



Bio-Inspired Synthesis of Bimetallic Nanoparticles and Their Applications: Review

Anjali Singh and Vijay Devra*

Department of Chemistry, Janki Devi Bajaj Government Girls College, Kota, Rajasthan, India

*Corresponding author E-mail address: v_devra1@rediffmail.com (Vijay Devra)

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Abstract: Bimetallic nanoparticles (BMNPs) have been the subject of extensive research, particularly in the last ten years. In this paper, we focus about the development of bimetallic nanoparticles using various plant extracts and microorganisms like bacteria, yeast, fungi, and algae, as well as their applications. Plants operate as a capping agent and a reducing agent (flavonoids, proteins, polyphenols, amino acids, alkaloids). Natural extracts provide a straightforward, economical, environmentally beneficial, and non-hazardous role in the biosynthesis of BMNPs. Transmission electron microscopy (TEM), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), Atomic force microscopy (AFM), Dynamic light scattering (DLS), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), UV-vis spectroscopy, and zeta potential were used to characterize the synthesized nanoparticles. Due to their potential applications in a number of sectors, including biosensing, imaging, nanomedicine, and catalysis, bimetallic nanoparticles (BMNPs) have recently attracted a great deal of attention. BMNPs combine the capabilities of two metals in a nanostructured system. This research will investigate the environmental uses of green produced bimetallic nanoparticles, including dyes, metal absorption, and other organic pollutants.

Keywords: Nanotechnology; bimetallic nanoparticles; green synthesis; plant extract; microbes; applications

1. Introduction

A fascinating area of research known as nanotechnology works with materials that have structural similarities to atoms and bulk materials, and at least one of their dimensions comes inside the 1–100 nm nanoscale range.^[1] The Greek word "nanos," which meaning "Dwarf," is where the term "nano" comes from.^[2] Due to the amazing optical, chemical, physical, mechanical, electrical, and magnetic capabilities of nanoparticles, research on them has increased significantly.^[3] This technology is useful to dangerous compounds because it can be processed in a reasonable amount of time, is practical, and has the capacity to perform extreme applications.^[4] Nanoparticles (NPs) can be categorised based on their origin, size, and structural composition. Natural nanoparticles, also known as nanoparticles with a natural origin, include substances including viruses, proteins, antibodies, and other nanoparticles that are linked to natural resources.^[5] Carbon nanotubes, metal nanoparticles, and nanomembranes are examples of artificial nanoparticles that are produced expressly for a purpose after adhering to the correct production procedures and essential techniques.^[6] BMNPs, a subclass of metallic nanoparticles, are of tremendous interest due to their characteristics.^[7] Core-shell, single alloy, sub-cluster, crown gem, hollow, hetero, and porous bimetallic nanoparticle architectures have been reportedly been studied. Core-shell nanoparticles are made of two or more nanomaterials, one of which

serves as the core and the other of which is situated around the centre of the core and is referred to as the shell.^[8] For the production of nanoparticles, there are two methods: "top to bottom" and "bottom to top" (Fig. 1).

The top-down method uses several physical and chemical techniques to reduce size starting with an appropriate starting material.^[9] While chemical technology uses dangerous and damaging chemicals that pollute the environment, physical technology involves the consumption of high energy, pressure, and temperature.^[10] The bottom-up method creates final particles in the nanoscale range by joining smaller entities and producing them via chemical and biological processes.^[11] The bottom-up method, which starts with simple molecules recognised as reaction precursors, is the most efficient method for synthesising nanoparticles. The size and synthesis of nanoparticles can be controlled depending on the subsequent usage by changing the precursor volume and reaction parameters like pH, temperature, extract concentration (Fig. 2).^[12]

2. Bio- inspired Synthesis of Bimetallic nanoparticles

Nanomaterials are biosynthesized using biochemical resources such as plant extracts and microorganisms such as bacteria, algae, fungi, viruses, and yeast. It is commonly recognised that biological

