Plant Stimulant to Nanotoxicity: Recent Advancements and Opportunities

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Abstract: Nanotechnology has come a long way showing major contributions in the field of agriculture and food production. The use of nanoparticles (NPs) is increasing day by day as they possess better solubility, enhanced magnetic and optical properties, and better surface to charge ratio. The affirmative effects due to the use of NPs have been explained, including enhanced germination, increased root and shoot length, and the overall increase in plant biomass along with improvement in physiological parameters like photosynthetic activity. Recently, the toxicological effects of NPs in agriculture have become a matter of concern. The current review focuses on the generation of reactive oxygen species (ROS), oxidative damage and defense mechanism in response to phytotoxicity caused by the use of NPs. The other aspects in this review include the effect of NPs on macromolecule concentration, plant hormones and crop quality. The review also discusses the future prospects of NPs on plant phytotoxicity and growth. Furthermore, it also discusses the possible measures which can be taken for plant protection and growth while using NPs in agriculture.

Keywords: Nanoparticles, nanotoxicity, plant hormones, phytotoxicity, plant stress, defense mechanism.

1. INTRODUCTION

Currently, nanotechnology is an exceptionally promising field due to its wide applications in biotechnology, pharmaceutical sciences, drug targeting, nano-medicine and other research areas. In fact, Vance et al. [1] reported a 30-fold increase in nano-based products between 2011 and 2015, and an estimated global market of over $1 trillion in 2015 [2]. Progress in the field of nanotechnology is immense, however, the adverse environmental impacts of nanoparticles (NPs) have raised concerns regarding their use in agriculture. There are several reports regarding the toxicity of various NPs [3, 4]. However, much is still unknown and some information is still lacking. Therefore, nanotoxicology is a mounting concern and regarded as a new research area in toxicology. The toxicological properties of NPs will help us to determine the extent of the environmental as well as social threats. Inherent properties of NPs comprise of size, shape, surface area, surface charge, crystal structure, coating, and solubility/dissolution, which greatly govern the NPs behavior, fate and transport, and toxicity. Additionally, environmental factors such as temperature, pH, ionic strength, salinity, and organic matter may influence the toxic behavior of the NPs.

Plants are the primary producers and play a major role in the ecosystem by interacting directly with the soil, water, and atmospheric compartments of the environment. They easily make their way into the human life cycle. The NPs can easily translocate and cause bioaccumulation. There are many reports on the desirable and undesirable impacts of NPs on plants and their wide applications in agricultural sciences. Although the toxic effects of NPs are well known, yet numerous positive effects of NPs in terms of seed germination, growth promotion and enhancement of metabolic rate cannot be overlooked. The toxic effects such as suppression of plant growth, inhibition of chlorophyll synthesis, photosynthetic efficiency, exerted by NPs are stated in Fig. (1). These positive and negative effects of NPs vary from plant to plant species, and type of NPs used and their concentration. Modern nano-biotechnological tools have a great potential to increase food quality, global food production, plant protection, detection of plant and animal diseases, monitoring of plant growth nano-fertilizers, nano-pesticides, nano-herbicides and nano-fungicides [3, 4]. There are several reports that prove the use of various nanomaterials in plant protection and growth enhancement. The use of NPs like silver (Ag), gold (Au), zinc (Zn), copper (Cu) and silica is well documented. The NPs help in target delivery in plants, thus triggering various pathways that increase the metabolite content of the plant as well as biomass. Extensive work has carried out in agriculture by making use of NPs, but many setbacks have also observed over the years due to the use of nanomaterials. For NPs synthesis, two approaches are nor-
nally employed, which include a) making use of chemical synthesis b) green approach by making use of natural products. Various nano-enabled strategies have been proposed to improve crop production to meet the growing global demands for food, feed and fuel [5]. Hazardous effects make the use of NPs less favorable in the agriculture sector as NPs can make a direct entry into the food chain via crop consumption. The small size of NPs is a boon, but sometimes these NPs cross the plant barrier and deposit in the plant system, hence making their way to the bio-cycle. Entry into the bio-cycle can cause serious issues like cytotoxicity, genotoxicity and phytotoxicity [6-8]. This review provides an impact of the use of NPs on various plants through various routes. This review also explains the mechanism of NPs induced toxicity, giving an insight into the plant system.

2. TYPES OF NANOMATERIALS USED FOR PLANT GROWTH AND TRANSPORT IMPLICATIONS IN AGRICULTURE

NPs can be diverse in nature, making them applicable in many fields. In this section, greater emphasis is laid on the use of recently used NPs in the field of plant protection.

