



Nanotechnology: A double-edged sword for future smart agriculture and phytopathological management in plants

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Abstract

Modern technology that would boost agricultural outputs might therefore preserve the country's sustainable living standards by enhancing food security. With nanotechnology, it's possible to produce foods of exceptional quality that also could increase the bioavailability of nutrients during the third decade of the twenty-first century which found usage in a variety of industries such as medical science, pharmaceuticals, food, and energy conservation. Nanotechnology is the synthesis, designing, characterizing, and utilization of assemblies, tools, and systems via directing the morphology and size variation at nanometer level (1 - 100 nm). Nanochemicals, nanopesticides, and nanofertilizers do increase yielding capacity without damaging agricultural land or irrigation water. Nanomaterials (NMs) are aimed protect crops from pests, microbial and fungal pathogens thereby lowering nutrient losses. Nanotechnology holds the potential to monitor soil quality in agricultural fields and sense crop health. Metallic Nanoparticles (Cu, Zn, Ni, Zn Fe, Ag, Al, Ti, and Al) can impinge on plant development, metabolism, and stress tolerance. This paper examines the role that nanoparticles (NPs) be playing while regulating oxidative stress, ROS turnover to mitigate abiotic stress in plants thereby emphasizing the advantages and of nanotechnology for better sustenance of future agriculture practices.

Keywords: Agriculture, Food security, Nano chemicals, Nanotoxicology and nano sensors, Oxidative burst, and ROS.

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Contribution of this paper to the literature

When applied to plant tissues, nanoparticles can be engineered to enhance resistance to pathogens by inducing the creation of defensive chemicals, which in turn stimulates a plant's inherent defense mechanisms. Before being widely used, nanoparticles' potential harm to non-target creatures, such as helpful bacteria and pollinators, needs to be carefully assessed.

1. Introduction

Nanotechnology is the "synthesis, designing, characterizing, and utilizing of assemblies, tools, and systems" [1] through tailored morphology and size variation on the nanoscale from 1 to 100 nm. The one nanometre is defined as one billionth (10^{-9}) of a metre which is a hint that technology can be used at that size scale. Nanoscience and nanotechnologies are deemed novel strategies in developmental study relating to the discovery of miracles and the action of substances on the atomic, molecular, or macromolecular scales, at which their capabilities differ considerably from those at the bulk stage. In this case, the physical, chemical, and biological properties of final materials and the bulk material are very different. Changes to properties resulting from working at the nanoscale lead to the introduction of novel materials with interesting architectures, advanced tools and additional beneficial products. Increase use of "nanoscience" "nanotechnology" by scientists to create nanomaterials (NMs) with a plethora of physical, chemical and biological strategies has contributed to the discipline of "nanoscience" and "nanotechnology" as per Zhao, et al. [2]. All of these techniques have a few drawbacks regarding isolation and purification of nanoparticles from similarity micro emulsions and the high use of surfactants. One kind of green technology that is useful is, simple to implement, fast, environmentally friendly, and time-efficient is the development of nanoparticles (NPs) from plant cell extraction. Figure 1, is showing various ways that eco-friendly formulated NPs that can enhance sustainability and the possibilities for agriculture areas, such as improved fertilization, less toxic environmental contaminants, as improved PGRs, and improved pesticides. Among other aspects of daily life, nanomaterials (NMs) are extensively utilized in industry, medicine, pharmaceuticals, and crop protection for sustainable agriculture. Oxidative stress is one type of situation where the excessive creation of reactive oxygen species (ROS) affects the cell's redox balance. NMs can cause oxidative stress, but they can also ameliorate it, which can lead to phytotoxicity [3]. Plant cells often accumulate ROS through a variety of mechanisms, including environmental stress [4] and stress from heavy metal build-ups [5-7]. The most frequently reported cases have been biotic stress, salinity stress [8] and physiochemical-cum-allopathy-induced stress [9]. Oxidative stress produces reactive oxygen species (ROS), which harm biological materials by altering their structural and functional characteristics and inducing genotoxicity [10] coupled with altered plant metabolism and ultimately triggers cellular death via both short-range and long-range apoptotic signaling cascades [11-13]. (This whole paragraph is rewritten as per instructions)

Plants frequently produce heat shock proteins (HSPs), which function as chaperones and are crucial in granting biotic and abiotic stress resistance, in order to combat different levels of biotic and abiotic stress cum signaling cascades both in cellular and organelles microcosm. Additionally, by favorably influencing the antioxidant enzyme systems in plants, HSPs usually decrease the levels of reactive oxygen species (ROS) and increase membrane stability. In addition, it employs ROS as a signal to molecules to stimulate HSP production. HSP also improves plant immunity by accumulating and stabilizing pathogenesis-related (PR) proteins under a variety of biotic stressors [14]. Therefore, the purpose behind this study is to provide light on how NMs, such as nanopesticides, nanochemicals and nanofertilizers, are used in agricultural soil to promote the safe growth and development of both cash and food crops. This chapter also discusses how NMs control oxidative stress in plants and how plants use antioxidants as defense mechanisms [15] to rid of themselves from reactive oxygen species.

2. The World of Nano-Agrochemicals

2.1. Nanopesticides

Because they keep pests (including rodents, ants, mice, cockroaches, aphids, crickets, and caterpillars) under control and preserve crop growth and development, pesticides are essential to agriculture. The term "nanopesticide" describes pesticides that are applied as agrochemical ingredients (AcIs) that have two or three dimensions and a nanostructure that ranges from 1 to 200 nm. The technique of enclosing active molecules in a nanoscale protective shell is known as nanoencapsulation (Figure 1). Effective pest management uses nanoencapsulated pesticides, although residue accumulation must be avoided. Various forms of copper oxide-based nanomaterials (CuONPs), zinc oxide-based nanopesticides (ZnONPs), magnesium hydroxide-based nanopesticides (MgOHONPs), and magnesium oxide-based nanopesticides (MgONPs) were developed to control pathogenic and

pest attack. Manufacturers of pesticides are increasingly using encapsulated nanopesticides. Light, humidity, and temperature are examples of environmental factors that can cause them to release the active components in a regulated way [16].