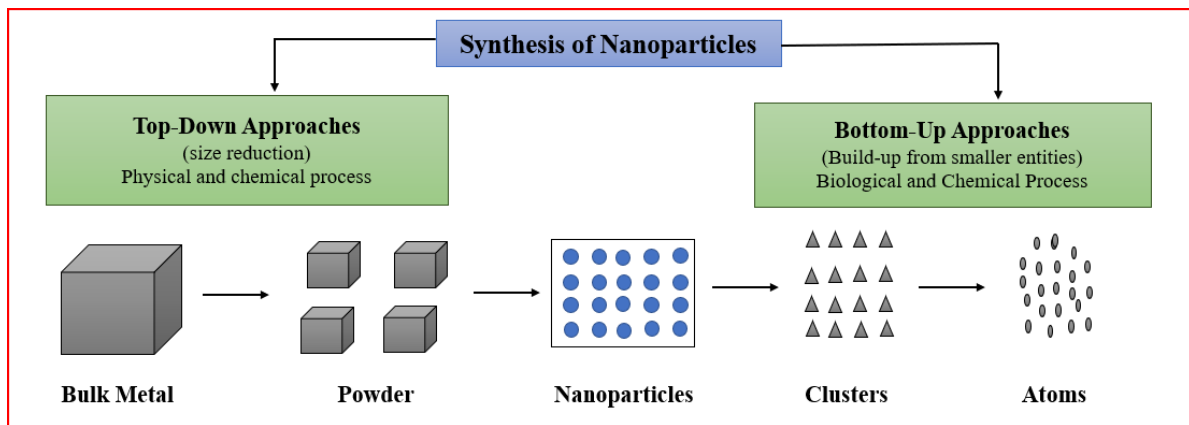


Fig. 1. Top Down and bottom-up Approaches

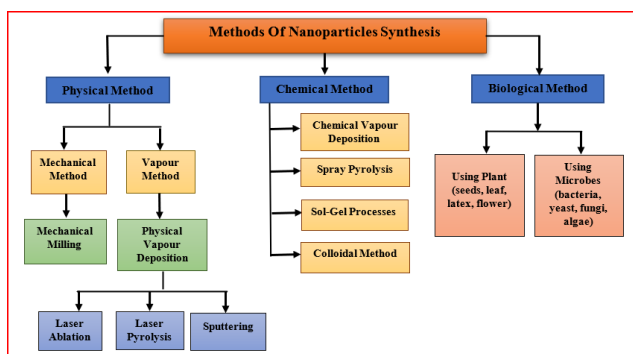


Fig. 2. Various methods for Synthesis process of Nanoparticles

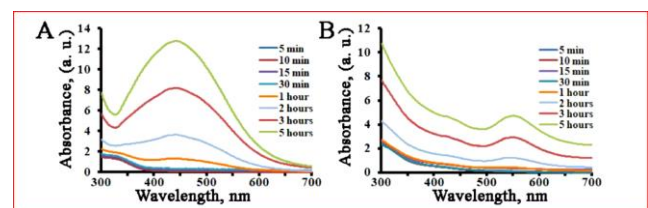


Fig. 3. AgNPs (A) and AuNPs (B) UV-visible spectra as a function of reaction time after being generated with *L. erythrorhizon* callus extracts.^[22]

organisms, like bacteria and living cells, are the best examples of devices with parts operating at the nanoscale level and doing many functions with extremely high efficiency, from energy generation to extracting target material.^[13] The bio-reduction and stabilisation or capping of metal ions to metal nanoparticles may be facilitated by a variety of bioactive compounds found in plant extracts, such as flavonoids, proteins, polyphenols, amino acids, alkaloids, enzymes, and reducing sugars.^[14]

2.1. Synthesis of bimetallic nanoparticles using Plant extract

Numerous researchers have reported the use of different plant extracts in the green production of metal nanoparticles. Because they are easier to produce and more readily available, plant extracts are thought to be more beneficial than microbes.^[15] Bimetallic nanoparticles are created by the reduction and co-reduction of precursor salts.^[16] Numerous plant components, including seeds, flowers, leaves, latex, fruits, etc., are employed in the synthesis of bimetallic nanoparticles (Table 1). Proteins, phenolic acids, alkaloids, carbohydrates, terpenoids, and other plant extracts called "bioactive polyphenols" play a significant role in decreasing and then stabilising metal ions.^[17] Fe-Pd bimetallic nanoparticles were made using a green method using grape leaves, which might serve as a reducing and capping agent. Fe/Pd nanoparticles with diameters ranging from 2 to 20 nm were discovered to be multi-porous and semi-spherical using transmission electron microscopy (TEM) and scanning electron microscopy (SEM).^[18] The biogenic manufacture of gold, silver, and the bimetallic alloy Au-Ag nanoparticles from aqueous solution is described in this report as a reducing and stabilising agent for

