Nanoparticles in Eliminating Contamination and Seed Germination

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Owing to their minuscule size, nanoparticles (NPs) acquire novel and unique properties that differ from their bulkier counterparts, giving rise to breakthrough technology with application-based solutions in many sectors of agriculture and plant biotechnology.

crops genetic engineering in vitro cultures nanoparticles regeneration seedlings secondary metabolites

1. Introduction

The release of nanoparticles (NPs) into the environment has raised concern because of their toxic effects on the environment and human health [1]. Moreover, the release of NPs into the environment could result in their entry and accumulation in agricultural soils from bio-solids impregnated with NPs through the application of sewage sludge for agricultural purposes [2]. Thus, the application of NPs in plant tissue cultures is promising, as this technique is used to screen different aspects of plants' growth and development, as well as to engage in genetic manipulation, bioactive compound production and plant improvement [3]. It has been noted that NPs have a positive impact because of their reduced size, elevated reactivity, mass-to-area ratio and other physico-chemical properties, but the negative effects of NPs have also been noted, which mainly depend on the type of metal, dissolution power and plant species [4][5].

2. Efficiency of Nanoparticles in Eliminating Contamination

The production of healthy plantlets is a prime concern behind the technique of plant tissue culture but microbial contamination is a common problem faced during this procedure. Conventionally, antibiotics are employed to eliminate microbes, but their frequent application can negatively affect plant tissue growth, e.g., antibiotics like carbenicillin and cefotaxime inhibit plant cell growth, organogenesis and embryogenesis [6][7]. Reports suggest that streptomycin and chloramphenicol interact with protein synthesis, rifampicin hinders nucleic acid synthesis and penicillin inhibits cell-wall membrane synthesis [8][9]. There is also the risk of a decreased genetic stability and lower regeneration capability of plants when a high level of antibiotics is used [10]. Nanomaterials are an alternative because of their distinctive features, which have been shown to possess antifungal and antibacterial properties that restrict microbial growth in in vitro cultures resulting in the successful mass propagation of selected species [11].

Silver nanoparticles (AgNPs) have been considered one of the better options, as the anchoring and penetration of Ag ions into microbes alter the cellular signals, via dephosphorylation, of key peptide substrates on tyrosine [12][13]. Another study suggested that Ag^+ ions cause a reduction in DNA replication, as well as inactivate the thiol group in proteins, that ultimately reduces microbial growth [14]. Similarly, Min et al. [15] reported that AgNPs restrict the growth of sclerotium-forming phytopathogenic fungi and, hence, can become an alternative to pesticides. AgNPs have been employed to reduce contamination during in vitro cultures of *Olea europaea* L. [16], *Nicotiana tabacum* L. [17][18], *Gerbera jamesonii* Bolus ex Hook.f. [19], *Solanum tuberosum* L. [11], almond x peach (G x N15) hybrid rootstock [20], *Rosa hybrida* L. [21], *Vitis vinifera* L. [22], *Vanilla planifolia* Jacks. ex Andrews [23], and *Phoenix dactylifera* L. cv. Sewi and Medjool [24]. In addition, combined treatment with nanosilver and nano-iron particles was reported to have a significant effect on decreasing the contamination rate in *Fragaria* × *ananassa* L. cv. Roby Gem [25]. Similarly, biosynthesized silver, chitosan, and selenium NPs were tested for their antimicrobial potential for the in vitro multiplication of three olive cultivars (Koroneiki, Picual, and Manzanillo). Of all the three NPs, AgNPs showed the best antimicrobial properties in all cultivars [26]. However, some studies have also suggested that the concentrations of AgNPs played a crucial role in culture growth as higher concentrations might induce adverse effects on explant response [2][11]. The phytotoxic effect of higher AgNPs has been observed in crop plants of *Phaseolus radiatus* L. and *Sorghum bicolor* (L.) Moench [27]. Whereas in tomato and potato plants, it has been reported that lower concentrations of AgNPs with longer exposure time effectively reduced the contamination without hampering explant viability [28].

