# Chapter 9
# Nanobiofertilizers: Applications, Crop Productivity, and Sustainable Agriculture

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## 1 Introduction

Agriculture is an inevitable sector that provides raw materials mainly for food and feed industries and other sectors like fuel, furniture, and feedstock industries. Agricultural productivity is challenged by different reasons such as unavailability of space, plant diseases, and abrupt climatic changes in environmental conditions. These severe issues demand a technique for reducing the inevitability of old techniques and developing modern practices that focus on improved agricultural productivity (Yunlong & Smit, 1994). Nanotechnology can be used for the sustainable growth of agriculture as it is a new, smart, and innovative technique with different applications (Tilman et al., 2002). The use of nanotechnology in agriculture is made possible by making necessary advancements in isolating and characterizing nanomaterials in a particular way forming nanoparticles with remarkable properties (Bandyopadhyay et al., 2013). The physical and chemical properties of NPs depend on unusual optical, physical, and biological features corresponding to materials employed in the synthesis of organic, inorganic, metal, and hybrid nanoparticles. Biofertilizers are mainly composed of live formulations of beneficial microbes that, when applied to seed, leaf, or soil, enhance plant growth by providing increased nourishment for the plants (Nanjwade et al., 2011; Thomas et al., 2013). Nanomaterials (NMs) are effective in agricultural fields with specific compositions,

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sizes, and properties that can be of natural or synthetic origin (Puri et al., 2009). Different NMs have enhanced earlier plant germination as well as plant production through the modulation of plant gene expression and associated biological pathways. It also depends on the plants and varies with different stages of plant growth, method of administration, and exposure time. Agricultural practices like the use of fertilizers and pesticides enhanced productivity but created serious and even life-threatening aftereffects. There arises the importance of practices that increase growth and yield-reducing issues like nanotechnology, where different techniques like nanoformulations of agrochemicals, nano biosensors, nanodevices, and nanoarrays are utilized (McLoughlin et al., 2011; Mir et al., 2018). The entry of metal complexes into the cell is facilitated by the movement of negatively charged compounds through the membrane with a negative charge (Tandy et al., 2006). There are different examples explaining the importance of NPs in the agricultural field. The compound aluminum (Al) oxide has a phytotoxic effect on root elongation, but loading this nano-Al with different percentages of phenanthrene reduced this inhibitory impact, suggesting slightly reduced root elongation in the presence of NP-coated phenanthrene (Yang & Watts, 2005). On the other hand, the seed treated with titanium dioxide ($TiO_2$) NPs enhanced the physiological properties of spinach, increasing the germination rate, chlorophyll, plant dry weight, and photosynthesis rate (Yang et al., 2006).

Biofertilizers are biological compounds with live microbes that are applied to seeds, plant surfaces, or soil and that promote plant growth through various mechanisms. Biofertilizers are products that, when added to the soil, contain microorganisms that are essential for soil fertility and plant growth. Biofertilizers colonize the rhizosphere, or interior, of plants when applied to the leaf surface, seeds, or soil and promote growth by controlling the amount or availability of primary nutrients to the plant host. Organic fertilizers contain chemicals and live microorganisms that provide nutrients to plants through natural processes such as nitrogen fixation, phosphorus solubilization, and the production of growth-promoting chemicals. They help to bring back the natural nutrient cycle, thereby increasing organic matter in the soil. Applying biofertilizers can improve soil sustainability and health while growing healthy crops. Biofertilizers may reduce the need for synthetic fertilizers and pesticides, but they cannot completely replace chemical fertilizers (Kole et al., 2013).

The process involving polymeric materials in which microbes are entrapped to produce beads that are permeable to various gases, nutrients, and metabolites to maintain cell viability is called encapsulation. Encapsulation provides good protection of the active substance against aggressive environmental influences. For the encapsulation process, different polymers like gelatine, starch, cellulose, etc., are used. Bioformulations are found in liquid and solid forms, but dry formulations are preferred over wet formulations because of their increased shelf life and ease of storage and transport. Micellar-enhanced ultrafiltration is used to separate organic compounds like thuringiensin dissolved in aqueous streams (John & Boppart, 2011).

Biofertilizers are formulations comprising one or more microorganisms that can enhance the productivity of soil by fixing atmospheric nitrogen and solubilizing phosphorus, which in turn stimulates plant growth. The integration of biofertilizers with nanoparticles to improve the growth of plants can be defined as nanobiofertilizers. Different strains of bacteria rely on different mechanisms, such as nitrogen fixation, potassium or phosphorus solubilization, phytohormone production, and degradability, in order to improve the uptake of nutrients, soil fertility, and yield improvement. The use of biological fertilizers is a mainstream scientific activity in developing sustainable agriculture, as they help overcome the shortcomings caused by chemical-based farming methods. The stability of biofertilizers can be enhanced by using nanoformulations resistant to desiccation, heat, and radiation (Zulfiqar et al., 2019).

## 2 Objectives

This chapter revolves around the formulation of different nanobiofertilizers involved in plant growth and stress mitigation. The mechanism of action of nanobiofertilizers employed in the application of plants and the synthesis and characterization of different nanoparticles are discussed. The entire chapter gives an idea of why application of nanobiofertilizers is helpful in sustainable agriculture and crop productivity.