2.1. Copper Nanoparticles (CuNPs)

Nanotechnology offers a wide range of applications for the agrochemical sector, providing plant protection that can moderate the quantities and frequency of the usage of chemical fertilizers for getting a concentrated effect on the target pest. The CuNPs are used extensively in the agriculture sector for multiple applications. Different ways have been reported for the synthesis of CuNPs due to their considerable importance. Recently, CuNPs synthesized by hydrothermal method using biocompatible surfactants like polyethylene glycol (PEG1000), tetra ethylene glycol (TEG) and polysorbate 20 (Tween 20) were tested against plant pathogen bacterial strains. It was found that CuNPs acted well against all bacterial strains. Although the CuNPs penetrated the plant system, yet they were reported to be non-toxic, making them even a better candidate to be used for plant protection [9]. Another property of the CuNPs was explored and it was seen that the use of these NPs even in lesser concentration enhanced the rice seedlings growth when prepared using the laser ablated technique. Extensive studies were carried out and conclusions were made based on the findings that exposure to lower concentrations (5, 10, 20, and 50 μM) of copper oxide nanoparticles (CuO NPs) was enough to enhance growth (in terms of fresh and dry weight and length) of rice seedlings. However, it was reported that higher concentrations (100, 200, and 500 μM) of CuO NPs decreased plant growth (in terms of length, fresh weight and dry weight) significantly (p<0.05) [10]. The CuO NPs have wide applications in agricultural, chemicals and food sectors. The CuO NPs have been reported for their application in the agricultural sector by increasing productivity either by aiding in the growth of the plant or an increase in the overall biomass of the plant. The application is not restricted to growth only, but a more inclusive approach like a controlled release of elements for better growth and highly efficient farming can also be expected when making use of CuO NPs. The controlled release may increase the overall productivity of agro products. Moreover, the use of CuO NPs for the controlled release of micronutrients in lettuce resulted in increased transpiration and stomatal conductance. Hence, a positive impact occurred due to the accumulation of CuO NPs in the lettuce roots. Application of CuO NPs also resulted in biomass increase by 16.3-19.1%, germination improvement by 14.5 % and a two-fold increase in the root and stem length compared to the control group. Results showed the development of new micronutrient fertilizer and crop protection agents based on CuNPs [11].

2.2. Silver Nanoparticles (AgNPs)

The AgNPs can be used for several purposes in the agricultural field. The use of nanotechnology can confirm and certify food security via improving crop production. The AgNPs have the ability to enhance the growth and yield of different plants by enhancing biomass. Sadak et al. (2019) reported the use of AgNPs for enhancing growth in fenugreek (Trigonellafoenum graecum). The use of AgNPs in varying concentrations (20, 40, and 60 mg/L) was verified.

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Fig. (1). Toxicity caused by NPs on plant germination, macromolecule concentration, plant hormones and crop quality. (A higher resolution/colour version of this figure is available in the electronic copy of the article).
2.3. Zinc Oxide Nanoparticles (ZnO NPs)

The use of ZnO NPs in tomato plants resulted in increased growth, photosynthetic efficiency, and antioxidant system. Exposure of tomato plants to ZnO NPs for 45 days resulted in increased growth. Antioxidant properties enhanced by 8 mg/L ZnO NPs exposure for 30 minutes. The beneficial properties of ZnO NPs show their potential use in the agricultural sector [15]. The use of ZnO NPs trigger specific pathways in the plant system leading to an increase in certain enzymes. The enzymes could help in an increase in a number of factors such as chlorophyll, carotenoids, proteins that ultimately increase biomass. The impact of ZnO NPs exposure studied in cotton plants displayed a positive impact on total biomass over the control. The results suggest that bioengineered ZnO NPs interact with meristematic cells and stimulate biochemical pathways responsible for plant growth [16]. The use of ZnO NPs and FeNPs on the wheat (Triticum aestivum) growth and cadmium (Cd) accumulation in wheat plant resulted in decreased root height and dry weight of the wheat plant. The toxicity of Cd was greatly reduced after the use of ZnO NPs and FeNPs. Exposure to these NPs also caused an increase in nutrient content [17]. ZnO NPs were also reported to improve the agronomical characteristics of rice [18].