Titanium dioxide (TiO_2) is another NP that has gained attention due to its antimicrobial potential, as it has photocatalytic properties to eliminate contamination from various sources, but its toxicity against microbial growth depends on the intensity and wavelength of light with concentration and particle size [9]. TiO_2 reacts with water molecules and forms free radicals like OH, HO_2, and H_2O_2 which in turn results in the oxidation of bacterial cells, suggesting that the photo-activation of TiO_2 via UV irradiation retards the bacterial growth [29][30]. It has been evaluated that the addition of TiO_2NPs in the Murashige and Skoog (MS) [31] medium enhanced the microbial resistance during the micropropagation of tobacco [17], *S. tuberosum* [9], and *Hordeum vulgare* L. [32]. Zinc oxide nanoparticles (ZnONPs) have eliminated nine strains of bacteria (*Bacillus megaterium*, *Cellulomonas uda*, *C. flarigena*, *Corynebacterium panrometabolum*, *Erwinia cypripedii*, *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., and *Staphylococcus* spp.) and four fungal species (*Aspergillus* spp., *Candida* spp., *Fusarium* spp., and *Penicillium* spp.) which increased difficulties during banana micropropagation [33]. Thus, it can be observed that although nanomaterials at higher concentrations have been proven as toxic for plant growth, they can be employed as disinfectant agents for the in vitro multiplication of various economically important crops. The majority of the reports used Ag, TiO_2, and Zn-based NPs for the inhibition of microbial growth during in vitro propagation, but new types of NPs should also be assessed. In this regard, various kinds of advanced nanomaterials like graphene, graphite, quantum dots, carbon nanotubes, polymer dendrimers, and atomic clusters will provide enough scope for the study; along with this, evaluations of concentrations, sizes, and types of NPs on various crop species and type of explant are also needed [34].

3. Influence of Nanoparticles on Seed Germination

Seed germination is a crucial stage for crop development since young seedlings are more vulnerable to biotic and abiotic stresses [35]. Therefore, lots of efforts to improve the efficiency of seed germination are published from time to time with new technological interventions. Studies to analyze the effect of NPs have been conducted during the last few years, and it was observed that genotype, variety, seed age, and environmental conditions determined the response to NPs [36]. Yasur and Rani [37] and Hatami [38] suggested that the water uptake during seed germination is critical because seeds are relatively dry and requires a substantial amount of water to initiate cellular metabolism and growth. The positive effects of NPs on germination begin with the high capability of NPs to penetrate the seed coat and promote water uptake along with the absorption of nutrients in the seed [39]. Mehrian et al. [40] documented that NP treatment accelerated seed germination from better water uptake by the seeds during the initial days, whereas a decrease in germination efficiency was noted as time passed because of the breakdown of stored nutrients or alternations in permeability properties of the cell membrane. Similarly, Rizwan et al. [41] noted that NPs can penetrate through the seed coat and affect the development processes of embryos through stimulation of the enzymes of metabolic processes. During the radicle appearance stage of seed germination, root apex tissues come in contact with NPs, which then move into the rhizodermis through the apoplast with endocytosis. In the root, they flow towards the plant secretory tissue using symplastic pathways and translocate to other plant organs. However, it has been noted that NPs at a high concentration result in a perforation of the cell wall and penetrate the protoplast and damage the root cell vacuoles. This triggers more production of reactive oxygenspecies (ROS) and it causes a blockage of electron transfer which induces oxidative stress. NPs also up-regulate the genes involved in cell division and carbon/nitrogen metabolism, and the negative effects observed in seedling growth are due to chromosomal aberrations and mitotic abnormalities. This leads to a decrease in cell division of the root meristem, hormonal imbalance, ROS over-production, and increased levels of lipid peroxidation [42]. The increased oxidative stress, in turn, increases hydrogen peroxide (H_2O_2) contents, activities of malondialdehyde (MDA), catalases (CAT), peroxidases (POD), and superoxide dismutase (SOD), as well as the production of compounds having antioxidant activities like phenolics and flavonoids [43]. Many studies have documented that NPs exert positive or negative influences on seed germination, seedling biomass as well as biochemical and metabolite contents. In the present research, researchers have taken only those examples where NPs were added into the media and not where seeds were placed on filter paper or water agar media after sonication treatment with NPs.

