



## Biosynthesis, Mechanisms, and Antibacterial and Photocatalytic Applications of NiO Nanoparticles: A Comprehensive Review

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### ABSTRACT

This review explores the use of nickel oxide nanoparticle (NiO NPs) for their use in antibacterial and photocatalytic applications. The interest in this area is driven by the need to combat antibiotic resistance and environmental pollution. The focus of this article is the “green” biosynthesis of NiO NPs; as opposed to traditional chemical and/or physical methods of synthesis. Green biosynthesis is defined as the use of biological entities (i.e., plants, microbes, algae), which utilize natural chemicals found within these organisms to synthesize metal oxide nanoparticles. They act as reducing/capping agents, which affect the morphology, crystallinity and particle size of the NiO NPs produced. Biosynthetic NiO NPs demonstrated significant bactericidal action against a variety of bacterial species; specifically, both gram-positive and gram-negative bacteria. Biosynthetic NiO NPs also showed effectiveness against drug-resistant strains of bacteria such as *Staphylococcus aureus* and *Escherichia coli*. These results were attributed to the generation of reactive oxygen species (ROS) and disruption of bacterial cell membranes. The reviewed literature further demonstrates that biosynthesized NiO NPs show significant potential for environmental cleanup. Various investigations report effective degradation of organic dyes and pollutants such as methylene blue under visible light exposure, highlighting the viability of green synthesis routes for producing photocatalytically. Future research efforts should be focused on standardizing the synthesis protocols developed to date, conducting in vivo toxicity testing on biosynthetic NiO NPs, developing heterojunctions using biosynthetic NiO NPs for improved sunlight-driven photocatalytic activity, and exploring various continuous-flow systems for large-scale industrial production of biosynthetic NiO NPs.

**Keywords:** NiO nanoparticles, Biosynthesis, Antibacterial activity, Photocatalytic degradation.

### 1. Introduction

A nanomaterial has at least one dimension measuring less than approximately 100 nanometers. The use of nanoparticles is important due to their existence of interesting phenomena such as a remarkable increase in the surface-to-volume ratio, a significant change in surface energy, enhanced reactivity, and customizable properties, making them highly attractive for numerous applications

[1]. Both organic and inorganic nanoscale particles are used in biomedical and environmental research.

Among the vast array of nanoparticles, metal oxide nanoparticles have captured significant attention due to their multifaceted applications in catalysis, energy storage, sensing, and biomedical fields. Metal oxides, including nickel oxide (NiO), titanium dioxide (TiO<sub>2</sub>), zinc oxide (ZnO), and iron oxide (Fe<sub>2</sub>O<sub>3</sub>), among others, exhibit distinct

properties. For instance,  $\text{TiO}_2$  nanoparticles are renowned for their photocatalytic activity, rendering them invaluable in environmental remediation and solar energy conversion [2].  $\text{ZnO}$  nanoparticles exhibit excellent antibacterial properties, making them suitable for biomedical and healthcare applications [3]. On the other hand,  $\text{Fe}_2\text{O}_3$  nanoparticles find extensive use in magnetic resonance imaging (MRI) and drug delivery systems due to their magnetic properties and biocompatibility [4].

Among these, nickel oxide (NiO) presents a compelling compromise of properties for sustainable nanotechnology. Unlike  $\text{ZnO}$ , which can suffer from photocorrosion, NiO demonstrates robust chemical stability [5]. Furthermore, the relative abundance and lower cost of nickel precursors, coupled with the intrinsic magnetic and p-type semiconducting properties of NiO, offer unique advantages for recyclable photocatalysts and multifunctional therapeutic agents [6]. The biosynthesis of NiO NPs is particularly strategic, as it can mitigate potential toxicity concerns associated with conventional methods while enhancing biocompatibility through natural capping agents.

The pursuit of efficient and environmentally friendly synthesis methods has driven the exploration of organic or green substances as precursors for the fabrication of metal oxide nanoparticles. By harnessing the power of natural resources, researchers aim to mitigate the ecological impact associated with conventional chemical synthesis routes while concurrently enhancing the biocompatibility and sustainability of the resulting nanoparticles. Successful synthesis of metal oxide nanoparticles with tailored properties and improved performance characteristics has been achieved through the utilization of plant extracts, microbial enzymes, agricultural waste, and other organic sources.

In the context of this review, the focus will be on nickel oxide (NiO) nanoparticles, which have garnered significant attention due to their

versatility in catalysis, energy storage, and biomedical fields. NiO nanoparticles possess exceptional electrochemical properties, making them ideal candidates for energy storage devices such as lithium-ion batteries and supercapacitors [7]. Moreover, their catalytic activity has found applications in various industrial processes, including gas sensing, hydrogen production, and environmental remediation. In biomedicine, NiO nanoparticles show promise in drug delivery, bioimaging, and cancer therapy, owing to their biocompatibility and tailor-made surface functionalities [8].

Integration of NiO nanoparticles in photocatalytic and antibacterial applications further underscores their potential in addressing pressing societal challenges. Numerous studies have demonstrated the antibacterial activity of biosynthesized NiO nanoparticles against multidrug-resistant bacteria, showcasing their effectiveness as potential antimicrobial agents [9]. Moreover, their photocatalytic properties have been harnessed for the degradation of organic pollutants in wastewater treatment and environmental remediation [5]. In recent years, the green synthesis of metal oxide nanoparticles has drawn considerable attention. Compared to standard synthesis methods, their protocols are also inexpensive and environmentally friendly. By conducting a meticulous review of the existing literature, this work aims to delve deeper into the intricate realm of NiO nanoparticle biosynthesis, as well as provide an in-depth examination of their evaluation in photocatalysis and antibacterial activities. Through the elucidation of various synthesis methodologies, characterization techniques, and performance evaluations associated with NiO nanoparticles synthesized via organic or green routes, this review seeks to offer valuable insights into their potential applications in real-world scenarios. Furthermore, it endeavors to unravel the underlying mechanisms governing their antibacterial and photocatalytic properties.

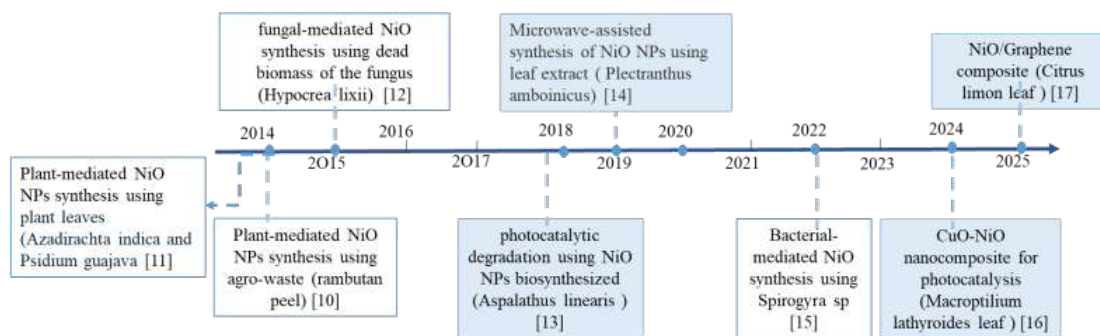


Fig. 1- Timeline of major milestones in the green biosynthesis of nickel oxide (NiO) nanoparticles (2014–2025).