2.2. Nanofungicides

The primary reason for the reduction in crop yields and profits is fungal infections. Therefore, it is essential to concentrate on creating fungicides that have an inhibitory activity in order to control fungal diseases that are lethal for food crops and to essentially protect and extend the shelf life of harvested commodities. Currently, nanofungicides are highly sought after due to their superior solubility, permeability, low dose requirement, and low phytotoxicity. These substances can be used to treat plant diseases and are both safe and environmentally friendly. NPs increase a plant's resilience to illness by activating its defenses or rendering microbes incapable of surviving. The antifungal properties of nanoparticles can help in the development of pesticides based on them [17]. Due to its many advantages over other nanoparticles, such as Cu, Zn, Au, ZnO, Al₂O₃, and TiO₂, numerous researchers have thoroughly studied the manufacturing of silver nanoparticles. Nanofungicides that are representative of the environment are favored over non-target areas or organisms that could potentially reduce soil fertility and sustainability either directly or indirectly.

2.3. Nano-insecticides

On agricultural land, chemicals called insecticides are being sprayed to either kill insects or prevent them from acting destructively toward crop plants. However, many chemicals are extremely harmful and toxic to biological sources as well as aquatic and terrestrial ecosystems. Modern nanoscience research is now creating nanotechnology-based botanical insecticides with active components made from essential oils or plant extracts [18]. For instance, organic extracts from different plant materials like neem leaf (*Azadirachta indica* A. Juss), leaf decoction of *Citrus limon* [(L.) Osbeck], extracts of acacia gum (*Acacia Senegal* (L.) Wild and peels powder of pomegranate (*Punica granatum* L.) were used in the green synthesis of MgOH [19, 20]. Experiment demonstrates that, in addition to their pesticidal activities [21] these botanical nanopesticides have a significant impact on plant growth and development that would boost high-yield organic farming during field trials [22].

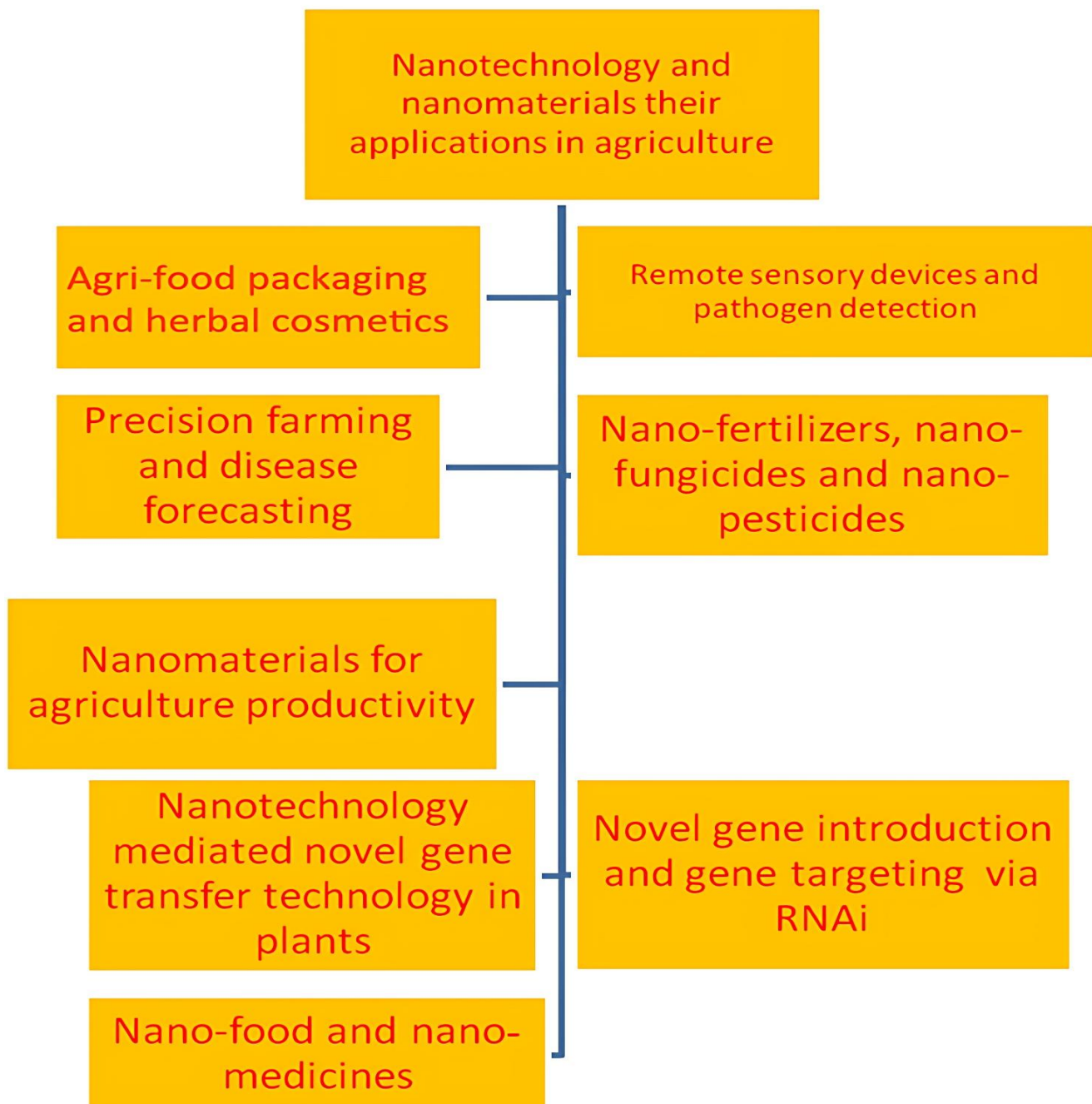


Figure 1. Schematic flow chart of different classes of nanomaterials (NMs) shows wider applications for sustainable agriculture.