3.1. Silver Nanoparticles (AgNPs)

In the majority of the studies, NPs' effect has been evaluated under in vivo conditions [44], but few were tested under in vitro conditions on the culture media. It is also observed that most reports suggested the usage of AgNPs (**Table 1**), e.g., Lee et al. [27] recorded a negative effect of AgNPs on *P. radiates* and *S. bicolour* seedling growth. Similarly, the growth of *Physalis peruviana* L. seedlings also decreased along with chlorophyll content, but biomass in terms of fresh (FW) and dry weights (DW) was increased. It was also revealed that the seedling growths were not much affected in soil as compared to the agar-based medium. This might be due to changes in the physico-chemical properties of NPs in the soil, as pore water harbours a range of electrolytes that increase the aggregation of AgNPs in soil. These aggregates were larger than the pore size of plant root cells and thus failed to pass through the cells. Greater aggregation may be the principal reason for the reduced phytotoxicity of AgNPs in soil. Thus, the relative germination index is extensively used as an indicator of phytotoxicity, and root growth is one of the sensitive biomarkers for the phytotoxicity assay [45]. Zaka et al. [46] compared AgNPs, gold nanoparticles (AuNPs), and copper nanoparticles (CuNPs) for *Eruca sativa* Mill. and observed that AgNPs increased seed germination, shoot and root lengths, and seed vigour index, whereas the other two adversely affected these parameters (**Table 1**). Further evaluation unveiled that all the NPs affected the biochemical milieu of the plants differently (**Table 2**). In another study, green synthesized AgNPs using *Curculigo orchioides* Gaertn. were found to exert a positive influence on seedling growth and biomass of *Oryza sativa* L. cv. Swarna. When the germinated seedlings were biochemically analyzed, an increase in chlorophyll, flavonol contents and enzymes (POD, SOD, CAT, APX, and GR) activities, and a decrease in phenolics, flavonoids, H_2O_2, and MDA contents were observed. The gene expression analyses revealed that the SOD gene was down-regulated, whereas genes for CAT and ascorbate peroxidase (APX) were up-regulated after AgNP treatment [47]. Similarly, increased seed germination, seed vigour index, shoot and root lengths, and fresh and dry biomass in *Pennisetum glaucum* (L.) R. Br. after the addition of AgNPs in the medium was reported [48]. The maximum germination was recorded at 40 ppm; at this concentration of AgNPs, mild activities of 2,2-Diphenyl-1-picrylhydrazyl (DPPH), SOD activities and proline content were recorded that significantly increased at higher dose of AgNPs. On the contrary, phenolic contents were higher at optimum germination concentration (40 ppm) and lower at higher concentration, whereas flavonoids were lower at 40 ppm and increased at high levels. AgNPs positively influenced the germination and seedling traits of *Brassica oleracea* L. var. *sabellica* ‘Nero di Toscana’ and *Raphanus sativus* L. var. *sativus* ‘Ramona’, whereas these traits were decreased in *Solanum lycopersicum* L. ‘Poranek’. One of the reasons behind decreased growth *S. lycopersicum* might be due to the presence of AgNPs in plasmodesmata, precluding the transport of nutrients that led to a reduction in plant biomass [49]. Recently, Tomaszewska-Sowa et al. [50] observed the effect of AgNPs and AuNPs on *Brassica napus* L., and revealed that application of both NPs decreased shoot and root lengths of seedlings irrespective of treatment time. However, total chlorophylls, carotenoids, anthocyanins, free sugars, and H_2O_2 contents were higher, but no major change in phenolics was found. The seed germination of *N. tabacum* was carried out using CTAB- and PVP-coated AgNPs, and coating with CTAB showed a positive influence whereas coating with PVP failed to show any positive effect on germination rate and biomass [51]. Similarly, positive influences of AgNPs have been also documented in *Brassica juncea* (L.) Czern. var. pusajaikisan [52], *Hylocereus undatus* (Haw.) Britton and Rose [53], and *P. vulgaris* [54] (**Table 1**).