2.4. Iron Oxide Nanoparticles (FeNPs)

The FeNPs have been used extensively in the field of agriculture for the purpose of disease detection, target delivery and many more due to their super capabilities, which include superior magnetism [19] and high surface free energy. The typical size of FeNPs ranges between 5-250 nm. Varying the size of FeNPs great application can be endeavored in the field of plant protection and growth enhancement. Recently, it was reported that the use of Ferric oxide (Fe3O4) NPs of varying sizes (8-50 nm) can help in enhanced root length, plant height, biomass, and chlorophyll content of wheat. The Fe3O4 NPs were translocated into the leaves. The Fe3O4 NPs of size 20-40 nm were considered as the optimum and were suggested to be used as a nanofertilizer [20]. Another report suggested the use of Fe3O4 NPs for increased metabolite content in pumpkin. The Fe3O4 NPs (10 and 40 nm) increased phloem sap-metabolites, which were related to oil metabolic pathway. Metabolites, including methoxy acetic acid 4-tetradecyl ester, cicosane, and heneicosane showed an increased percent of 19.7 %, 5.46% and 7 %, respectively [21]. By adopting the sol-gel auto-combustion method, mineral substituted NPs were developed. Calcium and magnesium substituted strontium nano-hexaferrites (Sr0.96Mg0.02Ca0.02Fe12O19, SrMgCa nano-HF) were synthesized and their impact was studied on barley (Hordeum vulgare L.) at the concentration of 125 to 1000 mg/L for three weeks. Germination rate and biomass increased by about 20%. Chlorophyll pigments showed an enhancement of about 33-42% when compared to the untreated control. The nano-HF treatment (250 mg/L) was the most effective. It suggested that the developed NPs have the potential for use in mineral deprived plants along with seed germination [22]. Several advancements have been made in the synthesis of FeNPs over time. Recently, Ca,Mg,Ni1.25Fe2O4 (x < 0.05) NPs were synthesized hydrothermally and used in the catalytic application. These NPs had a high surface area, and low cytotoxicity when examined against HCT116 and MCF-7 [23]. H. Tombuloglu et al. reported the usage of superparamagnetic iron oxide nanoparticles (SPIONS), particularly the magnetite nanoparticles of size 15-20 nm and showed no toxicity at all when used in the growth of barley plant [24]. Later, it was suggested that SPIONs ~12.5 nm in size alone were not capable of Cucurbita pepo growth but could be proved effective when used in conjugation with EDTA [25]. Magnetic NiFe2O4 NPs with size 12.5 ± 0.5 nm were used in barley and phytotoxicity was not reported for concentration lesser than 1000 mg/ml [26]. The MnFe2O4 magnetic NPs (MnFeNPs) with size 14.5 ± 0.5 nm were synthesized hydrothermally and were used as an effective delivery system for anti-cancer drugs [27]. Another investigation concluded with the use of Fe3O4 NPs with size ~13 nm in barley. Results suggested that 500 mg/L was enough to enhance the fresh weight (FW) by ~19% and ~88% for leaf and root tissues in comparison with control. No phytotoxic effect was recorded even at a high dose of the Fe3O4 NPs [28]. Recently, spinel copper ferrite (CuFe2O4) NPs were synthesized by a simple sol-gel combustion technique and doped with different concentrations of rare earth elements like Cerium (Ce3+). These NPs showed potential anti-bacterial property, thereby proving their application as protective covering over plants [29].
2.5. Silica Nanoparticles

Currently, silica NPs are in use for plant growth. The silica NPs are in use for their properties like controllable size, which helps in the release of the entrapped chemicals for target delivery in plant, composition, morphology, porous structure and pore size, surface chemistry that can be modified for even better applications, and dispersibility. Due to large scale properties, it becomes important to elaborate the role of silica NPs in this section [30]. Alsaleedi et al. reported the use of silica NPs for improved growth in the cucumber plant [31]. It showed that another application of mesoporous silica NPs is the targeted release of hormones and drugs in plants. It also showed that thiol gated mesoporous silica NPs (MSNPs) helped in the controlled release of abscisic acid (ABA), one of the key phytohormones. The ABA entrapped inside the pores of the MSNPs. An intracellular glutathione (GSH) responsive phytochemical delivery system used for thiolated MSNPs was tested on the model plant Arabidopsis thaliana. The MSNPs developed a particle diameter of ~20 nm and pore size of ~2.87 nm. Modification of the developed particles was done with decanethiol gatekeepers through GSH-cleavable disulfide linkages. The developed MSNPs significantly reduced the leaf stomatal aperture and water loss. Drought resistance developed in the plants due to silica NPs. This led to the conclusion that silica NPs show potential in delivering encapsulated biomolecules and deliver the desired phytochemicals in a controlled manner [32]. The silica NPs had also explored as nanofertilizers, nanoemulsions and nano-pesticides due to their positive impact after the usage on the plant. Application of nanofertilizers is one of the promising fields for increasing resources and efficiently using the available resources and reducing environmental pollutions to the maximum. Recently, the use of chemical fertilizers for growth and early development of plants for better productivity has largely contributed to the increased use of fertilizers, making it hazardous for both humans and plants. It also contributes to the soil damage. Hence, it is necessary to develop better and efficient chemical fertilizers as and when required. Nano fertilizers can be used in place of chemical fertilizers as their use overcomes the traditional problems faced by the use of chemical fertilizers. Herein, we describe the work of Kalteh et al. w.r.t the use of silica NPs as nanofertilizers. In this study, silica NPs helped in increasing the proline level, which was due to tolerance induction. Hence, silica NPs application helped in the reduction of the pollution effects originated from salinity in basil [33]. Counteracting against plant disease is necessary as it can greatly reduce the wastage of the production from agriculture. Herein, nanotechnology can greatly influence disease management in agriculture. In 2018, Derbalah et al. reported the use of MSNS compared to metalexyl, which is a fungicide. The MSNS was effective against Alternaria solani under laboratory and greenhouse conditions. The effectiveness was mostly attributed to the particle size, shape and porosity, which cumulatively acted for managing tomato early blight [34].