Figure 1 shows how this field has evolved quickly by presenting a timeline of the main events in the green biosynthesis of NiO nanoparticles.

**2. Biosynthesis of NiO Nanoparticles: Methods, Parameters, and Outcome Control**

This review was conducted using a systematic literature review (SLR) to compile and analyze recent research on the biosynthesis of NiO nanoparticles for antibacterial and photocatalytic applications. The search was conducted in major scientific databases using specific keywords to identify relevant studies published in the last decade. Selected articles were evaluated for their emphasis on biosynthesis methods using biological materials, such as plant extracts. The review focused on these bio-based synthesis approaches, assessing their efficacy and potential applications in both antibacterial and photocatalytic fields. This comprehensive analysis provides valuable insights into the potential of bio-based NiO nanoparticle synthesis to address pressing environmental and biomedical challenges.

**2.1. Synthesis of metal oxide nanoparticles**

Over the past two decades, the manufacturing

processes for metal oxide nanoparticles have been developed. Today, there are many biological, physical, and chemical synthesis routes to obtain nanoparticles adapted to the needs of the user. For example, for biological applications, reagents that are toxic to the human body are avoided because they are likely to remain on the surface of the nanoparticles at the end of the synthesis [18]. And for electronics, syntheses without or with few organic molecules are preferred to preserve the conductivity of the material. The equilibrium between command over nanoparticle characteristics, expense, and ecological effect differs considerably across the synthesis techniques. Highly pure nanoparticles with excellent control over size and shape are produced by physical approaches, like pulsed laser ablation or vapor phase deposition. However, these methods are energy-intensive and costly, and are rarely scalable [19]. Chemical methods, such as sol-gel, hydrothermal, or co-precipitation, offer good reproducibility, fine size distributions, and rapid reactions. They also allow easy dopant integration. However, these techniques frequently involve toxic solvents and hazardous waste, while complicating surfactant removal [20]. Green synthesis is different. Using plant extracts,

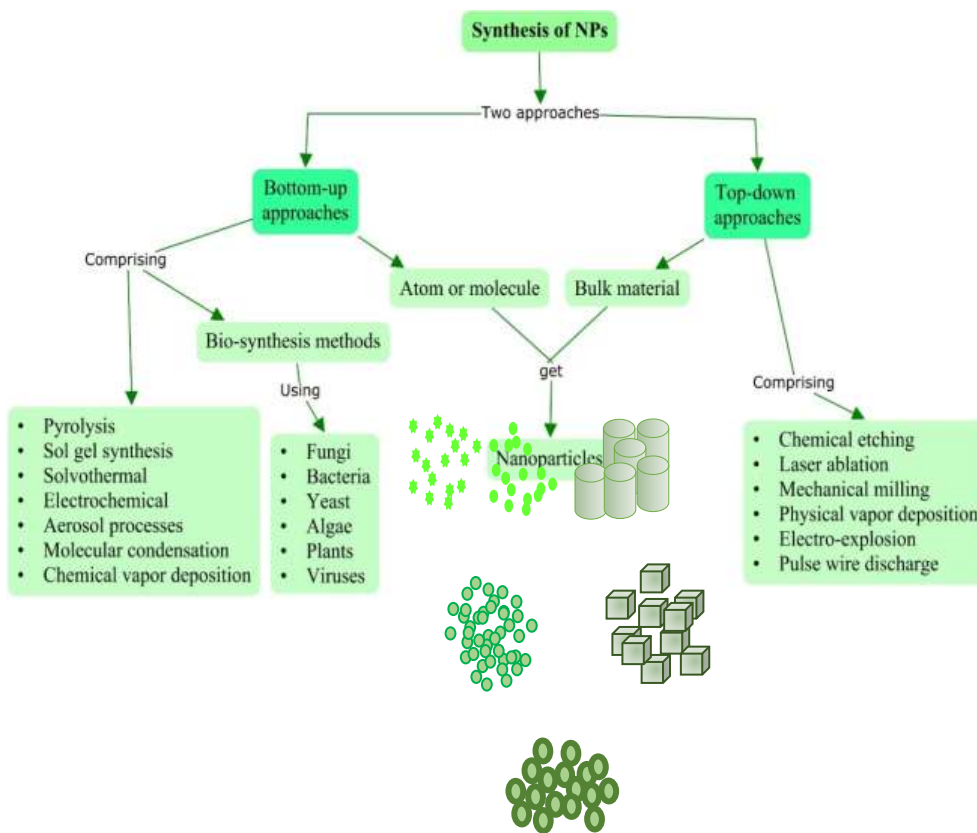


Fig. 2- An overview of the different techniques for the synthesis of nanoparticles.

microorganisms, or agricultural waste, it is eco-friendly, economical, and yields biocompatible nanoparticles under mild conditions. But control is limited. Particle size and shape are harder to manage, batch variability occurs due to seasonal changes in biological sources, and reaction times are often longer [21]. Despite these disadvantages, green synthesis remains the most sustainable and environmentally friendly approach. The different possible synthesis routes for the production of nanoparticles (NPs) are summarized in Fig. 2.

## 2.2. Nickel oxide (NiO) nanoparticles (NPs)

NiO NPs are nanoscale particles composed of nickel and oxygen atoms. They possess several unique properties due to their nanoscale size. One key property is their high surface area to volume ratio, which enhances their reactivity and catalytic potential [22]. In terms of classification, nickel oxide nanoparticles fall under the category of metal oxide nanoparticles, where the metal element in this case is nickel. They also exhibit semiconducting behavior, making them useful in electronic and optoelectronic applications. Nickel oxide nanoparticles (NiO-NP) have gained increasing popularity due to their exceptional range of properties, which make them well suited for a variety of applications. One of their greatest strengths lies in their chemical stability [6], which makes it resistant to both corrosion and oxidation. This property makes it adaptable to environments with aggressive conditions, like acidic or alkaline solutions. Depending on size and structure, NiO NPs may exhibit superparamagnetic, ferromagnetic, or antiferromagnetic behavior [23, 24]. As a result, it has proven to be beneficial in applications such as data storage devices, magnetic