Table 1. Effects of various NP on seed germination ofdifferent crops under in vitro conditions.

Plant	Nanoparticle (NP) Treatment	Parameters	Reference
Brassica juncea var. pusa jaikisan	AgNPs	Enhancement in the growth of seedlings in terms of shoot FW, shoot and root length, and vigor index	[52]
Brassica napus	AgNPs/AuNPs	Deceased shoot and root lengths, as well as shoot FW and DW	[50]
Brassica nigra	ZnONPs	Increased shoot length and shoot DW, decreased root length, shoot FW, root FW and DW	[55]

Plant	Nanoparticle (NP) Treatment	Parameters	Reference
Brassica nigra	CuONPs	Delayed seed germination, decreased plantlet length, and their FWs and DWs	[4]
Brassica oleracea var. *sabellica* ‘Nero di Toscana’	AgNPs	Increased germination response, shoot and root lengths, as well as biomass	[49]
Cicer arietinum	CuONPs	Decreased shoot and root lengths, FWs and DWs of shoot and root, increased lignifications in root cells	[56]
Eruca sativa	AuNPs, CuNPs and AgNPs	AgNP-increased seed germination, shoot and root lengths, and seed vigourindex; AuNP-and CuNP-decreased seed germination, shoot and root lengths, and seed vigour index	[46]
Glycine max hybrid S42-T4	MWCNTs	Early and better germination, increased shoot, root and leaf lengths, shoot and root FWs and DWs	[57]
Hylocereus undatus	AgNPs	Increased germination, shoot number, shoot, and root lengths, cladode size, and FW	[53]
Hordeum vulgare hybrid Robust	MWCNTs	Early and better germination, increased shoot, root, and leaf lengths, shoot and root FWs and DWs	[57]
Linum usitatissimum cv. Barbara	ZnONPs	Increased shoot and root length, as well as their FWs and DWs	[58]
Nicotiana tabacum	AgNPs	Increased germination and dry biomass	[51]
Oryza sativa cv. Swarna	AgNPs	Increased shoot and root length, FWs and DWs of shoot and root	[47]
Pennisetum glaucum	AgNPs	Increased germination, seed vigour index, shoot and root lengths, and fresh and dry biomass	[48]
Petroselinum crispum	TiO_2NPs	Increased germination, shoot and root lengths, and their FWs	[59]
Phaseolus radiatus	AgNPs	Adverse effect on seedling growth	[27]
Phaseolus vulgaris	AgNPs	Increased seed germination, shoot and root length, their FWs and DWs, number of axillary buds, adventitious buds and leaves	[54]
Physalis peruviana	AgNPs	Decreased shoot and root lengths, chlorophyll content, but increased FW and DW	[60]

Plant	Nanoparticle (NP) Treatment	Parameters	Reference
Raphanus sativus var. sativus 'Ramona'	AgNPs	Increased germination response, shoot and root lengths, and seedling biomass	[49]
Solanum lycopersicum var. Poranek	AgNPs	Decreased germination response, shoot and root lengths, and seedling biomass	[49]
Sorghum bicolour	AgNPs	Adverse effect on seedling growth	[27]
Vigna radiata	CuONPs	Decreased shoot and root lengths and their FWs, increased lignifications in root cells	[61]
Zea mays hybrid N79Z 300GT	MWCNTs	Early and better germination, increased shoot, root and leaf lengths, shoot and root FW and DW	[57]

AgNPs: silver nanoparticles; AuNPs: gold nanoparticles; CuNPs: copper nanoparticles; CuONPs: copper oxide nanoparticles; DW: dry weight; FW: fresh weight; MWCNTs: multi-walled carbon nanotubes; TiO_2NPs: titanium dioxide nanoparticles; ZnONPs: zinc oxide nanoparticles.

