

Green synthesized metal nanoparticles as an ecofriendly measure for plant growth stimulation and disease resistance[☆]

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ABSTRACT

Nanotechnology, as an advanced technology plays a vital role in various aspects of plants. In recent past, nanoparticles synthesized via biological routes received attention than that of other means of synthesis due to their cost effectiveness and ecofriendly nature. This review is aimed at providing a comprehensive compilation of information on ecofriendly nature of green synthesized nanoparticles especially in plant disease resistance and plant growth enhancement. According to the available literature, among the different biological sources used for green synthesis of nanoparticles, plant based green synthesis has become more popular in recent years because of their non-toxic nature, higher availability and accessibility. The effects of green synthesized nanoparticles are primarily dose dependent and vary with the characteristics of nanoparticle and the plant. Furthermore, application of such nanoparticles at optimum concentration has notably inhibited the number of phytopathogens and favoured the growth and yield attributes of crop plants. Using the green synthesized nanoparticle in combination with other materials were found to have synergistic effects in disease resistance and causes growth enhancement in crop plants. Even though, numerous inherent benefits are there in the green synthesized nanoparticles, constraints such as toxicity at higher concentrations and unsafe disposal to the environment may limit the continuous application and opens new avenues for future studies.

1. Introduction

National as well as global issues such as inadequate arable land, climate change, disease outbreaks, political conflicts have challenged the food security in the present era. Increasing the agricultural food production by expanding the cultivable land is practically impossible which would likely to cause other environmental issues such as deforestation, global warming, and loss of biodiversity. Agriculture is challenged by many factors such as less availability and poor efficiency of inputs, higher cost of capital items, biotic and abiotic stresses, and weakened preference among young. At this juncture, utilization of advanced technologies helps combat the challenges in agriculture and increase the crop production. Nanotechnology is one such fast-moving technology that has found successful in addressing several challenges in many fields including agriculture.

Nanotechnology deals with nanomaterials whose one dimension at least is at nanoscale. These engineered particles are of sizes ranging from 1 to 100 nm (Hossain et al., 2019; Shaikhaldein et al., 2021). Owing to the small size and higher surface area to volume ratio,

nanoparticles interact with biological components efficiently.

Nanoparticles interact with biological systems by means of several aspects including uptake, distribution and internalization. At first, uptake of nanoparticles occurs either via inhalation or ingestion or dermal exposure. Followed by absorption, nanoparticles interact with extracellular biomolecules and distributed throughout the body via the circulatory or vascular systems. Then, these particles interact with cells where the plasma membrane is the main interface and internalized into the cells (endocytosis). Internalization of nanoparticles is carried out via passive diffusion, where no any receptors involved. Endocytosis is influenced by size, surface properties, such as porosity and charge, and composition of nanoparticle (Brandelli et al., 2020). As soon as the particles are internalized by the cell, they are enclosed within a membrane-bound vesicle eventually degraded by hydrolytic enzymes.

They are adorned with specific features such as antimicrobial activity, catalytic activity and growth promotion of plants. As a result, the use of synthetic materials especially, pesticides and fertilizers have been reduced as the same functions are attained with nanoparticles. They have affected the growth and development of plants, depending on the

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properties of nanoparticles and the type of crop plant (Ma et al., 2010). Potential benefits of nanoparticles have paved the way for scientists to focus more on the production of nanoparticles and their application to sustainably counteract the challenges in agricultural crop production.

Nanoparticles are synthesized by a number of methods and those are broadly grouped into physical, chemical and biological methods. These methods utilize different sources as reducing agents and or electron donors during the synthesis. Even though all three methods are in practice over the years, application of physical and chemical means at large scale has been limited in agriculture and health sectors (Li et al., 1999). This is due to the risks and challenges associated, such as the use of potentially toxic chemicals (Some et al., 2019; Ahmed et al., 2017), requirement of expensive equipment and machinery (Ahmad and Jaffri, 2019; Hossain et al., 2019), requirement of larger laboratory space (Ahmad and Jaffri, 2019), rigorous processing conditions like elevated temperature, pressure (Ahmad and Jaffri, 2019; Hossain et al., 2019) and high energy (Kowshik et al., 2003). Furthermore, the time consumed (Kausar et al., 2022; Some et al., 2019), formation of harmful side products (Kowshik et al., 2003), high cost involved (Kausar et al., 2022; Ahmad and Jaffri, 2019; Some et al., 2019; Ahmed et al., 2017), harmful effects on the environment (Hamouda et al., 2019; Ahmed et al., 2017) exacerbates the challenges associated with their synthesis. As a result, the need for an alternative means of synthesis being particularly environmentally friendly is pronounced and has opened new avenues for the development of green technologies in synthesizing them.