Cannabis sativa. Ag-Au nanoparticles can also be employed as powerful antibiotics and anti-leishmanial agents.^[19] The pulp of the Palmyra fruit was used in the current investigation to perform and analyse the biosynthesis of bimetallic nanoparticles.^[20] The absorption peak at 400 nm, which can be attributed to the Ag-Cu NPs' absorption, can be seen in the UV-visible spectra of the toddy fruit extract-stably produced Ag-Cu NPs at about 558 nm.^[21] UV-visible spectra of AgNPs and AuNPs produced under various circumstances using callus culture extracts from *Lithospermum erythrorhizon*. (Fig. 3) The production of the nanoparticles was first studied using UV-Vis spectroscopic spectroscopy between 300 and 700 nm.^[22] The creation of bimetallic Au-Ag dendrites with chemosensitive activity was demonstrated using the seed-mediated method. The glucose oxidase and horseradish peroxidase-based dendritic Au-Ag bimetallic nanoparticles were used to accurately and specifically detect d-glucose.^[23]

2.2. Synthesis of bimetallic nanoparticles using Microbes

Microbes like bacteria, fungi, yeast, algae, and other biocompatible reagents are crucial in the biogenic synthesis of bimetallic NPs (Table 2).^[63] The co-precipitation technique was used to create Cu-Ag bimetallic nanoparticles for usage in applications to fight bacterial infections.^[64] One-celled yeast is an easy-to-manage microbe that may produce a variety of enzymes under laboratory settings. It concentrates its quick growth by ingesting minimal nutrients.^[65] *S. platensis* is used in the extracellular biosynthesis of silver, gold, and bimetallic nanoparticles. Protein is the most likely biomolecule in charge of the reduction and capping of greenly produced bimetallic nanoparticles, according to Fourier transform infrared spectroscopic investigations.^[66] Silver, gold, and bimetallic nanoparticles were produced naturally by the Gulf of Mannar's marine red alga,

Table 1. The green synthesis of bimetallic nanoparticles using plant part (leaf, fruit and fruit latex, seed)