...ourdi and ...hlorophyll ...per oxide ...hey have ... H_2O_2 and ...increased ...*ietinum* L. ...entrations (50–500 mg/L), and elevated H_2O_2 generation, MDA level, and POD activity along with increased lignifications in roots were observed. Further expression analysis revealed that *CuZn-SOD*, *CAT*, and *APX* genes were up-regulated in roots but no change was found in shoots [56]. Similarly, CuONPs, when used for the treatment of *Brassica nigra* (L.) K. Koch, delayed the germination of seedlings and decreased plantlet length and biomass significantly [55]. ZnONPs in the media containing seeds of the same plant negatively influenced seedling growth, shoot FW, and reduced stem diameter as the NP amount increased in the media. However, the treatment increased free radical scavenging activity, total antioxidant capacity, total reducing power, phenolics, and flavonoid contents in the shoot and root of the seedling (**Table 2**) [55]. Moreover, in seeds of *Linum usitatissimum* L. cv. Barbara, different concentrations of ZnONPs (1, 10, 100, 500, and 1000 mg/L) were tried, and 100 mg/L concentrations proved beneficial in terms of shoot and root lengths as well as seedling biomass, further higher concentrations adversely affected seedling growth [58]. In another study, treatment with multi-walled carbon nanotubes (MWCNTs) showed a positive influence on germination, seedling lengths, as well as biomass in *Glycine max* (L.) Merr. hybrid S42-T4, *H. vulgare* hybrid Robust, and *Zea mays* L. hybrid N79Z 300GT [57]. Unlike the spherical shapes of other NPs, MWCNTs are the allotropes of carbon that are arranged in an elongated, tubular manner with many rolled sheets. Its unique features like functional group, diameter, length, and solubility make its penetration inside the seed coat convenient and it is efficiently translocated in plants [62]. Similar observations have been well documented previously where MWCNTs improve germination, plant growth, and agronomic traits by penetration, and increasing the water and nutrient uptake [63][64].

Table 2. Biochemical changes in seedlings and cultures after NP treatment.

Plant	Nanoparticle (NP) Treatment and Culture Type	Biochemical Changes	Reference
Brassica juncea var. pusa jaikisan	AgNPs, shoots	Increased chlorophyll and decreased MDA, H_2O_2, and proline content, increased CAT, GPX, and APX activities	[52]