sensors, drug delivery, and MRI (magnetic resonance imaging) [6]. Furthermore, NiO also possesses interesting optical properties, capable of a wide bandgap and strong absorption in the ultraviolet range. This characteristic allows it to be used in optoelectronics and solar cells, where it harnesses its capability to produce electron hole pairs to facilitate photochemical reactions [24]. Bulk NiO has a typical band gap ranging from 3.6-4.0 eV; however, it is common for NiO NPs to have a larger band gap (sometimes even above 4.0 eV) because of the Quantum Confinement Effect created through its nano-scale size. The ability to adjust this band gap based on particle size and shape provides for efficient use of visible light [5]. Additionally, the surface defects and oxygen vacancies that are present create intermediate energy states that enhance the separation of electron-hole pairs, thereby boosting the overall photocatalytic activity. Finally, NiO boasts excellent thermal stability, making it suitable for high temperature applications, including fuel cells and catalytic processes. With its diverse range of properties, it's no surprise that NiO has attracted interest and found a wide range of applications in catalysis, energy, environmental remediation and medicine in recent times.

The statistical data analysis in Fig. 3 illustrates the increasing trend of research papers published in the field of NiO nanoparticles. These data were collected from the "Scopus database" and limited to the years between 2014 to 2025 using the search term combination ("nickel oxide" OR NiO) AND (nanoparticles OR NPs) AND (green OR bio OR plant OR eco)". The research also concluded that the total number of publications will increase significantly from 2016 to 2024.

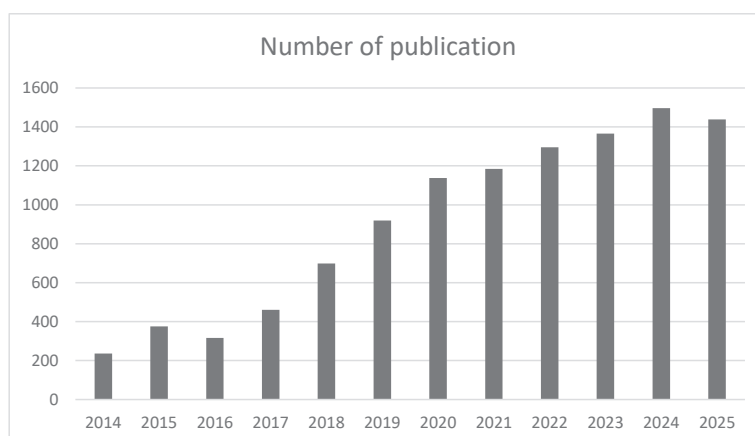


Fig. 3- Number of publications found in the Scopus database using the search term: "nickel oxide or NiO, nanoparticles or NPs and green or bio", allocated to year of publication (obtained 29/11/2025).

In terms of the environment, the use of nanomaterials is envisaged for the reduction of pollutant emissions, the treatment of effluents in particular by photocatalysis and the purification of gases, the production of ultra-pure water from sea water, wastewater, and air treatment [25]. The treatment of polluted water has become one of the major concerns of the human being, given its importance for the survival of every living species on this earth. Different treatment methods are developed to meet the daily needs of the frequent users, including settling, filtration, and adsorptions. Among the most used processes, adsorption which is the method of purification represents an option for the treatment of various types of effluent. Because of their dual-action mechanism, which combines adsorption and photocatalytic activity, NiO NPs are highly effective photocatalysts against dye pollutants in the field of environmental restoration. First, NiO NPs participate in adsorption, acting as molecular magnets to attract dye molecules to their surface and facilitate close contact for the subsequent photocatalytic destruction [7]. When the activated NiO NPs come into contact with light, they release reactive species such as hydroxyl radicals that effectively break down the adsorbed dye molecules. Their effectiveness in water treatment, air pollution control, and energy conversion has displayed significant potential, primarily due to

their ability to generate reactive oxygen species and maintain stability [26]. The results of these studies indicate that NiO nanoparticles possess favorable photocatalytic characteristics and can potentially be used as an effective solution for removing dyes from water, with a removal efficiency of up to 99.6% for Methylene blue (MB) dye [8].

### 2.3. Biosynthesis of NiO nanoparticles

The chemical synthesis of nanoparticles often involves the use of toxic chemicals, such as hydrazine and sodium borohydride as reducing agents, or toluene, methanol, and chloroform as volatile solvents. While these substances can produce high-purity nanoparticles, their toxicity raises significant environmental and safety concerns [27]. As a result, the development of alternative, greener synthesis methods has become increasingly important.

The utilization of biological sources, in particular plant extract, in synthesizing metal oxide nanoparticles involves the active participation of phytochemicals, which play a significant role in the chemical process [26]. By understanding the different roles played by phytochemicals in biosynthesis, researchers can optimize and customize the nanoparticle's properties, opening up new possibilities for use in various fields, such as medicine, electronics,

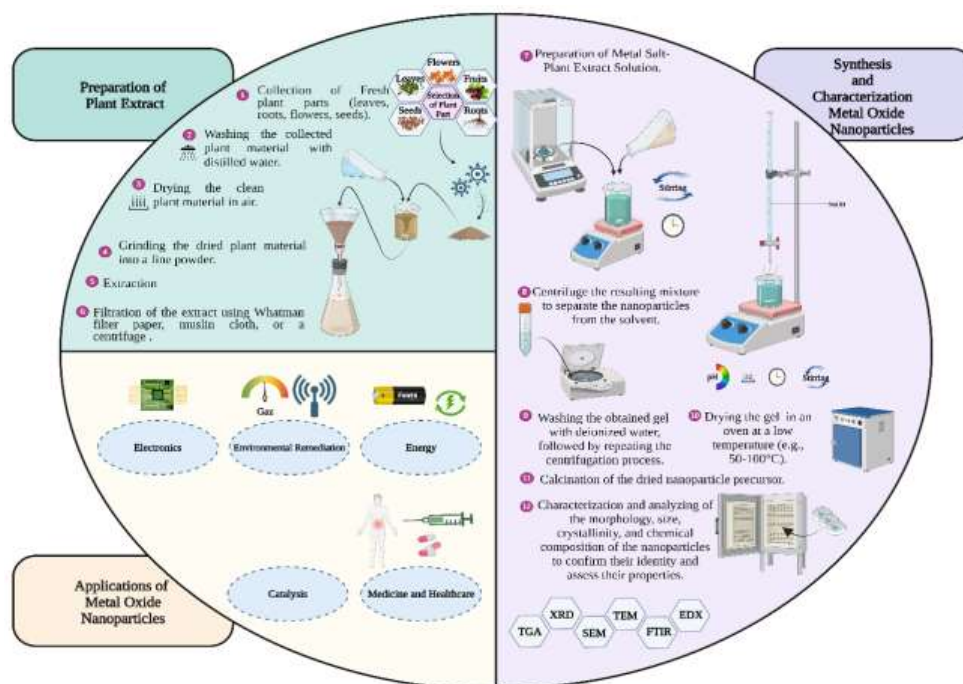


Fig. 4- Key steps in the biosynthesis of NPs using plants.

