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A Review of the Effect of Nanoparticles on Reactive Oxygen Species Production in Different Plants

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ABSTRACT

Engineered Nano Materials are designed nanoparticles used in industry, agriculture, electronics, textiles, and other fields. In agriculture, nanoparticles are used in different parts of a plant's life cycles and their effect on seed, germination, plant growth and development, and pathogen determination are considered. Reactive oxygen species production can break seed dormancy or even increase guard cells when the pathogen infects the plants. Engineered Nano Materials produce Reactive Oxygen Species, which can also damage cell membranes through lipid peroxidation, disrupting cell metabolism. However, some of these nanoparticles can also activate more antioxidants and protect chlorophyll and carotenoids from Reactive Oxygen Species. Elements in nano size can have different effects from their actual size. Nanoparticles can change the cell physiologically and stop its antioxidant defense system. In this review, the effects of nanoparticles on oxidative stress and also its effect on improving the negative impact of other stresses are discussed. Future studies should explore how nanoparticles impact ROS production in diverse plants, including medicinal varieties, in various environments. Improved research methods are crucial for a clearer understanding of these effects on plant health.

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Introduction

Dividing bulk materials into much smaller parts can cause special physical and chemical properties [1]. These minute parts are called nanoparticles (Nps). Particles that have at least one dimension less than 1-100 nm (10^{-9}) in diameter at molecular or atom levels are nanoparticles [2-4]. Nanoparticles can be divided into different parts through their dimension, morphology, and core material [5]. According to dimension, nanoparticles are classified into four categories; non-dimensional, in which all dimensions are in nanoscale; one-dimensional, two-dimensional, and three-dimensional [5]. Nanoparticles are considered because of their multi-dimensional potential and also their small size which leads to greater surface reactivity (in comparison with bulks) and concentration [3; 6-8]. These exclusions cause special physiological properties, which cannot be seen in larger size particles [9]. Engineered Nano Materials are used in a wide variety of products and new applications continue to be developed [8-10]. Engineered Nano Materials (ENMs) are divided into four groups; carbon-based Nps (graphene, carbon dots) [11]; metal oxide and metal-based Nps (Silver (Ag), Gold (Au) Nps) [12]; Nanosized polymers [13] and Nps and bulk composites [9; 14]. Nanoparticles ingress into plants via two primary routes: foliar application, where they are sprayed onto leaves, and root absorption through fertilization. The penetration of nanoparticles occurs through various plant entry points, such as the cuticle, stomatal openings, trichomes, and wounds when administered on leaf surfaces. Alternatively, when nanoparticles serve as fertilizers, they access the plant through the root tips, lateral roots, and rhizomes, facilitating their absorption. The process of nanoparticle penetration into plants is significantly influenced by several factors. These include the physicochemical characteristics of the nanoparticles themselves, the developmental stage of the plant, the specific species of the plant, root secretions, and the underlying soil characteristics, encompassing pH levels, presence of salt ions, and other soil properties [15]. In this article, the effects of nanoparticles on antioxidant defense systems such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX), and non-enzymatic antioxidants such as ascorbic acid (AA), phenolic compounds, glutathione (GSH), flavonoids, carotenoids in some plant families and the impact of improving the negative effects of other stresses such as salinity and drought are discussed.

Engineered nano materials in agriculture

In agriculture, Engineered Nano Materials (ENMs) are used in different parts of a plant’s life cycle. These treatments can be exposed to the plants in two ways: they can be either sprayed on foliar or entered through roots, and dissolved in water [16]. Recently, the effects of nanoparticles on seeds, germination, plant growth and development, and pathogen determination have been studied and identified [4]. For instance, they are used in growing plants to promote germination and plant development [17]. Factors that determine the extent of the impact of Nps are shape, surface, size, and properties of Nps [11], time of exposure (plant age) [12], plant species and soil type [13; 18].

Nanoparticles can either increase or decrease plant growth, photosynthesis, and plant resistance to biotic or abiotic stresses, through their distinctive properties [4; 19]. For instance, small nanoparticles can penetrate the cell membranes and disrupt cellular function, but larger Nps or particles need phagocytose to enter the cell [2; 6]. Generally, the smaller the Np, the greater its toxic effect [20] because the smaller sizes have greater reactivity [3].

Reactive oxygen species in plant

Reactive oxygen species (ROS) are aerobic metabolism products in plants [3]. ROS are produced permanently in chloroplasts, mitochondria, peroxisome and other parts of the plant cell by metabolic processes such as respiration and photosynthesis [4; 14; 21; 22]. They are by-products of metabolic pathways such as electron transport systems [23]. These ROS are also produced in plants not only in response to biochemical changes but also stresses, called oxidative stress which eventually causes one of these two results: sustainability with abiotic stress generated by ROS signals [11] and cell death stimulated by ROS [3; 4; 12; 24; 25]. In low concentrations, ROS act as a signaling molecule involved in growth and defense, but in higher concentrations caused by stresses, they damage different compounds of the cell such as proteins and limit plant growth [4]. A high level of ROS can also disrupt biochemical and physiological processes [26]. For instance, a low concentration of ROS in *Arabidopsis thaliana* exposed to Ag NPs causes root elongation, whereas a higher concentration will stop the process [27]. ROS are Oxygen-containing molecules that are too reactive and can easily take electrons from more stable compounds [24].

ROS have two general categories: free radicals such as superoxide (O_2^-) and hydroxyl radicals (OH^-), and non-free radicals such as hydrogen peroxides (H_2O_2) and singlet oxygen (O_2) [3; 28]. These are the by-products of many biochemical processes in plants [13]. OH^- and H_2O_2 are the main free radicals in terms of increasing oxidative stress [29]. H_2O_2 can convert to more OH^-, which can cause more toxicity in plants because they cannot be detoxified by any known enzymatic system [3; 30]. OH^- is also more reactive than other ROS, so it can react to any biological cell in the plants [3].