## 3 Encapsulation in Nanoparticles

Encapsulation of microorganisms beneficial for plants has shown an increase in the availability of nitrogen, phosphorus, and potassium in the root area. In the past decade, techniques have been standardized to create beads that coat or entrap microbial cells with polymeric materials to maintain cell viability by rendering them permeable to nutrients, gases, and metabolites (John & Boppart, 2011). Encapsulation is divided by size into macroencapsulation (a few millimeters to a few centimeters in size) and microencapsulation (size 1–1000 μm, generally less than 200 μm) (Nordstierna et al., 2010). The active agent involved is protected by encapsulation using starch or cellulose from harsh environmental factors (Chang et al., 2000; Cheze-Lange et al., 2002). The utilization of different dyes also helps in increasing the viability of microbes (Cohen et al., 1990). Although wet formulations have better shelf life and storage transport properties, they are less preferred compared to dry formulations (Burges & Jones, 1998). The increasing demand for new formulations to substitute chemical pesticides and fertilizers has attracted the attention of researchers and new avenues in this direction are being explored to create cheaper and more effective technologies. Micellar-enhanced ultrafiltration (MEUF) is an example for an advanced technique used to separate dissolved organic compounds like thuringiensin from aqueous streams of Bt-based products commercially (Tzeng et al., 1999). For these plant growth-promoting bacteria-based formulations, in situ product removal (ISPR), which is biochemical product removal during the fermentation process, has been successfully applied in the removal of Bt toxin proteins (Agrawal & Burns, 1996), whereas crossflow microfiltration (CFM) has been utilized for the extraction of all kinds of proteins and the harvest of recombinant yeasts (Hwang & Chang, 2004). Macroencapsulation technology has advantages over microencapsulation (Desai et al., 2022). Encapsulation adequately protects the active ingredient from aggressive environmental influences. Cellulose gelatine, starch, and other polymers are currently used for drug encapsulation (Amiet Charpentier et al., 1998; Chang et al., 2000; Cheze-Lange et al., 2002). Protection can be improved to some extent by coating the capsule with a dye (Cohen et al., 1990; Schoebitz et al., 2013).

### *3.1 Nanoemulsions*

A wide range of natural and synthetic ingredients such as oil, surfactants, cosurfactants, weighting agents, ripening inhibitors, thickeners, or gelling agents are used to create a simple and highly efficient pharmaceutical delivery system for encapsulation. Nanoemulsions are about ten to several hundred nanometers in size. Nanoemulsions have been shown to be beneficial for the bioavailability of some types of essential substances by increasing their bioactivity in agrochemicals. There are also stable particle aggregations and gravitational separations.

### *3.2 Nanolipid Carriers*

Nanolipid carriers are formulations of solid lipids and oils called nanostructured lipid carriers. They are advanced lipid-based nanocarriers that perform better than classical nanoemulsions due to lower leakage of entrapped bioactive ingredients and improved control of the size and release process.

## 4 Formulation of Nanobiofertilizer

The formulations comprising one or more microorganisms that can enhance the productivity of soil by fixing atmospheric nitrogen and solubilizing phosphorus, which in turn stimulates plant growth, are called biofertilizers (Kole et al., 2013). Therefore, the integration of biofertilizers with nanoparticles to improve the growth of plants can be defined as nanobiofertilizers (Simarmata et al., 2016). Nanobiofertilizers can be effectively employed for improving nutrient utilization and soil fertility and thereby increasing yields through increased nitrogen fixation, potassium and phosphorus solubilization, phytohormone production, and detoxification. The advancements in biofertilizers are one of the major scientific endeavors for the development of sustainable agriculture, as they help to overcome the shortcomings associated with chemical-based farming techniques (Zulfiqar et al., 2019).

The stability of biofertilizers can be enhanced by using nanoformulations with resistant to desiccation, heat, and radiation (Jampílek & Kráľová, 2017). Hydrophobic silica nanoparticles added to the water-in-oil emulsion showed an improvement in the delivery of biofertilizers to soil and plants (Kaushik & Djiwanti, 2017). Nanobiofertilizers are capable of solving the limitations of biofertilizers, but this promising technology requires further research and development (Zulfiqar et al., 2019). Inoculation of nanoparticles and biofertilizers enhances plant growth and stress tolerance. In conclusion, nanobiofertilizers have become an economically and ecologically sustainable, highly versatile, and long-lasting agricultural tool (Sharma et al., 2023) (Fig. 9.1).

## *4.1 Bioformulations*

A formulation is a mixture of active and inert substances, whereas a bio-preparation is a formulation of microorganisms to preserve them, deliver them to their destination, and enhance the activity of biofertilizers. Inert media include fine clay, peat,

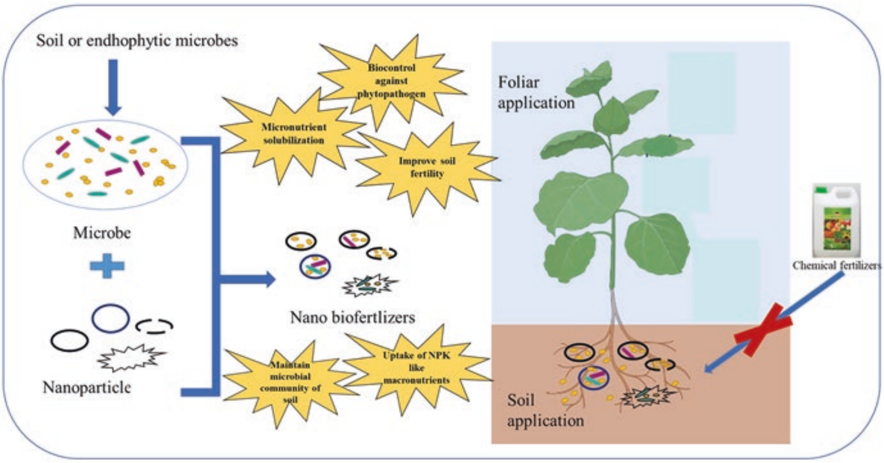


**Fig. 9.1** Beneficial microorganisms, such as bacteria, fungi, and algae, are incorporated into nanoparticles in nanobiofertilizers, a category of fertilizer based on nanotechnology. These biofertilizers can aid plant growth and development in a few ways, including better nutrient uptake, increased pest and disease resistance, and increased tolerance to environmental stressors. Here are a few possible uses for nanobiofertilizer. By offering sustainable and environmentally benign substitutes for traditional fertilizers and pesticides, nanobiofertilizers have the potential to completely transform the agriculture sector

vermiculite, alginate and polyacrylamide beads, diatomaceous earth, talc, vermiculite, properties, some additives such as gums, silica gel, methylcellulose, and starch preparations are available in solid and liquid formulations.