3. Synthesis of Nanoparticles

Using a variety of processes, including chemical, biological and physical, nanoparticles can be manufactured which have been aimed to be environmentally safe, biodegradable but reproducible in activities, with lower toxicity, higher efficacy and upholding strong antimicrobial, antifungal, antibacterial and antiviral characteristics. These qualities vary depending on the techniques of production (Figure 1). The environmentally friendly methods (Green synthesis) of producing nanoparticles from plant extracts and with active molecules from biological microorganisms [23] had been a great initiative to its truest sense. These methods are pollutant-free, environmentally beneficial and eventually would produce to little toxic waste [24]. Recently, TiO₂ NPs were produced utilizing *Carica papaya* [25]. Microorganisms such as bacterial and fungal cells are referred to as "bio-reactors" in the context of nanoparticle manufacturing [26].

Figure 2 Illustrates the synthesis of different various formulations used for types of different Nano-particles for commercial uses reported till date.

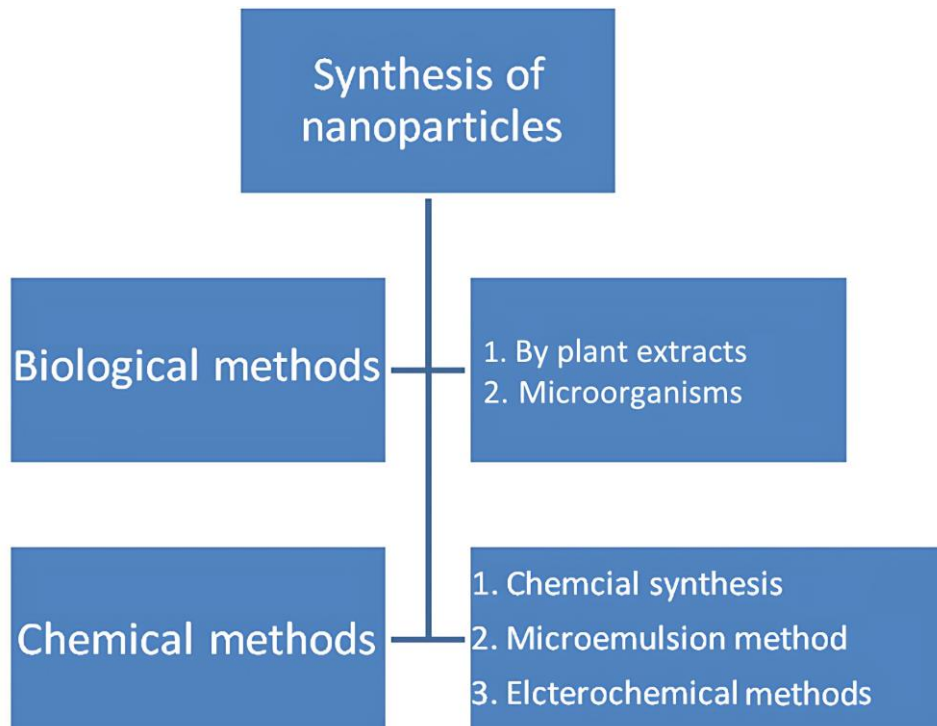


Figure 2. Synthesis of different types of different Nano-particles reported till date.

3.1. Biological Methods

3.1.1. By Plant Extracts

Recently, many ways to synthesizing nanoparticles from plant extracts have been explored. Plant-derived nanoparticles are becoming increasingly important in biological applications. Copper nanoparticles (Cu NPs) can be biosynthesized from *Magnolia sp*, *Syzygium aromaticum* and *Zinziber officinale* plant extracts [27]. *Azadirachta indica* and *Citrus lemon* were employed to synthesize gold-based nanoparticles (AuNPs) and silver-based nanoparticles (AgNPs) [28].

Table 1 Illustrates the Biosynthesized Nanoparticles that are currently being in us employing different plant extracts.

Table 1. Biosynthesized Nanoparticles by using plant extract.

Bio-Synthesized nanoparticles	Plant extract	Plant extract	References
CuO NPs	<i>Magnolia sp</i> , <i>Syzygium aromaticum</i> and <i>Zinziber officinale</i>	Antibacterial	Yaqub, et al. [29] and Yashwant, et al. [30]
Au NPs	<i>Eclipta alba</i> , <i>Nepenthes khasiana</i>	Antibacterial	Zhang, et al. [31] and Bhau, et al. [32]
AgNO ₃ NPs	<i>Azadirachta indica</i> , <i>Musa acuminata peel</i>	Antibacterial	Kumari, et al. [33] and Maruthai, et al. [34]
TiO ₂ NPs	<i>Carica papaya</i>	Antifungal	Saka, et al. [25]
Fe ₂ O ₃ NPs	<i>Mentha spicata</i>	Antibacterial, cytotoxic and anticancerous	Umar, et al. [35]

3.1.2. By Microorganisms

Verticillium sp., *Phomas p.*, *Fusarium oxysporum*, *Phaenerochaetechrysosporium* and *Aspergillus flavus* are some instances of fungi that are used to make silver nanoparticles. (AgNO₃-NPs) [36]. However, some microorganisms, such as *Clostridium versicolor* and *Bacillus subtilis*, are additionally employed in the synthesis of silver nanoparticles (AgNO₃-NPs) [37]. Plant viral capsids are also employed as bio-templates for nanoparticle synthesis, such as Tobacco Mosaic Virus (TMV), which is used to biosynthesize Ag and Ni NPs.