Plant	Type of Alloys	Size (nm)	Shape	Applications	Ref.
Leaf					
<i>Gloriosa superba</i>	Ag-Au	10	Spherical	Antibacterial and Antibiofilm activities	[24]
<i>Ocimum basilicum</i>	Au-Ag	3-25	Spherical	Antidiabetic and Antimicrobial activity	[25]
<i>Mirabilis jalapa</i>	ZnO/Ag	19.3 - 67.4	Spherical	Antioxidant and Antibacterial activity	[26]
<i>Piper pedicellatum C. DC</i>	Ag-Au	3-45	Spherical	-----	[27]
<i>Commelina nudiflora</i>	Au-Ag alloy	20-80	Spherical	Antibacterial activity	[28]
<i>Gardenia jasminoides</i>	Ag-Fe	13	Spherical	Antimicrobial activity	[29]
<i>Anacardium occidentale</i>	Au-Ag Core-shell	6-10	Spherical	Bio reduction	[30]
Tea Leaves	Au-Ag	20-200	Spherical	Wastewater purification	[31]
<i>Cacumen Platycladi</i>	Au-Pd alloy	7.4	Spherical	Bio reduction	[32]
Eucalyptus	Fe-Ni	20-50	Spherical and irregular shaped	Catalytic activity	[33]
Golden rod (<i>Solidago canadensis</i>)	Ag-Au alloy	25.9	Spherical	Electrocatalyst and Biosensor activity	[34]
Mahogany (<i>Swietenia mahogani JACQ.</i>)	Ag-Au alloy	50	Spherical	Medical and packaging applications	[35]
<i>Moringa oleifera</i>	Ag-Au	11-25	Hexagonal, triangular, spherical	Anticancer activity	[36]
	Ag-Cu	87	Globular in shape	Antibacterial activity	[37]
Palm tree (<i>Phoenix dactylifera</i>)	Cu-Ag	26	Spherical	Antibacterial activity	[38]
Sago pondweed (<i>Potamogeton pectinatus</i>)	Au-Ag alloy	10.6 ± 5	Spherical	Antibacterial activity	[39]
<i>Senna occidentalis (coffee senna)</i>	Ag-Ni	20	Pseudo-cubic	Optical activity	[40]
<i>Stigmaphyllon ovatum</i>	Ag-Au	15	Spherical	Anticancer activity	[41]
Persimmon (<i>Diopyros kaki</i>)	Au-Ag	50-500	Cubic	-----	[42]
Aloe Vera	Ag-Cu	61	Spherical	Antibacterial activity	[43]
Bay Leaves (<i>Laurus nobilis L.</i>)	Ag-Au Core-shell	8±3	Spherical	Use in Biomedical field	[44]
<i>Boerhavia diffusa</i>	Co-Ni	10	Irregular shapes	Simultaneous production of Biohydrogen and Bioethanol	[45]
<i>Canna Indica</i>	Ag-Ni	9.1 ± 1.1	Quasi-spherical	Antimicrobial and Antibacterial activity	[46]
<i>Catharanthus</i>	Ag-Pd Core-shell	15-30	Quasi-spherical	Antioxidant and anticancer activity	[47]
<i>Cyclea peltata</i>	Fe-Cu Core-shell	45-50	Spherical	Degradation of dye	[48]
<i>Pulicaria undulata</i>	Au-Ag alloy	20-50	Spherical	Catalytic activity	[49]
<i>Azadirachta Indica</i>	Au-Ag Au-core Ag-shell	50-100	Spherical	Antibacterial activity	[50]
Fruit and Fruit latex					
Chinese wolfberry	Au-Ag alloy	15	Spherical	Photocatalytic activity	[51]
Kei-Apple (<i>Dovyalis caffra</i>)	Au-Ag	5-24	Spherical	Anticancer activity	[52]
<i>Kigelia africana</i> fruit	Ag-Cu	10	-----	Antimicrobial activity	[53]
<i>Achras sapota</i> Linn.	Ag-Cu	20-40	Spherical	In vitro toxicity	[54]
<i>Artocarpus heterophyllus</i> fruit latex	Ag-Au	15	Spherical	Antioxidant and antibacterial activity	[55]
<i>Moringa oleifera</i> (drumstick)	Ag-Cu	9	Spherical	Antimicrobial and antibacterial activity	[56]
Oak fruit hull (Jaft)	Ag/ZnO	19.2	Spherical	Photocatalytic activity	[57]
<i>Persea americana</i> (avocado)	Ag-Au alloy	44-55	Spherical	Antibacterial, Antifungal and Antioxidant	[58]
<i>Terminalia chebula</i>	Ag-Pd	20-70	Spherical	Anticancer and Antimicrobial activity	[59]
Triphala	Ag-Au	40-70	Spherical	Antimicrobial activity	[60]
Seed					
<i>Lawsonia inermis</i> seed	Au-Ag	15-35	spherical and irregular	Photocatalytic activity	[61]
<i>Madhuca longifolia</i>	Au-Ag	34-66	Spherical	Antimicrobial activity	[62]