#### 4.1.1 Solid Formulations

Granules

Granules are dry preparations with an active ingredient content of 5–20%, a binder, and a carrier (Brar et al., 2006). They are divided into coarse particles (size range 100–1000 μm) and microgranules (size range 100–600 μm). The granules are formulated to be non-clumping, dusty, and free-flowing and break easily, releasing the active ingredient. The pellets are nonbreathable and safe and are mainly used for soil treatment. A concern with granular dosage forms is related to storage and extended shelf life (O'Callaghan et al., 2005). The most commonly used pellets are wheat flour pellets or corn flour. Granules are made from gelatinized corn starch, gluten, cottonseed, and sugars, gelatine or gum acacia, sodium alginate, and diatomaceous earth. Although granulated formulations are very effective, their use is also insufficient due to the UV inactivation of the active ingredient (Bailey et al., 1996).

Wettable Powders

Wettable powders (WPs) consist of active ingredients (50–80%), bulking agents (15–45%), dispersants (1–10%), and surfactants (3–5%) to achieve desired efficacy formulations (Brar et al., 2006). These dry formulations are readily miscible with water and can be easily added to normal water just before application. WPs have a longer shelf life by controlling moisture content, which ensure a firm marketplace. Agricultural substances and business waste by merchandise consisting of bagasse–sand–molasses mixtures, corn cob–sand–molasses, compost/farm manure mixture, cow dung–sand mixtures, diatomaceous earth, fly ash, inert charcoal, natural cakes, sawdust–sand–molasses mixtures, and wheat bran–sand mixtures also can be used to put together powder formulations (Khan et al., 2007).

Dust

Dust is also one of the oldest types of formulations, which contains a very finely ground mixture of active ingredients (usually 10%) and particles with sizes in the range of 50–100 μm. They also have a longer shelf life and are more effective, but they still have some handling and application issues.

### 4.1.2 Liquid Formulations

Liquid formulations, also called aqueous suspensions, consist of suspensions of biomass in water, oil, or a mixture of both (emulsions) (Schisler et al., 2004). Typical liquid formulations contain 10–40 active ingredients 1–3% suspension composition, 1–5% dispersant, 3–8% surfactant, and 35–65 °C plus liquid (oil or water) (Brar et al., 2006). The liquid formula can be the following genres.

#### Suspension Concentrates

Suspension concentrates (SCs) are formed from solid active ingredients with poor water solubility and reasonable stability. They are nondusty and easy to use compared to WPs.

#### Oil-Miscible Flowable Concentrate

This is a stable suspension of active ingredients in a fluid intended for prior dilution in an organic solvent (Singh & Merchant, 2012).

#### Ultralow Volume Suspension

They are ready-to-use suspensions with ultralow volume equipment, and air or soil spray equipment, and create a very fine spray (Singh & Merchant, 2012).

#### Oil Dispersion

Oil dispersion (OD) is a stable suspension of the active ingredients in solvents or oils that are insoluble in water (Michereff et al., 2009). OD has confirmed its growing importance over the past decade. Some protective measures are required when handling fungi containing OD formulations. As with long-term storage, the active ingredient (conidia) may be suspended or solidified at the bottom of the container (Butt et al., 2001). The oil evaporates much less, so it has a longer exposure and can be applied as an emulsion (oil in water) (Luz & Batagin, 2005) or, in some cases, as an inverse emulsion (water in oil) (Batta, 2007).

### 4.2 Formulations for Nutrient Uptake

Microbial inoculants serve as an effective method of supplying nutrients to plants as they greatly reduce the use of chemical fertilizers, leading to an increasing number of commercially produced biofertilizers for various crops (Berg, 2009; Trabelsi & Mhamdi, 2013).

Nitrogen is an essential plant macronutrient required in large quantities, but only a very small amount is provided by nitrogen fertilizers to the soil, and only a very small percentage of it is utilized in agricultural systems, even when the amount of application is remarkably increased (Vitousek et al., 2009). Nitrogen-fixing capability is limited to very little, and some others depend on symbiotic fixation of nitrogen by rhizobia (leguminous association) and Frankia (nonlegume association) (Franche et al., 2009). Humans are now synthetically fixing nitrogen at twice the rate of natural processes. Therefore, the role of rhizobia in sustainable crop production is confirmed, and it can be used as inoculum with nanoformulations to envisage agronomic practices for better nitrogen supply (Gupta et al., 2004; Arora & Padua, 2010). In legume inoculation, powdered granular or liquid formulations contain peat as carrier material. *Azoarcus, Achromobacter, Burkholderia, Gluconacetobacter, Herbaspirillum, Klebsiella*, and *Serratia* have been identified as potent endophytic nitrogen-fixing strains that can be used as microbial inoculum in preparation (Franche et al., 2009).