2. Green synthesis of nanoparticles

Green synthesis of nanoparticles is termed as biosynthesis of nanoparticles as it employs an array of biological sources. Plants (root, stem, leaf, fruit, seeds (Sana et al., 2020), microorganisms (bacteria, fungi, algae (Hossain et al., 2019; Kausar et al., 2022) and biopolymers (starch, alginate, cellulose, chitosan, proteins (Altaf et al., 2021) have been used for this purpose.

Green synthesis of nanoparticles is considered as a harmless means of nanoparticle synthesis. Compared to physical and chemical methods, this method provides many advantages (Fig. 1). However, few drawbacks including ecological imbalance resulted due to exploitation of natural biological sources and seasonal variation of phytochemicals may limit the green synthesis of nanoparticles (Altaf et al., 2021).

Green synthesis of nanoparticles requires three essential components namely reducing agents, solvent and capping agents. Biomolecules either naturally present or produced by plants and microbes such as polyphenols, flavonoids, terpenoids, tannins, alkaloids, polysaccharides, protein, amino acids, vitamins act as reducing agents in nanoparticle synthesis (Ibrahim et al., 2020; Hossain et al., 2019). These reduce the metal ions to zero valence state, and thereafter the functional groups exist in these primary biopolymers and phytochemicals help in stabilizing the synthesized nanoparticle. Among the three biological routes, nanoparticle synthesis utilizing plants is preferred because of their greater abundance, safety (Loomba and Scarabelli, 2013), non-toxicity and availability of broad range of phytoconstituents that act as reducing agents (Uddin et al., 2021).

Nanoparticle synthesis take place in a three-step process (i) reduction of metal ions, primarily indicated with the colour changes (ii) clustering and (iii) stabilization of nanoparticles (Some et al., 2019). Fig. 2 illustrates the simplified procedure of nanoparticle synthesis. Accordingly, several physical (temperature, time, ratio of precursor chemical to biomaterial) and chemical factors (pH, molarity of precursor chemical, concentration of extracts) influence the synthesis of green nanoparticles. For an example, slight alkaline pH favours the formation of smaller sized nanoparticles due to faster reactions while at a higher pH, agglomeration of nanoparticles occur (Haroon et al., 2019). Therefore, processing condition should be optimized to obtain desirable morphological features as well as the composition for

effective production and efficient utilization during bulk synthesis of nanoparticles.

3. Nanoparticles as an antimicrobial agent

Diseases caused by pathogenic bacteria, fungi and viruses are one of the key challenges in crop production. Their management using conventional pesticides possess many drawbacks including high cost, toxicity, biomagnification, environmental persistency and resistance development against the applied agrochemicals. This has urged the scientists to seek for alternative measures in plant disease management.

In this context, several metal-based nanoparticles engineered via green synthesis have found potential for antimicrobial activities and widely employed to manage diseases caused by bacterial and fungal pathogens. Properties of nanoparticles such as their small size, high surface area to volume ratio and zeta potential, differ with the biological source used. This influences the nature of interaction of nanoparticles with the microbial surfaces, in turn causing potential antimicrobial effects. In particular, small sized and less negative nanoparticles interact more with more negatively charged microbial cell membranes due to strong electrostatic attraction (Some et al., 2019) and would express greater antimicrobial activity. Antimicrobial effect of nanoparticles is a resultant of associated microbial factors (e. g. sensitivity) and inherent characteristics of nanoparticles (e. g. type, concentration). For an example, fungal phytopathogens had lesser mycelial growth upon exposure to nickel oxide nanoparticle (NiONP) synthesized using *Berberis balochistanica* stem extract. Depending on sensitivity of microbes the mycelial growth has been < 10 mm, < 20 mm, < 30 mm respectively in *Alternaria alternata*, *Aspergillus niger* and *Fusarium oxysporum* at 500 µg/ml (Uddin et al., 2021). Silver nanoparticles (AgNPs) synthesized using endophytic bacteria (*Pseudomonas poae* strain CO) isolated from garlic plants (*Allium sativum*) have shown mycelial growth inhibition of 80.56% and 85.78% in PDA and PDB growth media respectively at 20 µg/ml NiONPs (Ibrahim et al., 2020).