Reactive oxygen species function in plants is essential. For instance, enough ROS production can break seed dormancy or even increase guard cells when the plants are infected by the pathogen [13; 31]. ROS is also the main factor in producing greenhouse gasses, which will decrease in response to a lack of oxygen [32]. However, ROS also prevents plant germination and seedling growth and photosynthesis when its concentration is higher than usual [33]. A low concentration of ROS acts as a signaling molecule, whereas a high concentration can cause oxidative damage [34]. For example, a high concentration of H_2O_2 can cause toxicity and oxidative stress in plants [35]. Thus, plants have an effective antioxidant system that can control ROS concentration and protect the plants from oxidative stress [8; 13; 23]. Because of the existence of ROS, antioxidant defense systems will be produced permanently to disrupt ROS production [16].

Antioxidant defense systems are divided into enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX) [11], and non-enzymatic antioxidants such as ascorbic acid (AA), phenolic compounds, glutathione (GSH), flavonoids, and carotenoids [12; 28; 36; 37]. In addition, proline acts as a non-enzymatic antioxidant and decreases ROS effects [38].

Catalase is the first known antioxidant with a vital role in eliminating ROS in stressed plants [3]. Peroxidase (POD) can increase plant resistance against pathogens [33]. Superoxide dismutase acts as a ROS remover in the chloroplast, mitochondria, peroxisome, and cytosol. It can convert superoxide to H_2O_2 and O_2 [35; 39], and the produced H_2O_2 can be eliminated by ascorbate peroxidase, catalase, and peroxidase to protect the plants [40; 41]. Phenols and flavonoids are also antioxidants that can eliminate free radicals and protect the antioxidant system [42; 43]. Moreover, anthocyanin and anthocyanidins can protect plants from ROS damage [34; 44]. When the balance between ROS and the antioxidant's defense system is disturbed, oxidative stress will occur [45].

As still creatures, plants are affected by many biotic and abiotic stresses [19]. Studies have shown that abiotic stresses such as Nps and heavy metals can overproduce ROS and cause oxidative stress in plants [8; 22; 36]. In other words, a high level of ROS is a sign of resistance to abiotic and biotic stresses [31]. Aggregation of ROS is a common response to almost all abiotic stresses, which in high concentration can eliminate half of the crop yield in plants [14]. Therefore, although ROS is a common product of a plant's metabolism, it can also cause oxidative stress with a negative impact on its growth [40]. Oxidative stress can affect cellular organelles and biomolecules such as protein, carbohydrates, lipids, secondary metabolites and DNA, and disorder cell death plan [7; 8; 24]. For instance, increasing ROS can affect protein structure, function negatively and cause cell toxicity [22].

Engineered nanoparticles (ENPs) produce ROS which can also damage cell membranes through lipid peroxidation, disrupting cell metabolism [43; 46]. Zinc oxide nanoparticles (ZnO NPs) change the antioxidant enzyme and damage chloroplast which can produce ROS and cause oxidative stress [47]. Since NPs can cause necrosis and yellow pigments on leaves, researchers must pay greater attention to NP concentration [48]. Molybdenum nanoparticles (Mo NPs) can cause oxidative stress in soybean roots [41]. Silver nanoparticles (Ag NPs) can overproduce ROS, which damages chloroplast, lipid, and DNA macromolecule instructions [49].

However, some of these NPs can also activate more antioxidants and protect chlorophyll and carotenoids from ROS [26; 50]. Engineered nanoparticles (ENPs) can activate antioxidants to strengthen the defense system of plants and eliminate ROS [33]. For example, Cadmium sulfide (CdS) nanoparticles overproduce ROS in the roots of *Vicia faba* L., but these NPs activate glutathione which eliminates overproduced- ROS [51]. Exposure to different NPs can improve growth and decrease Cadmium (Cd) in the plants compared to controls [52]. Molybdenum (Mo) Engineered Nano Materials (ENMs) improve superoxide dismutase, peroxidase, and catalase activities, which decrease oxidative stress caused by ROS in Chinese cabbage [41]. NPs improve antioxidant activity, whereas a higher concentration of these NPs decreases the activity [53]. Phosphorus (P) NPs can increase ascorbate and glutathione content in plants.

Many studies need to be conducted in this field of study because their uses as fertilizers and pesticides in plants will increase in the future [54]. Researchers can even use NPs to reduce the effects of other stresses in the plants but these NP features might also cause oxidative stress.

Produce reactive oxygen species in plants and metal

Reactive oxygen species have different effects on plants. Cadmium (Cd) elements damage the activity of antioxidant enzymes [55]. With the exception of this element, other elements might play a protective role against oxidative damage, including an increase in chlorophyll content and phenolic compounds to protect the plant's primary antioxidants [56]. An increase in chlorophyll may help plants exude ROS, and phenolic compounds are important because of their protective role against oxidative damage [56]. They can also increase autophagy[1] mechanisms caused by oxidative stress [57]. However, ROS are known for their distinct function of acting as signaling molecules when exposed to stress [7].

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[1] Autophagy is also a defense and repair mechanism to reduce the damage caused by increasing of ROS produced by $nTiO_2$.

Elements in nano size can have different effects from their actual size. Nanoparticles can change the cell physiologically and stop its antioxidant defense system [8]. This is exemplified in the suppression of plant growth by reducing photosynthesis and stimulating ROS accumulation, which results in oxidative damage in the plants under nZnO treatments [58]. They can also stimulate oxidative stress due to greater ROS production, which can activate the antioxidant defense system [8]. Stimulation of enzymatic and non-enzymatic antioxidant systems by selenium (nSe) nanoparticles reduced the symptoms of oxidative stress [59]. In addition, the antioxidant defense system was activated to protect the plants from oxidative stress caused by Copper oxide nanoparticles (CuO Nps) (12 mg/L) treatment [29].

Titanium dioxide nanoparticles (TiO_2 Nps) directly affect the activity of peroxidases and other antioxidant enzymes, and reduce their activities. This is due to the capability of these nanoparticles to suddenly change the activity of enzymes and disrupt them [60]. It has also been shown that the synthesized nanoparticles can inhibit DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals in a concentration-dependent manner, and increasing the concentration of nanoparticles increased the inhibitory activity [61]. Table 1 presents the effects of NPs on different plant families (Table 1).