Phosphate is probably the least available plant nutrient found in the rhizosphere because it is inorganically fixed and forms organic complexes (Eswaran et al., 1997). In average soils, the phosphorus content is much lower, and only 0.1% of it is available for plants (Achal et al., 2007). It was observed that the application of phosphate fertilizers does not meet the needs of the plant. Mineralization and immobilization of the organic conversion of insoluble phosphorus into a form accessible to plants is a biological process in the soil, such as the microbial activity of phosphate solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF) (Fankem et al., 2006; Khan et al., 2007). The development of microbial inoculum containing phosphate solubilizing microbes (PSM) and the use of PSM have helped increase yields in many plants. Commercialization as biopreparations has not been very successful due to quality control and the development of reliable and pollution-free bioproducts, while field performance is open to various environmental influences (Khan et al., 2009). *Pseudomonas* spp., *Bacillus* spp., *Aspergillus* spp., and *Penicillium* spp. are mainly used in PSB-based biofertilizers (Sharma et al., 2013). However, later products such as phosphobacterin, P Sol B®, and FOSFOSOL® received a lot of attention due to their success.

Potassium intake is as important as nitrogen and phosphorus for balanced plant growth. This macronutrient participates as an enzyme activator in several physiological reactions, such as protein synthesis, photosynthesis, and starch synthesis, and contributes to resistance to diseases and insects (Rehm & Schmitt, 2002). In the world, India ranks fourth in terms of total potassium consumption after the United States, China, and Brazil. It was found that “instant” K in the soil is dissolved by some bacteria with the release of organic acids, which increases the concentration of K in the soil solution (Meena et al., 2014). The ability to dissolve K-rich minerals such as mica, illite, and orthoclase is of great interest in the development of probiotics able to provide soluble K to plants. Biofertilizer K has been tested in several countries, notably China and Korea (Sheng & Lin, 2006). Most of the development of potassium-based biofertilizers has involved the use of these PSBs, which can also dissolve potassium-containing minerals. *Frateuria aurantia* has recently been recognized as a very efficient K-mobilizing bacterium and has been used in the commercial production of the biofertilizers Symbion-K, Biosol K, and K Sol B (Ahmed & El-Araby, 2012).

### *4.3 Formulations for Biocontrol*

About 1400 biocontrol products are commercially available worldwide (Marrone, 2007), and new products are registering day by day. The formulations of different biofertilizers depend on different factors like the type of microbe, viability, and virulence of the strains, and whether the amount of inoculum is sufficient to create an impact on plants. The goal is to ensure that the agent is delivered alive, is functional, and has the potential to be effective in the field (Ash et al., 2010). Many researchers are elucidating the mechanism in detail and the methods of preparation (Burges & Jones, 1998; Couch, 2000).

### *4.4 Consortia-Based Inoculants*

Most of the biological formulations contain a single strain; mixed cultures with other microorganisms serve as a better approach for the total growth and development of plants. In case of legumes, the use of rhizome co-inoculation with mycorrhizae gave substantial results. This co-inoculation not only upgraded the plant's nutritional status but also increased drought tolerance in alfalfa (Ardakani et al., 2009), soybean (Song et al., 2012), broad beans, chickpeas (Tavasolee et al., 2011), and pigeon peas (Bhattacharjee & Sharma, 2012). The combination of PSB and rhizobia in legumes promotes plant growth (Messele & Pant, 2012). The technique that provides a faster and more continuous supply of nutrients for growth is the integrated application of PSB with the co-culture of K-soluble bacteria. In the recent times, conjugate nanobiofertilizer formulations are being developed by researchers as sustainable agriculture practices and several patens are being awarded (Paikray & Malik, 2010). A conjugated biological formulation with nine strains from the genera *Azotobacter* spp., *Bacillus* spp., *Frauteria* spp., and *Streptomyces* spp., formulated as a wetting powder and found to be beneficial to gram black (Maiyappan et al., 2010). In a similar study, the bioconjugates of *Burkholderia* sp. MSSP and three other PGP bacteria were tested to enhance the growth of *Cajanus cajan*. In this

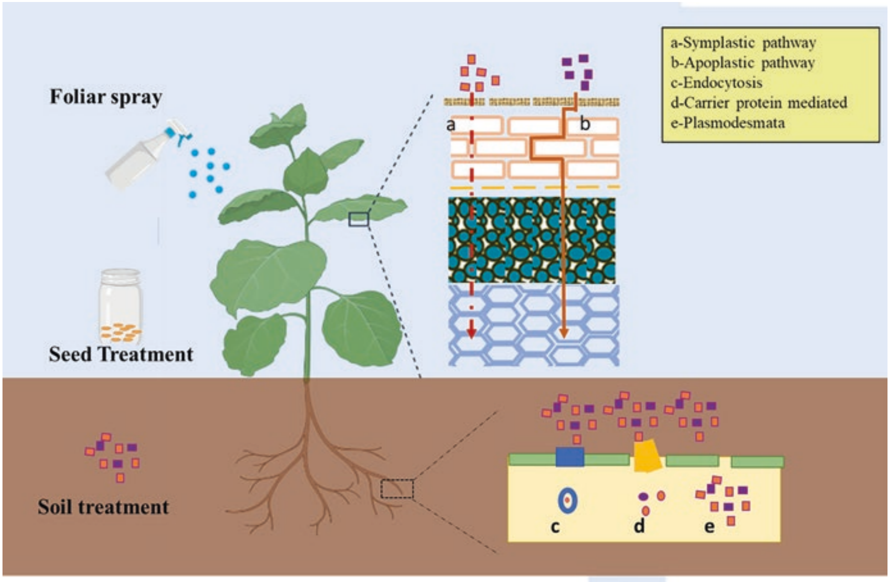


**Fig. 9.2** Depending on the technique of delivery, the mechanism of action and uptake of nanoparticles can differ. In general, various parameters such as nanoparticle size, shape, surface charge, and concentration, as well as plant species and ambient conditions, can influence nanoparticle uptake and the mechanism of action. These criteria must be considered when selecting the best nanoparticle and application method for a certain crop and growing condition. Furthermore, the safety and potential environmental implications of nanoparticles must be carefully assessed in order to ensure their long-term and responsible use in agriculture

study, different materials like bagasse, sawdust, cocoa peat, rice husk, wheat bran, charcoal, rock phosphate, and whey paneer were used as liquid carriers and the results confirmed growth enhancement in pigeon pea (Pandey & Maheshwari, 2007). The combined inoculation of AMF and Rhizobium fungi facilitated a higher accumulation of N and P in the shoots of common pea plants compared with inoculation of both separately. Cyanobacteria, microalgae, and Azotobacter populations can be considered the best candidates for biostimulants and biofertilizers for plants (Zayadan et al., 2014). BioGro is a conjugated biofertilizer with *Pseudomonas fluroscens*, a soil yeast, and two PGPR Bacillus strains widely used in Vietnam (Fig. 9.2).