Plant	Nanoparticle (NP) Treatment and Culture Type	Biochemical Changes	Reference
Brassica napus	AgNPs/AuNPs, shoots	Increased chlorophylls, carotenoids, anthocyanins, free sugars, H_2O_2 contents, no change in phenolic content	[50]
Brassica nigra	ZnONPs, shoots and roots (seedling), callus	Increased free radical scavenging activity, total antioxidant capacity, total reducing power, phenolic, and flavonoid contents	[55]
Brassica nigra	CuONPs, seedling and roots (from leaf and stem derived callus)	Seedlings increased free radical scavenging activity, total phenolic, and flavonoid content, decreased total antioxidant and reducing potential; Roots increased free radical scavenging activity, total antioxidant and reducing potential, total phenolic, and flavonoid contents	[4]
Brassica oleracea var. *sabellica* 'Nero di Toscana'	AgNPs, leaves	Decreased chlorophyll, carotenoid, and anthocyanin contents, no change in phenolic, protein contents and SOD activities, increased GPOX activity	[49]
Campomanesia rufa	AgNPs, shoots	No significant difference in SOD activity	[65]
Caralluma tuberculata	AgNPs, callus	Increased PAL and free radical scavenging, SOD, POD, CAT, APX activities, total phenolics, and flavonoid contents	[66]
Cicer arietinum	CuONPs, seedling	Increased H_2O_2 generation, MDA content, POD activity, and lignification in roots	[56]
Cichorium intybus	Fe_2O_3NPs, hairy roots	Increased hairy root growth, total phenolic, and flavonoid contents	[67]
Corylus avellana cv. Gerd Eshkevar	AgNPs, cell suspension	Increased CAT, APX, H_2O_2, PAL activities, decreased SOD and POD activities, and total soluble phenol content	[68]
Corylus avellana cv. Gerd Eshkevar	AgNPs, cell suspension	Increased MDA, total phenolic, anthocyanin, and flavonoid contents	[69]
Cucumis anguria	AgNPs, hairy roots	Increased total phenolic and flavonoid contents, and antioxidant activities	[70]
Eruca sativa	AuNPs, CuNPs, and AgNPs, seedling	AuNPs decreased total antioxidant capacity, total phenolic and flavonoid contents, increased DPPH, SOD and POD activities, no change in protein content; CuNPs decreased total antioxidant capacity,	[46]

Plant	Nanoparticle (NP) Treatment and Culture Type	Biochemical Changes	Reference
		DPPH activity, protein content, increased total phenolic, and flavonoid contents, SOD and POD activities; AgNP decreased total antioxidant capacity, DPPH activity, decreased total phenolics and flavonoid contents, POD activity, increased SOD activity, no change in protein	
Fragaria × ananassa cv. Queen Elisa	FeNPs, shoots	Increased chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, total carbohydrates, total protein, and total free proline and iron contents, decreased H_2O_2 and MDA content, higher SOD and POD activities	[71]
Linum usitatissimum cv. Kerman Shahdad	ZnONPs/TiO_2NPs, cell suspension	Increased PAL and CAD activities, and total phenol content	[72]
Linum usitatissimum cv. Barbara	ZnONPs, seedling and callus	Increased ROS production, membrane lipid peroxidation, protein carbonylation and 8-oxo guanine formation, SOD, POD, radical scavenging activities, total phenolics, and flavonoid contents	[58]
Maerua oblongifolia	AgNPs, shoots	Higher chlorophyll, total protein and proline contents, and increased activities of antioxidant enzymes	[73]
Momordica charantia	AgNPs, cell suspension	Increased MDA, H_2O_2, total phenolics and flavonoid contents, and antioxidant activity	[74]
Musa paradisiacal cv. Grand Nain	ZnNPs and ZnONPs, shoots	Higher proline, chlorophyll, and antioxidant enzymes activities	[33]
Musa spp.	AgNPs, shoots	Increased chlorophyll content	[75]
Nicotiana benthamiana	CH-ZnO, callus	Increased chlorophyll, carotenoid, proline contents and PAL and AO activities, decreased MDA and H_2O_2 levels	[76]
Nicotiana tabacum cv. Bright Yellow-2	ZnONPs, cell suspension	Decreased dehydrogenase, oxidoreductase SOD, POD and APX activities, increased GR, PAL, protease, caspase-like and acid phosphatases activities, and total phenolic content	[77]
Oryza sativa cv. Swarna	AgNPs, seedling leaves	Increased chlorophyll and flavonol contents and POD, SOD, CAT, APX and GR activities,	[47]