Poaceae

In *Triticum aestivum L.*, copper nanoparticles (Cu Nps) reduce oxidative stress and increase growth. In contrast, CuO Nps inhibit the growth of these plants [12] [62]. These plants cause oxidation by absorbing more chromium (Cr), but when they are exposed to Cu Nps (69 nm), more Cr could be lost and oxidative stress decreases [12]. A significant increase in H_2O_2 and malondialdehyde (MDA) was observed in wheat treated with nickel oxide nanoparticles (NiO Nps) [63]. An increase in ROS production and alterations in genes, which are involved in repairing DNA mismatch, and cell division of wheat and *Zea mays* seedlings were observed when Titanium dioxide nanoparticles (TiO_2 Nps) were the treatment [47]. In addition, nSe motivated ROS in wheat and changed the expression of specific gene patterns [59]. TiO_2 Nps reduced the content of malondialdehyde and total antioxidant activity (TAA) in wheat roots, followed by high concentrations of $nTiO_2$ causing oxidative damage; therefore, ROS accumulation in the roots of the plants was higher than in its leaves [64; 65]. High concentrations of nTiO2 caused oxidative damage in wheat roots. Therefore, ROS accumulated in these plant roots was greater than in their leaves [64].

When wheat was treated with ZnO Nps at a concentration of 100 mg/L, the activity of superoxide dismutase and peroxidase enzymes increased [52]. In these plants, toxicity was observed in addition to the production of H_2O_2 and malondialdehyde in aluminium oxide nanoparticles (Al_2O_3 Nps) treatment [62]. Treatment of zinc nanoparticles (Zn Nps) reduced the level of ROS in wheat cultivated in acidic soil, and as a result, the activity of catalase enzymes decreased [66]. Wheat plants treated with magnetic nanoparticles showed no significant difference in H_2O_2 production compared to controls, but the activity of superoxide dismutase increased in these plants [67].

Reduction of ROS and improvement of antioxidant defense system were the results of biogenic Cu Nps treatment in *Triticum aestivum L.* [12]. Generally, nanoparticles such as TiO_2, Al_2O_3, ZnO, and ZnO_2 (Zinc peroxide) reduced superoxide dismutase activity in wheat [68]. The presence of Ag Nps in stems and roots of Wheat plants caused oxidative stress whereas, 10 mg/L of Ag Nps produced less ROS than other concentrations of these treatments in the germination of wheat seeds [13; 69]. In addition, a decrease in cadmium (Cd) was observed in the presence of ferric oxide (Fe_2O_3) Nps in these plants [55].

Table 1. Summary of the effect of nanoparticles on some species.

Plant species	Type of nanoparticle	Concentrations	Size	Effects	Family
Poaceae	Increased activity of GPX, POD, APX, CAT and SOD enzymes	-	0.5, 1 and 5 gr.L	TiO_2	*Triticum aestivum*
Poaceae	Reduced oxidative stress	-	5, 50 and 150 mg/L	TiO_2	*Triticum aestivum*
Poaceae	Caused toxicity	-	335 to 570 mg/kg	CuO	*Triticum aestivum*
Poaceae	Increased levels of H_2O_2 and MDA	-	50 mg/mL	Al_2O_3	*Triticum aestivum*
Fabaceae	Activating the main defense mechanism of AOX	8 nm	Maximum 500 mg/L	CuO	*Faseolus vulgaris L.*
Fabaceae	Increased oxidative stress	50 nm	0 to 500 mg/L	CuO	*Glycin max*
Fabaceae	Accumulation of ROS, increase in antioxidant enzymes activity and MDA	-	Not mentioned	TiO_2	*Pinto bean*
Fabaceae	Reduced CAT and POX activity	-	Not mentioned	Magnetic Iron Nps	*Vigna radiata*
Fabaceae	Reduced MDA and H_2O_2	-	25, 50 ppm	Ag	*Brassica juncea*
Fabaceae	Caused DNA and oxidative damage and increased ROS	-	2000 mg/L	CeO_2	*Soybean*
Asteraceae	Production of phenols and flavonoids but no oxidative stress	-	10, 100 and 1000 mg/L	TiO_2	*Stevia rebaudiana*

Plant species	Type of nanoparticle	Concentrations	Size	Effects	Family
Asteraceae	Increased oxidative stress	-	50, 100 and 1000 mg/L	TiO_2	*Lactuca sativa*
Polygonaceae	Increased CAT activity	-	100 mg/L	ZnO	*Fagopyrum esculentum*
Polygonaceae	Increased H_2O_2	-	Not mentioned	Au	*Candida albicans*
Lamiaceae	Increased proline during oxidative stress	-	Not mentioned	ZnO	*Salvia rosmarinus*
Lamiaceae	Increased H_2O_2 production	-	250 and 500 mg/mL	MWCNTs (Multi-Walled Carbon Nanotube)	*specific*
Brassicaceae	Increased oxidative stress	50 nm	0 to 500 mg/L	CuO	*Brassica juncea*
Brassicaceae	Increased oxidative stress	50 nm	0 to 500 mg/L	CuO	*Brassica napus*
Brassicaceae	Redused ROS accumulation	10-0.6 nm	51 mg/L	PNC	*Arabidopsis thaliana*
Liliaceae	Increased CAT enzyme	-	Not mentioned	ZnO	*Oryza sativa* L.
Araceae	increase ROS amount	-	10 mg/L	Ag	*Spirodela polyrhiza*
Amaryllidaceae	Increased SOD activity	-	2000 mg/L	Al_2O_3	*Allium cepa*
Hydrocharitaceae	Production of H_2O_2, GSH, CAT, GR and GSSG and increase in oxidative stress	-	250, 500 and 750 mg/L	TiO_2	*Hydrilla verticulata*
Ranunculaceae	Increased antioxidant enzymes activity	5 to 8 nm	Not mentioned	NiO	*Nigella arvensis*
Ranunculaceae	Increased antioxidant compound	5 nm	Not mentioned	Al_2O_3	*Nigella arvensis*

The activity of Calcium (Ca) channels increased with 1000 mg/L treatments of nanoparticles in *Zea mays*, which was possibly due to the expression of defense-related genes in response to oxidative stress activated by Ag Nps treatment [70]. Reactive oxygen specie production was increased by exposing *Zea mays* plants to Ag Nps and the activity of antioxidant enzymes such as H_2O_2 was enhanced [70]. There was an increase in H_2O_2 and a decrease in malondialdehyde in the nanoceria treatment in maize plants [71]. There was also an increase in H_2O_2 in these plants treated with ceric oxide nanoparticles (CeO_2 Nps) stress [33]. Simultaneously, with an increase in the activity of catalase and ascorbate peroxidase enzymes in maize plants treated with CeO_2 Nps, the amount of extra H_2O_2 decreased [72].