## 5 Synthesis of Nanoparticles

Nanomaterials can be synthesized by using physical, chemical, and biological approaches. The top-down approach describes physical or chemical processing that converts bulk material into nanoform, for example, by grinding, milling, etc. The other method of synthesis is the bottom-up method, in which smaller building

**Table 9.1** Nanoparticle synthesis methods

| Type of NP synthesis | Method |
|---|---|
| Physical synthesis | Thermal decomposition, ball milling, lithography, laser ablation and sputtering |
| Chemical synthesis | Sol-gel method, chemical vapor decomposition, spinning, and pyrolysis |
| Biological synthesis | Microbial incubation, plant-based biosynthesis |

blocks are assembled together to create functional nanoscale materials. The bottom-up approach mostly involves chemical processing, while the top-down approach involves physical breaking (Raliya et al., 2018). The nanoscale fertilizer produced thus gives high productivity, nutrient enrichment, enhanced soil fertility, more microbial diversity, and nutrient mobilization, reducing the demand for fertilizers. The most common approaches used for the synthesis of nanoparticles are chemical reduction by organic and inorganic reducing agents. The chemical synthesis approaches employed in the synthesis of nanoparticles include chemical vapor deposition (CVD), chemical precipitation, and sol-gel technique (Tarafder et al., 2020). Various physical synthesis techniques, including gas condensation, planetary ball mills, vibrating ball mills, low-energy tumbling mills, and high-energy ball mills, were explored. Physical synthesis methods are commonly used method because of ease in synthesis and less time-consuming (Uhm et al., 2007). Biological synthesis is a process where different microbes, like bacteria and fungi, are utilized in green nanosynthesis. The biosynthesis of NPs uses different plant extracts or microbial extracts. It is also reported that plant waste is employed as a reducing agent for the synthesis. These green chemistry biosynthetic pathways reduce the risk of contamination at the source level, where reagents are eco-friendly (Tarafder et al., 2020).

A cost-effective and ecofriendly approach for the synthesis of nanoparticles is green synthesis, which is devoid of toxic chemical usage. The combined amalgamation of extracts of organisms and metallic salts leads to production of nanoparticles via green synthesis. This can be done through two different methods based on their composition: a) plant-based and b) microbe based methods. Plants based method is more convenient as the plant material can reduce the metallic ions quickly (Table 9.1).

## *5.1 Physical Synthesis or Top-Down Synthesis*

### 5.1.1 Thermal Decomposition Method

Thermal decomposition is an energy-consuming process in which particles are chemically decomposed by heat (Salavati-Niasari et al., 2008). The temperature for chemical decomposition depends on specific temperature at which the element used for nanoparticle synthesis is chemically decomposed. As an example, paramagnetic polyethylene glycol is used to synthesize gadolinium oxide nanoparticles through thermal decomposition (Ijaz et al., 2020).

### 5.1.2 Ball-Milling Method

It is a simple, inexpensive mechanical method that uses large-sized substances to produce nanoparticles. In this method, kinetic energy is transferred from the medium used for grinding to the material to be destroyed. Materials with enhanced properties, like metals and alloys, are used to form nanoparticles in industrial scale. Alloys of different metals are used to increase the properties of nanoparticles according to their usage. In ball milling model, different milling techniques are used, like horizontal oscillatory milling, ultrasonic wave-assisted ball milling, and planetary ball milling (Ijaz et al., 2020).

### 5.1.3 Lithography

Lithographic methods are capable of making micron-sized particles, which require energy-intensive and expensive equipment. There are different lithographic techniques, like electron beam lithography, photolithography, soft lithography, focused ion lithography, nanoimprint lithography, and dip-pin lithography. Compared to typical lithography, nanoimprint lithography is a unique method. This is done through template synthesis: a template material like a latex sphere is synthesized and coated with soft polymeric material. However, top-down synthesis destructs the coating material (Ijaz et al., 2020).

### 5.1.4 Laser Ablation

A simple method for synthesizing nanoparticles is to irradiate various metals immersed in solution with laser light and condense plasma to produce nanoparticles (Amendola & Meneghetti, 2009). This is a traditional top-down chemical approach and differs from metal-to-nanoparticle reduction. The main advantage of laser ablation techniques is that they do not require any stabilizing agent or chemical (Ijaz et al., 2020).

### 5.1.5 Sputtering

Sputtering is the ejection of particles for the deposition of nanoparticles (Das et al., 2016). The easy deposition of a thin NP layer can be facilitated by annealing. The size and shape of nanoparticles are determined by factors such as temperature, layer thickness, annealing time, and substrate (Shah & Gavrin, 2006). Various types of nanoparticles are synthesized by sputtering (Ijaz et al., 2020).

## *5.2 Bottom-Up Method*

The bottom-up method is a constructive process where the reversal of the top-down method occurs. In this method, nanoparticles are constructed from small subunits. Bottom-up methods include different techniques like chemical vapor deposition (CVD), sol-gel, spinning, pyrolysis, and biological synthesis (Ijaz et al., 2020).