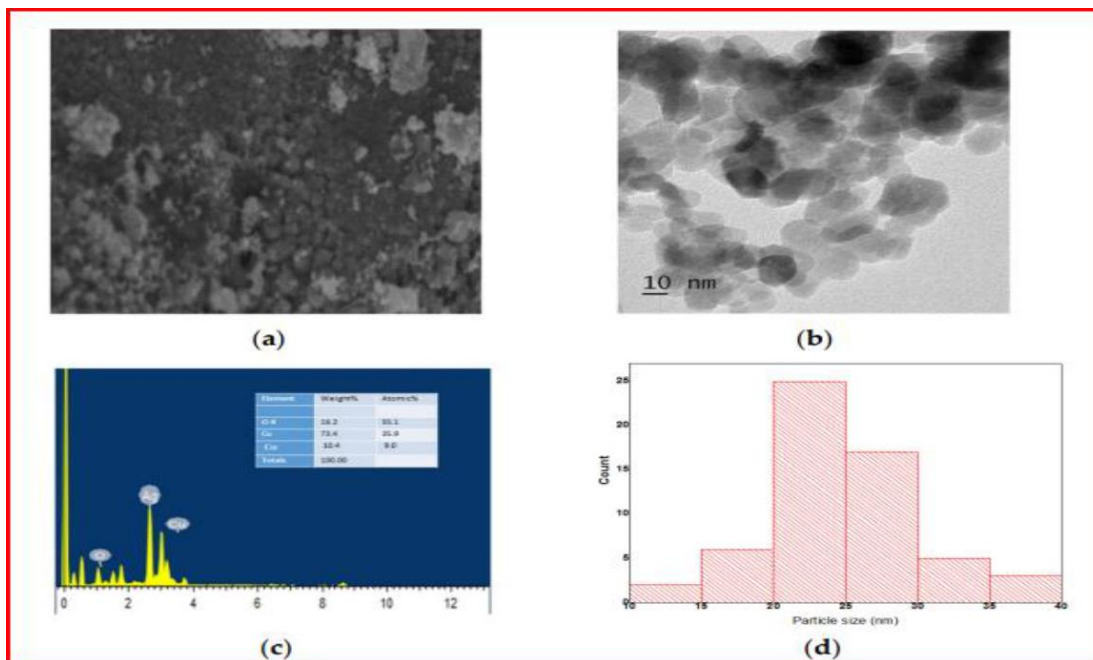


Fig. 4. Images of Ag-Cu bimetallic nanoparticles-(a) SEM, (b) TEM, (c) EDS and (d) the particle size histogram.^[68]

Table 2. The green synthesis of bimetallic nanoparticles using micro-organism (algae, fungi, bacteria, yeast)

Microbes	Type of Alloys	Size (nm)	Shape	Applications	Ref.
Algae					
Red algae	Au-Ag	20-40	Spherical	Antibacterial activity	[67]
Gracilaria species					
<i>Chlorella acidophila</i>	Au-Ag	5-45	Spherical	Potential applications in sensor	[73]
Green marine alga <i>Ulva reticulata</i>	Au-Ag	57-230	Star shaped	Biological activity	[74]
<i>Spirulina platensis</i>	Au-Ag	17-25	Spherical	Biomedical and Biotechnological activity	[66]
Diatom	Ag-Pd	56	Spherical	Degradation of azo dyes for wastewater treatment	[75]
<i>Chlamydomonas reinhardtii</i>	Ag-Au alloys	10-20	Round shaped		[76]
Fungi					
<i>Neurospora crassa</i>	Au-Ag alloys	3-90	Spherical	Surface plasma enhanced applications	[77]
<i>Oyster mushroom</i>	Au-Pt	16	Icosahedral shape	Anticancer activity	[78]
<i>Aspergillus terreus</i>	Ag-Cu	20-30	Spherical	Antioxidant, Antibacterial, and Cytotoxic activity	[68]
<i>Trichoderma reesei</i>	Au-Ag	25-40		Antimicrobial activity	[79]
<i>Fusarium oxysporum</i>	Au-Ag alloy	8-14	Spherical		[80]
<i>Fusarium semitectum</i>	Au-Ag alloy	10-34	Spherical	Possible optoelectronics and medical applications	[81]
Bacteria					
<i>Bacillus safensis</i> LAU 13	Ag-Au alloy	13-80	Ring shape	Antifungal, Dye Degradation, Anti-Coagulant and Thrombolytic Activities	[82]
<i>Escherichia coli</i>	Au-Ag core-shell structure	1-2	Spherical	Antibacterial	[83]
<i>Deinococcus radiodurans</i>	Au-Ag	149.8	Spherical	Nanomedicine for Malachite Green detoxification	[84]
Bacterial Auxin	Fe-Mn	100	Spherical	Plant Biofertilizer	[85]
<i>Shewanella oneidensis</i> MR-1	Pd-Pt	20-100	Flower shape	Catalytic reduction of 4-nitrophenol	[86]
<i>Cupriavidus necator</i> cells,	Pd-Au	5-200	Spherical	Reducing p-nitrophenol to p-aminophenol	[87]
Yeast					
<i>Pichia pastoris</i>	Au-Pd	5-100	Flower like shape	Antimicrobial activity	[88]

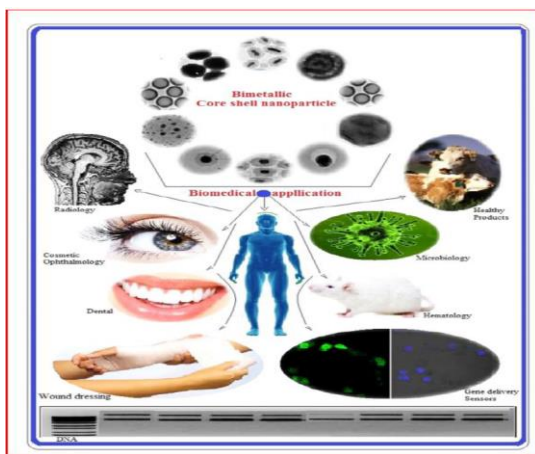


Fig. 5. Applications of biosynthesized bimetallic and core-shell NPs in biomedical fields.^[8]