and environmental remediation. In this process, plant extracts act as reducing agents, capping agents, and/or stabilizers [8, 27]. As reductants, certain phytochemicals like flavonoids and phenolic compounds are used for their antioxidant properties. They donate electrons to metal ion precursors, leading to their reduction into metal nanoparticles. For example, hydroxyl groups in flavonoids and phenolic compounds can donate electrons to metal ions, facilitating their reduction [28]. As capping and stabilizing agents, they are often used to functionalize the surface and prevent particle aggregation [29]. These agents prevent the growth of nanoparticles, controlling their size, and shape, preventing particle aggregation or agglomeration [30]. They act by creating repulsive forces between nanoparticles, keeping them dispersed and separate from each other.

Several key parameters influence the biosynthesis

process, including [31]:

- The plant species used (each with its own reducing agents)
- The quantity of plant extract
- The precursor concentration
- The duration of the reaction
- The pH of the solution
- Calcination time and temperature

Characterization is a critical aspect of nanoparticle research, focusing on factors such as size, morphology, crystallinity, charge, and composition [31]. Common techniques for nanoparticle characterization include ultraviolet-visible spectroscopy (UV-Vis), scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR).

Fig. 4 provides a comprehensive overview of the biosynthesis process of nanoparticles using plants. It is divided into three main sections: preparation of plant extracts, synthesis protocol, and characterization.

Table 1- The various sources used by the researchers for the biosynthesis of NiO nanoparticles

Year	Parts	Green sources	Precursor	Size (nm)	Crystalline structure	Ref
2016	Yeast	Rhodotorula Mucilaginosa	NiCl <sub>2</sub> .6H <sub>2</sub> O	5.5	Spherical	[32]
2019	/	Chitosan	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	10–30	Spherical	[33]
2020	Leaves	Ananas Comosus	NiCl <sub>2</sub> .6H <sub>2</sub> O	5.75	Cubic	[34]
2020	/	Egg White	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	67.5	Cubic	[35]
2021	Leaves	Capparis Decidua	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	16.75	Cubic	[36]
2021	Root	Curcuma Longa	Ni(OCOCH <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O	28	Spherical	[37]
2021	Gum	Arabic Gum	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	59	Spherical	[38]
2021	Stem	Berberis Balochistanica	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	31.44	Rhombohedral	[39]
2021	Algae	Marine Algal	NiCl <sub>2</sub> .6H <sub>2</sub> O	32.64	Spherical	[40]
2021	Seed	Nigella Sativa	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	10–50	Spherical	[41]
2022	Algae	Sargassum Wightii (Marine Brown)	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	48.11	Hexagonal	[22]
2022	Leaves	Phytolacca Dodecandra L'herit	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	14.18	Cubic	[42]
2022	Leaves	Camellia Sinensis	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	26	Spherical	[43]
2022	Cell free (biomass)	Spirogyra Sp	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	27.7	Cubic	[44]
2023	Flower Buds	Syzygium Aromaticum	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	39.7	Cubic	[9]
2023	Leaves	Moringa Oliefera	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	9.38	Cubic	[28]
2023	Leaves	Psidium Guajava	NiCl <sub>2</sub> .6H <sub>2</sub> O	21.20	Cubic	[30]
2023	Bacterial strain	Shewanella Species	NiCl <sub>2</sub> .6H <sub>2</sub> O	20	/	[45]
2023	Leaves	Acacia Nilotica	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	16	Cubic	[46]
2024	Lichen	Parmelia Perlata	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	40–50	Cubic	[47]
2024	Leaves	Azadirachta Indica (Neem)	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	10.5	Cubic	[48]
2020	Leaves	Stevia	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	20 - 50	Spherical	[49]
2025	Leaves	Salvia officinalis	Ni(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	16	Spherical	[50]
2026	Leaves	Indian Almond (T.catappa)	NiSO <sub>4</sub> .6H <sub>2</sub> O	20	Spherical	[51]

Analysis of the compiled literature (Table 1) reveals key trends in biosynthesis outcomes. Plant-mediated synthesis predominantly yields NiO NPs with spherical or cubic morphology, with sizes typically ranging from 10 to 50 nm. This range, however, highlights a central challenge: while biosynthesis is effective, achieving precise and uniform control over size and shape is less straightforward than with top-down physical methods. The diversity of biological sources, from leaves (e.g., Neem, Psidium Guajava) to non-traditional agents like egg white or sheep dung, directly influences the phytochemical profile acting as capping agents, thereby affecting nucleation, growth, and final nanoparticle characteristics such as crystallinity (cubic vs. hexagonal) and surface functionality [26].

#### 2.4. Factors influencing the morphology of biosynthesized NiO NPs

The nano-sized metallic oxide objects have extraordinary properties that are quite different from bulk materials. Most of the physical and chemical properties of these nanoparticles depend on their size and shape. To obtain non-agglomerated nanoparticles with well-controlled average size and narrow size distribution, the development of synthesis methods is imperative. The efficiency of the nanoparticle synthesis technique is measured by the quality of the particle size control obtained, the crystalline quality, and the morphology of the nanoparticles as well as the quality of their surface state [52]. The biosynthesis of nanoparticles (NPs) is highly sensitive and depends on several key operational parameters, regardless of the technique

used. Factors such as pH, precursor concentration, reaction time, volume ratio, agitation, light exposure, temperature, and aeration must be carefully managed [53]. Youcef Messai et al. [54] show that pH has no significant effect on the evolution of the average size of NiO NPs crystallites; this could imply that other factors influence crystallite size, and that pH alone is insufficient to fully explain the result. Optimizing a biological method is a complex and time-consuming process that demands substantial resources. The use of living organisms adds another layer of complexity, making it difficult to standardize and replicate protocols, as the internal conditions can vary widely between species and even among individuals of the same species.

#### 2.5. Morphological and Structural Hallmarks

The biosynthesis parameters discussed directly govern the resulting nanoparticle characteristics. As evidenced by the data in Table 1, plant-mediated synthesis consistently produces NiO NPs with defined morphologies, primarily spherical and cubic, and sizes typically within the 10-50 nm range. These morphological and structural attributes are routinely confirmed using characterization techniques such as Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) for direct visualization, and X-ray Diffraction (XRD) for determining crystalline phase and size. The prevalence of the cubic structure across many studies suggests a thermodynamic preference under common biosynthesis and calcination conditions.