In *Zea mays* plants, an increase in H_2O_2 as well as lipid peroxidation under CeO_2 Nps treatment was observed which eventually led to an increase in ROS [73].

Furthermore, these nanoparticles increased the activity of catalase and ascorbate peroxidase enzymes in *Oryza sativa* [73] and the amount of H_2O_2 increased in *Zea mays* by using these nanoparticles [22]. Therefore, spraying nanoceria was very effective in reducing ROS and increasing enzymatic activity in these plants, which led to a decrease in lipid peroxide [74].

Silver nanoparticles can induce tolerance mechanisms of oxidative stress in rice seedlings [74; 75]. Production and accumulation of ROS as well as oxidative stress were observed under CuO Nps treatment in *Oryza sativa,* which more precisely increased the activity of antioxidant enzymes, the amount of H_2O_2 and malondialdehyde [18; 76-78]. In addition, a significant increase in malondialdehyde and superoxide dismutase production was seen in the presence of Ag Nps in rice [77; 79]. Multi-walled carbon nanotubes can produce ROS in these plants [72].

In *Oryza sativa* plants, ROS increased and oxidative stress was observed in CeO_2 and Ag Nps treatment [75; 80]. In addition, carbon nanotubes can produce ROS in these plants [80]. As previously mentioned, CuO Nps increased the H_2O_2 accumulation in rice leaves and barley plants [8]. Alteration in superoxide dismutase and catalase activity was observed simultaneously with the increase of ROS in *Oryza sativa* exposed to CuO Nps treatment [81]. In addition to superoxide dismutase, peroxidase and catalase in both rice species under ZnO Nps stress, the activities of ROS and glutathione reductase were increased. [82]. The production of antioxidant enzymes such as H_2O_2, O_2, and hydroxide (OH^-) was induced in rice by ZnO Nps treatment [83; 84]. Moreover, these nanoparticles managed the effects of oxidative stress by increasing the inhibitory enzyme, superoxide dismutase, in these plants [85]. In *Hordeum vulgare*, excessive increases in ROS and oxidative stress were observed when they were exposed to NiO Nps [22]. Ni@Fe_2O_3 heterodimer nanoparticles (Nps) reduced growth parameters of barley (*Hordeum vulgare*) plants such as chlorophyll content, but they had no effects on phytotoxicity.

Brassicaceae

Reactive oxygen species were produced in *Brassica juncea* when exposed to gold nanoparticles (Au NPs) [40]. 29% increase in ROS was observed in *Brassica juncea* plants treated with Au nanoparticles (100 ppm) [7]. Malondialdehyde and H_2O_2 were reduced in these plants treated them with 25 and 50 ppm of Ag Nps [86]. H_2O_2 increased in these plants by 28% and 19%; respectively, by treating with CuO and TiO_2 nanoparticles [73]. Moreover, treatment of just CuO Nps increased malondialdehyde activity and ROS in *Brassica juncea* [87]. Increasing antioxidant capacity was evident in these plants in the treatment of Ag nanoparticles [88].

Iron oxide nanoparticles treatment increased antioxidant enzymes such as superoxide dismutase, catalase, and ascorbate peroxidase in *Brassica nigra* [89]. In addition, the treatment of these nanoparticles in *Raphanus sativus L.* leaves had the least amount of antioxidant activity [90]. A decrease in chlorophyll under the influence of Ag Nps was observed in *Brassica rapa* [56]. When these plants were treated with CeO_2 Nps, the amount of H_2O_2 increased [33]. The increase in H_2O_2 was evident in the presence of graphene in *Brassica oleracea* [87].

An increase in the activity of superoxide dismutase, peroxidase, and catalase enzymes was seen in *Brassica napus* when using TiO_2 Nps [90]. By reducing the levels of H_2O_2 and malondialdehyde, oxidative stress was also reduced in *Brassica napus* plants, which were exposed to Fe_2O_3 nanoparticles [4]. However, no exposure of antioxidant enzyme activity was observed when *Brassica napus* was exposed to TiO_2 Nps [65]. Superoxide dismutase activity was increased in *Brassica. napus* and *Arabidopsis thaliana* using ZnO and TiO_2 nanoparticles, respectively [91; 92].

Autophagy decreased ROS through the destruction of organelles which produced ROS [93]. For instance, 0.1 and 0.5 mM of $nTiO_2$ decreased autophagy in wild Arabidopsis [57]. CuO Nps reduced oxidative stress and leaf growth in *Arabidopsis thaliana* [62]. Different concentrations of Cu Nps accumulated ROS in the leaves and roots of *Arabidopsis* [62]. Then, Ag Nps caused toxicity and produced ROS in these plants [94]. The maximum increase in anthocyanin was observed in *Arabidopsis thaliana* with Ag Nps presentation [95]. In addition, polyacrylic acid nanoceria (PNC) in the chloroplast of this plant enhanced the excess light of the plants, so by protecting vulnerable chloroplast systems and chlorophyll pigments from oxidative damage, the plant absorbed excess light [14]. In other words, PNC enhances photosynthesis and inhibits ROS in *Arabidopsis thaliana* L. [96]. For example, the 24 µM of nanoceria destroyed both $O_2^{\circ-}$ and H_2O_2 in the chloroplasts, and 51 mg/L of PNC-coated nanoceria reduced significantly ROS whereas, the expression of ROS-related genes in these plants increased in concentrations of 50 and 300 mg/L of Ag Nps [27; 30] . Furthermore, in this plant, nanoceria with lower concentrations reduces leaf ROS levels by 52% [96].

However, in *Arabidopsis* plants treated with spherical Ag Nps, the level of oxidative stress reached its highest level. On the other hand, decahedral Ag Nps caused the highest growth promotion and the lowest oxidative stress in these plants [95]. Generally, the expression of antioxidant enzyme genes and their transcripts in response to ROS increased in Arabidopsis, treated with Ag Nps [49; 97]. If the concentration of the CeO_2 and Indium (III) oxide (In_2O_3) nanoparticles treatment increased, the amount of H_2O_2 in the leaves also increased [40]. The disruption of cellular metabolism and an increase in ROS was observed in the CuO

Nps treatment in the plants [78]. In these plants, there was a decrease in biomass and an increase in ROS with CuO Nps [81]. Furthermore, higher levels of anthocyanins were observed in Arabidopsis plants using CuO Nps [34].

