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REVIEW ARTICLE

The potential of nanomaterials associated with plant growth-promoting bacteria in agriculture

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Abstract

The impacts of chemical fertilizers and pesticides have raised public concerns regarding the sustainability and security of food supplies, prompting the investigation of alternative methods that have combinations of both agricultural and environmental benefits, such as the use of biofertilizers involving microbes. These types of microbial inoculants are living microorganisms that colonize the soil or plant tissues when applied to the soil, seeds, or plant surfaces, facilitating plant nutrient acquisition. They can enhance plant growth by transforming nutrients into a form assimilable by plants and by acting as biological control agents, known as plant growth-promoting bacteria. The potential use of bacteria as biofertilizers in agriculture constitutes an economical and eco-friendly way to reduce the use of chemical fertilizers and pesticides. In this context, nanotechnology has emerged as a new source of quality enrichment for the agricultural sector. The use of nanoparticles can be an effective method to meet the challenges regarding the effectiveness of biofertilizers in natural environments. Given the novel sustainable strategies applied in agricultural systems, this review addresses the effects of nanoparticles on beneficial plant bacteria for promoting plant growth.

Keywords Endophytes · Nanobiofertilizer · Nanoparticles · Plant growth · Rhizobacteria

Introduction

Population growth associated with economic development has led to increased demand for food (Ma et al. 2018). For this reason, the indiscriminate use of chemical fertilizers and pesticides to achieve high crop yields has caused depletion of soil microbial communities, contamination of water and soil, loss of biodiversity, and an imbalance in ecosystem function (Vance 2001; Vessey 2003; Gurikar et al. 2016). Considering environmental degradation and limited land resources, alternatives that can partially or even wholly replace chemical inputs in agriculture have never been more critical.

Sustainable agriculture demands addressing the concerns of agriculture and the environment (Tilman et al. 2002). High crop yields with fewer environmental degradation can be achieved through the effective use of biofertilizers, biopesticides, and ecologically conscientious water use, and soil maintenance. For these purposes, the application of plant growth-promoting bacteria (PGPB) is a sustainable practice to benefit plants. Rhizospheric bacteria are symbiotic free-living soil PGPB associated with plant roots,

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while endophytic PGPB colonize leaves, flowers, or inner tissues of the host plant (Glick 2020). Rhizospheric and endophytic bacteria promote plant growth through different mechanisms, including those involving nutrient uptake, plant stress resistance, and protection against phytopathogens (Redman et al. 2002; Waller et al. 2005; Ryan et al. 2008; Castro et al. 2018; Batista et al. 2018). Therefore, biofertilizers consisting of microorganisms have increasingly shown economic potential for use in organic farming. The global plant biostimulant market is estimated to grow by 12% per year and worth USD 3.0 million by 2022 (Meticulous Market Research 2017).

Nevertheless, many factors cause destabilization and inconsistency of microbial inoculants in the field, hindering biofertilizer efficacy. In recent years, nanotechnology has been widely used for precision agriculture. Due to their small sizes and unique properties compared to those of their bulk materials, nanoparticles (NPs) are being studied for use in addressing some obstacles of biofertilizers, such as reproducibility, storage stability, dehydration, and temperature sensitivity (Bansal et al. 2014; Duhan et al. 2017).

Nanostructured materials have been indicated to increase the potential of PGPB as inoculants—so-called nanobiofertilizers. Silicon, zinc, titanium, and gold NPs have been reported to increase the number of bacterial cells and improve the beneficial properties of PGPB in plants (Dimkpa et al. 2012a; Karunakaran et al. 2013; Rangaraj et al. 2014; Palmqvist et al. 2015; Shukla et al. 2015; Zand et al. 2020). The use of nanomaterials is broadly considered the next technological and scientific step to support humankind's development (Singla et al. 2020).

In this context, nanotechnology applied to beneficial microorganisms constitutes a promising approach to revolutionizing the agricultural sector by optimizing crop systems and providing economic and environmental benefits. Therefore, this article aims to provide an overview of the potential advantages of formulations using inorganic NPs associated with PGPB for plant development.

Nanotechnology for sustainable agriculture

Nanotechnology involves materials with unusual properties that appear either due to the quantum confinement effects or the existence of exceptionally reactive surfaces, which occur only at a nanometric scale. Compared with the macroscopic level, the nanoscale level brings about material properties that are different and novel due to the reduced size, greater surface area-to-weight ratio, and shape of nanomaterials (Roduner 2006; Gutiérrez et al. 2011). In terms of their advantageous properties, nanomaterials have high reactivity and improved bioavailability/bioactivity, adherence, and surface effects (Gutiérrez et al. 2011). NPs can be formed by only a single element (such as silver) or by a mixture of elements, such as those composing oxides (TiO_2, SiO_2, ZnO) (Dinesh et al. 2012).

Innovative strategies are increasingly necessary for sustainable agriculture (Prasad et al. 2017). Approaches based on nanotechnology constitute efficient tools to overcome challenges in the agricultural and food industries, such as the growing demand for food, food safety, plant disease, and climate change (Biswal et al. 2012). Recently, researchers have started testing nanomaterials with relatively few negative environmental impacts to improve crop production. Within the agricultural field, nanotechnology is applied in several areas: seed science, nanofertilizers, nanoherbicides, water management, nanoscale carriers, biosensors, agricultural engineering, and animal science (Koul 2019). For example, smart agricultural systems developed with nanomaterials enable high nutrient absorption by plants, delivery and controlled release of molecules in target tissues, early detection of diseases, and protection from the environment (Suman et al. 2010; Tarafdar et al. 2013; Bhattacharyya et al. 2016). Nanostructured materials have been used in association with agricultural inputs (nanofertilizers and nanopesticides) to promote targeted delivery and release in plants at relatively low doses per application, consequently decreasing the byproducts that could remain in the soil and disrupt the ecosystem (Varma et al. 2017).

Nanoencapsulations with specific bacterial strains can be inoculated into seeds, which are referred to as smart seeds, to decrease seeding rates, ensure correct field stands, and improve crop performance. Smart seeds can also be dispersed over the field and scheduled to germinate under appropriate temperature, moisture, and soil pH conditions (Chinnamuthu and Boopathi 2009; El-Ramady et al. 2018).