2.6. Graphene Nanoparticles

Graphene nanomaterials (GNM) are a few-layer-graphene, graphene oxide (GO), reduced graphene oxide (RGO), and graphene nanosheets (GNS). The GNM has significantly advanced in recent years and used in many fields, including energy storage, electronic devices and batteries, biosensors, cell imaging, drug delivery, and tissue engineering [35]. Normally, the GNM released into the environment during their production, transport, consumption, and disposal. There have been considerable research reports regarding the phytotoxicity of GNS, but far less research on the realistic release amount and concentration in the environmental media (air, water, and soil). Miralles et al. elaborated the release pathways of engineered nanomaterial into the natural environment as delivery systems in agriculture, as biosensors, and as a release from medical and cosmetic applications [36]. It was reported that 1.6 mg/kg of graphene was the maximum release amount from graphene-polyethylene composite films when applied in food packaging [37]. Many groups have reported and expressed concern about the potential human health and ecological risks resulting from the manufacture and use of GNS [38, 39]. Nowadays, the effects of GNS on humans, aquatic organisms, and their effects on plants have investigated [40]. The NPs are gradually enriched to higher levels of the food chain, leading to toxic effects in organisms [41]. Therefore, it is important to study and understand the hazards of nanomaterials that include toxicity, mutagenicity and toxic impacts on ecosystem services.

3. NANOPARTICLES PHYTOTOXICITY

The nanoparticles (NPs) may be made up of organic, inorganic or hybrid materials and confined with dimensions ranging from 1 to 100 nm (at the nanoscale) [42]. The NPs come into the environment via either natural process or different anthropogenic process. Due to the rapid use of NPs in different fields, they are accumulating in the different compartments of the environment, such as freshwater, air, soil, etc. [43]. Among them, the major amount of the NPs sinks in the soil surface, from where it reaches to the different parts of the plants like shoot, roots and other plant tissues. These NPs in different concentrations induce cytotoxicity to the plants by inhibiting seed germination, regulating the plant growth, cause morphological and physiological changes [42, 44-46]. Furthermore, they also affect the plant growth hormones, defense mechanism and respiratory mechanisms of plants [42].

3.1. Effect of NPs on Germination and Seedling Growth

The NPs toxicity to the plant species is due to the metal or polymeric coating that forms the NPs. The effects of these NPs vary on different plant species. The NPs produce severe effects on seed germination and seedling development. A number of reports are available showing the phytotoxic effects of NPs on seedling development of plants. Ma et al. reported that the NPs from the lanthanide series, such as lanthanum oxide (La2O3), gadolinium oxide (Gd2O3), and ytterbium oxide (Yb2O3), severely inhibit root growth in different plants like radish, tomato, rape, lettuce, wheat, cabbage, and cucumber at the concentration of 2000 mg/L [46]. Similarly, Yin et al. reported that exposure to 40 mg/L of gum Arabic (GA) coated silver nanoparticles (GAAgNPs) significantly reduced the germination rate of three species and enhanced the germination rate of one species of wetland
plants [47]. A similar study conducted by D. Lin and B. Xing reported germination inhibition in ryegrass and corn at a concentration of 200 mg/L of nano-sized Zn (zinc, 35 nm) and ZnO (zinc oxide, 20 nm), respectively [44]. The root growth of radish, rape canola, ryegrass, lettuce, corn, and cucumber species was also inhibited upon exposure to 2000 mg/L nano-sized Zn and ZnO [45].