In addition, *Arabidopsis* exposed to 30 nm CuO Nps showed that ROS amount was dependent on nanoparticle concentrations, and regulation of gene expression was seen for both enzymatic and non-enzymatic antioxidant defense systems [8]. Among these plants, those treated with 200 mg/L TiO_2 Nps × 10 mg/L TC (Tetracycline) showed an increase in catalase activity [91]. In these plants, using In_2O_3 Nps did not increase the amount of H_2O_2, but the amount of O_2^- slightly increased by using In_2O_3 Nps [40]. TiO_2 Nps alone increased ascorbate peroxidase activity in Arabidopsis [98].

Malondialdehyde levels were increased by using Ag Nps in the *Crambe abyssinica* wild plant [73]. In the treatment of CeO_2 Nps, the levels of catalase and H_2O_2 in *Raphanus sativus* increased and decreased, respectively [73].

Fabaceae

Molybdenum-based nanomaterials (nano-Mo) increased superoxide dismutase activity in soybean roots. According to experiments, nano-Mo aggregation was able to increase superoxide dismutase and peroxidase levels and remove ROS in these plants [41] . ROS production and peroxidase activity was significantly increased in soybean under CuO Nps stress [8; 99]. In these plants, catalase activity increased with exposure to CeO_2 Nps but did not increase significantly with exposure to TiO_2 Nps [100]. In addition, TiO_2 Nps produced oxidative stress and ROS in *Lens culinaris* medik [101].

Cicer arietinum was protected by Cu-Zn Nps. These nanoparticles showed inhibitory effects on ROS [102]. An increase in ROS production as well as catalase and peroxidase activities were observed in *Cicer arietinum* treated with CuO Nps [8; 88]. These plants treated with ZnO Nps (900 mg/kg soil) increased ROS and ascorbate peroxidase activities and decreased catalase activities [66].

There was an increase in oxidative stress and antioxidants in response to TiO_2 Nps treatment in *Vicia faba* plants [90; 103]. The treatment of silver particles and silver nanoparticles had negative effects on *Vicia faba*, including loss of leaf tissue, a disorder of photosynthesis, and an increase in ROS [49]. In the presence of Ag Nps in these plants, the total amount of phenol was increased [98]. Carbonic anhydrase (CA) enzyme activity was increased in these plants due to $nTiO_2$ Nps treatments, which was probably additional protection by exposing water deficit stress against oxidative stress [39].

Cadmium sulfide (CdS) Nps caused excessive production in *Faba bean* roots, but the same nanoparticles removed the extra ROS in the leaves and roots by activating the glutathione (GSH) enzyme [51]. In beans, the activity of antioxidant enzymes was increased in the TiO_2 Nps stress in addition to the increase in oxidative stress [47]. In the case of bean plants, the amount of malondialdehyde and catalase increased with Zn Nps treatment [66]. In the presence of TiO_2 Nps, *Faba bean* leaves caused an antioxidant (particle size dependent) response [65].

The 335 to 570 mg/L of CuO Nps concentration caused toxicity in *Mung bean* plants [104]. In this way, these nanoparticles, even in low concentrations, reduced the growth of roots and stems of these plants by producing ROS and lipid peroxidation [1].

Injection of CuO Nps into the Alfalfa plant by hydroponic method causes a reduction in root length and a decrease in catalase activity in both roots and stems [5]. Ascorbate peroxidase (APX) activity was increased in the treatment of engineered copper nanoparticles (Cu ENPs) in Alfalfa root in a hydroponic medium [54]. Simultaneous increases of ROS and H_2O_2 and lipid peroxidation were observed in *Vigna radiata* plants when they were treated with Ag Nps [97].

Oxidative stress and increase in H_2O_2, ascorbate peroxidase, and lipid peroxidation were observed in the presence of ZnO Nps in *Pisum sativum* plants treated with these nanoparticles; whereas, silica nanoparticles regulated superoxide dismutase and catalase activity in these plants [7; 80; 105; 106]. However, no change in catalase activity was reported under the stress of ZnO Nps [45]. Chlorophyll content was reduced in *Pisum sativum* by Ag Nps treatments [56]. The antioxidant compounds and catalase activity in the *Lupinus termis* plant were increased by 21.3 nm of ZnO Nps [107]. The abundance of antioxidant compounds and some defense enzymes against oxidative damage in *Phaseolus vulgaris* were altered by nanoceria [108]. An increase in catalase activity and oxidative stress was observed in *Trigonella foenum-graecum* by Al_2O_3 Nps treatment [109]. Another example is the *Glycyrrhiza glabra* which showed positive effects on phenolic compounds when treated with ZnO and CuO Nps [110].

Solanaceae

Reactive oxygen species production increased in the roots of *Lycopersicum* plants exposed to Mo Nps and NiO Nps, which caused superoxide dismutase, glutathione, ascorbate peroxidase, and catalase in the plants to reduce turbulence [80; 111]. In tomatoes, cell death increased as a result of an increase in ROS and superoxide dismutase in the presence of NiO Nps [63]. In addition, tomato plants caused oxidative stress due to NiO Nps [33]. In another report, the presence of ZnO Nps in these plants reduced the chlorophyll content and ROS but increased the activity of antioxidant enzymes [107]; [108]. An increase in H_2O_2 was also observed in tomatoes by spraying Cu-Zn Nps. (102) Superoxide dismutase activity also increased when these plants were treated with TiO_2 Nps [97]. The results showed that different concentrations of graphene (a type of carbon allotropes) increased H_2O_2 in tomatoes [87]. In these plants, Ag Nps and NiO Nps caused DNA damage [11]. In addition, silver nanoparticles motivated oxidative stress in tomatoes; as a result, phenols increased in the plants, which was an appropriate method to clean and eliminate ROS [112].