### 5.2.1 Chemical Vapor Deposition (CVD) Method

Chemical vapor deposition involves usage of reaction chamber in which thin layering of gaseous reactant is added onto the substrate. When in contact with the heated substrate, gas combines with substrate to form a chemical reaction. As a result of this reaction, a thin film of product is produced on the surface of the substrate, which is subsequently recovered and used. The nanoparticles obtained will be hard, strong, uniform, and highly pure, making CVD a very advantageous method. The major disadvantage of CVD is the requirement of special machinery and the production of highly toxic gas as byproducts (Shah & Gavrin, 2006).

### 5.2.2 Sol-Gel Method

The sol-gel method is a combination of condensation and hydrolysis reactions with colloids formed from solid particles suspended in a continuous liquid, and gels are formed by dissolving solid macromolecules in a solvent. The sol-gel method is the most preferred method, where suitable chemical solutions such as metal oxides and chlorides used in the sol-gel process act as precursors. The precursor is dispersed in the host liquid by stirring, sonication, or shaking. The final product is separated from the solid phase and liquid phase by using filtration, sedimentation, and centrifugation, and nanoparticles are recovered (Saberi-Rise and Moradi-Pour, 2020).

### 5.2.3 Spinning

Nanoparticles are synthesized using a rotating disk whose physical parameters are controlled, called a spinning disk reactor. The reactor is made devoid of oxygen by filling it with nitrogen or inert gas to avoid chemical reactions. The liquids such as water and precursors are pumped inside the chamber or reactor. The nanoparticles synthesized through this are characterized by various factors such as disc surface, liquid/precursor ratio, disc rotation speed, liquid flow rate, and location of the feed. The particle sizes ranged from 3 to 12 nm (Smith Nigel et al., 2006).

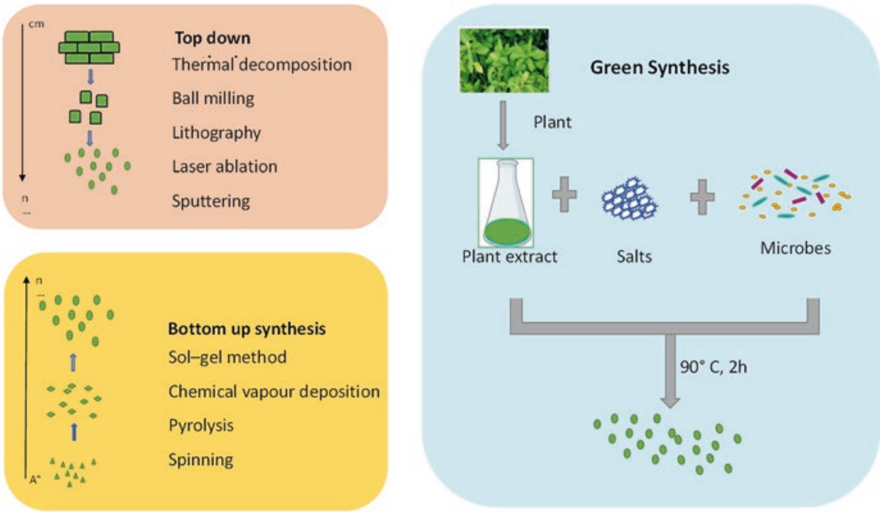


**Fig. 9.3** Nanobiofertilizers are a sort of nanotechnology-based fertilizer that incorporates beneficial plants and microorganisms into nanoparticles, such as bacteria, fungi, and algae. Choosing an acceptable method is influenced by a few factors. To ensure the safety and environmental sustainability of the final nanobiofertilizers, the synthesis process must be properly developed and carried out

### 5.2.4 Pyrolysis

Pyrolysis is a widely used industrial method for the synthesis of nanoparticles. In this process, the precursors are burned with a flame. The precursors may be in liquid or vapor form. The precursor is transferred into the furnace at high pressure to recover nanoparticles. In order to produce a high temperature, a laser or plasma is used instead of a flame. The high temperature makes it easy to evaporate (Sourice et al., 2015) (Fig. 9.3).

## 6 Characterization of Nanoparticles

Nanoparticles can be characterized by qualitative or quantitative methods.

**Qualitative Analysis**

1. Fourier transform infrared spectroscopy
2. UV–visible spectrophotometry
3. Scanning electron microscope
4. Atomic force microscopy
5. X-ray diffraction

**Table 9.2** Qualitative and quantitative characterization of nanoparticles

| No | Technique | Type of analysis | References |
|---|---|---|---|
| 1. | FTIR | Qualitative | Jabeen et al. (2018), Joshi et al. (2019), Kamnev et al. (2021), Khalofah et al. (2021), Rahman et al. (2021), Tarafder et al. (2020) |
| 2. | SEM | Qualitative | Jabeen et al. (2018), Joshi et al. (2019), Kamnev et al. (2021), Rahman et al. (2021), Sotoodehnia et al. (2019), Tarafder et al. (2020) |
| 3. | TEM | Quantitative | Saleem and Khan (2023), Sotoodehnia et al. (2019) |
| 4. | XRD | Qualitative | Jabeen et al. (2018), Joshi et al. (2019), Tarafder et al. (2020) |
| 5. | HAADF | Quantitative | Joshi et al. (2019), Mejías et al. (2021) |
| 6. | UVS | Qualitative | Jabeen et al. (2018), Joshi et al. (2019) |
| 7. | AFM | Qualitative | (Joshi et al. (2019), Rahman et al. (2021) |
| 8. | ICP-MS | Quantitative | Rahman et al. (2021), Tarafder et al. (2020) |

**Quantitative Analysis**

1. Transmission electron microscopy
2. Annular dark-field imaging
3. Inductively coupled plasma-mass spectrometry (Table 9.2)