3.2. Chemical Methods

There are several commercial chemical methods for synthesis of NPs that incorporated chemical reduction, microemulsion and electrochemical approaches [38]. Michael Faraday initially discovered the chemical reduction process in 1857. This approach is excellent for producing nanosized copper nanoparticles (Cu NPs). On the other

hand, the electrochemical approach is utilized to create metal nanoparticles. It is accomplished by sending an electric current between electrodes.

4. Employment of Nanoparticles Designed for Plant Disease Management

4.1. Cytoprotectant Nanoparticles

Applied directly to seeds, leaves, or roots, nanoparticles, which have a size range of 10 to 100 nm, have special properties that shield plants against pests and diseases. An extensive investigation has been conducted into the antibacterial, antifungal, and antiviral properties of metal nanoparticles, employing copper, silver, zinc oxide, and titanium dioxide. Silver nanoparticles, particularly those produced using "green synthesis," have shown significant antifungal effectiveness against a range of fungal-infections and decreased the risk of viral infection. Silver-based nanoparticles provide challenges in terms of manufacturing, toxic interactions against soil-borne, growth-promoting rhizospheric flora. Other routinely used metal nanoparticles, such as copper and titanium dioxide, are being investigated for their antibacterial properties. Furthermore, chitosan nanoparticles have good biological characteristics and have rendered viral resistance and antibacterial activity against a variety of diseases. Chitosan has the ability to function as both solitary nanoparticles and nanocarriers for increased delivery [39].

4.2. Anti-Fungal and Fungal-Like Pathogen Killer Nanoparticles

Nanotechnology provides promising options for treating fungal diseases in plants through a variety of ways, including detection, nutrition augmentation, nano-fungicide transporters and direct impacts of nanoparticles (NPs) on fungal pathogens. Nanoscale nutrients, such as copper, when coupled with other elements, have been proven to increase plant nutrient uptake and immune responses to fungal diseases. Standard copper-based fungicides (such as copper oxide, copper sulphate, copper oxychloride, dicopper chloride trihydrate, cuprous oxide, copper octanoate, copper sulfide, and copper hydroxide) have been replaced by copper-based nanomaterials, displaying superior efficacy in reducing fungal symptoms and boosting crop output [40]. Furthermore, nanomaterials mixed with organic chemicals, such as chitosan-coated iron oxide NPs, have demonstrated efficacy in suppressing post-harvest fungal infections on fruits and vegetables [41]. Furthermore, nanomaterials mixed with organic chemicals, such as chitosan-coated iron oxide NPs, have demonstrated efficacy in suppressing post-harvest fungal infections on fruits and vegetables [41]. Nanoencapsulation of fungicides in particles such as mesoporous organosilica has demonstrated enhanced efficiency and stability, resulting in considerable decreases in fungal lesion sizes when compared to established fungicides [42]. These nanotechnology-based techniques provide a secure and more effectual method of addressing fungal diseases in agriculture.

5. Plant Disease Control and Management: Applications of Nanomaterials

Pesticides and herbicides of various varieties have long been used to control disease. The application of nanoparticles in the management of plant diseases has shown to be highly advantageous in the long run. Nanotechnology offers several methods of controlling plant disease. Nanotechnology has made significant advances in plant disease management. In the future, it might be used to diagnose diseases caused by bacteria, viruses, fungi, insect pests, pathogenic nematodes and so on. It might well be utilized as a diagnostic tool for diseases caused by bacteria, viruses, fungus and insects. These can be used as biosensors and nano-analytical instruments. Nanoparticles have the potential to offer protection against bacteria, viruses, insects and fungi. Different metallic NPs like, Ag, Cu, ZnO and TiO₂ exhibit strong antibacterial and antifungal activities [43]. Different types of nanoparticles (both metallic and green) are used in the management of disease in plants. All things considered, nanoparticles with a variety of modes of action and applications for various pathogens and crops provide potential answers for managing plant diseases brought on by bacteria, fungus, and viruses.

6. Bio-Efficacy of Metallic NPs against Plant Pathogens

6.1. Zn NPs (Zinc Nanoparticles)

Because of their low toxicity, zinc nanoparticles (Zn NPs) can be used to treat a wide range of illnesses. It possesses viricidal and bactericidal effects. Numerous pathogenic fungus, such as *Mucor plumbeus*, *Botrytis cinerea*, and *Penicillium expansum*, have been demonstrated to be effectively combatted by zinc nanoparticles (Zn NPs) [44]. The antifungal and antibacterial properties of zinc oxide nanoparticles (ZnO NPs) against pathogens including *Pseudomonas aeruginosa* and *Botrytis cinerea* have piqued curiosity. Their simple synthesis and photocatalytic actions on plant pathogenic fungi make them useful for disease management and detection [45]. Significant antibacterial actions against bacteria, fungus, and spores have been demonstrated by zinc oxide nanoparticles that makes them useful in the treatment of disease. Pathogen growth is finally inhibited by these nanoparticles, which also might bring forth oxidative stress, damage pathogen cell structure, and prevent DNA replication. Thanks to their exceptional catalytic, optical, and physical properties, zinc (Zn) nanoparticles—in particular, ZnO NPs—are widely employed in a variety of industries, including food, medicines, chemicals, and agriculture. *Sclerotinia sclerotiorum*, *Penicillium expansum* and *Staphylococcus aureus* are some examples of bacterial and fungal pathogens which are being inhibited to grow vegetatively. They also display concentration-dependent micronutrient releasing characteristics [45].