The concentration of nanoparticles is an important factor. The inhibitory effects of most of the nanoparticles are primarily dose dependent, i.e. higher the concentration higher the inhibition. Meanwhile effective concentration varies with the type of nanoparticle (Table 1). For an example, the biogenically synthesized zinc oxide (ZnO) and copper oxide (CuO) using lemon peel extract showed a dose dependent antifungal activity against *Alternaria citri*. Minimal inhibitory concentrations (MIC) were of 80 mg/ml and 90 mg/ml respectively for ZnO and CuO while minimal fungicidal concentration for both nanoparticles was 100 mg/ml (Sardar et al., 2022). Furthermore, in terms of biological source and the type of nanoparticles used, *Dickeya dadantii* that causes bacterial stem and root rot in sweet potato was inhibited by 200% and 1000% respectively upon the application of AgNPs synthesized with cell free culture supernatant of plant growth promoting bacteria *Pseudomonas rhodesiae* strain (G1) (Hossain et al., 2019) and ZnONPs synthesized with lemon fruit extract (Hossain et al., 2019).

In addition to phytopathogens, green synthesized nanoparticles have potential antimicrobial activity against certain microbes that are pathogenic to beneficial insects. For an example, AgNPs synthesized using aqueous leaf extract of *Morus indica* showed 50% and 100% growth inhibition of *Escherichia coli* K12 and *Staphylococcus aureus* that cause Falchieria disease in silkworm (*Bombyx mori* L.) (Some et al., 2019). Moreover, supplementary feeding of biosynthesized AgNPs at 10 µg/L favoured the survival and weight of larvae and pupae of silkworm (Some et al., 2019). Thus, application of AgNPs either on diseased or healthy mulberry leaves can enhance the silk production.

Moreover, green synthesized nanoparticles were found potential not only in inhibiting the phytopathogens *in vitro* but also in *in vivo* and field conditions, where the inhibitory effects can vary with pathogen and exposure time (ex: pre, along with and post infection). For an example, maximal growth inhibition was observed 6 days after incubation for *Penicillium* (92%) and *Fusarium* (89%) when treated with 400 µg/ml



Fig. 1. Benefits of green synthesis method of metal nanoparticles El-Naggar et al 2017; Iravani, 2011. Mittal et al 2013, Murugan et al., 2014, Velusamy et al., 2016.

of AgNPs synthesized using *Azadirachta indica* leaf extract, while for *Aspergillus* (91%) it was after 2 days (Harooun et al., 2019). Further, the exogenous application of AgNPs synthesized using *Moringa oleifera* leaves at a concentration of 30 ppm to kinnow mandarin plants, reduced the incidences of canker disease and *Alternaria* brown spot. However, the disease incidence was found to rise after 25 days in the first case (Hussain et al., 2019) and after 5 days in the latter (Hussain et al., 2018).

All the above-mentioned examples implied the potential of nanoparticles as antimicrobial agents. Further, rather than using a single nanoparticle, combinations of nanoparticles have found synergized antimicrobial activity. In particular, a wide zone of inhibition (25–53 mm) observed upon exposure of a nanocomposite of ZnO and CuO prepared with lemon peel extract implied better antifungal activity of nanocomposite, meanwhile single application of ZnO and CuO nanoparticles respectively resulted the zones of inhibition of 21.5–51.5 mm and 18.5–50 mm (Sardar et al., 2022). A nanocomposite made of chitosan and two metal nanoparticles of Cu and ZnO (CS-Zn-CuNPs) showed higher antifungal activity with a 1.7 cm zone of inhibition at 90 µg/ml and its application reduced gray mold disease caused by *Botrytis cinerea* and lowered the fruit rot percentage over the control (Al-Dhabaan et al., 2017). Furthermore, Kumar et al. (2022) has

reported that mancozeb loaded nanocomposite prepared with chitosan biopolymer and carrageenan completely inhibited *Sclerotinia sclerotiorum* and *Stemphylium lycopersici* in *in-vitro* conditions at 1.5 ppm. This nano formulation showed enhanced disease control efficacy for *S. lycopersici* (73%), *Alternaria solani* (79%) and *S. sclerotiorum* (77%) compared to commercial fungicide in tomato and potato treated with 10 ppm of polymeric nanoparticle after disease outbreak.