## 7 Types of Nanobiofertilizer

Biofertilizers include different bacteria for nutrient uptake and solubilization. Nitrogen-fixing bacteria are essential for plant growth and development because plants cannot convert atmospheric nitrogen to ammonia. *Azotobacter, Rhizobium*, and *Azospirillum* are important examples of nitrogen-fixing bacteria. Azotobacter is an aerobic bacterium in alkaline soils that has found increasing application in large-scale nitrogen fixation. Rhizobium forms symbiotic bonds with the roots of legumes and is therefore a useful biofertilizer for legumes. *Bacillus, Pseudomonas*, and *Aspergillus* are primarily phosphate-solubilizing microorganisms. They accelerate plant growth by increasing plant access to phosphorus. Apart from these, the commercialized biofertilizer industry focuses on potassium-mobilizing biofertilizers, zinc-dissolving biofertilizers, and NPK-mobilizing microbes. Different forms of nanobiofertilizers and their applications can effectively alleviate plant biotic and abiotic stress and improve plant nutritional value (Giri et al., 2023). Biofertilizers in agriculture have several drawbacks, including short crop-specific shelf life, instability in the field due to lack of defined environment, need for special storage conditions, easy drying, and uncharacteristic dosage. Apart from the shortcomings of essential biofertilizers, they are helpful for sustainable agriculture, have improved stress tolerance, and enhance soil fertility, which is inevitable to remedy nutrient deficiencies. To overcome these limitations, formulations based on nanoparticles were developed. NPK can be formulated together with these nanoparticles individually or in consortia to find better ways to improve cultivation practices (Tables 9.3 and 9.4).

## 8 Advantage Over Conventional Methods

Nano-formulated biofertilizers are more stable than regular biofertilizers and biostimulants due to deactivation by drying, heat, and UV light. Microbial-derived nanoparticles are more stable, nontoxic, cheaper, and environmentally friendly compared to chemically derived ones. Nanobiofertilizers promoted plant growth and nutrient quality by maintaining soil fertility through nitrogen fixation, phosphate solubilization and mobilization, siderophore generation, and plant hormone synthesis. Plant yield and quality are improved by increasing photosynthesis, nutrient uptake efficiency, photosynthetic accumulation, and nutrient transfer. Depletion of soil nutrients through leaching, gasification, soil erosion, and competition with other organisms enhances nutrient uptake and assimilation by plants. A large area can be treated with a small amount of nanobiofertilizer compared to chemical fertilizers. Rhizobium, which promotes plant growth, acts as a bio-organic component in nano-biofertilizers, assists in nitrogen fixation and phosphate solubility, and aids in soil fertility restoration. Nanomaterials help release nutrients slowly and stably according to plant needs in a synchronous mode and also act as resistance agents. Nanoclay-coated *Trichoderma* sp. and *Pseudomonas* sp. are used as an antifungal agent and also provides plant resistance to abiotic stress (Ali et al., 2021) (Table 9.5).

**Table 9.3** Different plant growth promoting bacteria can be used for production of biofertilizers

| | |
|---|---|
| *Nitrogen fixing* | |
| Free living | *Azotobacter* |
| Symbiotic | *Rhizobium* |
| Associative symbiotic | *Azospirillum* |
| *Phosphorous solubilizing* | |
| Bacteria | *Pseudomonas striata* |
| Fungi | *Penicillium* spp. *Aspergillus spp* |
| *Phosphorus mobilizing* | |
| Arbuscular Mycorrhiza | *Glomus spp* |
| Ectomycorrhiza | *Amanita* spp. |
| *Plant Growth Promoting Bacteria* | |
| Plant Growth Promoting Rhizobacteria | *Pseudomonas, Azospirillum, Azotobacter, Bacillus, Burkholdaria, Enterobacter, Rhizobium, Erwinia, Mycobacterium, Mesorhizobium, Flavobacterium* |

**Table 9.4** Nanoparticles with PGPR on their respective host and influence on plant growth and stress mitigation

| Sl | Nanobiofertilizers | Plant | Microbe | Response | References |
|---|---|---|---|---|---|
| 1. | Silicon dioxide ($SiO_2$ NPs) | *Triticum aestivum* | *Azospirillum brasilense, Bacillus* sp.*, and Azospirillum lipoferum* | Drought resistance | Akhtar and Ilyas (2022) |
| 2. | Iron/zinc oxide NPs | *Triticum aestivum* | *Azospirillum, Pseudomonas and, Azotobacter* | Enhanced yield and growth in water deficit areas | Seyed Sharifi et al. (2020) |
| 3. | Zinc NPs | *Phaseolus vulgaris* | Rhizobium | Enhancement of nutrient uptake and plant growth | Morsy et al. (2017) |
| 4. | Silver NPs | *Solanum tuberosum* | Mixture of Azospirillum and Azotobacter-Nitroxin | Total yield increment of tubers | Davod et al. (2011) |
| 5. | Nano zeolite | *Zea mays* | Bacillus | Plant growth | Khati et al. (2018) |
| 6. | Zn NPs | *T. Aestivum* | Biochar | Heavy metal stress | Bashir et al. (2020) |
| 7. | Fe NPs | *Trifolium repens* | Pseudomonas fluorescens | Heavy metal stress | Daryabeigi Zand et al. (2020) |
| 8. | Ti NPs | *Triticum secale* | *Azospirillum brasilense, A. caulinodans and, Azotobacter chroococcum* | Heavy metal stress | Ghooshchi (2017) |
| 9. | | *Sorghum bicolor* | *Azotobacter* | Carbohydrate and chlorophyll content | Eliaspour et al. (2020) |
| 10. | Ag-nanoparticles | *Allium cepa* | *Bacillus pumilus and Pseudomonas moraviensis* | Salinity stress | Jahangir et al. (2020) |
| 11. | Silver nanoparticles | *Zea mays* | *Bacillus cereus* | Bioinoculant and growth stimulator | Kumar et al. (2020) |
| 12. | Ag-nanoparticles | *Cucumis sativus* | *Pseudomonas putida Pseudomonas stutzeri* | Enhance the antioxidant and defense enzyme activities to enable the plant in the tolerance of different stresses | Nawaz and Bano (2020) |