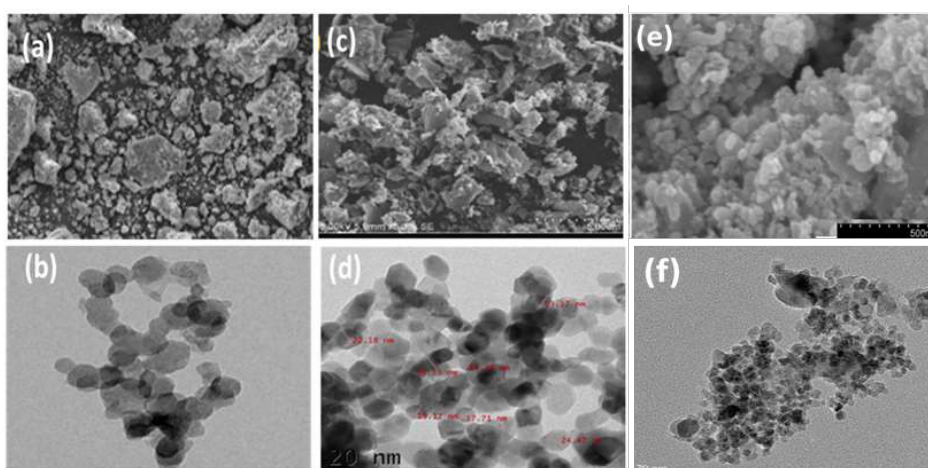


Fig. 5- SEM and TEM micrographs of biosynthesized NiO NPs from selected studies. (a) SEM and (b) TEM of NiO NPs from *Curcuma longa* root extracts (28 nm) [37], (c) SEM and (d) TEM of NiO NPs from *C. gigantean* leaves extract (31 nm) [55], (e) SEM and (f) TEM of NiO NPs from *Stevia* leaf extract (20 nm) [49].

To give a real sense of what biosynthesised NiO NPs actually look like, we compiled eight typical SEM and TEM images from four studies in Table 1 (root, leaf and seed extracts only). Figure 5 shows how different they look, even though they are all 'green' NiO. *Curcuma longa* root produces fairly large particles measuring around 28 nm [37]. *Calotropis gigantea* leaves produce particles of a similar size (~31 nm) [55]. *Stevia rebaudiana* leaf extract produces particles measuring from 20 to 50 nm with notably spherical morphology [49]. You can literally see the organic coating in some of the TEM shots. This variation in particle size and morphology is not random but rather reflects the differing phytochemical compositions inherent to roots versus leaves of distinct plant species, which influence reduction kinetics and surface stabilization during nanoparticle formation.

## 2.6. Surface and Optical Traits

Beyond size and shape, the surface chemistry of biosynthesized NiO NPs is a critical property imparted by the biological extract. Fourier Transform Infrared (FTIR) spectroscopy often confirms the presence of organic functional groups (e.g., -OH, C=O) from phytochemicals on the NP surface. This biological corona enhances colloidal stability, improves biocompatibility, and can influence interfacial interactions in applications. Optically, biosynthesized NiO NPs typically exhibit a bandgap energy between 3.2 and 4.0 eV, as determined by UV-Vis spectroscopy [13]. This positions them as promising visible-light-active photocatalysts, as their electronic structure allows for the absorption of photons to generate electron-hole pairs necessary for redox reactions [56].

These inherent properties: size, shape, crystallinity, and bandgap, are the fundamental determinants of the photocatalytic and antibacterial efficacy discussed in the following sections.

## 3. Photocatalytic Applications of NiO biosynthesis

While the field of photocatalysis shows promise, there is still much to discover regarding the potential of nanomaterials. An area of particular interest within this field is the effective removal of dyes from wastewater, where nanomaterials play a vital role in minimizing environmental impact. This emphasizes the significant need to persistently conduct thorough research in order to further progress and development in this rapidly evolving domain [57]. Research and development in the field of photocatalysis is continuing apace. The aim is to develop more efficient, more stable, less costly, and more environmentally friendly photocatalysts for use in industrial wastewater treatment. Dyes

are widely used in sectors such as printing, food, cosmetics, and textiles. However, once discharged into the environment, these dyes become a major source of wastewater pollution, endangering aquatic life and challenging traditional treatment methods. Photocatalysis is the solution to this problem. Photocatalysts, made from metallic oxide (MO) NPs, react chemically with light to break down dyes into less harmful substances.

MO nanoparticle-based photocatalysts utilize light energy (UV-light) to initiate chemical reactions on their surface. The process begins with the absorption of photons by the nanoparticles, which excites an electron from the valence band to the conduction band, creating electron-hole pairs ( $e^-/h^+$ ). These holes migrate to the surface and react with water molecules, forming hydroxyl radicals ( $OH^\cdot$ ), which are potent oxidants that break down dye molecules into less harmful compounds [5]. Meanwhile, the remaining electrons on the nanoparticle surface react with dissolved oxygen to produce superoxide anions ( $O_2^{\cdot-}$ ). This sequence of reactions effectively degrades dyes, as illustrated in Fig. 6.

Several research papers [8, 23, 55] have investigated how NiO NPs perform as photocatalysts against a range of dyes, including Methyl Orange (MO) dye, Rhodamin B, azo dyes, and Methylene Blue (MB) dyes. The results of these studies indicate that NiO nanoparticles possess favorable photocatalytic characteristics and can potentially be used as an effective solution for removing dyes from water. It is important to note that the specific degradation mechanism of dye solutions by NiO nanoparticles may vary depending on factors such as the properties of the dye molecules, the size and surface characteristics of the NiO nanoparticles, and the reaction conditions. The degradation mechanism of dye solutions by NiO NPs in photocatalysis involves several key steps and can involve the following steps:

a) Light absorption: The process involves the absorption of light photons, which leads to the formation of electron-hole pairs within the nickel oxide (NiO) nanoparticles. These nanoparticles have the ability to absorb light energy, primarily in the ultraviolet (UV) or visible range, depending on their specific bandgap energy. As a result of photon absorption, electron-hole pairs are generated within the NiO nanoparticles [42, 58].

b) Generation of reactive species: Similar to other metal oxide photocatalysts, NiO NPs can generate reactive oxygen species (ROS) such as hydroxyl radicals and superoxide radicals, by