6.2. AgNPs: (Silver Nanoparticles)

Silver NPs are the most effective ones. It exhibits antibacterial, antiviral, nematocidal and antifungal properties reported till date. Silver nanoparticles (Ag NPs) have been found to have viricidal activity, shielding the Faba bean plant from BYMV. Tomato Mosaic Virus (ToMV) and Potato Mosaic Virus (PMV) resistance had been reported to be effectively provided out of foliar spraying of silver nanoparticles (Ag NPs). Additionally, it had been proved that AgNPs have a viricidal effect on the Banana Bunchy Top Virus (BBTV), as seen by the decreased viral infection in banana plants treated with AgNPs. It has the ability to inhibit the growth of *E. coli*, *P. aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. When applied to *Staphylococcus aureus* it had exhibited strong antibacterial and

antimicrobial properties [46]. According to Yakout and Mostafa [47] poisonous bacteria like *Xanthomonas axonopodis* and *Phytoplasma aurantifolia* can be identified with the use of silver nanoparticles (AgNPs), which effectively would change color to brown rapidly from yellow. Silver nanoparticles are effective in treating illnesses like Sun Hemp Rosette Virus (SHRV) in *Cyamopsis tetragonoloba* and late blight in tomatoes because they have shown efficiency against a variety of pathogens, encompassing different genera of viruses and fungi. The unique structure, size, and electrical/optical activity of silver nanoparticles (Ag NPs) make them interesting antibacterial and antifungal agents. They affect membrane integrity, cause cellular content leakage and do prevent pathogens from producing ATP and adhering to cell membranes. Furthermore, they oxidize lipids and proteins, cause cellular and ROS-mediated cytotoxicity, altering phosphotyrosine levels that would harm mitochondria [48]. Ag NPs have been shown to effectively suppress the growth of numerous bacterial and fungal infections, including dangerous species like *Bipolaris sorokiniana* and *Magnaporthe grisea*. Additionally, they reduce the titers of tobacco mosaic virus (TMV) and bean yellow mosaic virus (BYMV) in afflicted plants. Strong antibacterial action was demonstrated by silver nanoparticles (Ag-NPs) made from plants such as *Azadirachta indica* (neem, Meliaceae) and *Calligonum comosum* (arta, fire bush, Polygonaceae) [49] against plant bacterial and fungal diseases. Ag-NPs also shown antifungal action against different crop diseases and nematicidal activity against root-knot nematodes, indicating their potential for surface sterilizing of diseased seeds without compromising seed germination. On perennial ryegrass, silver nanoparticles effectively decreased leaf spot and gray leaf spot without causing any phytotoxicity and greatly decreased their antifungal activity. AgNPs would successfully produce relief following conidial inoculation, reducing diseases that differed significantly between three hours before and twenty-four hours after the treatments. When compared to the water control, the majority of silver formulations administered to plants three hours prior to spore inoculation considerably reduced both phytotoxicity and pathological symptoms. The most successful pre-inoculations were those with AgNO₃ (25 and 50 ppm), Ag(p) (200 ppm), and Ag(e) (50 ppm), which allowed for less than 7% foliar damage by *B. sorokiniana* and *M. grisea* in extremely disease-conducive environmental circumstances, whereas control plants treated with water suffered more than 70% damage. On the other hand, more than 50% of the foliar damage was caused by the inability of delayed treatments of silver preparations 24 hours after spore inoculation to effectively reduce both diseases. Silver ions were thought to be very effective after prolonged release because they inhibit microbial respiration and metabolism in addition to causing physical injury inside the cellular microcosm [46].

6.3. FeNPs (Iron Nanoparticles)

Iron nanoparticles (Fe₂O₃ NPs) are less harmful and therefore suitable for everyday use. It is highly reactive and has antiviral properties against TMV. Khan, et al. [50] revealed that Fe₂O₃ NPs have the capacity to limit the proliferation of *Phytophthora infestations*. Iron oxide (Fe₂O₃) NPs and magnesium oxide (MgO) NPs also demonstrate antibacterial efficacy against various pathogens, with smaller NPs showing higher penetration and effectiveness.

6.4. NiNPs (Nickel Nanoparticles)

Cucumber plants treated with Ni NPs exhibited antiviral action, as well as an increase in leaf count and dry weight. When treating *Staphylococcus aureus* (exhibiting Methicillin-resistance) infections, it exhibited antibacterial efficacy. Ni NPs had been made from an organic extract of the *Ocimum sanctum* plant and exhibit antibacterial effectiveness against *Bacillus subtilis* and *E. coli* [51].

6.5. TiONPs (Titanium Nanoparticles)

It possesses the ability to oxidize biomolecules, which contributes to its significant antiviral activity. It has been found that treating *Vicia faba* L. with TiO₂ NPs reduces viral infection caused by Broad Bean Stain Virus (BBSV) [52]. Strong antibacterial activity against bacterial phytopathogens and viruses, such as Turnip Mosaic Virus (TuMV), had been demonstrated by titanium dioxide (TiO₂) nanoparticles (NPs) produced using sustainable methods (TuMV) [53].