Plant	Nanoparticle (NP) Treatment and Culture Type	Biochemical Changes	Reference
		decreased phenolics, flavonoids, H_2O_2 and MDA contents	
Oryza sativa cv. IR64	AgNPs, shoot	Decreased MDA, proline and H_2O_2 levels	[78]
Pennisetum glaucum	AgNPs, seedling	Increased DPPH, proline, SOD, POD, and CAT activities, total phenolics and flavonoid contents	[48]
Phoenix dactylifera	MWCNTs, shoots	Increased flavonoid, chlorophylls and carotenoid, nutrient contents, decreased phenolics and tannin contents, SOD, GPOX, and GR activities	[79]
Phoenix dactylifera cv. Hayani	AgNPs, somatic embryos	Increased chlorophyll content	[80]
Physalis peruviana	AgNPs, seedling derived shoots and shoots	Seedling derived shoots- increased CAT and APX activity, and decreased chlorophyll content, SOD and MDA activities;Shoots- no change in SOD, APX and MDA levels, decreased CAT activity	[60]
Raphanus sativus var. sativus 'Ramona'	AgNPs, leaves	Increased carotenoid, phenolic contents, and SOD activity, decreased chlorophyll, anthocyanins, protein contents, and GPOX activity	[49]
Saccharum spp. cv. Mex 69-290	AgNPs, leaves	Increased N, Ca, Mg, Fe, Cu, Zn, Mn, and decreased P, K, and B content, higher total phenolics, ROS and lipid peroxidation contents, and antioxidant activity	[81]
Simmondsia chinensis	MWCNTs, shoots	Increased total tannin content and antioxidant activities, decreased phenolics and flavonoid contents	[82]
Solanum lycopersicon	Fe_3O_4NPs, shoots	Increased proline content and osmotic potential	[83]
Solanum lycopersicum	ZnONPs, callus	Increased Na, N, P, K, and Zn ionic, protein contents, SOD and GPX activity	[3]
Solanum lycopersicum var. Poranek	AgNPs, leaves	Increased chlorophyll, anthocyanins, phenolics, protein contents and SOD and GPOX activities, decreased carotenoid content	[49]

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Plant	Nanoparticle (NP) Treatment and Culture Type	Biochemical Changes	Reference
Solanum tuberosum	SiO_2NPs, leaves	Increased antioxidant enzymes activity and expression of proteins	[84]
Solanum tuberosum cv. White Desiree	AgNPs, shoots	Increased total chlorophyll, carotenoids, proline, total flavonoids, phenolics, lipid peroxidation and H_2O_2 contents, decreased anthocyanins	[85]
Vanilla planifolia	AgNPs, shoots	Higher chlorophyll, increased elements like N and B, no change in P, Ca and Mg, and decreased K, Fe, Cu, Zn, Mn, and B contents, higher total phenolics, ROS and lipid peroxidation contents, and antioxidant activity	[23]
Vigna radiata	CuONPs, seedling	Decreased chlorophyll and increased proline contents, H_2O_2 and MDA contents in root, no change in carotenoid, H_2O_2 and MDA contents in shoots	[61]

AgNPs: silver nanoparticles; APX: ascorbate peroxidase; AO: ascorbate oxidase; AuNPs: gold nanoparticles; CAT: catalase; CH-ZnO: chitosan-zinc oxide nanoformulation; CuNPs: copper nanoparticles; CuONPs: copper oxide nanoparticles; DPPH: 2,2-diphenyl-1-picrylhydrazyl, FeNPs: iron nanoparticles; Fe_2O_3NPs/Fe_3O_4NPs: iron oxide nanoparticles; GPX: guaiacol peroxidase; GPOX: glutathione peroxidase; GR: glutathione reductase; H_2O_2: hydrogen peroxide; MDA: melondialdehyde; MWCNTs: multi-walled carbon nanotubes; PAL: phenylalanine ammonia lyase; POD: peroxidase; ROS: reactive oxygen species; SiO_2NPs: silicon dioxide nanoparticles; SOD: superoxide dismutase; TiO_2NPs: titanium dioxide nanoparticles; ZnNPs: zinc nanoparticles; ZnONPs: zinc oxide nanoparticles.

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