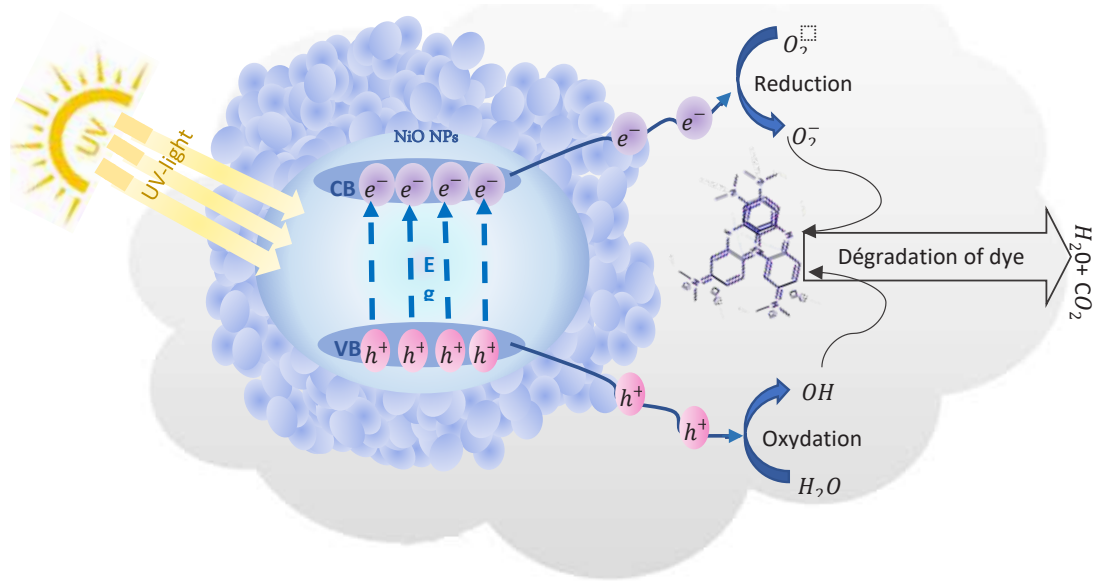
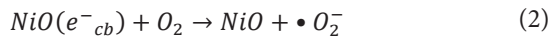
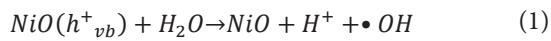
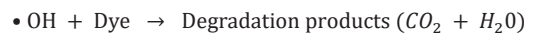
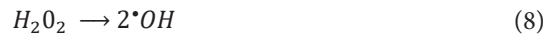
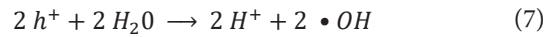
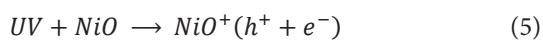


Fig. 6- Photocatalytic mechanism of NiO NPs.

the recombination of the electron-hole pairs or by interactions with water or oxygen molecules [46, 59]. The holes in the valence band (VB) can react with water molecules to produce hydroxyl radicals (eq.1), while the electrons in the conduction band (CB) can react with dissolved oxygen to form superoxide radicals (eq.2). Subsequent reactions lead to the formation of hydroxyl radicals, as shown in eqs. 3 and 4.



c) Pollutant degradation: The generated reactive species, especially hydroxyl radicals, are highly oxidative and can react with organic pollutants present in the wastewater. The hydroxyl radicals attack the organic molecules, breaking their chemical bonds and leading to the degradation of the pollutants into smaller and less harmful compounds, such as carbon dioxide and water [60, 61]. The photocatalytic mechanism for NiO nanoparticles is described in eqs. 1–12:



In order to use light to remove dyes from water, the scientists looked for the best nanoparticles. Giannakis et al. [62] showed that certain NPs based on semiconductors with energy gaps ranging from 1.7 to 5 electronvolts are highly efficient photocatalysts. Their ideal band gap allows electrons to receive the necessary energy from light to participate in the degradation of pollutants. Sunil Kumar and his team [23] investigated how well lime-made NiO NPs could break down methylene blue with light. They found that after 90 minutes of light exposure, the nanoparticles degraded almost all the dye (99.64%). The dye molecules first stick to the NiO NPs, and then the light breaks them down. In another study, Shubha and her colleagues [63] made NiO NPs using sheep dung as fuel. When exposed to ultraviolet (UV) light, these nanoparticles eliminated an impressive

97.7% of the methylene blue dye. Regular light wasn't nearly as effective, only removing 19% of the dye in the same amount of time (100 minutes). They also found similar results with another dye, malachite green. UV light broke down almost all (96.8%) of the dye with the NiO NPs, while regular light only degraded 18%. The study by Sundaresan and Ravichandran [64] used *Evolvulus alsinoides* leaf extract to create NiO nanoparticles. They tested how well these nanoparticles could break down another dye called Rhodamine B (RhB) with light. The nanoparticles were able to get rid of up to 85% of the RhB dye after 90 minutes of UV light exposure.

Diallo et al. [13] conducted a study on the use of biosynthesised NiO nanocrystals produced using *Aspalathus linearis* (Rooibos) leaf extract, which is native to the Western Cape Province of South

Africa. The researchers focused on the degradation of methylene blue (MB) when exposed to UV light. They discovered that NiO nanoparticles synthesized at higher temperatures (400 °C) exhibited significantly better photocatalytic performance than those synthesized at lower temperatures (300 °C). According to them, the improved efficacy of NiO-400°C can be attributed to its enhanced crystallinity, which facilitates more efficient generation and separation of electron-hole pairs when exposed to light. These electron-hole pairs actively contribute to the degradation process. In addition, the presence of oxygen vacancies in NiO-300°C resulted in the re-coloring of degraded MB (LMB) back to its original form under visible light. Several studies (Table 2) demonstrate that NiO nanoparticles synthesized from biological materials hold significant potential for using light to clean polluted water.

Table 2- Application of biosynthesized NiO nanoparticles as a photocatalyst

Biological source	Average size (nm)	Band-gap (eV)	Irradiation source	Environmental application	Degradation efficiency %	Degradation time	Ref
Limonia accidissima natural fruit juice	25	3,4	UV light	Methylene blue	99,6	90 mn	[23]
Moringa oleifera leave	21.6	3.32	UV-light	Safranin dye	92,75	180mn	[28]
				Methyl orange	98,02		
Polymer : Arabic (gum)	59	3.30	UV light	Methylene blue	82	270 mn	[38]
<i>Shewnella</i> spp	20		Sunlight	Methylene blue	82.36	4h	[45]
C.Gigantea (leaves)	31	3,4	UV-light	Methylene blue	98	180 mn	[55]
<i>Andrographis paniculata</i> (leaves)	24	3.51	Sunlight	Evans blue	88.13		[65]
<i>Rheum turkestanicum</i> (root)	15		UV light	Rhodamine blue	95	30h	[66]
<i>Punica granatum</i>	25	3,5	Visible light	Methylene orange	90	60mn	[67]
<i>Lantana camara</i> (leaves)	21		UV light	Methylene blue	85	120mn	[68]
of <i>Salvia hispanica</i> L. (chia) seeds	30	3,47		Methylene blue	83	150 mn	[69]
<i>Tinosphora cordifolia</i>	19,5	3,14	UV-light	Tetracycline (TC)	99.4	30mn	[70]
<i>Trachyspermum ammi</i> (seed)	29.8	3.1	UV-light	Allura red	91.73	90mn	[49]
Aloe vera (AV) leaf	29	3,05	UV light	Methylene orange	82	120mn	[71]
<i>Citrus medica</i> leaf	12,1	3.1	visible light	Methylene orange	98	60mn	[72]
<i>Alpurnia Aurea</i> leaf	17.61		visible light	Malachite green	98.17	30 mn	[73]
Olive Leaf	17	5,2	solar light	Crystal violet dye	95	40 mn	[74]
<i>Pseudochrobactrum</i> sp	14,51	3.70	solar light	4-nitrophenol	65	25 mn	[75]