The work carried out by Rui et al. assessed the phytotoxic effects of silicon oxide (SiO$_2$) NPs on Bt-transgenic cotton and observed significant inhibition in the plant growth and the shoot and root biomass of the same [48]. In the case of rice, the possible effects of different sized Ag NPs (20, 30-60, 70-120 and 150 nm) were investigated at different concentrations (0.1, 1, 10, 100 and 1000 mg/L) upon seed germination and seedling growth. The results revealed a decrease in seed germination and seedlings growth with increasing sizes and concentrations of AgNPs [49]. Another study related to two different coating and sized AgNPs, i.e., 20 nm polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNPs) and 6-nm GA-AgNPs suggested that the PVP-AgNPs had no effect on germination of plant species while GA-AgNPs exposure significantly reduced the germination rate of three species and enhanced the germination rate of one species [47]. Immediately after plant uptake, NPs translocated to different plant parts and got accumulated, causing severe damage to them. Accumulation in plant parts leads to the degradation of crop quality, including reduction in seed germination rate, fresh and dry biomass, as already mentioned. Additionally, the process of photosynthesis is altered (affecting chlorophyll synthesis), chromatin condensation is enhanced and the transpiration rate is reduced. P.M.G. Nair et al. also mentioned that exposure to 0.5-1.0 mg/L AgNPs not only reduced root and shoot biomass, but also decreased chlorophyll and carotenoids content in rice [50]. A similar outcome reporting decline in chlorophyll content was also reported in Pisum sativum on exposure to ZnO NPs [51]. A recent study of Alquraidi et al. demonstrated a decrease in chlorophyll a and b pigments in Coriandrum sativum plants treated with CuNPs. Graphene based NPs also greatly affect the plant germination. Delayed germination was observed in rice seeds treated with 50 mg/L of graphene for 3 days as reported by Nair et al. [52]. A similar toxic effect was reported though the overall germination was not affected and delayed initiation in germination was observed [53]. It is reported that a concentration of 500 mg/mL and 400 mg/mL was enough to enhance the growth in rice and wheat plant [53-54]. The GNM can have toxic effects like loss of morphology and decrease in growth parameters, such as root and shoot length, root number, root diameter, and biomass production. Negative effects on the shoot height and root length of rice seedlings were observed when exposed to RGO [55]; in addition, the root diameter and the number of cells in the transverse section significantly decreased.

### 3.2. Effect of NPs on Macromolecule Concentration

Basically, lipids, proteins, polysaccharides and nucleic acids are four main classes of macromolecules found in plants. However, mixed polymers like glycoproteins, which contain both sugars and amino acids in the covalent linkage, are also known. The NPs are able to interact with macromolecules and alter their function and activities in the plant system. It is important to mention that there are several studies measuring the phytotoxicity of NPs in terms of oxidative stress and membrane damage. However, particular studies emphasizing the effect of NPs on macromolecular content in plants are still missing. In this regard, this section will highlight some studies on the effect of NPs on macromolecular alterations. Rico et al. conducted a study to evaluate the effect of cerium oxide CeO$_2$NPs on macromolecule composition in rice seedlings [56]. Fatty acid composition in rice roots exposed to 0-500 mg/L CeO$_2$NPs showed a significant variation. Either the fatty acid (lauric acid, myristic acid, valeric acid, stearic acid, oleic acid, linolenic acid and linoleic acid) content significantly increased or decreased by CeO$_2$ NPs treatments, except for palmitic acid. On the other hand, lignin content reduced significantly only in 500 mg/L CeO$_2$ NPs treated roots as compared to control roots. The rest of the treatments (62.5, 125 and 250 mg/L) did not significantly change lignin content. Rico et al. cited the reason that elevated peroxidase activity and hydrogen peroxide (H$_2$O$_2$) content work as controlling factors in lignin synthesis. However, the reverse happened in this case, where even enhanced peroxidase and H$_2$O$_2$ could not prevent a decline in lignin biosynthesis at 500 mg/L CeO$_2$ NPs treatment [57]. Vannini et al. carried out a proteomic study on Eruca sativa roots after exposing to AgNPs, and observed significant changes in proteins related to redox regulation and disturbed cellular homeostasis of the plant [57]. Another study conducted by Mustafa et al. exposed early stage soybean to three different AgNPs of the size 2, 15 and 50-80 nm at a concentration range of 0.2-20 ppm. Proteomic studies made it clear that changes in proteins under AgNPs exposure were mainly related to stress, cell signaling and cell metabolism. Furthermore, an important enzyme, i.e. glyoxalase, exhibited a decline in response to AgNPs [58]. Similar work of Mustafa et al. also observed greater changes in proteins related to the glycolysis pathway on the exposure of soybean to aluminum oxide (Al$_2$O$_3$) NPs [59]. Proteome studies provide a link between gene expression and cell metabolism, which elucidate molecular pathways of plants exposed to NPs stress [60].

Nucleic Acid is another target of NPs. The NPs enter into cell organelles at the cellular level, resulting in the production of ROS. These ROS cause cytotoxicity and genotoxicity like membrane damage, chromosomal aberrations and DNA damage. Several researchers have accounted for the effect of NPs on DNA damage. Recent work of Alquraidi et al. (2019) confirmed the genotoxic effects of CuNPs in C. sativum plants via Random Amplified Polymorphic DNA (RAPD) analysis. Genotoxicity resulted in the alteration of C. sativum genome, which was confirmed by different banding patterns of RAPD. Mattiello et al. reported genotoxicity in Hordeum vulgare plants treated with CeO$_2$ and titanium oxide (TiO$_2$) NPs. In addition, CeO$_2$ NPs induced modifications resulted in chromatin aggregation in nuclei of root and shoot cells [61]. Atha et al. also reported mutagenic DNA lesions in different plants as an indicator of DNA damage on exposure to CuO NPs [62]. Lee et al. also assessed the genotoxic effects of ZnO and CuO NPs on buckwheat. DNA assays via RAPD displayed different DNA polymorphisms at 2000 and 4000 mg/L of ZnO and CuO NPs in treated plants of buckwheat when compared to control [63].
3.3. Effect of NPs on Plant Hormones