Nanomaterials in biofertilizer formulations

Plant survival depends on multiple aspects of the community to which it belongs (Lundberg et al. 2012). It is well known that PGPB support plant health by several direct and indirect mechanisms. The direct mechanisms include the uptake of essential nutrients from the soil, including nitrogen, phosphorous, iron, and the synthesis or regulation of plant growth-related hormones, such as auxin, cytokinin, or gibberellin (Glick 2012; Gond et al. 2015; White et al. 2015). Indirectly, PGPB help plants endure biotic stress by producing antibiotics, antioxidant enzymes, and other molecules; balancing reactive oxygen species; lowering nutrient availability for pathogens; synthesizing pathogen-inhibiting volatile compounds; and promoting induced systemic resistance in the plant (Santoyo et al. 2012; Glick 2020; Li et al. 2016; Srivastava et al. 2016).

Since crop plants can take up only 30–50% of chemical fertilizers, a substantial amount of input remains in the soil, polluting groundwater. Consequently, fertilizer efficiency has decreased over the years due to saturation (Mózner et al. 2012). Biofertilizers consist of live or latent microorganisms (inoculants) in a formulation that provides easy handling and extended storage, acting as a delivery instrument of microbes to increase nutrient availability for plants (Sahu and Brahmaprakash 2016; Kour et al. 2020). PGPB, such as nitrogen fixers, phosphorus solubilizers, potassium solubilizers, and biocontrol agents, are used worldwide in agriculture as inoculants (Mohammadi and Sohrabi 2012; Sahu and Brahmaprakash 2016). N-fixing inoculants dominate the global biofertilizer market, as plants cannot convert atmospheric nitrogen into usable nitrogen, an essential nutrient for plant survival (GVR 2020). The genera *Azospirillum*, *Acetobacter*, *Azotobacter*, and *Pseudomonas* include species most commonly used as inoculants. Moreover, *Pseudomonas* and *Bacillus* species are potent biocontrol agents and plant growth promoters under stress conditions (Praveen Kumar et al. 2014; Kumar and Verma 2018). The advantages of biofertilizers over chemical fertilizers include increased sustainability, low environmental impact, improved soil fertility, and increased accessibility to marginal and small farmers (Thomas and Singh 2019). Therefore, microbial biofertilizers can reduce or replace chemical fertilizers in agriculture and alleviate adverse impacts.

Although PGPB are promising and are already being used commercially as inoculants in cropping systems, their practical application may not coincide with the expected performance in plants due to problems concerning their stability in the soil, field applications, and delivery systems (Koul 2019). As many factors influence bacterial colonization in plants, generally, bacterial populations decline quickly after inoculation, decreasing bacterial activity in the rhizosphere. To prevent this decline in the field, PGPB need either an appropriate microenvironment or physical protection for a prolonged period (Bashan et al. 2014; Timmusk et al. 2017). Furthermore, a minimum number of bacterial cells is a critical factor for obtaining a positive plant response. For example, the bacterium *Azospirillum brasiliense* needs to be at 10^6–10^7 cells/plant (Bashan 1986). For these reasons, the application of PGPB in crop systems requires peat or liquid carriers to stabilize and support the bacteria during storage and transport (Namasivayam et al. 2014). The most crucial portion of the biofertilizer is the carrier, which originates from organic or inorganic compounds or is synthesized from specific molecules (Smith 1992; Malusá et al. 2012).

Alternative materials must be explored to preserve bacterial inoculum quality and efficiency, as well as reduce production costs and adverse impacts (Herrmann and Lesueur 2013). For these purposes, nanostructured materials may be a modern and efficient approach that may enhance the stability and targeting of beneficial microorganisms in agriculture. The incorporation of nanoformulations can provide stable and reproducible biofertilizers by increasing their resistance to desiccation, heat, and UV radiation (Vandergheynst et al. 2007; Ghormade et al. 2011). This approach involves integrating microbial cells into inorganic or organic NPs, delivering the bacteria under regulated conditions to target tissues at specific times (Timmusk et al. 2018).

The large specific surface area of NPs with a negative charge and different groups mostly determines the specificities and interactions between microorganisms and NPs, independent of the NP material. Despite the total negative charge, there may be positive charges and hydrophobic sites on the surface of cells and solid particles, promoting adhesion between microorganisms and nanomaterials (Breznak et al. 2012; Kurdish 2019). Aggregation and binding patterns can occur because NPs induce hydrophobic regions on the bacterial cell membrane or because NPs can attach to hydrophobic spots already present on the bacteria (Hayden et al. 2012). However, interactions of particles with bacteria occur not only by electrostatic attraction but also by bacterial surface chemical reactions, such as those associated with phospholipid membrane exposure (Palmqvist et al. 2015). The lipopolysaccharide (LPS), lipoteichoic acid (LTA), proteins, and phospholipids of bacterial cells are perhaps the most important biomolecules interacting with NPs (Jiang et al. 2010). The method of NP transport into bacterial cells depends on the size of the compounds, and there is still no specific model to predict PGPB and NP interactions (Shukla et al. 2015).

It has been suggested that NPs affect the plant-PGPB system through direct and indirect mechanisms. In the direct mode, the NPs enhance nutrient availability, while in the indirect one, NPs stimulate the PGPB and consequently improve the growth-promoting effects (Kalia and Kaur 2019). The mechanisms of which NPs indirectly benefit the plant–microbe association include increased cell viability and bacterial growth rate, help plants to endure adverse conditions by inducing the secretion of specific compounds in microbes, support microbial interaction by providing a high surface area, enhanced plant beneficial features of microbes, and protection of inoculants in the field when applied as carriers (Ghalamboran and Ramsden 2012; Karunakaran et al. 2013; Abdel Latef et al. 2018; Gudadhe et al. 2018).