In tobacco, a reduction of ROS was observed with Ag Nps [56]. In addition, in the ZnO Nps treatment, ROS and oxidative stress were produced in these plants [53] [68]. The same results were observed in all cellular parts of tobacco with Fullerene (a kind of nanotube) treatment [113]. Production of ROS was seen in *tobacco* leaves using Iron (II, III) oxide (Fe_3O_4) Nps, which in turn increased the activity of peroxidase and catalase [31]. The production of ROS increased catalase and peroxidase due to the presence of Fe_3O_4 Nps in the tobacco plant [31].

At the concentration of 100 mg/L of nZnO, ascorbate peroxidase activity increased in *Hyoscyamus reticulatus*, but the high level of ZnO NPs decreased the activity [114]. Reactive oxygen species were produced in *Solanum tuberosum* plants with both Ag Nps and Ag ion treatments, but more reactive oxygen species were produced in the plants, treated with Ag Nps [115]. At a concentration of 10 mg/L of Ag Nps, the level of ROS increased and the highest amount of antioxidant enzymes such as catalase, ascorbate peroxidase, and superoxide dismutase was shown in these plants [115].

Higher levels of anthocyanins were also found in *Solanum melongena*, treated with NiO Nps [34]. In addition, these nanoparticles increased malondialdehyde activity in *Lycium barbarum* [34]. Superoxide dismutase and catalase activities increased by TiO_2 and Al_2O_3 nanoparticles in *Hyoscyamus niger* and *Nicotiana tabacum* plants, respectively [87; 109].

Cucurbitaceae

In *Cucumis sativus* root, peroxidase activity increased compared to controls when the plants were treated with ZnO and CuO nanoparticles [8]. In addition, the use of ZnO Nps increased catalase activity in these plants [79]. While TiO_2 Nps increased catalase activity and decreased ascorbate peroxidase activity in *Cucumis sativus* [76]. ROS-related enzymes such as catalase and peroxidase increased when cucumber under hydroponic conditions were treated with CuO Nps [116]. There was also an increase in malondialdehyde with the same conditions in these plants [87].

Increased respiration and activation of the defense antioxidant system were seen through an increase in malondialdehyde in the presence of Ag Nps in *Cucumis sativus* [117; 118]. The impact of lanthanum (III) oxide (La_2O_3) Nps in these plants was demonstrated in the increase in ROS and eventually cell death [33]. The oxidative stress caused by TiO2 Nps in these plants was another example. DNA damage was observed in *Cucurbita pepo,* using the same nanoparticles [80; 97]. In *Cucumis melo,* Se-Np-mediated improved its antioxidant properties [119].

Araceae

Accumulation of ROS due to Ag Nps reduced photosynthesis in *Spirodela polyrhiza* [94]. In these plants that were exposed to 0.5 - 5 mg/L of Ag^+ or 1-5 mg/L of Ag Nps, ROS accumulation was significantly higher than in the controls [120]. In addition, these plants produced more ROS when exposed to Engineered Nanoparticles [121]. The highest amount of superoxide dismutase enzyme was observed in *Spirodela polyrhiza* treated with 75 mg/L of ZnO Nps [79]. In general, there was an increase in antioxidants in these plants under the TiO_2 Nps treatment [103].

Higher levels of superoxide dismutase were observed in *Spirodela punctata*, treated with ZnO and Ag nanoparticles (0.01 and 1000 mg/L). Because of the high level of H_2O_2, superoxide dismutase was produced [122].

Reactive oxygen species in *Lemna minor* was increased by CuO Nps [11]. Ferric oxide (Fe_2O_3) Nps also produced oxidative stress in *L. minor* [2]. In addition, the accumulation of ROS due to Ag Nps inhibited plant growth and caused cell death in these plants [120]. The amount of H_2O_2 in *Lemna minor* treated with CuO Nps was 6 times higher than the controls [11]. An increase in ROS and oxidative stress were observed in *Lemna gibba* with Ag Nps treatment [113]. Iron oxide nanoparticles (Fe2O3) applied to *Lemna gibba* L plants for 7 days also produced a substantial amount of ROS, disrupted the activity of photosystem II, and resulted in a decrease in chlorophyll content [96].

Asteraceae

Phytotoxicity was created by the release of CeO_2 Nps ions in *Lactuca sativa* [3]. In addition, during the injection of copper(II) hydroxide ($Cu(OH)_2$) Nps, the Fenton reaction produced more ROS in these plants [16]. Se Nps-mediated improved the antioxidant properties of *Lactuca sativa*; whereas, $Cu(OH)_2$ nano toxins in these plants reduced antioxidants [16; 119]. Ascorbate peroxidase activity was increased in all copper treatments in these plants' roots in a hydroponic medium [54]. Interestingly, lettuce did not alter its antioxidant activity when exposed to TiO_2 Nps [65].

After flavonoid reduction, total phenol content and total antioxidant capacity in *Stevia rebaudiana* were reduced under 100 mg/L of ZnO Nps [48]. Moreover, there was an increase in superoxide and H_2O_2 in *Tanacetum vulgare* treated with CuO Nps [123]. Another example is the increase in the amount of superoxide dismutase treated with CeO_2 Nps in *Asparagus lettuce* [62]. Pb Nps can inhibit ROS production in *Globe artichoke* plants [124].

Amaryllidaceae

Treatment of Ag and NiO nanoparticles caused DNA damage in *Allium cepa* [11]. In addition, an increase in O_2^- and $H_2O_2^-$ levels were observed in onions in the presence of Ag Nps, which caused an increase in ROS production [7]. Furthermore, in another study on the roots of *Allium cepa*, it was found that the contact of the plant with silver nanoparticles increased the production of ROS and the activity of different antioxidant enzymes in this plant [96]. ROS produced by NiO Nps (100 to 500 mg/L) disrupted intracellular homeostasis in *Allium cepa* [125]. Malondialdehyde levels also increased in the presence of NiO Nps in these plants [34].

In the case of other nanoparticles, it can be stated that the levels of glutathione peroxidase (GPX), catalase, and superoxide dismutase enzymes increased in *Allium cepa* roots treated with Ag Nps, Zn^{2+} and 1000 mg/L Zn [21]. TiO_2 Nps temporarily stimulated oxidative stress in *Allium cepa* roots [126]. Through the penetration increase of DCF (2′,7′-dichlorofluorescein diacetate) by ZnO Nps, ROS production also increased in these plants [37].