Plant hormones are organic compounds produced by plants as by-products of metabolism. Plant hormones are responsible for the regulation of physiological responses during plant growth. The role of hormones in plant development and stress adaptation is an outcome of various synergistic and antagonistic interactions between hormones. When plants are exposed to different types of stresses, different hormonal pathways are either upregulated or downregulated [64, 65]. Some studies regarding the production of plant hormones in response to NPs stress have been highlighted recently. Vinkovic et al. performed ultra-high-performance liquid chromatography electrospray for hormonal analysis in pepper plants and reported a significant elevation in total cytokinin levels in pepper tissue in response to AgNPs treatment [66]. A similar study of Wang et al. observed upregulation in six genes of ethylene signaling pathways in cucumber after exposure to silver sulfide (Ag2S) NPs [67]. A contrast study was also reported by Le Van et al. where the production of plant hormones was downregulated in the presence of NPs. There was a reported decrease in indole-3-acetic acid (IAA) and ABA in transgenetic cotton after exposure to CuO NPs [68]. A significant inhibition in phytohormones (IAA, ABA) production in root tissue of rice on exposure to FeNPs was also mentioned earlier [69]. A decrease in phytohormones concentration was evident in rice seedlings exposed to carbon nanotubes [70]. Furthermore, T.Vinkovic et al. mentioned the effect of ZnONPs on five hormones, i.e. ABA, cytokinins, auxins, salicylic acid and jasmonic acid, in Arabidopsis thaliana. With an increase in NPs concentration, synthesis of auxins and cytokinins was suppressed in shoot apical meristems. In contrast, cis-zeatin (cytokinin) enhanced by 280% and 590% after exposure to 20 and 100 mg/L of ZnO NPs. The ABA content upregulated in leaves and apices, whereas salicylic acid elevated in leaves and roots. Jasmonic acid was suppressed in the presence of NPs [66].

3.4. Effect of NPs on Crop Quality

For the past few years, NPs are used as nanopesticides and nanofertilizers in agriculture. The use of NPs in agriculture includes NPs like nanoeolites and hydrogels, which improve the soil quality and used as nanosensors to monitor soil and plant health [71]. However, there is a debate over the use of NPs in agriculture as some NPs like AgNPs are known to release Ag ions in soil and affect biomass accumulation in soil. Therefore, it is essential to know the role of a particular NP for its effective use in agriculture [65]. The NPs are known to affect the crop quality by producing a change in amino acid, non-reducing sugars, fatty acids and phenolic content in plants. Certain studies have proved the same. Rico et al. carried out a study where the rice plants grown in soil treated with CeO2 NPs were harvested. Experimental results showed that rice grains obtained from CeO2-treated plants had less sulphur, glutelin, valeric acid, palmitic, lauric acid, Fe and starch. Moreover, antioxidants in rice grains produced from CeO2 NPs treated plants were less, except for flavonoids. The study concluded that CeO2 NPs compromise rice quality [56]. Titanium oxide NPs significantly reduced the biomass of rice and suggested that TiO2 NPs would have a mixed effect on quality as well as quantity of rice [72]. In another study, exposure to CeO2 NPs (800 mg/kg) in soil caused a 31.6% decrease in cucumber yield [73]. More recently, the application of CeO2 and ZnO NPs reduced maize yield by 38% and 49%, respectively. Exposure to these NPs also transformed the corn quality by altering mineral elements in kernels and cobs. Homologous results were also observed in soybean plants, on applying CeO2 and ZnO NPs in soil [74]. In addition, CeO2 NPs also changed phenolic content, non-reducing sugars and protein fractionation in cucumber fruits [73]. The grain quality, as well as quantity, was affected on the application of different NPs in time and dose dependent manners. Further studies are required for the proper understanding of whether the decrease in grain/fruit yield and quantity indicates NPs toxicities in plants.