Nanomaterials can positively impact beneficial bacterial traits, such as the production of secondary metabolites and nitrogen fixation (Fig. 1). For example, copper oxide NPs (CuO NPs) increased IAA synthesis in the plant growth-promoting bacterium *Pseudomonas chlororaphis*, and zinc oxide NPs (ZnO NPs) increased siderophore production, probably induced by ion release in the bacterium (Dimkpa et al. 2012a, b). Silver NPs (AgNPs) increased the nitrogen

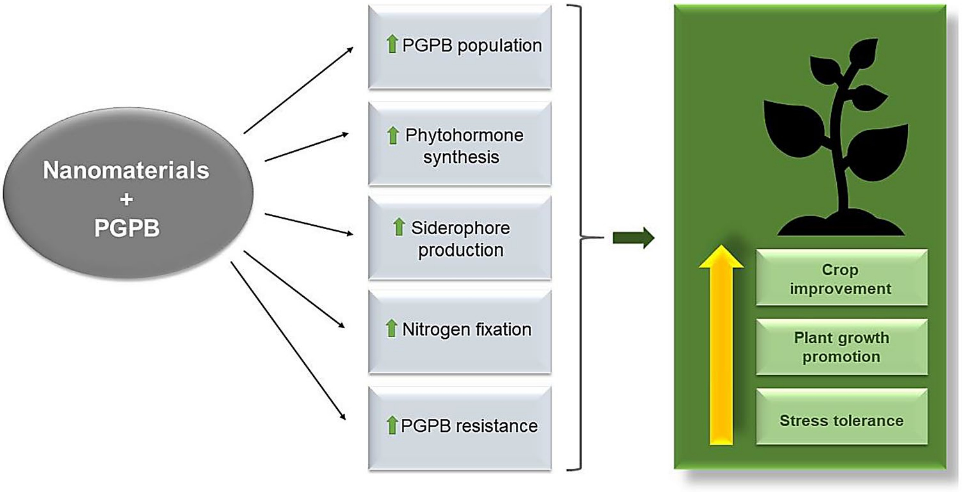

Fig. 1 Positive effects of NPs on beneficial bacteria and plant growth

fixation potential in *Nitrosomonas europaea* by upregulating the expression of the nitrification-related genes *amoA1* and *amoC2* (Yang et al. 2013). However, these studies also showed negative impacts of NPs on beneficial bacterial traits, indicating that their effects on bacteria are unpredictable and depend on several factors. Indeed, there are various mechanisms through which nanomaterials influence bacterial metabolism, although they are not well understood. These mechanisms apparently include ion interactions, changes in gene expression, and modification of cell membranes.

The association between NPs and PGPB can also improve the suppression of phytopathogens. Since NPs increase the number of bacterial cells, PGPB have an advantage over phytopathogens in the competition for niches and nutrients. Additionally, by increasing the production of secondary metabolites of PGPB and nutrient uptake, plants are more resistant to phytopathogen attack. Silica NPs, in particular, increase the thickness and mechanical resistance of plant cell walls, providing extra protection against herbivory and entry of phytopathogens (Djamin and Pathak 1967; Kaufman et al. 1979; Cui et al. 2020). It is well documented that several beneficial bacteria exert antagonistic activity against microorganisms harmful to plants in their natural environments, either by competing or by synthesizing antimicrobial biomolecules (Sturz et al. 2000; Berg et al. 2005; Ryan et al. 2008; Quecine et al. 2014). Therefore, it is crucial to conduct experiments testing bacteria under laboratory, greenhouse, and field conditions to confirm their antagonistic properties. In this way, NPs improve the bacteria already selected for biocontrol. In fact, it is difficult to predict whether NPs also stimulate phytopathogens. Thus, large-scale studies on target bacteria, NPs, phytopathogens, and plants are necessary to ensure the safety and effectiveness of nanobiofertilizers.

There is a lack of studies regarding plant growth-promoting bacterium-NP-plant interactions at the molecular level. Thus, the plant molecular activity triggered by nanomaterials associated with PGPB is still poorly understood. Physiological studies involving NPs and plants have demonstrated that small-size NPs can move through the symplast, such as through plasmodesmata, whereas larger NPs accumulate in the apoplastic space (Jha and Pudake 2016). These studies did not include bacteria, so the effects might differ when introducing PGPB into the system.

Rhizophagy is a mechanism in which plant roots internalize and break down microbes to extract nutrients (Paungfoo-Lonhienne et al. 2013). White et al. (2012, 2014) found that plant roots produce reactive oxygen, which degrades cell walls, membranes, proteins, and other molecules of the symbiotic bacteria in the periplasmic spaces of cells to extract nitrogen from the bacteria. These findings indicated that plants absorb nutrients from microbes as part of a microbivory process (White et al. 2019). In the rhizophagy cycle, as proposed by White et al. (2018), free-living microorganisms obtain nutrients from the soil and become intracellular/endophytic after entering the plant through its root tips; the host plant then extracts those nutrients through an oxidative process. After the nutrients are exhausted, the surviving microbes leave the plants through root hairs and recharge in the rhizosphere, starting the cycle again (White et al. 2018). Following this logic, we hypothesize that some of the NPs attached to the bacterial surface remain within plant tissues in this process.

TiO_2 nanoparticles

Titanium dioxide (TiO_2) is an oxide formed from a transition metal and is one of the most commonly used semiconductors for photocatalysis (Gupta and Tripathi 2011). It is also present in many everyday products, such as paint, paper, plastics, ceramics, cosmetics, and food packaging (Jameel

2015). Some of the reasons for its widespread use include its chemical stability, handling safety, and biocompatibility (Shi et al. 2013). TiO_2 exists as three different crystalline phases: anatase, rutile, and brookite. The first two involve tetragonal structures and are the most common (Noman et al. 2019). Considering the photocatalytic activity of TiO_2, anatase is preferred over rutile because the former adsorbs less oxygen and has a higher Fermi level. However, due to its bandgap being close to 3.0 eV, which varies for each phase, this material can be activated only under UV radiation (Tanaka et al. 1991). Because TiO_2 nanoparticles have a larger surface area than bulk titanium dioxide does, they have a greater photocatalytic capacity.