6.6. Gold Nanoparticles

Because of their physiochemical characteristics, they have high antibacterial actions. Thus, AuNPs have been employed as biosensor components to diagnose various types and intensity of different plant disease. Using the Surface Plasmon Resonance (SPR) method, Au NPs play a crucial role in identifying the pathogen causing Wheat Kernel Bunt disease, as well as late blight of potato and tomato caused by *Phytophthora infestation* using an Au NP-based Lateral Flow Strip Biosensor [54]. Au nanoparticles were utilized as the detecting tags for phytopathologists during field trials of cash crops. Gold nanoparticles (AuNPs) have been widely used in numerous detection technologies, including strip-based DNA sensors for bacterial and viral infections. They also provide extremely immunospecific sensors for specific infections such as *Leptosphaeria maculans* and *Pseudocercospora fijiensis* [54]. AuNPs enhance the detection of viruses utilizing DNA hybridization assays and Label-Free Colorimetric Biosensing Techniques, such as Cucumber Green Mottle Mosaic Virus (CGMMV) [55]. Gold nanoparticles exhibit potent antifungal properties against pathogens like *Aspergillus flavus* and *Aspergillus niger* and they have been utilized in combating wheat stem rust and other fungal diseases.

6.7. CuNPs (Copper Nanoparticles)

It has excellent potential for plant disease control with strong wide spectrum antibacterial actions. Fungicides made from Cu NPs had been having the capability to avert the growth of *Phytophthora infestations*. Cu NP-based Bordeaux mixtures lowered the *Xiphinema* index. According to reports, Cu NPs have had antibacterial activity against *Xanthomonas compestriv. vesicatoria* in tomatoes [56]. *Phomopsis sclerotoides*, the causal source of cucumber root rot disease, was inhibited most effectively by CuO NPs [57]. Copper nanoparticles (CuNPs) are used in nano-biosensors to quantify salicylic acid, which is a molecular marker of biotic stress for several plant diseases such as mungbean leaf spot and bacterial blight disease [58]. Owing to the antibacterial characteristics of copper

nanoparticles, the development of bacteria like *Escherichia coli* and *Staphylococcus aureus* as well as fungus like *Phytophthora infestans* had been effectively inhibited. Copper nanoparticles (Cu NPs) exhibit antifungal capabilities, especially when combined with nanochitosan. Copper oxide (CuO) NPs were mainly explored for managing plant bacteria demonstrating high bactericidal activities against pathogens like *Escherichia coli* and *Staphylococcus aureus* (MRSA, methicillin-resistant) [59].

Figure 3 Describes the different Classes of synthesized nanoparticles using metallic and non-metallic inorganic components.

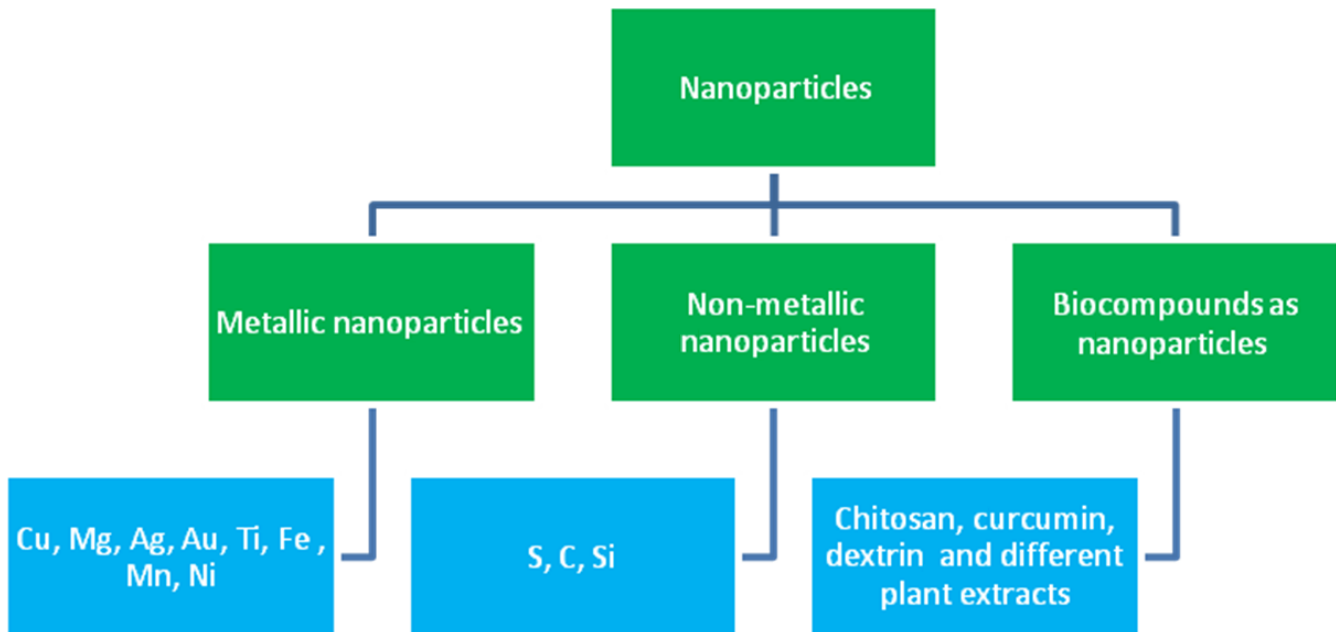


Figure 3. Classes of synthesized nanoparticles.

6.8. MgNPs (Magnesium Nanoparticles)

When it comes to broad-range both Gram-positive and Gram-negative bactericidal activities, MgNPs possess potent antibacterial activities. It has been proven that MgO NPs have antibacterial properties against *Ralstonia solanacearum*. MgO NPs might have strong antibacterial properties against *Ralstonia solanacearum*-induced tomato bacterial wilt, according to recent studies [60].