Amaranthaceae

Titanium dioxide nanoparticles (TiO_2 Nps) in *Spinacia oleracea* had different effects. These nanoparticles reduce oxidative stress caused by UVB radiation in these plants [126]. TiO_2 Nps were involved in the activation of the antioxidant system in these plants [90]. In addition, these nanoparticles destroyed monomers, which can be the secondary result of ROS production in *Spinacia oleracea* [108]. According to previous reports, TiO_2 Nps in these plants reduced oxidative stress by increasing superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase [127].

In the presence of nano anatase *Spinacia oleracea* eventually reduced and then eliminated ROS by increasing the electron transfer rate and activating superoxide dismutase, catalase, ascorbate peroxidase, and glutathione peroxidase (GPX) [128]. When these plants were exposed to intense light, TiO_2 Nps treatment slowed down the production of free radicals and also increased superoxide dismutase, peroxidase, and catalase in the plant's chloroplasts [129]. Also, an increase in superoxide dismutase, catalase, ascorbate peroxidase, and guaiacol peroxidase (GPOX) enzymes was seen in TiO_2 Nps-treated *Spinacia oleracea* [7].Another example, the highest ROS intensity was found in *Salicornia* roots in a culture medium with a concentration of 1000 mg/L ZnO Nps [36].

Lamiaceae

The phenolics of *Rosmarinus officinalis* plants were increased by spraying nanoparticles [130]. Proline increased in these plants with ZnO Nps treatment during oxidative stress [18]. In addition, iron (Fe) Nps increased flavonoids and decreased oxidative stress in the *Dracocephalum moldavica L.* plant [44]. Another instance is that TiO_2 Nps had various effects on *Salvia officinalis*, including the production of ROS and oxidative stress and plant yield and growth change [110]. Furthermore, TiO_2 Nps produced oxidative stress and ROS in *Melissa officinalis* [18].

Algae and moss

CuO nanoparticles decreased photosynthesis in *Chlamydomonas Reinhardtti* algae due to ROS accumulation [94]. ROS production increased by Titanium dioxide nanoparticles TiO_2 Nps treatment, either with or without UV radiation, in Algae cells [131]. The amount of ROS in *Thalassiosira pseudonana* algae exposed to ZnO-Go (ZnO-conjugated graphene oxide) was higher than the amount of ROS in other types of NH (CNT (carbon nanotubes),TiO_2-Go (Titanium dioxide nanoparticles conjugated graphene oxide), and TiO_2-CNT (Titanium dioxide nanoparticles - carbon nanotubes)) in which the algae cells were treated [132]. ROS production increased with exposure to Engineered Nanoparticles in freshwater algae [121]. In addition, the antioxidant defense response was affected by TiO_2 Nps in algae [133]. Humic acid (HA) can act as an antioxidant by reducing oxidative stress. HA reacts with ROS and reduces the toxicity of ZnO Nps in algae cells [46]. Higher concentrations of ROS were also observed at higher concentrations of ZnO Nps nanoparticles [46].

After UV radiation, ROS were produced with nNiO treatment in *Chlorella vulgaris* algae [134]. Furthermore, Cu Nps produced ROS and damaged the cell membranes in these plants [135]. AF-Ag Nps (Allium fistulosum_silver nanoparticles) + SRHA (Suwannee river humic acid)(environmentally related concentration of DOM (Dissolved organic matter)) on *Chlorella vulgaris* also acted as an antioxidant and reduced ROS [136]. ROS and oxidative stress were caused in tiny algae when they were exposed to nNiO [134]. ROS had a slight increase when algae were exposed to TiO_2 Nps with UV radiation [131].

In other species

Decreased CO_2 uptake might occur in the presence of Cerium oxide nanoparticles (CeO_2 Nps) as well as TiO_2 Nps. An increase in enzymes such as catalase and ascorbate peroxidase was reported in *Coriandrum sativum* when they were treated with CeO_2 Nps [73]. These nanoparticles also increased the total phenols in *Bacopa monnieri* seeds [56; 112]. In another report, the amount of H_2O_2

increased with graphene treatment in *Malabar spinach* [87]. The amount of H_2O_2 also increased with graphene treatment in these plants [87].

An increase in H_2O_2 and malondialdehyde was observed in the presence of TiO_2 Nps in *Linum usitatissimum* [87]. No significant changes in H_2O_2 levels were observed in *Hydrilla verticillata* treated with TiO_2 anatase and TiO_2 rutile compared to controls; whereas TiO_2 Nps treatment increased the amount of H_2O_2 and glutathione reductase (GR) in these plants [116; 133; 137]. Also, an increase in superoxide dismutase activity was reported in *Abelmoschus esculentus* leaves exposed to $nTiO_2$ [138].

In *Annona muricata* L. leaves, which were treated with Cu NPs and Ag NPs, high variability of flavonoids was observed [99]. Catalase activity was increased in *mulberry* leaves containing Ag Nps solution due to the dominance of H_2O_2 [139]. An increase in chlorophyll under the influence of these nanoparticles was observed in *Potted pelargonium* [56].

Following the increase in the production of saponins, phenols, and iridoids in *Nigella sativa*, the amount of several antioxidant enzymes in these plants were also increased when the plants were treated with Al Nps [33]. Moreover, peroxidase resists the plants from pathogens and wound healing, which was increased by exposure to engineered Al and NiO Nps [33]. In these plants, the amount of H_2O_2 tripled in three to six hours by treatment of Cu Nps [140]. Another species, *Nigella arvensis*, was treated with Al_2O_3 Nps and increased H_2O_2 amounts [33]. In addition, the combination of TDZ (thidazuron) + NS (nano-silver) reduced H_2O_2 levels in *graveolens pelargonium* [127].

In *Rosa hybrida*, a decrease in malondialdehyde and H_2O_2 levels was observed under NS treatment [141]. By the same treatment, an increase in the activity of protective enzymes such as ascorbate peroxidase, catalase, and superoxide dismutase, as well as an increase in malondialdehyde and free radicals such as O_2^- and H_2O_2 in *Paeonia lactiflora* were reported [141]. The amount of malondialdehyde and H_2O_2 increased in Fragaria treated with Se-Nps compared to Fragaria under salinity stress [119]. Moreover, in *Scrophularia kakudensis*, with an H_2O_2 increase, the activity of antioxidant enzymes increased in the high concentration of nano-ZnO treatment, and therefore ROS production decreased through the increase in CAT [114]. The activity of CAT and MSI (Membrane Stability Index) increased in *Trachyspermum ammi L.* which were sprayed with Fe_2O_3 Nps under non-saline conditions [142].