There are different synthesis methods for obtaining titania nanoparticles: deposition methods, oxidation methods, hydrothermal methods, and sol–gel methods (Baetzold 1981; Seifried et al. 2000; Feng et al. 2005; Chen 2009). Hydrothermal and sol–gel methods are the most commonly used for controlling the size and shape of nanoparticles. Hydrothermal synthesis is defined as crystallization above room temperature and under high pressure within a solvent that can be aqueous or nonaqueous. With this process, the final material properties can be controlled by adjusting the pH, temperature, duration of synthesis, type of solvent, or the amount of each reagent added (Liu et al. 2014). The synthesis is usually carried out in an autoclave that can withstand the extreme conditions used. Among the advantages, the robust control of size, purity, and low agglomeration predominate (Chen et al. 2015; Mamaghani et al. 2019). The sol–gel synthesis method is a wet chemical method and allows the synthesis of high-purity nanoparticles at a relatively low temperature and allows increased stoichiometry control of doping. This method involves the conversion of a solution with a titanium precursor into an inorganic solid formed by nanoparticles. The solution (sol), consisting of colloidal particles dispersed in a liquid, is formed by hydrolysis or solubilization of the precursor, and condensation leads to the formation of a gel. Typical precursors include metal oxides and metal chlorides (Akpan and Hameed 2010; Macwan et al. 2011).

Evidence that titania NPs have adhesive effects on bacteria was reported by Park et al. (2008), who found that *Pseudomonas* spp. presented adhesion and bioluminescence rates that were higher on surfaces with nanostructured titania than with conventional titania. Thus, it can be hypothesized that nanotitania could be used to guide bacteria to a specific site (Palmqvist et al. 2015). TiO_2 nanoparticles synthesized by the sol–gel method have been demonstrated to help plant growth-promoting rhizobacteria (PGPR) attach to plant roots (Table 1). Bacteria in the presence of nanoparticles form stable and thicker layers than do those grown within a self-produced biofilm (Timmusk et al. 2018). Additionally, the biomass of wheat seedlings increased when double inoculations of PGPR were performed with titanium NPs in peat soil under adverse conditions caused by drought, salt, and fungal pathogens. These findings can be explained by the increased bacterial colonization in the presence of NPs (Timmusk et al. 2018). Likewise, Palmqvist et al. (2015) reported that titania NPs helped the plant growth-promoting bacterium *Bacillus amyloliquefaciens* attach to oilseed rape (*Brassica napus*) roots and protected plants against the fungal pathogen *Alternaria brassicae* (Palmqvist et al. 2015). Zand et al. (2020) tested TiO_2 nanoparticles in association with the rhizobacterium *Pseudomonas fluorescens* to promote phytoremediation in soils contaminated with cadmium (Cd). The nanoparticles were synthesized by the sol–gel method in which $TiCl_4$ was used as a precursor and ranged in size between 10 and 40 nm. White clover (*Trifolium repens*) seedlings were exposed to PGPR and different doses of TiO_2; the results showed that the coapplication of bacteria and NPs at concentrations up to 500 mg/kg improved *T. repens* plant growth and increased plant biomass. The Cd uptake and accumulation capacity also increased under the same conditions in both the roots and the shoots. However, at a concentration of 1000 mg/kg TiO_2, the effect was inhibitory (Zand et al. 2020). Furthermore, titanium NPs at a concentration of 0.01% benefited broad bean plants in saline soil by increasing either enzymatic activity or the solubility of sugars and amino acids. These findings show that TiO_2 nanoparticles can also be useful for growing crops in contaminated soils (Abdel Latef et al. 2018).

SiO_2 nanoparticles

Silicon is the second most common element on Earth after oxygen and is very abundant in the soil (Wainwright et al. 1997). Plants naturally demand silica to help respond to biotic and abiotic stresses (Ma 2004); these responses include enhancing water-use efficiency and the photosynthetic potential of plants and increasing both the mechanical strength and the rigidity of leaves, thus preventing them from falling over and preventing pathogen attack (Datnoff et al. 1997; Jian et al. 2006; Namaganda et al. 2009). Plants produce silica bodies by absorbing soluble silica from the groundwater and transporting it to different tissues through the vascular system.

Silicon oxide (SiO_2) nanoparticles, also known collectively as silica, are among the most widely used nanoparticles (Neethirajan et al. 2009). They can be either extracted from the environment or synthesized. When extracted, they are in a mineral silica form with a crystalline phase (such as quartz), differing from synthetic nanoparticles, which are amorphous. The advantage of synthesizing these NPs is the purity of the final material, as the extracted NPs have impurities from other metals (Rahman and Padavettan 2012).

Table 1 Published studies on the use of nanoparticles with PGPB in plants

Nanoparticles	Synthesis	Concentration	PGPB	Plant Species	Findings	Reference
CuO	Commercial	50 mg/kg	Soil microbiota	Wheat (*Triticum aestivum*)	Increased N_2 fixation Increased nitrification Increased plant growth	Guan et al. (2020)
SiO_2	Extracted from *Equisetum telmateia*	0.05 ppm 0.07 ppm	*Pseudomonas stutzeri* *Mesorhizobium* spp.	Land cress (*Barbarea verna*)	Increased shoot and root dry weight Increased nitrogen and phosphate in the soil	Boroumand et al. (2020)
TiO_2	Sol–gel, with $TiCl_4$ used as a precursor	100 mg/kg 250 mg/kg 500 mg/kg 500 mg/kg 1000 mg/kg	*Pseudomonas fluorescens*	*Trifolium repens*	Coapplication of bacteria and NPs promoted the growth of *T. repens* and increased its biomass Enhanced Cd uptake and Cd accumulation capacity, promoted soil phytoremediation 1000 mg/kg TiO_2 caused inhibitory effects on plant growth	Zand et al. (2020)
Alginate-SiO_2 Carbon nanotubes	Method of Tu et al. (2015)	–	*P. fluorescens* *Bacillus subtilis*	UCB-1 pistachio	Increased plant biomass and bud length	Pour et al. (2019)
TiO_2	Captigel Sol–gel approach	50 µg/mL	*Bacillus thuringiensis*, *Paenibacillus polymyxa*, and *Alcaligenes faecalis*	Wheat (*Triticum aestivum*)	Double inoculants with NPs stabilized bacterial attachment to the roots Increased biomass of plant in peat soil under adverse conditions	Timmusk et al. (2018)
TiO_2	Commercial	50–400 µg/mL	*Pseudomonas aeruginosa* *P. fluorescens* *Bacillus amyloliquefaciens*	–	200 µg/mL decreased phosphate solubilization and production of iron-binding siderophore molecules 200 µg/mL reduced the production antibiotics by *P. aeruginosa* and *P. fluorescens* All the effects were dependent on the concentration	Haris and Ahmad (2017)