(continued)

**Table 9.4** (continued)

| Sl | Nanobiofertilizers | Plant | Microbe | Response | References |
|---|---|---|---|---|---|
| 13. | Gold NPs | | *Pseudomonas fluorescens, Bacillus subtilis* | Plant growth promotion | Shukla et al. (2015) |
| 14. | Ag-NP | *Withania somnifera* | *Bacillus mojavensis* | Improves growth, photosynthetic attributes, gas exchange parameters, and Alkalo-Polyphenol contents | Danish et al. (2022) |
| 15. | Bio fabricated Ag-NPs | *Saccharum officinarum* | *Fusarium oxysporum* | Antifungal activity against phytopathogens | Amna Mahmood et al. (2021) |
| 16. | Green nanoparticles | *Cuminum cyminum* | | Restrain<br>Restrain fusarium wilt<br>Restrain fusarium wilt by Antioxidant defense system | Thummar et al. (2022) |
| 17. | Silver nanoparticles | *Saccharum officinarum* | *Bacillus* sp. *Strain AW1−2* | Antifungal activity against *Colletotrichum falcatum* Went | Ajaz et al. (2021) |
| 18. | Silver nanoparticles | | *Fusarium oxysporum* | Antibacterial potential | Ilahi et al. (2022) |
| 19. | Silver nanoparticles | *Triticum aestivum* | | Strong fungicide against *Bipolaris sorokiniana* | Mishra et al. (2014) |
| 20. | Silver nanoparticles | *Linum usitatissimum* | *Comamonas testosteroni* | Salinity stress tolerance | Khalofah et al. (2021) |
| 21. | Silver nanoparticles | *Zea mays* | Rhizospheric bacteria | Biomass enhancement | Sillen et al. (2015) |
| 22. | Silver nanoparticles | *Zea mays* | *Pseudomonas fluorescence, Bacillus cereus* | Growth of maize and bioremediation of heavy metals under municipal wastewater irrigation | Khan and Bano (2016) |
| 23. | Titanium dioxide nanoparticles | *Trifolium repens* | *Bacillus thuringiensis Azotobacter chroococcum* | Promote phytoremediation of cadmium-polluted soil | Zand et al. (2020) |
| 24. | Nano zeolite and nano chitosan | *Trigonella foenum-graecum* | *PS2-KX650178 and PS10-KX650179* | Improve soil fertility | Kumari et al. (2020) |

(continued)

**Table 9.4** (continued)

| Sl | Nanobiofertilizers | Plant | Microbe | Response | References |
|---|---|---|---|---|---|
| 25. | Titania ($TiO_2$) nanoparticles | | *Bacillus thuringiensis* | Total plant growth promotion | Timmusk et al. (2018) |
| 26. | Green molybdenum nanoparticles | *Triticum aestivum* | *Bacillus* sp. *Strain ZH16* | Improved growth by nutrients supply, ionic homeostasis and arsenic accumulation | Ahmed et al. (2022) |
| 27. | Alginate – bentonite coating enriched with titanium nanoparticles | *Phaseolus vulgaris* | *Bacillus subtilis Vru1* | Against *Rhizoctonia solani* | Saberi-Rise and Moradi-Pour (2020) |

**Table 9.5** Advantage of nanobiofertilizers over chemical and nanofertilizers

| Chemical fertilizer | Nanofertilizer | Nanobiofertilizer |
|---|---|---|
| Enhanced yield | Efficient usage of fertilizer | Increased nutrition status in plants |
| Improved quality of yield | Proper uptake from soil to increase yield | Promoted plant growth |
| Imbalanced fertilization | Required amount of fertilization | Slow release of nutrients |
| Decreased soil organic matter | Least impact on soil organic matter | Increase nutrition status in soil |
| Reduced yield after a period of time | Extend the duration of supply without affecting the yield | Enhanced plant resistance |

# 9 Conclusion

Chemical fertilizers have been used for years to increase the productivity of agricultural activities. However, chemical fertilizers have been associated with adverse effects such as environmental toxicity and long-term overuse of chemical fertilizers. This has led to the need for new, nontoxic, environmentally friendly alternatives to improve agricultural productivity without the associated side effects. To ensure the biosecurity of agriculture, it is recommended to use nanobiofertilizers instead of chemical fertilizers. Biofertilizer ingredients contain beneficial microbes with PGPR properties that supplement crop nutrients by increasing nitrogen fixation and dissolving complex organic matter into simpler forms for easy plant availability. Although they have some serious problems, such as poor shelf life, external stability, and performance in various environmental conditions, nanoparticle formulations have superiority in all of them. Encapsulation of nanomaterials extended their shelf life and showed controlled release of biofertilizers when needed. It is an environmentally friendly, renewable approach that can boost nutrient use efficiency, enrich beneficial microbial communities in soil, improve the activity of related signaling cascades, facilitate improved soil fertility and yield, and contribute to crop disease resistance. Chemical fertilizers are widely used in the agricultural sector, are the most expensive inputs in agriculture, and have various negative effects on crop production, including environmental pollution. We need environmentally sustainable strategies that improve nanotechnology and offer solutions through nanobiofertilizers that have a promising future in the field of sustainable agriculture. Nanobiofertilizers are potential nutrient enhancers that allow a slow and continuous release of nutrients into plants during the plant's growing season. Nanobiofertilizer can have several advantages for plants, such as slow and targeted release of nutrients.

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