Gracilaria sp. *Gracilaria sp.* had an absorption peak at 419 nm for 100% Ag, 536 nm for 100% Au, and 504 nm for Ag-Au (1:1) concentration.^[67] The spherical and dispersion of aggregated Ag-cu bimetallic nanoparticles of 20–30 nm sizes were visible in the SEM and TEM images (Fig. 4a and 4b). Without any contaminants, the EDS revealed the existence of Ag, Cu, and O. (Fig. 4c). The particle size histogram reveals that, with 11 counts, the particle size 20–22 nm was the most prevalent (Fig. 4d).^[68] *Spodoptera frugiperda* is being controlled via the myco-synthesis of zinc oxide and titanium dioxide bimetallic nanoparticles. When used on *S. frugiperda* larvae, BMNPs demonstrated considerable toxicity and hindered the larvae's ability to feed. These investigations demonstrated that *S.* demonstrated effective larvicidal action against *S. frugiperda* larvae after 48 hours of treatment at 100 g/ml.^[69] For use in direct methanol fuel cells, bimetallic Pt-Ru nanoparticles were effectively created by bio-reducing Pt (IV) and Ru (III) in an aqueous solution using the reducing bacterium *Shewanella algae* (*S. algae*).^[70] *Exiguobacterium aestuarii* bacteria used in the biogenic manufacture of bimetallic Au-Se nanoparticles.^[71] *Shewanella oneidensis* MR-1 bacteria are used in the microbial manufacture of Pd-Fe₃O₄, Au-Fe₃O₄, and PdAu-Fe₃O₄ nanocomposite as well as the catalytic reduction of nitroaromatic chemicals. After the catalytic reaction, the magnetic nanocomposite is easily recoverable via magnetic degassing.^[72]

3. Applications of Bimetallic Nanoparticles

3.1. Environmental Applications

The process of cleansing the environment with nanoparticles that promote microbial activity is replaced by nano-bioremediation.^[89] Due to their environmental friendliness and lack of use of harmful ingredients in the synthesis or stability of nanostructures, green synthesis of nanostructures has gained appeal.^[6] UV-vis spectroscopy was used to investigate the catalytic activity of nickel-supported iron oxide magnetic nanoparticles (Ni/Fe₃O₄ BMNPs) towards the degradation of the malachite green (Mg) dye. The removal of hazardous contaminants, quick reaction times, and gentle reaction conditions are the method's main benefits.^[90] The catalytic activity of the created copper-silver nanoparticles was used to analyse the

methylene blue dye's degradative process. 82% of the dye was removed, it was discovered.^[38] Investigations were done on the orange II degradation caused by the iron-palladium nanoparticles' catalytic activity. Bimetallic Fe/Pd NPs produced via green synthesis were more effective in removing Orange II at 12 hours than Fe NPs (16.0%). Less than 2.0% of Orange II was eliminated while all precursors, such as grape leaf extract, Fe²⁺, and Pd²⁺, appeared to have no effect.^[91] Bimetallic Fe-Cu nanoparticles produced by biosynthesis were examined for methyl green dye degradation and degradation kinetics. The outcomes demonstrated that the BMNPs could efficiently degrade methyl green dye by as much as 82% in 105 minutes, and that this process followed pseudo second order kinetics with an R² of 0.9862.^[48] The reduction of nitrophenols and organic dyes for water treatment results in the biosynthesis of Cu-Ag and Cu-Ni bimetallic nanoparticles supported by plants.^[92] Environmentally harmful methyl orange and 4-nitrophenol are catalytically decomposed by the green, one-step synthesis of Au-Ag bimetallic nanoparticles made from *Lawsonia inermis* seed extract.^[61] Utilizing the created Ag-Cu BNPs, the dyes methyl orange (MO), Congo red (CR), methylene blue (MB), and rhodamine B (RhB), as well as the fluoroquinolone antibiotics ciprofloxacin (CIP) and levofloxacin (LVO), were all destroyed.^[3]