Table 1 (continued)

Nanoparticles	Synthesis	Concentration	PGPB	Plant Species	Findings	Reference
ZnO	Commercial	200 µg/mL	*P. aeruginosa* *P. fluorescens* *B. amyloliquefaciens*	–	Inhibited IAA production and phosphate solubilization in the three bacteria Reduced the production of antibiotics by *P. aeruginosa* and *P. fluorescens* The three bacterial species produced increased amounts of siderophore All the effects depended on the concentration	Haris and Ahmad (2017)
TiO_2	Captigel Sol–gel approach	50 µg/mL	*B. amyloliquefaciens*	*Brassica napus*	The NPs promoted bacterial attachment to plant roots Improved colonization and protected the oilseed rape from a pathogen	Palmqvist et al. (2015)
Au	Citrate reduction method, with $HAuCl_4$ used as a precursor	6.25 µg/mL	*P. fluorescens*, *B. subtilis*, *Paenibacillus elgii*, and *Pseudomonas putida*	–	Increased the growth of *P. fluorescens*, *B. subtilis*, and *P. elgii* No impact on *P. putida*	Shukla et al. (2015)
Fe, Zn, Mn	Commercial	4 L/ha 8 L/ha	*Azotobacter* spp. *Pseudomonas* spp.	Spring wheat (*Triticum aestivum*)	Increased spike length, spike number, seed number, seed number per spike, seed weight, and number of days until physiological maturity	Mardalipour et al. (2014)
SiO_2	Rice husk using alkaline treatment followed by acid precipitation	0.5 g/kg	Phosphate solubilizers Nitrogen fixers Silicate solubilizers	Maize (*Zea mays* L.)	Increased bacterial populations, total biomass, and soil nutrient contents	Rangaraj et al. (2014)
SiO_2	Rice husk using alkaline treatment followed by acid precipitation	10 mg/L	*Bacillus megaterium* *Bacillus brevis* *P. fluorescens* *Azotobacter vinelandii*	Maize (*Zea mays* L.)	Increased bacterial viability and the total soil bacterial population Increased N, P, and K contents Caused 100% seed germination	Karunakaran et al. (2013)
ZnO	Commercial	500 mg/L	*Pseudomonas chlororaphis*	–	Inhibited IAA production by the bacteria	Dimkpa et al. (2012b)
CuO	Commercial	200 mg/L	*P. chlororaphis*	–	Enhanced IAA production by the bacteria	Dimkpa et al. (2012b)

Silica NPs are extensively used in various fields due to their ease and low cost of synthesis; large amounts of silica can be obtained, and their morphology and size, as well as their biocompatibility, chemical inertness, and large surface area can be controlled (Rao et al. 2005; Jeelani et al. 2020). One of the main applications is their use in controlled-release systems, allowing the loading of various compounds, such as drugs, DNA, RNA, proteins, fertilizer components, and pesticide ingredients. In addition to allowing the controlled output of molecules, silica NPs can improve the efficiency, specificity, bioactivity, and biocompatibility of molecules (Hom et al. 2009; Xu et al. 2019). One way to better control release is by synthesizing mesoporous silica NPs, as the pores hinder molecules from exiting (Narayan et al. 2018; Karaman and Kettiger 2018). The use of silica NPs in agriculture has also been reported to increase maize seed viability when present in smart pesticide formulations and augment bioremediation (Yuvakkumar et al. 2011; Kumari and Singh 2016; Liang et al. 2020).

The most commonly used methods to synthesize SiO_2 nanoparticles include reverse microemulsion, flame synthesis, and sol–gel methods. In reverse microemulsion synthesis, spherical micelles are formed by a surfactant dissolved in an organic solvent. When in contact with water, the polar heads organize themselves to form reverse micelles, which are microcavities containing water. The nanoparticles then infiltrate these microwells after the addition of the silicon precursor. This method's disadvantages include high costs and difficulty removing the surfactant from the final material (Yoo and Pak 2013). Flame synthesis, also known as chemical vapor condensation, is based on the flame decomposition of metal–organic precursors. The most commonly used process involves reacting oxygen and hydrogen with a precursor of silicon, silicon tetrachloride ($SiCl_4$). Disadvantages of this method include the difficulty in controlling the NP size and morphology (Shekar et al. 2012). Finally, the sol–gel method is based on the hydrolysis and condensation of a silicon precursor, an alkoxide, in the presence of a catalyst, either an acidic or basic one. The most widely used sol–gel method is the Stöber method. The precursor used is tetraethylorthosilicate (TEOS, $Si(OC_2H_5)_4$), which is added to a solution containing water and ethanol. Hydrolysis, catalyzed by an ammonium hydroxide base, occurs with the nucleophilic attack of water on the alkoxide, forming silanol groups ($Si(OH)_4$). The subsequent condensation of these groups results in Si–O–Si chains, creating the three-dimensional structure of silica (Stöber et al. 1968). The great advantage of the Stöber method is the production of dispersed NPs with a spherical shape and the ability to control their size. In the sol–gel synthesis method, the NP sizes depend on the concentration of the reagents and temperature. Their final sizes are based on the TEOS/NH_3 molar ratio; the higher the ratio is, the smaller the diameter (Bailey and Mecartney 1992). Arantes et al. (2012) conducted a study using chemometrics to investigate the influence of each reagent on the final size of NPs. In all experiments where the ammonia amount was increased, the NPs obtained also increased in size (Arantes et al. 2012). In mesoporous silica, a surfactant is added to the reaction medium, and the solution is heated before adding TEOS. Cetrimonium bromide (CTAB) is the most commonly used surfactant, and the temperatures used are close to 80 °C (Parangi et al. 2014).