6.9. Se and SiNPs (Selenium and Silicon Nanoparticles)

Both silicon nanoparticles (Si NPs) and selenium nanoparticles (Se NPs) had exhibited strong antibacterial action against a wide range microbial disease [61]. Growth and Infections Plant viruses such as the Papaya Ringspot Virus (PRV) and the Tomato Yellow Leaf Curl Virus (TYLCV) had been experimentally inhibited by silicon oxide (SiO₂) nanoparticles (PRSV) [62].

6.10. Pt and Pd NPs (Platinum and Palladium Nanoparticles)

According to Chlumsky, et al. [63] platinum nanoparticles (Pt NPs) and palladium nanoparticles (Pd NPs) could augur potent antibacterial activities against a variety of food-borne pathogen strains as well as bacteria including *Escherichia coli* and *Streptococcus mutans*.

6.11. Al₂O₃ NPs (Aluminum oxide Nanoparticles)

Aluminum oxide (Al₂O₃) NPs had shown promises in controlling fungal pathogens by stimulating electrostatic attraction with cell membranes, increasing ROS production and damaging cellular structures [64].

6.12. CeO₂ NPs (Cerium oxide Nanoparticles)

Cerium oxide (CeO₂) NPs exhibit antibacterial properties and have the potential to regulate phytopathogenic activity [65].

6.13. Ni NPs (Nickel Nanoparticles)

The antibacterial efficacy of nickel nanoparticles (Ni NPs) had been comparable against a range of diseases, including viruses [66].

6.14. Chitosan Nanoparticles

Chitosan nanoparticles had shown effectiveness in impeding the spread of viruses within plants and boosting the host's immune response. They could strongly enhance fruit resistance and modulate various physiological processes in plants, thereby aiding in disease management [67].

Overall, nanoparticles offer diverse mechanisms for combating plant pathogens, including induction of systemic resistance, inhibition of DNA replication, disruption of cell structure and modulation of cellular signalling pathways. Their antibacterial qualities make them useful instruments in the creation of successful plant disease management plans. However, the use of cadmium nanoparticles (Cd NPs) is limited due to their toxicity to humans, animals and plants.

7. Nanoparticles as “Strategic Weapons” against Plant Pathogens - the Mechanism of Action

Nanotechnology presents various promising avenues for managing plant diseases, primarily through the direct application of nanoparticles to foliage, seeds, or soil. Nanotechnology is transforming plant disease detection by allowing improved tools to identify pathogens quickly and accurately. Nanoparticles, with their small size and distinct features, improve biosensor selectivity, sensitivity and detection limits. When it comes to pathogen detection, metal nanoparticles—such as those made of gold, silver, copper, zinc oxide, and other metals—augur distinct benefits over enzyme-related testing systems. These advantages include their bigger surface area-to-volume ratios and simpler physical sensor-mediated detection processes [68].

8. Cellular Uptake and Mechanism of Nano-Toxicity

Engineered nanoparticles (ENPs) are absorbed by cells through endocytosis, non-endocytosis, and passive transport [69]. Small, positively charged and hydrophobic ENPs are primarily transported *via* diffusion, whereas perisorption can occur *via* intestinal microvilli or enterocyte cells. Endocytosis routes, including as receptor-mediated endocytosis and caveolae/lipid raft-dependent endocytosis, transport ENPs to lysosomes, where they might induce intracellular toxicity due to metal ions. Transporter proteins or ion channels are used to absorb the ions created during ENP breakdown. ENPs can cause toxicity and disturb cytophysiology once they enter inside the cell by causing membrane lipid peroxidations [70] and DNA damage plus protein injury [8] and oxidative stress via ROS outbursts, followed by other mechanisms that halt the normal physiological states [71]. ENPs' form, size, and coating composition are examples of their physicochemical characteristics that affect their toxicity. Smaller ENPs may cause more toxicity and particle form also influences nanotoxicity. Positively charged ENPs may be more harmful and bioaccumulative due to their affinity for cellular membranes [72]. Dietary exposure to ENPs might cause cellular stress, affecting eating behavior and lipid peroxidation levels. A multitude of processes, such as direct cellular membrane destruction, disruption of ATP generation, and DNA replication, are the direct outcomes of nanomaterial toxicity [73]. When organisms consume free nano-ions, morphological changes occur, such as cytoplasm contraction and DNA condensation [74]. Nano-ions also interact with respiratory enzymes and transport proteins, resulting in proton leakage and affecting cellular activities [75]. Nanotoxicity causes oxidative stress by producing reactive oxygen species (ROS), which damage DNA and cause cell death. The chemical composition of nanomaterials impacts the amount of ROS produced. ROS predominantly target DNA, causing various forms of oxidative DNA damage [76]. However, the conversion of less reactive species into more reactive radicals (Figure 4) sharply worsens cellular damage. Ramos-Zúñiga, et al. [77] study the transition of less reactive species into more reactive radicals, particularly the hydroxyl radical, which can cause severe cellular damage Ramos-Zúñiga, et al. [77].

Figure 4 Illustrates the detailed processes during Nanoparticle toxicity which has a general mechanism. (1) Accumulation and dissolution of nanoparticles and entry inside the cell. (2) The concentration of nanoparticles on the cell surface causes depolarization and membrane rupture. (3) Nanoparticles' entry inside the cells causes sustained release metallic ions as a result of NP dissolution. The release of ions would produce the following effects: (4) ROS outburst, membrane depolarization via lipid peroxidation and protein oxidation; (5) Reduced ATP generation; (6) disruption of endomembrane systems and collapse of photosynthesis by chloroplast assembly (7) Mitochondrial membrane depolarization leading to apoptosis.