Green synthesized nanoparticles have received potential as antimicrobial agents via two mechanisms; cell membrane damage (Hossain et al., 2019; Sable et al., 2018; Sardar et al., 2022) and oxidative stress development (Ali et al., 2021; Duran et al., 2016; Onodera et al., 2015; Reidy et al., 2013). Nanoparticles attached to microbial surfaces and especially those with the size lesser than microbial cell membrane pores easily penetrate the membrane and damage the cell with the release of toxic ions from nanoparticles. The smaller, less negatively charged green synthesized AgNPs (Hossain et al., 2019) and ZnONPs (Sardar et al., 2022) nanoparticles inhibit the fungal pathogens in this manner. In addition, nanoparticles induce morphological changes such as pores and pit formation and increase the permeability of microbial cell membranes by altering the membrane transport systems. This eventuates to leakage of cell constituents causing cell death. The NiONPs (Uddin et al., 2021) and AgNPs (Saleem et al., 2017; Dakal et al., 2016)

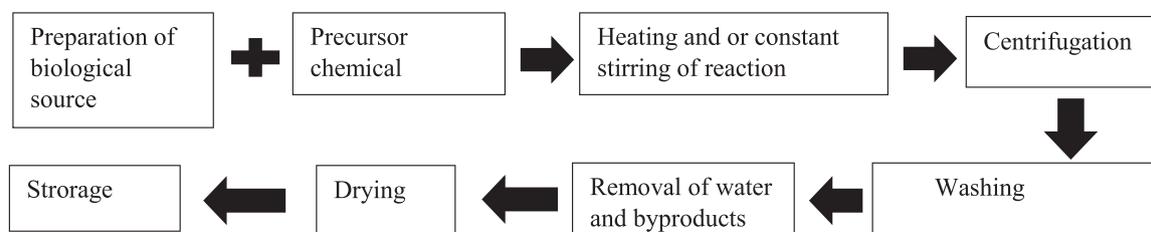


Fig. 2. Schematic representation of green synthesis of nanoparticles.

Table 1
Antimicrobial effect of green synthesized nanoparticles.

Nanoparticle	Biological source	Morphology	Effective concentration/ dose	Inhibitory effect of nanoparticle		Reference
				Microorganism	Indicator	
AgNP	Cell free culture supernatant <i>Pseudomonas rhodesiae</i>	Spherical 20–100 nm diameter	50 µg/ml	<i>Dickeya dadantii</i>	Optical density at 600 nm	Hossain et al., 2019
AgNP	<i>Moringa oleifera</i> leaves	Rectangular 8–28 nm	30 ppm	<i>Xanthomonas axonopodis</i>	Disease incidence	Hussain et al., 2019
AgNP	<i>Moringa oleifera</i> leaves	Rectangular Average size-21.64 nm	30 ppm	<i>Alternaria alternata</i>	Disease incidence	Hussain et al., 2018
AgNP	Leaf extract of <i>Morus indica</i>	Quasi-spherical Average particle size-54 nm	80 µg/ml	<i>Escherichia coli</i> K12	Optical density	Some et al., 2019
AgNP	Rice leaf extract (<i>Oryza sativa</i>)	Hydro dynamic particle size- 36–107 nm	10% (v/v)	<i>Staphylococcus aureus</i>	Optical density	Adak et al., 2021
AgNP	<i>Bacillus licheniformis</i>	Various shapes (Triangular, hexagonal and spherical) Average size-77 nm	10% (v/v)	<i>Xanthomonas oryzae pv oryzae</i>	Zone of inhibition (diameter)	
AgNP	<i>Pseudomonas poae</i> strain CO	Spherical 19.8–44.9 nm	10% (v/v)	<i>Helminthosporium oryzae</i>	Growth inhibition	
ZnONP	Lemon peel extract (<i>Citrus limon</i>)	Spherical and elongated 14.57–49.88 nm	10% (v/v)	<i>Rhizoctonia solani</i>	Growth inhibition (in vitro)	
ZnONP	Lemon fruit extract (<i>Citrus limon</i>)	Cuboid, hexagonal prism, thin rods, near spheroid, irregular shape	20% 0.1 µg/µL	<i>Rhizoctonia solani</i>	% Infection (in vivo)	Elbeshhey et al., 2015
NiONP	<i>Berberis balochistanica</i> stem extract	Rhombohedral agglomerated shape Average size-31.44 nm	20 µg/ml	Bean Yellow Mosaic Virus	Disease severity	
CuONP	Lemon peel extract (<i>Citrus limon</i>)	Spherical shape 18.18–43.37 nm	100 mg/ml	<i>Fusarium graminearum</i>	% Inhibition of mycelium growth	Ibrahim et al., 2020
TiO ₂ NP	Lemon fruit extract (<i>Citrus limon</i>)	Near spheroid, irregular shape Average particle size-41.5 nm	50 µg/ml	<i>Alternaria citri</i>	Zone of inhibition (diameter)	Sardar et al., 2022
			500 µg/ml	<i>Dickeya dadantii</i>	Inhibition zone (diameter)	Hossain et al., 2019
			1000 µg/ml	<i>Alternaria alternata</i>	% Inhibition	Uddin et al., 2021
			100 mg/ml	<i>Fusarium oxysporum</i>	Zone of inhibition (diameter)	
			50 µg/ml	<i>Aspergillus niger</i>	% Inhibition	
			50 µg/ml	<i>Alternaria citri</i>	Zone of inhibition (diameter)	Sardar et al., 2022
			50 µg/ml	<i>Dickeya dadantii</i>	Zone of inhibition (diameter)	Hossain et al., 2019