3.5. Phytotoxicity of NPs-biochemical Basis (ROS, Oxidative Damage and Defense Mechanism)

Plants produce as well as scavenge ROS constantly in mitochondria, peroxisomes, chloroplasts, endoplasmic reticulum and plasma membranes. Reactive oxygen species include singlet oxygen (‘O2), superoxide anion, H2O2 and hydroxyl radical (OH). Sometimes, the equilibrium between ROS production and scavenging alters under stress conditions like applications of NPs in higher concentrations. Reactive oxygen species ultimately affect the membrane lipids, resulting in their peroxidation and causing oxidative stress. Oxidative stress is measured by lipid peroxidation, electrolyte leakage and propidium iodide fluorescence assay [43]. Plants inherit some defense strategies to overcome the toxicity caused by NPs stress. Under stress, either enzymatic or non-enzymatic defense machinery activates against stress and plants cope up with the NPs induced toxicity. However, when the stress factor crosses the limit, the detoxification mechanism also fails and leads to programmed cell death, i.e., apoptosis in plants. It has been observed that metal NPs cause more stress in plants in a dose-dependent manner than bulk metal particles [75, 76].

Gorczyca et al. reported a two-fold increase in electrolyte leakage in wheat seedlings exposed to AgNPs over control. CeO2 NPs (125 mg/L) resulted in an enhancement in electrolyte leakage and lipid peroxidation but produced no effect on H2O2 content [77]. The H2O2 content also elevated in roots, but only after exposure to 500 mg/L CeO2 NPs [56]. Similarly, H2O2 and malondialdehyde (MDA) content increased in barley treated with CuO NPs [78]. The ROS production in roots of tomato plants treated with NiO NPs over the control was also reported [79].

Rao et al. stated that Brassica juncea displayed tolerance against stress caused by CuO and TiO2 NPs, due to the coordinated and well-organized defense system by the activation of antioxidant enzymes. Loss of membrane integrity in root meristems of Allium cepa cells was reported on exposure to ZnO NPs [80]. Moreover, antioxidant machinery was deregulated by ZnONPs toxicity, which led to cell cycle arrest and cell death [81]. Jiang et al. observed enhancement in levels of ROS, peroxidase (POD), superoxide dismutase (SOD) activity and glutathione content in Spirodela polyrhiza, on the application of 6 nm AgNPs [82]. Antioxidant enzyme
activities in terms of guaiacol peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and malonyldialdehyde (MDA) content were significantly affected in Phaseolus vulgaris on exposure to TiO$_2$ NPs [83]. Enzymatic activities like SOD, ascorbate peroxidase (APX), and CAT were also elevated in wheat seedlings treated with 200 mg/L Al$_2$O$_3$ NPs, as compared to control [84]. Enzymatic activities like SOD and CAT were inhibited in leaves and roots of wheat by AgNPs treatment [77]. Likewise, CAT, glutathione reductase (GR) and dehydro ascorbate reductase (DHAR) activities also decreased in barley leaves treated with 500 ppm of CeO$_2$NPs, when compared to control [85]. Despite numerous studies, there is no clear evidence that could correlate disturbances in enzymatic activities with the chemical properties of NPs. It is not certain that alteration in enzymatic activities was because of enzyme interactions with NPs. Thus, the role of NPs in modulating antioxidant defense system needs further investigation.

4. FACTOR AFFECTING NPs PHYTOTOXICITY

The phytotoxic nature of NPs is primarily influenced by the shape, size, chemical composition, and coating material composition of NPs [86]. Apart from these plant species, surrounding rhizosphere or soil conditions also determine the phytotoxic behavior of NPs. Furthermore, phytotoxicity may also vary due to the NPs uptake mechanisms by plant roots and transportation up to the above-ground parts through the vascular system. Uptake of NP via soil starts with the root system, therefore the dissolution of NPs in soil or surrounding media also affects the phytoxic nature of the NPs. In this regard, different researchers have documented each and every parameter described as follows:

4.1. Plant Species

The phytotoxicity of NPs also attributed to the type of plant species. The type of seeds, distribution and dispersion of roots and solute transportation behavior of the plant also affect the phytotoxic nature of NPs. In this regard, Jain et al. reported that the presence of thick cuticle on testa and roots, pearl millet (xerophytic plant) was found to be relatively less sensitive to ZnO NPs as compared to wheat and tomato (mesophytic plants) with normal cuticle layer [87]. In another study, Azura et al. published data regarding the inconsistencies in terms of NP adsorption rate within the two varieties of paddy seeds. They found that the variation in the covering of seed with broad and short pores affects the adsorption rate of NPs [88]. Recently, Spielman-sun et al. explained the effect of surface charge of NPs translocation and leaf distribution in vascular plants with contrasting anatomy [89]. They observed that translocation efficiency also affected due to the charge vasculature of the leaves. Comparing leaf vasculature, CeNPs were able to move much further outside of the main vasculature in dicot plants than monocot plants, likely due to the larger airspace volume in dicot leaves compared to monocot leaves [89].

