories named intracellular and extracellular methods. In the extracellular method microorganism capture the metal ions at the periphery of cell and synthesize the metal nanoparticles by reduction in the presence of enzymes while during the intracellular method the microorganism transport metal ions into microbial cells where synthesis of metal nanoparticles occurs by reduction in the presence of enzymes. Singh et al. (2018) provided a summary of metal and metal oxide nanoparticles, along with their size and morphology, synthesized using diverse bio species such as bacteria, fungi, and yeast. The summarized information can be found in following Table 1.

Sr. No.	Species	Nanoparticles	Size (nm)	Morphology		
Bacteria						
1.	Bacillus cereus	Silver	20-40	Spherical		
2.	Pseudomonas proteolytica, Bacillus cecembensis	Silver	6–13	Spherical		
3.	Paracoccus haeundaensis BC74171T	Gold	20	Nearly spherical		
4.	Ureolytic bacteria	ZnO	10-15	Hairy shape		
5.	Bacillus sp. B2	Se	20-50	Spherical		
6.	Bacillus pumilus, Bacillus paralicheniformis and Sphingomonas paucimobilis	Silver	4-20	Spherical To oval		
7.	Lactobacillus casei	Silver	20-50	Spherical		
8.	Klebsiella pneumonia, Escherichia coli, Enterobacter cloacae	Silver	28–122	Spherical		

Table 1: Green synthesis of metal and metal oxide nanoparticles by using bacteria, fungi and yeast.

9.	Bacillus indicus	Silver	-	-
10.	Plectonema boryanum UTEX 485	Gold	< 10-25	Cubic, octahedral
11.	Bacillus subtilis 168	Gold	5-50	Hexagonal-octahedral
12.	Bacillus megaterium D01	Gold	< 2.5	Spherical
12			рН 7: 10–20	
13.	Shewanella alga	Gold	pH 2.5: 15–200 pH 2: 20	Triangular
14.	E. coli DH 5α	Gold	8–25	Spherical
15.	Desulfovvibrio desulfuricans	Gold	20-50	Spherical
16.	Rhodopseudomonas capsulate	Gold	10–20	Cancer hyperthermia
17.	Magnetospirillum magnetotacticum	Iron Oxide	47	_
18.	Aquaspirillum magnetotacticum	Iron Oxide	40–50	Octahedral prism
19.	Shewanella oneidensis	Uranium oxide	1–5	_
20.	Klebsiella aerogenes	Cadmium sulfide	20-200	_
21.	E. coli	Cadmium sulfide	75	Fluorescent labels
		Fungus		
1	Rhizopus nigricans	Silver	35-40	Round
2	Verticillium	Silver	21-25	Spherical
3	Aspergillus fumigates	Silver	5-25	Spherical
4	Phanerochaete chrysosporium	Silver	50-200	Pyramidal
5	Aspergillus flavus	Gold	Average 12	Nearly spherical
6	Aspergillus niger	Silver	20	Spherical
7	Fusarium semitectum	Silver	10-60	Crystalline spherical
8	Cladosporium cladosporioides	Silver	10-100	Spherical
9	Cariolus versicolor	Silver	25-75	Spherical
10	Fusarium solani	Silver	5–35	Spherical
11	Penicillium brecompactum	Silver	23-105	Crystalline spherical
12	Penicillium fellutanum	Silver	5-25	Spherical
13	Phomaglom erata	Silver	60-80	Spherical
14	Alternata alternate	Silver	20-60	Spherical
15	Trichoderma viride	Silver	5-40	Spherical
16	Verticillium luteoalbum	Gold	<10	Triangular, hexagonal
17	Rhizopus stolonifer	Silver, Gold	25-30, 1-5	Spherical
18	Trichothecium sp.	Gold	10–25	Spherical, rod-like and triangular
19	Fusarium oxysporum	Gold-silver alloy	8-14	Spherical
20	Aspergillus terreus	Zinc oxide	8	Spherical
21	Aspergillus flavus TFR7	Titanium dioxide	12–15	Spherical
22	Fusarium keratoplasticum strain (A1-3) and Aspergillus niger strain (G3-1)	Zinc oxide	100	Hexagonal and Nano rode respectively
		Yeast		
1.	MKY3	Silver	2–5	Hexagonal
2.	Saccharimyces cerevisae broth	Gold, silver	4–15	Spherical
3.	Saccharomyces cerevisiae	Silver	2-20	Spherical
4.	Saccharomyces cerevisiae	Selenium	75-709	-

### **FUTURE SCOPE**

The future scope of green synthesis of nanoparticles using plant extracts, microorganisms (such as bacteria and fungi), and other biological entities appears promising and holds great potential. As researchers continue to investigate and understand the underlying mechanisms of nanoparticle formation, it is expected that more efficient and controlled synthesis methods will be developed. The exploration of diverse botanical and microbial sources may lead to the discovery of new nanoparticle synthesis capabilities and the production of nanoparticles with unique properties. Advancements in nanotechnology, materials science, and biotechnology are likely to play a significant role in tailoring the size, shape, and surface properties of nanoparticles, enabling their targeted applications in various fields, including medicine, agriculture, and environmental remediation.

Moreover, the eco-friendly nature of green synthesis methods will likely gain further attention, as industries seek sustainable and environmentally conscious alternatives for nanoparticle production. As more research is conducted and knowledge expands, the potential for commercialization and real-world applications of biologically synthesized nanoparticles is expected to increase, contributing to a greener and more sustainable future.

### CONCLUSIONS

In conclusion, the green synthesis of nanoparticles using bacteria, fungi, and plants has emerged as a promising approach for the synthesis of nanoparticles. This approach is eco-friendly, cost-effective, and has significant potential for large-scale production. Bacteria, fungi, and plants possess unique biochemical pathways that can be exploited to synthesize *al* **15(5): 1559-1566(2023) 1564**  nanoparticles with specific sizes, shapes, and surface properties. Additionally, green synthesized nanoparticles exhibit excellent biocompatibility and have a wide range of applications in various fields such as medicine, agriculture, and industry. Therefore, the use of bacteria, fungi, and plants for green synthesis of nanoparticles is a sustainable alternative to conventional methods that involve toxic chemicals and high energy consumption.

However, there are some intrinsic drawbacks in green synthesis of nanoparticles using microorganisms as they are very sensitive to change in growth conditions such as temperature, pH, and nutrient availability. Slight change in growth conditions could lead to unfavorable results. Further, slow growth of microorganisms is also a major speed breaker in the progress of green synthesis. More researches are needed to control and optimize the growth conditions. The uprising field of genetic engineering is an effective solution to modify the microorganisms as per the requirements. It is expected that green synthesis approach will become more widely adopted and contribute to the development of a more sustainable and environmentally friendly future.

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