have acquired antimicrobial activity in this manner. On the other hand, nanoparticles like AgNPs, NiONPs, CuONPs have the ability to generate reactive oxygen species, thus cause breakages in DNA, impairment of DNA replication, protein destruction, lipid peroxidation, respiration disturbances leading to oxidative stress causing ultimate cell destruction (Kausar et al., 2022; Hossain et al., 2019; Sable et al., 2018; Logeswari et al. 2015).

4. Nanoparticles as growth stimulant

Facilitated attachment to surface, uptake, translocation and interaction of nanoparticles with and within the cellular components favour the growth and yield of crops and plants. Improvement in seed germination, nutrient uptake, enhanced production of photosynthetic pigments and other secondary metabolites, have been observed upon treating with green synthesized nanoparticles. So far, the effects on seed germination, photosynthesis, yield and product quality have been explored and documented by a number of scholars.

Smaller sized nanoparticles break and penetrate the seedcoat and promote the growth upon releasing of ions. Sable et al. (2018) documented the promotion of germination of bajara (*Pennisetum glaucum*) seeds by 48% over the control when treated with 0.2 ml of AgNPs synthesized using bacterial supernatant of *Bacillus subtilis spizizenii*. Further, Kausar et al. (2022) stated that the germination percentage of wheat (*Triticum aestivum*) variety 18 Elite line 5 was increased by 33% and 100% when exposed to 50 mg/ml and 25 mg/ml of CuNPs respectively and the germination index was doubled compared to the control at 25 mg/ml. Furthermore, biosynthesized CuONPs with leaf tissue of lavender (*Lavandula angustifolia*) and green tea (*Camellia sinensis* L.) recorded 2 folds greater germination indices than that of chemically synthesized nanoparticles in tomato (*Solanum lycopersicum* L.) and lettuce (*Lactuca sativa* L.) even at higher concentrations of 4000 µg/ml (Khalidari et al., 2021). When considering the effect of nanoparticles on photosynthetic pigments and other biochemicals, application of CuNPs synthesized using the extract of mint (*Mentha longifolia*) enhanced the contents of chlorophyll, carotenoids, phenols and soluble sugar in wheat (*Triticum aestivum*) varieties (Kausar et al., 2022). Similarly, higher concentration of chlorophyll (51.4 SPAD units) were recorded in wheat when 30 ppm of CuNPs prepared with onion extract (Hafeez et al., 2015). A ZnO NP concentration of 5 mg/L yielded the highest levels of chlorophyll a and chlorophyll b, carotenoids and soluble protein in *Maerua oblongifolia* (Sheikhaldein et al., 2021). Higher levels of chlorophyll a and b carotenoids, phenols were found in wheat varieties with the application of green synthesized CuNP at 50 mg/L, while higher level of total phenols was recorded at 100 mg/L.