The percentage of dye degradation was determined using the formula [65]:

$$D\% = \frac{C_0 - C_t}{C_0} \times 100 \quad (13)$$

In this equation, D% indicates the degradation percentage,  $C_0$  is the initial dye absorbance (mg/l), and  $C_t$  represents the absorbance of the dye at various intervals during the degradation process (mg/l).

The photocatalytic performance summarized in Table 2 cannot be dissociated from the NPs' physicochemical properties. For instance, smaller NPs (< 20 nm) generally offer a higher surface area for dye adsorption and ROS generation. The study by Diallo et al. [13] explicitly correlated enhanced crystallinity (achieved at higher calcination temperature) with improved charge carrier separation and thus higher MB degradation. Furthermore, the presence of plant-derived capping agents can sometimes mediate charge transfer or influence the adsorption step, underscoring the complex structure-activity relationship in biosynthesized catalysts.

Optimizing the catalytic activity of biosynthesized metal oxide nanoparticles requires a good understanding of how different parameters interact. To truly capture the optimal conditions for each application, it is often useful to combine laboratory experiments with numerical simulations. For example, Moussa Boudiaf and his team [41] found that NiO nanoparticles synthesized from *Nigella sativa* seed extract exhibit catalytic activity that varies with catalyst mass and solution pH. They observed that degradation is faster with 5 mg of NiO at pH 9 compared to 3 mg, highlighting the importance of these parameters in improving process efficiency. The pH plays a vital role in the photocatalytic performance of green-synthesized NiO nanoparticles, as observed by Siddique et al. [76]. Free electrons are involved in dye degradation, and the electrons will not generate rapidly at acidic nor neutral medium which may be the reason that dye degradation did not occur. It is noted from this study that alkaline condition favored dye degradation; this was attributed to pH factor since it control the surface charge of NiO as well as adsorption of cationic dyes. The maximum performance was achieved in the pH range between 7 and 9, where the dye-surface interaction is more applicable. At low pH, aggregation of nanoparticles and the restraining effect of radicals reduce degradation. Whereas, at pH greater than 9 partial hydrolysis

and formation of  $\text{Ni}(\text{OH})_2$  occurred.

#### 4. Antibacterial Applications of NiO bio synthesis

Metal oxide nanoparticles (NPs) have shown good antimicrobial activity, depending on several factors such as size, stability, and concentration in the growth medium. In general, the size of bacterial cells is in the micrometer range, while their outer cell membranes have nanoscale pores. Since nanoparticles can be smaller than these pores, they have the unique ability to cross the cell membrane [77]. As a result, metal oxide nanoparticles can be considered as antibacterial agents that can specifically target and inhibit the growth of bacteria, either by killing them (bactericides) or by stopping their growth and reproduction (bacteriostats). These antibacterial agents generally use one of three main mechanisms to combat bacteria [78].

-Inhibiting Cell Wall Synthesis: Some agents prevent bacteria from constructing their protective cell walls, leaving them vulnerable and ultimately causing them to burst and die.

-Disrupting Protein Production: Other agents interfere with the bacteria's ability to produce essential proteins, crucial for their survival.

-Inhibiting DNA Replication: Certain agents block the replication of bacterial DNA, preventing bacteria from reproducing and eventually leading to their death.

Fig. 7 provides an illustration of the antimicrobial mechanism of NiO nanoparticles (NPs).

Researchers are testing the antibacterial potential of biosynthesized NiO NPs in a controlled laboratory environment. The process starts with the preparation of NiO nanoparticles tailored to specific properties. Suitable bacterial strains are then selected for testing. Two types of bacteria can be used: gram-positive bacteria, which have a thick layer of peptidoglycan, and gram-negative bacteria, which have a thinner layer of peptidoglycan but an additional outer membrane [79]. Culturing the selected bacteria in appropriate growth media is essential to ensuring their viability during the experiments. Once the bacterial cultures are ready, they are exposed to different concentrations of nickel oxide nanoparticles to assess their antibacterial effects. These initial steps lay the groundwork for further investigations into the nanoparticles' ability to combat bacterial growth and pave the way for additional tests to understand their mechanisms of action and potential applications [80]. Commonly used methods to test the antibacterial potential of nanoparticles include:

-Disc diffusion assay: Nanoparticles diffuse from

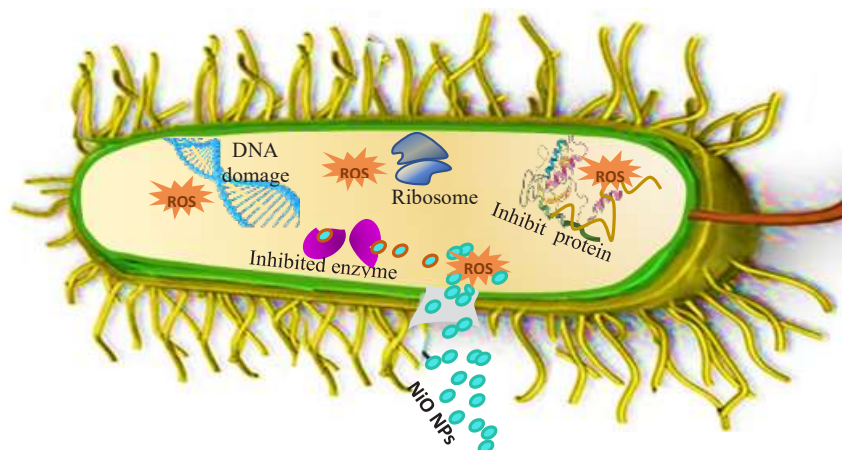


Fig. 7- The antimicrobial mechanism of NiO nanoparticles.

a filter paper disc onto an agar plate, inhibiting bacterial growth and producing a visible zone of inhibition.

-Broth microdilution and viable plate count assays: These help to determine the minimum inhibitory and bactericidal concentrations by exposing bacterial cultures to different nanoparticle concentrations.