4.2. Soil Properties

Recent studies suggested that soil property plays a major role in determining the phytotoxicity of NPs. The retention period of NP in soil and attraction forces of NPs with soil particle also influence the toxicity of NPs [90-96]. In this regards Du et al. and Larue et al. suggested that the TiO$_2$ NPs were retained in the soil for longer periods and adhered to cell walls of wheat [95, 96]. The dissolution of ZnO NPs in the soil enhanced the uptake of toxic Zn by wheat. In another study, Qiu and Smolders worked on the investigation of CuO NPs toxicity to barley (Hordeum vulgare) with reference to CuO bulk particles (CuO-BPs) and Cu acetate (Cu(OAc)$_2$) in six different soils [93]. The result suggested that there was no indication that nanoparticulate or colloidal Cu enhanced toxicity. Furthermore, Gao et al. measured the rate of dissolution of CuO NPs in bulk soil and stated that the roots affect the dissolution of NPs [94]. They observed that the Cu fraction increased from 17 mg/kg to 223 mg/kg in fresh treatments and from 283 mg/kg to 305 mg/kg in aged treatments of wheat plants over the growth period due to dissolution. They also investigated the dissolution rate via considering the presence of roots in the soil, and found that the presence of root may also have opposite and somewhat compensatory effects on NP dissolution.

4.3. Size of the NPs

The rate of NPs entry into the plant system depends on the size of NPs, where smaller ones enter easily and alter the metabolic pathways, whereas larger ones cannot enter the cells and do not affect the metabolic pathways. Another factor is the surface area. It has been reported that AgNPs with the greater surface area are more toxic to viruses, bacteria and fungi.

5. MECHANISMS OF NPs PHYTOTOXICITY

The recent studies show that the seed germination of test plants was significantly reduced in response to the different dose of NPs which contains different metallic ions like Ti, Zn, Si, etc. as its major constituents [7, 8, 15, 17], and possible mechanisms attributed to the phytotoxic properties of these NPs are explained in Fig. (2). Qian et al., Haverkamp and Marshall, Ma et al. reported that NPs are responsible for the accumulation of metal ions in the cytoplasm, which induce a reduction in mitochondria and blockage of DNA synthesis pathways [46, 90, 91]. This leads to the inhibition of the germination and growth of the seedlings. Maximum germination inhibition was observed in monocot plants due to the relatively smooth seed coat, smaller weight to volume ratio as compared to the other plant species. The chlorophyll content and percent respiration of the treated seedlings were reduced significantly due to the NPs presence, which may be due to the alteration in the leaf diffusibility due to the accumulation of NPs [48, 52, 69]. Furthermore, this accumulation also disturbs the transpiration rate and stomatal aperture, which lead to a change in the rate of photosynthesis and consequent inhibition in the development of roots, and shoots. The loss of cellular respiration of test plants was due to the mitochondrial damage, because of which the energy production hindered due to reduced ATP generation, which ultimately leads to growth retardations of plants.

It is also evident that the exposure of plants with NPs produces stress conditions that may reduce the activities of
carbohydrate and protein hydrolyzing enzymes. The decreased activity of enzymes may lead to the accumulation of carbohydrates and proteins in the roots of the treated sample. This observation is in agreement with earlier works that reported that the stress conditions affect macromolecular contents by reducing the activities of their hydrolyzing enzymes. Furthermore, the effect of NPs on the antioxidants enzyme defense system is also discussed in this paper. The work reported on the profiling of the different antioxidant enzymes APX, GPX and SOD suggested that all the enzymes were showing up-regulation in their activities. The elevated activities of the antioxidant enzymes reported due to the generation of ROS in response to the NPs dose. The altered activities of these antioxidant enzymes were also indicating the oxidative stress on the seedlings, which may be induced by the accumulation of NPs inside cells [78, 82, 83].

CONCLUSION AND OPPORTUNITIES

After examining the constructive and deleterious impacts of nanomaterials in plant protection, there is a need to develop naturally synthesised biocompatible nanomaterials. The innovative nanomaterial should not only be non-hazardous in nature but also has positive implications when used. The problems recently faced by the use of nanomaterials include genotoxicity, biotoxicity and phytotoxicity, which need to be overcome. The use of nanomaterials should be examined as it can be a potential hazard if neglected. Constant efforts are required in developing and establishing a more robust bioengineering nanomaterial for better application and lesser impacts on the environment. As reviewed in the literature, it can be observed that more attention is given to the synthesis of NPs using ways of processes than making efforts in systemic and beneficial ways, which could be used in implications of nanomaterials for better application in fields of plant protection and growth. The major challenge is developing a nanomaterial, which has a better biocompatibility. Controlled releases of NPs into the environment are another major drawback that needs to be overcome since the greater release of NPs contributes to bioaccumulation of the NPs into the plant system leading to phytotoxicity. Alternate efforts should be made in adopting and developing a system that would evaluate the release of NPs in the environment for their effective use.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Plant Stimulant to Nanotoxicity


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