The impact of nanoparticles on plant growth depends on the nanoparticle type, properties, concentration and the exposure time. Development stage of plant and their responsiveness to the nanoparticle i. e. susceptibility or tolerance (Hossain et al., 2019) also play a role. Among these factors, the concentration of nanoparticles on plant growth is of primary concern. As the concentration increases, nanoparticles favour the growth in a positive manner. However, beyond a certain level, higher concentrations pose a negative impact on growth attributes due to their accumulative toxic effect. The concentration at which maximum potential benefits attained can be considered as optimum/typical concentration in further applications of nanoparticles (Table 2). For example, the NiONPs synthesized using *Berberis balochistanica* stem extract exhibit non-inhibitory and stimulatory effects on seed germination and has shown higher relative germination rate of 3–8%. However, concentrations above 250 µg/ml showed inhibitory effects (Uddin et al., 2021). Furthermore, the effects of nanoparticles on seed germination are crop and or variety specific. CuNPs synthesized using aqueous extracts of mint (*Mentha longifolia* L.) showed maximum germination indices at 50 mg/ml in wheat (*Triticum aestivum*) variety 18-Elite lines 1, 3 and 6. Meanwhile these indices were maximum at 25 mg/ml in the Elite line 5 (Kausar et al. (2022).

Overall, nanoparticles pose either positive or negative effects on seedling and crop growth and yield, depending on the type and concentration of nanoparticle, application method (seed treated or foliar sprayed or both) and the variety. The green synthesized CuNPs at concentrations of 0.4 ppm and 30 ppm has shown better growth (leaf area, fresh weight, dry weight, root dry weight and chlorophyll content) and yield parameters. Beyond this concentrations, CuNPs had negative effects on growth and yield owing to higher absorption and accumulation of NPs in cells (Hafeez et al., 2015). Green synthesized CuONPs did not completely retard the growth of lettuce (*Lactuca sativa* L.) and tomato (*Solanum lycopersicum* L.) seedlings in contrast to the chemically synthesized CuO NPs at 4000 g/ml (Khalidari et al., 2021). Similarly, graphene oxide nanoparticles synthesized using *Nigella sativa* seed extract enhanced the length of roots and shoots, number of leaves, number of root nodules per plant, number of pods, and seeds per pod in mungbean (*Vigna radiata*) when applied to soil up to a concentration of 1200 mg/L (Mirza et al., 2022).

In addition to the vegetative growth parameters, the nanoparticles have effectively enhanced the yield parameters of crops. In particular, widely utilized AgNPs synthesized using *Moringa oleifera* applied at 30 ppm showed higher fruit weight (about 225 g/fruit) and yield/plant (410 g/plant) (Hussain et al., 2019). Mehmood and Murtaza (2017) recorded biological yields of 8.47 tons/ha, 8.24 tons/ha and 8.11 tons/ha respectively in pea (*Pisum sativum*) plants with AgNPs synthesized using bark extract of *Berberis lyceum* applied to seeds, foliar and both plant parts.

The clear and comprehensive mechanisms attributed to the stimulatory or the inhibitory effects beyond a threshold level of green synthesized NPs are not yet fully explored. According to the literature, nanoparticles have the potential to enhance the production of phytohormones, enzymes and stabilize the cell membranes. In particular, green synthesized ZnONPs promotes the synthesis of gibberellins and cytokinins which in turn boost the vegetative growth of plants (Tondey et al., 2021). AgNPs prevent the inhibitory effects of ethylene and improve the electron exchange efficiency and thereby help to increase the yield (Mehmood and Murtaza, 2017). Even though, the mechanism of nanoparticles in plants have been explained at cellular level to this extent, further studies are required to explore the molecular mechanisms behind their mode of operation.

5. Challenges, gaps and future prospects

Engineered materials of nanoscale referred to as nanoparticles have expanded their application in numerous fields including agriculture. Nanoparticles engineered via biological routes become more popular due to their greater potential towards growth and development of crop plants.