An experiment conducted with maize (*Zea mays*) seeds demonstrated that 50 nm silica NPs increased the viability of PGPB and their population in the soil. The NPs did not show a toxic effect, maintaining an optimal pH for the bacteria. Moreover, they increased the amount of nitrogen, phosphorus, and potassium (NPK), resulting in the germination of all maize seeds (Karunakaran et al. 2013). Rangaraj et al. (2014) also studied the effects of nanosilica on PGPB in maize. The results showed that the nanoparticles increased the bacterial population, total biomass, and soil nutrient contents. In both studies, silica NPs proved to be better than other sources of silicon. Nanosilica was also reported to benefit tomato (*Lycopersicum esculentum*) plants by increasing the germination rate, average germination time, seedling fresh weight, and dry weight (Siddiqui and Al-Whaibi 2014). In all these studies, the germination was better, indicating the potential of silica NPs for crop improvement.

Another way NPs can be used in agriculture is based on their encapsulating capacity. Pour et al. (2019) encapsulated *Pseudomonas fluorescens* and *Bacillus subtilis* in alginate-silica NPs and carbon nanotubes. This method helped to improve the root length and micropropagation of UCB-1 pistachio. Inoculation of explants with encapsulated bacteria increased plant biomass and bud length compared to those of the controls (Pour et al. 2019). Boroumand et al. (2020) extracted silica NPs from *Equisetum telmateia*; the researchers sprayed them with the phosphate-solubilizing rhizobacterium *Pseudomonas stutzeri* and *Mesorhizobium* spp. to test their effects on land cress (*Barbarea verna*) plant growth. The NPs improved bacterial growth when they were applied at concentrations of 0.05 and 0.07 ppm. When NPs were used simultaneously with both bacterial species, the dry weights of the shoots and roots were the highest recorded, and the nitrogen and phosphate contents increased in the soil, improving plant growth (Boroumand et al. 2020). Silica NPs (5–20 nm) have also shown a positive effect on *B. subtilis*, inducing an 85% increase in cytokinin synthesis (Kurdish et al. 2018).

The interaction between nanosilica and bacteria can be explained by the hydration properties of silica NP surfaces, facilitating their attraction to bacteria and consequently improving bacterial acid resistance (Gordienko and Kurdish 2007; Hirota et al. 2010). Furthermore, studies have shown an increase in the negative surface charge of some gram-negative bacteria when silicon dioxide particles were added to the media. It has been proposed that the increase in charge density is due to a change in porin conformation resulting from the adsorption of the particles by cell surfaces (Gordienko et al. 1993, 1999; Gordienko and Kurdish 2005, 2007).

Studies show that SiO_2 NPs can enhance the ability of bacteria to promote plant growth. The stimulating effect of mineral NPs on bacterial growth may be explained by the improved oxygen mass transfer and ion exchange processes in the media (Kurdish 2019). We hypothesized that the same effect might happen with other nanomaterials. Furthermore, NP attachment to the bacterial surface may alter the shape and size of cells, increasing growth (Phenrat et al. 2009).

ZnO nanoparticles

Zinc oxide (ZnO) is an n-type semiconductor; its nanoparticle properties include high chemical stability, nontoxicity, biocompatibility, and photothermal stability, and these particles can be produced at relatively low costs (Weldegebrieal 2020). The high capacity to absorb ultraviolet radiation makes zinc oxide a promising photocatalyst. This compound is considered an alternative to TiO_2 because it has a similar bandgap (3.37 eV) (Kołodziejczak-Radzimska and Jesionowski 2014). Zinc oxide NPs are very useful in medicine and food packing due to their biocompatibility, biodegradability, and antimicrobial properties (Akbar et al. 2019). Additionally, ZnO has piezoelectric properties, generating electrical tension under mechanical pressure, making it a viable material for sensors (Bhatia et al. 2016). These nanoparticles are classified as one-, two-, or three-dimensional based on their structure; one-dimensional examples include nanorods, needles, tubes, and wires. An example of a two-dimensional ZnO particle is a nanosheet, and a three-dimensional model is a flower (Wang 2004).

ZnO NPs can be synthesized by both physical and chemical methods. The most commonly used physical methods include laser ablation, characterized by the removal of atoms from a solid with an intense laser beam, and physical vapor deposition, in which the material in a vapor state is transferred to a substrate (Thareja and Shukla 2007; Laurenti et al. 2015). Among the chemical methods, the precipitation, sol–gel, and hydrothermal synthesis methods are most common. In precipitation synthesis, a reduction in zinc salt in solution occurs using a reducing agent that controls the size of NPs, followed by thermal treatment (Kołodziejczak-Radzimska et al. 2010). The zinc precursors used are zinc chloride and zinc acetate, while the reducing agent used is ammonium carbonate (López et al. 2017). The sol–gel method makes it possible to obtain ZnO nanoparticles repeatability and at low cost, in addition to allowing surface modifications (Al Abdullah et al. 2017). The hydrothermal method can be used for synthesizing nanoparticles of different shapes and sizes by varying the composition of the reaction mixture, resulting in materials with high crystallinity (Bharti et al. 2017).

ZnO NPs have been used in agriculture to improve food crop growth and increase yields. For example, ZnO NPs at a 1000 ppm concentration promoted seed germination and seedling vigor and increased root and stem growth in peanut (*Arachis hypogaea*) plants (Prasad et al. 2012). When these NPs interact with PGPB, various effects have been reported. The bacterium *Pseudomonas chlororaphis* and 500 mg/L ZnO NPs produced higher amounts of siderophores than did the controls in 24 h. An explanation for this would be the release of Zn^{2+} ions by NPs, which bind to siderophores. However, ZnO was shown to inhibit the production of IAA at 48 h. Nevertheless, this reduction cannot be explained by the release of cations since IAA production was shown not to be affected in a Zn^{2+} solution (Dimkpa et al. 2012a, b). Zinc oxide NPs also inhibited IAA production in the ZnS plant growth-promoting bacterial species *P. aeruginosa*, *P. fluorescens*, and *B. amyloliquefaciens*. The concentrations of NPs used ranged from 100 to 400 µg/mL, and as the concentration increased, the production decreased. The NPs also inhibited the phosphate solubilization of the three bacteria, which again proved to depend on zinc oxide concentration. Regardless, all the bacteria produced more siderophores in the presence of ZnO NPs than in the control conditions, and the increase in NP concentration led to increased production of this compound (Haris and Ahmad 2017).