Oxidative stress increased in *Fagopyrum esculentum* (Buckwheat) because of CuO Nps treatment [81]. CAT could also prevent ROS production by converting H_2O_2 to O_2 in these plants [114]. The high concentration of nano-ZnO caused a natural defense system against ROS in these plants [114]. However, plants of

Fagopyrum esculentum, treated with ZnO Nps in hydroponic culture, decreased CAT activity and increased ROS production [113].

An increase in the activity of antioxidants was observed in *Caralluma tuberculata* exposed to Ag Nps [99]. ROS catabolism activities increased with Ag Nps in *Ricinus communis* [75]. In addition, ROS and the amount of phenol increased in these plants treated with Ag Nps which depended on the concentration of nanoparticles [97; 112].

An increase in catalase and superoxide dismutase activity were observed in *Salvinia natans* exposed to ZnO Nps [33]. Excess electrons in the terrestrial plant, *Clarkia unguiculata*, might cause ROS [120]. In these plants, the level of antioxidant defense enzymes increased after a high level of TiO_2 Nps for seven days [133].

New researches

The use of nanoparticles on stressed plants helps to improve or worsen the effects of stress. The use of nanoparticles with antioxidant activities can eliminate ROS and protect plants from abiotic stress [4]. For instance, plants exposed to different types of nanoparticles (Nps) improved growth and reduced cd concentration in plants compared to controls [52].

Spraying CeO_2 Nps reduced oxidative damage in sorghum under drought stress due to a decrease in the level of superoxide anions (O^{2-}) and H_2O_2 radicals [4; 143]. Another example, under drought stress conditions, Sorghum plants sprayed with nanoceria showed a significant decrease in O^{2-}, H_2O_2, and malondialdehyde and increased activity of catalase, superoxide dismutase, and peroxidase compared to control [74]. Under salinity stress, nanoceria in germinated maize seeds increased the activity of antioxidant enzymes. Thus, the amount of ROS decreased [71]. Another species, Silicon (Si) Nps reduced significantly the oxidative stress caused by salinity in rice plants by reducing electrolyte leakage as well as malondialdehyde and H_2O_2 contents [144].

Soybean seedlings treated with Silicon dioxide nanoparticles (SiO_2 Nps) and salinity stress had a significant reduction in H_2O_2 and O_2. As a result, the combination of SiO_2 and TiO_2 nanoparticles showed an increase in superoxide dismutase and catalase in these plants [92; 145]. Catalase, peroxidase, and ascorbate peroxidase activities increased significantly in *Faba bean* plants when exposed to Si Nps and salinity stress [144]. In addition, the application of Cu Nps under salinity stress increased the activity of antioxidant enzymes in tomatoes, and these nanoparticles were able to control pathogens in these plants [42].

Foliar application of *Rosmarinus officinalis* with Fe Nps and Zn Nps reduced the toxicity caused by salinity stress and reduced the amount of H_2O_2 and malondialdehyde in these plants [130]. Maximum superoxide dismutase and catalase activities were observed in *Trachyspermum ammi L.*, which were nano-treated under salinity stress [142]. Another example, *Vigna radiata* plants produced more ROS when exposed to arsenic (As), but by adding green Ti Nps to these plants, their antioxidant defense system was strengthened and ROS was reduced [103].

The use of Si Nps for pumpkin seedlings caused the seedlings to maintain stable levels of O_2 and H_2O_2 by increasing the activity of antioxidant enzymes and this situation occurred while the seedlings of these plants were under salinity stress [144]. When *Dracocephalum moldavica* was under salinity stress, 200 mg/L of TiO_2 Nps decreased H_2O_2 and increased the activity of antioxidant enzymes [35]. In addition, flavonoids increased the plant's tolerance to salinity and also prevented oxidative stress. Foliar application of Fe Nps could increase the amount of flavonoids in these plants [44].

The combination of fluoride (F) solution with Ag Nps in *Cajanus cajan* reduced the amount of ROS by increasing the activity of antioxidant enzymes [88]. Ag Nps compensated for the negative effect of fluoride [88]. Another example, spraying $FeSO_4$ (ferrous sulfate) Nps on *Helianthus annuus* increased CAT activity, regardless of salinity treatment [145]. Cadmium treatment increased the toxic effects on *Vigna unguiculata* roots, but with using nanoparticles, this amount was significantly reduced [138]. Application of ferrous oxide (FeO) Nps in *Helianthus annuus* plant under Cr stress-activated protective mechanisms and increased root and branch growth, photosynthesis, and pigments and reduced chromium-induced oxidative stress [146].

In other examples, Fullerol (Fullerene carbon nanotube) both produced and destroyed ROS, which can affect the tolerance of abiotic stress, particularly drought stress [147]. For example, the application of Fullerol in *Brassica napus* had a positive effect on the activity of GR and ascorbate peroxidase in drought stress and their activity increased. The same conditions reduced the concentration of H_2O_2 [147]. In wheat plants treated with UV-B, the addition of Si Nps reduced the H_2O_2 increase in the leaves of the plants [148].

Conclusion and future perspective

This review illustrated the effects of NPs on ROS production and oxidative stress. ROS can disrupt plant function by stealing cells' electrons to stabilize themselves. The lack of electrons in cells will disrupt cellular functions. Nanoparticles can also cause ROS production in the plants, but its level differ from NPs to NPs, plant species to plant species. An increase in ROS should be measured, because it would affect every single part of the plant, and most of the time, negatively. However, ROS production can sometimes improve antioxidant defense systems to decrease oxidative stress. This action would help the plants to protect themselves from probable damages caused by ROS production. Recently researchers have used NPs in stressed plants. NPs could decrease ROS produced by other stresses such as salinity and drought. NPs could increase antioxidants, which can eliminate ROS produced by other stresses. If these NPs are used to decrease the negative effects of other stresses, most of the problems will be solved. Further research is required to investigate the effects of nanoparticles on ROS in medicinal plants because few studies have been done on these plants. Furthermore, the effects of nanoparticles on plant performance must be considered in future research.

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