Green synthesis of nanoparticles is considered as low-cost eco-friendly method as it utilizes the cheaper, harmless biological sources as reducing and capping agents. However, the utilization of biological sources may limit the large-scale commercial production of nanoparticles. The prevalence of plants vary seasonally and geographically. For example, Fenugreek (*Trigonella foenum-graecum*) (used to synthesize AuNPs), Andean blackberry (used to synthesize CuNPs) are distributed respectively in China (Aswathy Aromal and Philip, 2012) and in Ecuador, Colombia, and the Andean region of Central and South America (Kumar et al., 2017). Apart from the availability, the correct growth stage of plants at which sufficient phytochemicals are available is another limitation. Some plants require a number of years to attain physiological maturity and are utilized in the flowering stage (Sana and Dogiparthi, 2018). This may create significant economic loss in the primary production from those plants. To circumnavigate this limitation, biological sources that are available regardless of climate and geographical conditions could be considered as materials for green biosynthesis. One such example is *Malva sylvestris* which is used to synthesize CuONPs is widely available in Europe, Asia, and America

Table 2
Growth stimulation effect of green synthesized nanoparticles.

Nanoparticle	Biological source	Morphology	Crop	Concentration	Method of addition	growth/yield parameter	Percentage improvement (Approximate)	Reference
AgNP	<i>Moringa oleifera</i> leaves	Rectangular 8–28 nm	Kinnow mandarinine (<i>Citrus reticulata</i>)	30 ppm	Foliar spray	Fruit weight	28.5	Hussain et al., 2019
ZnONP	<i>Ochradenus arabicus</i>	Hexagonal 10–50 nm	<i>Maerua oblongifolia</i>	5 ppm	Tissue culture (Mixing with MS media)	Fresh weight	162.4	Sheikhaldein et al., 2021
NiONP	<i>Berberis balochistanica</i> stem	Rhombohedral agglomerated shape Average size-31.44 nm	Radish (<i>Raphanus sativus</i>)	31.25 µg/ml	Seed treatment	Shoot length Leaf number Seedling length	223.6 62.27 13.86	Uddin et al., 2021
AgNP	<i>Bacillus subtilis spitzizenii</i>	Spherical 2–5 nm	Bajara (<i>Pennisetum glaucum</i>)	2 ml	Seed treatment	Fresh weight	64.83	Sable et al., 2018
CuONP	(<i>Lavandula angustifolia</i>) and green tea (<i>Camellia sinensis</i> L.)	Spherical 50–100 nm	Lettuce (<i>Lactuca sativa</i> L.)	4 µg/ml	Seed treatment	Dry weight Leaf length Root length Shoot length	39.13 58.39 62.22 16.67	Khaldari et al., 2021
CuNP	<i>Mentha longifolia</i>	Spherical Average size-23 nm	Wheat variety 18 Elite line 3 (<i>Triticum aestivum</i> L.)	50 mg/L + 5 ml/day	Seed treatment and foliar spray	Root length Root length	50 153	Kausar et al., 2022
AgNP	<i>Berberis lycium</i>	Spherical 10–100 nm	Wheat variety 18 Elite line 5 (<i>Triticum aestivum</i> L.)	25 mg/L + 5 ml/day	Seed treatment and foliar spray	Shoot length Fresh weight Root length	20 95 134	Mehmood and Murtaza, 2017
CuNP	Onion extract (<i>Allium cepa</i>)	12–20 nm	Pea (<i>Pisum sativum</i>)	60 ppm	Seed treatment and foliar spray	Shoot length Fresh weight Seeds per pod No of pods per plant 100 seed weight Biological yield Green pod yield Leaf area	54 18 67 54 24 40 88	Hafeez et al., 2015
			Wheat (<i>Triticum aestivum</i> L.)	0.4 ppm	Blending with MS media	Fresh weight Dry weight Root dry weight Grains per spike	139 83 150 31	
			Wheat (<i>Triticum aestivum</i> L.)	30 ppm	Soil application	Spike per pot 100 grain weight Grain yield per pot	49 58 110	

(Kuppusamy et al., 2016). Utilization of agricultural waste is a sustainable means of overcoming the issue of using plant material for biosynthesis of NPs.