3.2. Bio-medical Applications

Nanomaterial solutions for the fundamental understanding of current and emerging disease, diagnostics, and therapy are made possible by BMNPs' synergistic anti-microbial efficiency.^[93] Since multidrug-resistant bacteria have emerged as a result of the extensive use of antibiotics, it is essential to search for safe and efficient antibacterial materials.^[94] Ag-Fe bimetallic NPs have different antibacterial properties depending on the microorganism's cell wall or cell membrane composition. However, the Ag-Fe bimetallic nanoparticles' antibacterial mechanism is still unclear.^[95] Similar to the actions of mono-metallic nanoparticles against yeasts and both Gram-positive and Gram-negative multidrug-resistant bacteria, Ag-Fe bimetallic nanoparticles demonstrated an antibacterial (bactericidal) synergistic impact between the two metals.^[29] Nanoscience is one of the most exciting fields of study today (Fig. 5). Bimetallic and core-shell NPs with superior qualities, when compared to single NPs, have a specific economic value in the engineering, pharmaceutical, and other industrial sectors due to their steadily improving robustness, performance, and range of applications.^[8] In the bacterial environment, bacteriostatic Ni²⁺ ions and bactericidal Cu²⁺ ions can produce cytotoxicity similar to that of a third-generation medication.^[96] With the ability to increase photothermal conversion efficiency in comparison to Au NPs alone and to improve its antibacterial capabilities against *E. coli*, Au-Ag NPs offer tremendous potential in the quick and colorimetric detection of H₂O₂ without TMB and peroxidase.^[83] Researchers investigated the antibacterial activity of NCFs with in situ produced AgNPs, CuNPs, and BMNPs (Ag-Cu) against Gram-positive (*S. aureus* and *B. licheniformis*) and Gram-negative (*E. coli*) bacteria.^[97] Numerous fungal strains have intensively investigated the use of silver metal for mycosynthesis, and the resulting nanomaterials have shown more antibacterial action than traditional drugs.^[98]

3.3. Application in Agriculture

The use of nanotechnology in many disciplines, including the food and agricultural industries, has completely changed how science and technology are developed.^[99] Different pests and pathogens that negatively affect plant growth are constantly affecting the worldwide agricultural industry, which leads to failed agricultural manufacture and issues with food security.^[100] Bimetallic Mn-Fe NPs derived from bacterial supernatant demonstrated the best effects on plant growth, particularly germination rate, root growth, and fresh weight in maize plants, and can therefore be employed as micronutrient nanofertilizers.^[85] Because of this, efforts have recently been concentrated on creating activated carbon from lignocellulose and agricultural waste. Hazelnut shell, olive stone, jojoba seed, coconut shell, bamboo, rice husk, sugarcane bagasse, and many other types of lignocellulose are among the numerous acceptable agricultural leftovers and materials that can be employed.^[101] The synergistic action of the two metals is related to the improvement of disease. For instance, the anti-tumour activity of Ag-Au composition is brought on by the synergism of noble metal ions.^[102] Mycosynthesized nanoparticles have demonstrated promise in applications for nanopesticides and nanofertilizers to advance sustainable agricultural practises.^[98] Additionally highlighted were the usage of nanoscale active components, promising properties like controlled release, and targeted delivery of nanofertilizers to improve agricultural output, crop quality, and various environmental effects.^[103] Bimetallic Fe-Zn nanoparticles enhance plant physiological characteristics and seed yield, making their application advantageous for developing nano-fertilizer industries.^[104]

4. Conclusions

An extensive field of research with several applications has been streamlined by nanotechnology. In comparison to more expensive physical or chemical procedures, many researchers favour the biological approach. Based on the current state-of-the-art, the described biogenic approaches are helpful for regulating the precise structure, size, and special features of BMNPs in order to expand the range of potential applications beyond monometallic nanoparticles. Bimetallic nanoparticle biosynthesis is thought to be risk-free, economical, non-toxic, environmentally benign, and energy-efficient. The creation of different metal nanoparticles by bacteria, fungi, algae, and yeast has received interest in order to better understand nanoparticle synthesis. The development of improved bimetallic nanoparticles, as well as the numerous variables impacting nanoparticle synthesis and the various characterization methods employed to do this, are the key themes of this paper. Bimetallic nanoparticles have a wide range of potential applications in the disciplines of nanomedicine, drug delivery, catalysts, anti-microbials, agriculture, and many more fields that need for further study. The creation of highly developed bimetallic nanoparticles will undoubtedly benefit civilization.

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Conflicts of Interest

The authors declare no conflict of interest.

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