In another study, ZnO NPs inoculated with PGPR in soybean plants promoted increased plant height, number of nodules, and grain weight. When the concentration of zinc oxide was increased, the dry weight of nodules per plant, pod number per plant, and grain number per plant also increased (Seyed Sharifi and Khoramdel 2016). Similarly, Gudadhe et al. (2018) reported that $ZnSO_4$ NPs, in association with the *Pseudomonas* spp. PGPB, increased nitrogen, phosphorus, potassium, and zinc contents in rice plants, promoting increased grain yield and seed nutrient contents (Gudadhe et al. 2018).

Other nanoparticles

Soils have a rich amount of natural mineral nanoparticles, which play an essential role in the physiological and biochemical processes of microorganisms due to their close interactions (Kurdish and Kigel 1997; Mishra and Kumar 2009; Kurdish 2010). Therefore, the effects of nanomaterials on PGPB have been explored to improve bacterial efficiency in plants. Kurdish et al. (2015) investigated the impact of soil nanomaterials on N fixation and P mobilization bacterial activity. The authors found that NPs of vermiculite and saponite promoted bacterial growth and that vermiculite NPs increased both the dehydrogenase activity in *Azotobacter vinelandii* and the peroxidase activity in *B. subtilis* at concentrations of 1.5 and 2.5 g/L. The formulation consisting of bacteria and nanomaterials enhanced grain yield and protein content and reduced lesions caused by diseases in wheat and barley plants. Furthermore, Chobotarov et al. (2017) reported that vermiculite NPs increased the accumulation of abscisic acid (ABA) in *A. vinelandii* and *B. subtilis* and 3-indoleacetic acid (IAA) synthesis in *B. subtilis*. The phytohormone ABA is a key regulator of plant tolerance to biotic and abiotic stresses (Zhu 2002; Fujita et al. 2006), while IAA is essential for plant growth and development (Waseem et al. 2018). Since mineral NPs are present in nature, as well as silica, their use can be considered a safer approach with vast prospective potential along with PGPB to enhance plant growth and tolerance to stressful environments and phytopathogens. However, it is necessary to investigate the interactions between different bacterial species and NPs of various sizes and concentrations under natural conditions. An increase in IAA synthesis was also shown in the plant growth-promoting bacterium *Pseudomonas chlororaphis* by CuO NPs at a concentration of 200 mg/L, possibly explained by ion release (Dimkpa et al. 2012b). Depending on their dose, these NPs are harmful to beneficial plant bacteria (Baek and An 2011). Hence, this finding highlights the dose-dependent effect of nanomaterials in bacteria. The application of CuO NPs to the soil of wheat (*Triticum aestivum*) increased microbial community health, enhanced nitrogen fixation, and decreased denitrification processes, which led to plant growth. A correlation was observed between increased nitrate concentration in the rhizosphere and an increase in the expression of genes related to N fixation (Guan et al. 2020). These findings suggest that CuO NPs can be involved in N fixation-related gene expression.

Gold NPs (AuNPs) are valuable materials due to their chemical inertness, resistance to surface oxidation, and low toxicity in natural environments (Shankar et al. 2004; Shukla et al. 2015). Shukla et al. (2015) endorsed the potential of these NPs in nanobiofertilizer formulations due to their growth-promoting effects on the beneficial bacteria *P. fluorescens* and *B. subtilis* as the AuNP concentration increased.

A successful nanobiofertilizer can also be achieved by mixing more than one bacterial species with various nanomaterials. Mardalipour et al. (2014) reported the benefits of different NPs associated with two PGPB in spring wheat plants. The nanobiofertilizer (Biozar®), which includes *Azotobacter*, *Pseudomonas*, and NPs of Fe, Zn, and Mn, improved agronomic traits and increased crop growth and yield.

AgNPs are effective at controlling plant diseases (Jo et al. 2009; Aguilar-Méndez et al. 2011; Abdellatif et al. 2016). These particles act as growth stimulants and enable plants to inhibit senescence induced by reactive oxygen species (ROS) under stress conditions (Karuppanapandian et al. 2011). Pallavi-Mehta et al. (2016) examined the effects of silver nanoparticles on the bacterial rhizosphere diversity and growth of wheat (*Triticum aestivum*), cowpea (*Vigna sinensis*), and Brassica (*Brassica juncea*) plants. While wheat showed only a negative effect, the cowpea showed a positive response in growth parameters and root nodulation, and Brassica exhibited a positive response for shoot parameters. Regarding the bacterial diversity in cowpea and wheat, the 75 ppm concentration reduced the population of N fixers and siderophore producers, while 50 ppm positively affected P solubilizers. In the case of Brassica, there was no change in diversity when either concentration was used (Pallavi-Mehta et al. 2016).

Chavan and Nadanathangam (2019) reported that, at low concentrations, AgNPs presented bactericidal activity against N-fixing, phosphate-solubilizing, and biofilm-forming bacteria (2–22 µg/mL). However, there was an increase in the abundance of the bacteria *Stenotrophomonas* spp. and *Pseudomonas* spp., which have a great capacity to promote plant growth (Chavan and Nadanathangam 2019). Khan and Bano (tested the influence of AgNPs and *Pseudomonas* spp. and *Bacillus cereus* PGPR on maize growth. The NPs attached to the bacteria improved the root area and length. Nanosilver particles also improved rhizobacterial bioremediation ability for Pb, Cd, and Ni (Khan and Bano 2016). AgNPs with *Pseudomonas putida* were also reported to benefit cucumber (*Cucumis sativus* L.) plants. These particles enhanced the activities of phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD), and catalase (CAT) and increased the flavonoid contents of cucumber leaves, thus improving plant tolerance to disease and stress (Nawaz and Bano 2019).