Green synthesized nanoparticles have acquired the potential in various applications due to their unique morphological features. On the contrary, the size and the shape of nanoparticles question their quality and limit the particles' benefits and applications. During the green synthesis, nanoparticles are produced in a wide range of sizes. Thus far, studies have not considered their relative proportions in determining the efficacy. The nanoparticles synthesized by physical method exhibits high purity and uniform particle size distribution (Xu et al., 2020). For efficient internalization, nanoparticles should be of smaller than the pores in the biological membranes. There could be possibilities of non utilization of synthesized nano particles, when the proportion of particles with undesirable size and shapes is high. At this juncture, screening of suitable materials, that would ensure uniformity in the morphological features of the particles is important. Uniform sized CuONPs (5–10 nm) have been synthesized using *Gloriosa superba* L. (Naika et al., 2015).

Processing condition also challenges the green synthesis of nanoparticles. Even though this method demands lesser energy compared to other methods, green synthesized nanoparticles require higher energy in certain aspects mainly during reaction (in the form of heat) and extraction. For example, AgNPs are synthesized at 600°C using *Ferula persica* root and leaf extracts (Nasiri et al., 2018), and CuNPs are synthesized at 800°C using guava fruit extract (Caroling et al., 2015). Short reaction time and simple extraction method are ideal for cost-effective large-scale synthesis of nanoparticles. To achieve the goal of making the extraction process as simple as possible, researches should also take into account the feasibility and simplicity of the extraction process. Utilization of certain plant extracts (ex. grape seed extract) have found successful with the less energy (< 100°C) during extraction in the synthesis of AgNPs (Ping et al., 2017).

Stability of nanoparticles in the environment is another challenging aspect in utilizing them. The stability of the suspension is largely determined by the size of the particles and affinity toward other environmental constituents. The metal nanoparticles are prone to oxidation in air due to their weaker stability in nature. These nanoparticles cannot be stored in normal environmental conditions for future use and hence are stored in specialized environment of inert gases such as Ar, N₂ (Leili et al., 2018; Nasrollahzadeh and Mohammad Sajadi, 2016). Capping of metal nanoparticles with PEG helps in stabilizing the metal colloid (Caroling et al., 2015). In contrast, nanoparticles of greater stability do not require specified storage conditions, rather can be stored at ambient conditions. The AgNPs synthesized using leaf extract was found to be stable in the aquatic environment (Sun et al., 2014). Stability of nanoparticles can be regulated by controlling the particle size and surface capping or through functionalization techniques (Sharma et al., 2014; Tejamaya et al., 2012). Furthermore, optimizing the processing conditions have an effect in producing stable nanoparticles. For example, stable and uniform-sized silver nanoparticles were formed at the optimized conditions of neutral pH, 75°C temperature, 60 min incubation time and 1 mM concentration of AgNO₃. Moreover, in the synthesis of nanoparticles, a combination of plant extracts yielded more stable nanoparticles compared to when individual plant extracts were used (Liaqat et al., 2022).

Even though, green synthesized nanoparticles offer a number of benefits, certain challenges may limit their applications. Cumulative toxicity is one such challenge. In particular, green synthesized nanoparticles show antimicrobial and growth stimulant effects at lower concentrations, however at higher concentration they have inhibitory effects on the target organism. In such conditions, coating of nanoparticles with biocompatible materials during the synthesis would help to suppress unnecessary toxic effects (Tariq et al., 2022). Coating of powdered AgNPs with biocompatible materials such as polyvinyl pyrrole repressed the phototoxicity of synthesized AgNPs (Karn et al., 2011).

Several studies conducted in these aspects implied that green synthesized nanoparticles can be used as fungicides or bactericides and growth enhancers. However, depending on the nanoparticle type, concentration and mode of application, their effects on the growth of plants vary. Therefore, efforts should be made to optimize the effects of nanoparticles on a broader spectrum rather than being specific either to nanoparticle or the type crop plant. The experiments conducted to investigate the effects of nanoparticles are mainly focused on short-term crops and it should be extended further on to perennial crops in the future. Moreover, direct disposal of the nanoparticles to the environment may threaten the beneficial micro and macro-organisms. Their frequent, continuous and excess usage could bio magnify their toxic effects on higher order organisms in the food web. Therefore, measures for the safe disposal of nanoparticles to reduce the toxic effects on ecosystem components is of prime importance.

Data Availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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