-Time-kill assay: This method tracks the rate and extent of bacterial killing over time, providing insight into the dynamics of antibacterial activity and helping to optimize nanoparticle concentrations for effective results.

In many studies, it has been reported the importance of carefully selecting the biological agent in the biosynthesis of NiO nanoparticles to achieve optimal antibacterial properties. Muhammad Hafeez et al. [81], tested the antibacterial activity of NiO nanoparticles synthesized using *Populus ciliata* leaf extracts. They evaluated the effectiveness against both Gram-positive bacteria (*Bacillus subtilis*, *Bacillus licheniformis*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*). *Bacillus subtilis* showed the larger inhibition zone than *Klebsiella pneumoniae*. The study also found that increasing the concentration of NiO NPs enhanced their antibacterial effectiveness. In a recent study, NiO nanoparticles biosynthesized with *Tribulus terrestris* demonstrated significantly greater antibacterial effectiveness against *E. faecalis*, while

showing moderate activity against *P. Aeruginosa* [82]. Samia Q. Alghamdi and colleagues [80] successfully utilized olive leaf waste for the green synthesis of NiO nanoparticles. The antimicrobial activity of these NiO-olive nanoparticles was tested against waterborne pathogens. They showed strong activity against Gram-positive bacteria and fungi, but only moderate efficacy against Gram-negative bacteria, which are more drug-resistant due to their protective outer membrane, higher levels of transport proteins, and a protective slime layer.

The antibacterial efficacy (Table 3) is profoundly influenced by NP morphology and surface charge. Smaller nanoparticles exhibit greater membrane penetration capability, while the organic corona from plant extracts can facilitate interaction with bacterial cell walls. The generally lower activity against Gram-negative bacteria (e.g., *Pseudomonas aeruginosa*) compared to Gram-positive ones highlights the role of the outer membrane as a barrier, an effect modulated by NP surface properties. Variability in inhibition zones for the same bacterium across studies often traces back to differences in NP size, concentration, and surface chemistry inherent to the biosynthesis method.

Table 3 displays a list of various biosynthesized NiO nanoparticles, along with their different zones of inhibition for both Gram-negative and Gram-positive bacteria.

Table 3- Antibacterial applications of biosynthesis NiO nanoparticles

	Precursor	Biological source	Bacteria	zone of inhibition (mm)	Average size (nm)	Band-gap (eV)	Ref
Gram-positive bacteria	Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Solanum trilobatum	Staphylococcus aureus	21	23.21	3.59	[83]
		Solanum trilobatum	Streptococcus pneumoniae	12	23.21	3.59	[83]
	NiSO <sub>4</sub> ·6H <sub>2</sub> O	Trigonella subenervis	Streptococcus pneumoniae	24.4 mm	26.43		[84]
	Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Jatropha gossypifolia	Staphylococcus aureus	6.5	36.94		[85]
	Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Piper betle	Bacillus subtilis	25		2.50	[86]
	Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Orange leaf	Staphylococcus aureus	32	20.34		[87]
	Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Aloe vera gel	Bacillus subtilis	23		3.2	[88]
	Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Acacia nilotica	Staphylococcus aureus	8.7	16		[46]
		Acacia nilotica	Bacillus subtilis	13.7	16		[46]
	Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Parmelia Perlata lichen	Staphylococcus aureus	19	40-50		[47]
	Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Moringa Oleifera leaves	S. mutans	14	9-43		[89]
	NiSO <sub>4</sub> ·6H <sub>2</sub> O	leaf Lagerstroemia speciosa, LS	Staphylococcus aureus	17.67	23.71	3.88	[91]
	NiSO <sub>4</sub> ·6H <sub>2</sub> O	Leaf Bombax ceiba, BC	Staphylococcus aureus	11.11	40.17	3.71	[91]
	NiSO <sub>4</sub> ·6H <sub>2</sub> O	root extracts of Piper chaba PC	Staphylococcus aureus	12	26.46	3.84	[91]
	Nickel (II) acetate	Curcuma longa root	E. coli,	19	28	2,6	[34]
	Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Acacia nilotica	Escherichia coli	11.5	16		[46]
	Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Acacia nilotica	Pseudomonas aeruginosa	10.5	16		[46]
	Gram-negative bacteria	Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Solanum trilobatum	Escherichia hermannii	9	23.21	3.59
Solanum trilobatum			Escherichia coli	18	23.21	3.59	[83]
Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O		Trigonella subenervis	Escherichia coli	20.1	26.43		[84]
Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O		Jatropha gossypifolia	Klebsiella species	3.5	36.94		[85]
Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O		Piper betle leaf	Escherichia coli	20	-	2.50	[86]
Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O		Orange leaf.	Escherichia coli	25	20.34		[87]
Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O		Aloe vera gel	Pasturella multocida	19		3.2	[88]
Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O		Arachis hypogaea (peanut) shell	Proteus vulgaris	10.25	20	3	[90]
Ni(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O		Moringa Oleifera	E. coli	13	9		[89]
NiSO <sub>4</sub> ·6H <sub>2</sub> O		leaf Lagerstroemia speciosa, LS	Escherichia coli	8.83	23.71	3.88	[91]
NiSO <sub>4</sub> ·6H <sub>2</sub> O	Leaf Bombax ceiba, BC	Escherichia coli	12.28	40.17	3.71	[91]	
NiSO <sub>4</sub> ·6H <sub>2</sub> O	root extracts of Piper chaba PC	Escherichia coli	14.47	26.46	3.84	[91]	

## 5. Conclusion

This review explains how green synthesis methods help reliably produce NiO NPs with clear structural and functional properties. Studies show that the phytochemical compounds serve as effective reducing and stabilizing agents. They directly affect the shape and crystallinity of the nanoparticles. The resulting NiO NPs have strong, broad antibacterial activity. They work even against resistant strains like *Staphylococcus aureus* and *Escherichia coli*. This is mainly due to producing

reactive oxygen species (ROS) and damaging cell membranes. In addition, their photocatalytic ability to break down pollutants such as methylene blue and industrial dyes under visible light has been consistently proven ; most recent papers report degradation rates regularly above 90 % in less than two hours.

That said, we are still a long way from seeing these particles used outside the lab on a large scale. The same plant collected in summer or winter, or grown in two different regions, can give

noticeably different results. Long-term toxicity studies in mammals are still very scarce. Also, techno-economic studies are needed to confirm the environmental and financial benefits of these green methods.

Future research should focus on:

- agree on simple, robust protocols to prepare and store plant extracts ,
- run proper in vivo toxicity studies,
- Developing NiO-based heterojunctions and nanocomposites to sunlight-driven photocatalysis,
- test continuous-flow bioreactors for industrial scale production.

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