It is worth mentioning that even though these findings show the potential of AgNPs to promote plant growth, they also warrant attention because of their harmful effects on some bacterial populations. Several studies have reported on the bactericidal activity of AgNPs and their adverse impacts on soil microbial diversity. Nevertheless, their toxicity seems to rely on bacterial species and synthesis methods of NPs (Dimkpa et al. 2011).

Industrial-scale production of nanoparticles

Two main approaches can be used to produce nanoparticles on an industrial scale, *bottom–up* and *top–down*. Bottom–up methods involve forming a complex and more structured arrangement of atoms, resulting in structures such as clusters and nanoparticles. On the other hand, top–down methods imply that the nanostructure is synthesized by removing atoms or crystalline planes. Both have advantages and limitations. The most common top–down approach for producing nanoparticles is the ball milling process. This method can produce large amounts of nanoparticles from cheap raw materials but with the disadvantage of presenting broad particle size distributions and irregular nanoparticles. Several oxides can be processed by ball milling, including several phosphates (Danelon et al. 2015). Other top-down routes, such as electron beam induce etching, produce only a few milligrams or less material, which is only feasible for exceptional (and expensive) applications. Bottom–up routes are simple and often use water as a solvent, which represents an environmental advantage. Metallic nanoparticles, such as silver and gold, can be easily synthesized on a large scale from water-soluble salts reduced by cheaper molecules, like citrates or alcohols (Gorup et al. 2011). Nowadays, natural extracts have emerged as an environmentally friendly method to produce metallic nanoparticles (Fernandes et al. 2018). Nonaqueous solvents have opened a new route for obtaining nanoparticles in cases where they can act as solvent and reactant. Silica and other oxides can be synthesized on an industrial scale from the hydrolysis of some alkoxides, usually using several small reactors instead of a large reactor.

Toxicological effects of nanoparticles on plant growth-promoting bacteria

Even though nanomaterials have the potential to increase the abundance of PGPB, they might negatively affect soil bacteria. As mentioned before, the shape of NPs seems to be one of the factors influencing their biological activity. For example, Dimkpa et al. (2011) found that spherical AgNPs did not damage the integrity of *Pseudomonas chlororaphis* membranes, while Pal et al. (2007) found that triangular AgNPs exhibited the strongest biocidal action in *Escherichia coli*. Thus, it has been proposed that AgNPs with different shapes may also have other modes of action regardless of having the same surface areas (Pal et al. 2007). In another study, Dimkpa et al. (2012b) reported that CuO NPs enhanced bacterial IAA production, while ZnO NPs reduced the synthesis of this phytohormone in the same bacteria. The authors suggested that NPs with different shapes (CuO NPs have a round shape, and ZnO NPs have elongated structures) were critical in the production of bacterial secondary metabolites (Dimkpa et al. 2012b).

Bacterial toxicity also seems to be dependent on the nature of the metal (Sinha et al. 2011) and the concentration of NPs (Hayden et al. 2012). Chavan et al. (2020) reported that TiO_2 NPs were more inhibitory to gram-positive bacteria, suggesting that the bacterial surface is primarily responsible for TiO_2 NP toxicity. Rousk et al. (2012) proved that Cu and Zn at more soluble forms were more toxic to soil bacteria than the metal oxide forms or nanoforms. Therefore, these NPs can be more harmful than the bulk material only when the metal dissolution is higher in this form, depending on the soil (Rousk et al. 2012).

The overall conclusion regarding the toxicity of nanomaterials is that the NP reactivity, toxicity, and uptake increase as the particle size decreases; positively charged NPs are more toxic to most living systems; NP toxicity is correlated with ionic dissolution; and rod-shaped and anisotropic NPs are more toxic even with less absorption (Singh et al. 2019).

Regarding phytotoxicity, NPs have either positive or negative impacts on plants. The toxic effects depend on plant species and NP composition (their nature, size, concentration, and exposure time) (Jha and Pudake 2016).

The introduction of nanomaterials into the environment might hinder biological organisms at many levels. The major obstacle involves several variables associated with nanomaterials, such as their material composition, size, shape, concentration, and interactions.

It is important to emphasize the evidence of agricultural improvements caused by the association of NPs and PGPB. Thus, there is an urgent need for in-depth investigations concerning lethal and sublethal doses on a case-to-case basis.

Conclusion and future perspectives

There is no doubt that the use of PGPB is a promising viable eco-friendly alternative to the use of chemicals in agriculture. However, knowledge of specific plant–microbe interactions and innovative approaches must be explored for the ability to use them in the field successfully.

Nanotechnology serves as an effective tool to overcome obstacles associated with microbial biofertilizers. Many nanomaterials have shown positive effects either on PGPB or on the growth of various plant species, making these materials promising components of biofertilizer formulations. The approach of using nanomaterials in association with beneficial bacteria in plants can increase bacterial viability and resistance in the environment, improving their field stability and consequently promoting plant growth. However, there are different effects of NPs on bacterial metabolism and concerns about the possible hazardous consequences of nanomaterials on the environment, food safety, and human health, so these effects must be investigated.

Further experimentation involving plant growth-promoting bacterium-NP-plant is necessary to evaluate the effectiveness of this complex system at the molecular level. Several factors are involved: the nature of bacteria and their metabolism, nanomaterials (their substance, synthesis method, shape, size, and concentration), plant species, and environmental interactions. In addition, nanomaterial characterization is also of great importance, since there are many combinations in which NP characteristics interact in different ways with the natural environment and cells.

Most of the experiments cited in this review were performed under controlled conditions, thus excluding abiotic and biotic interactions found in the field. Therefore, field experiments must be conducted to better understand and validate the previous findings.

The use of nanomaterials associated with PGPB can potentially increase yields in agricultural systems and address global food demands. Thus, any possible hazard needs to be investigated with sensitive risk assessment methods.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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