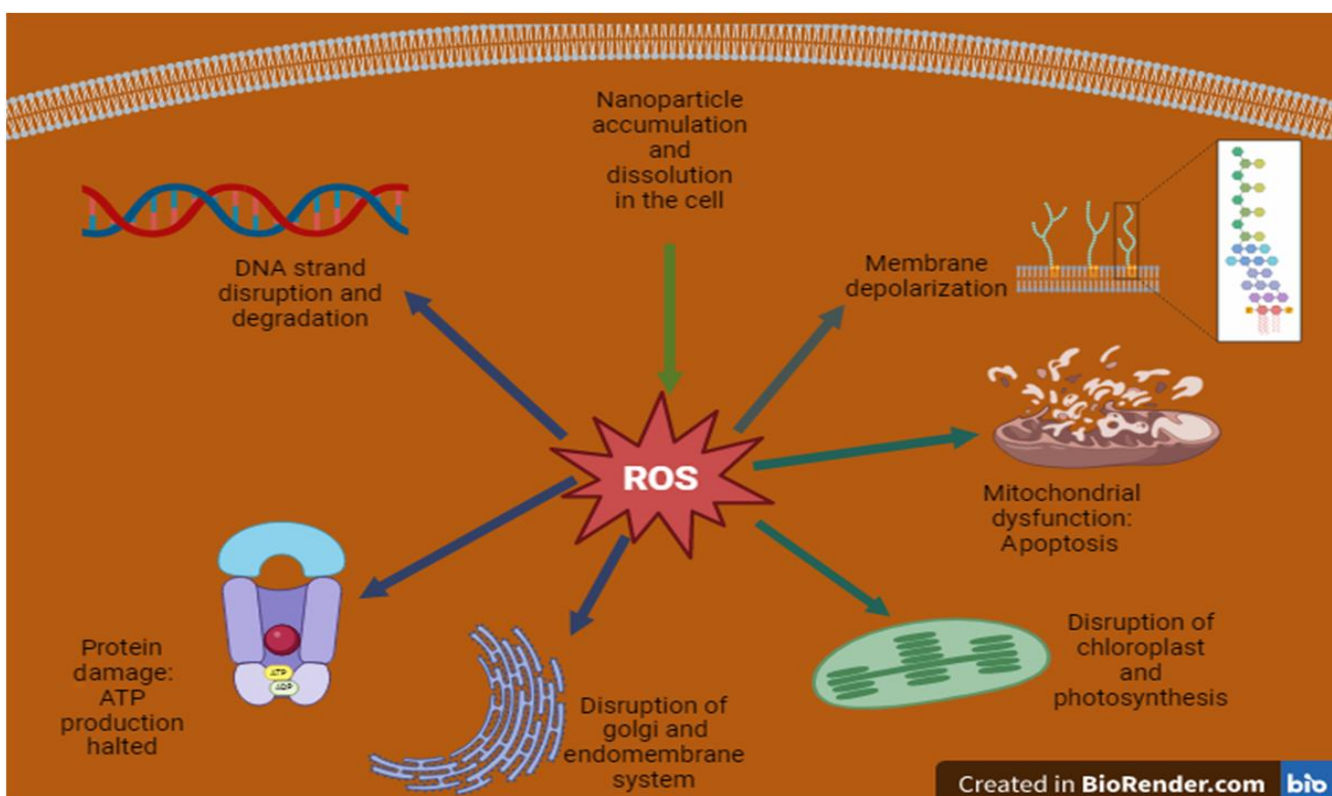


Figure 4. Nanoparticle toxicity has a general mechanism. (1) Accumulation and dissolution of nanoparticles and entry inside the cell. (2) The concentration of nanoparticles on the cell surface causes depolarization and membrane rupture. (3) Nanoparticles' entry inside the cells and sustained release metallic ions as a result of NP dissolution. The release of ions would produce the following effects: (4) ROS outburst, membrane depolarization via lipid peroxidation and protein oxidation; (5) Reduced ATP generation; (6) disruption of endomembrane systems and collapse of photosynthesis by chloroplast assembly (7) Mitochondrial membrane depolarization leading to apoptosis.

9. Cellular Toxicity via DNA Damage by Nanoparticles

The DNA damage within the microbial cells exposed to AgNP or ZnONP had been probed by electrophoretic analysis DNA strand breakage visualized within agarose gels [78]. Because they produce reactive oxygen species (ROS), zinc oxide nanoparticles (ZnO NPs) had been well known for their antibacterial properties. ZnO NPs caused concentration-dependent toxicity and membrane damage after being extensively absorbed into the bacterial cells. Studies on genotoxicity had shown that exposure to ZnO brought forth substantial DNA damage inside bacterial cells. Every observation showed that ZnO NPs caused substantial oxidation of proteins, DNA damage, and ROS over-production along with concurrent thiol group depletion. Moreover, ZnO NP exposure was found to downregulate a number of genes linked to the metabolic pathway and DNA repair, while simultaneously upregulating the expression of genes connected to the DNA damage responses [79]. CuI NPs generated ROS-mediated DNA damage for transcription suppression, as demonstrated by reporter gene assay, and they also caused ROS production in both gram positive and gram negative bacteria. In the presence of amine functional groups from diverse biological molecules, ROS most likely would have developed on the surface of CuI nanoparticles. Additionally, they cause harm to membranes [80]. According to Gordon, et al. [81] Ag ions would prevent DNA replication and deactivate SH-groups in functional proteins. Microorganisms' biological activities, including alterations in the structure and function of cell membranes, were known to be impacted by silver. Silver also would likely cause the inhibition of the expression of proteins involved in ATP generation [82]. It follows that oxidative stress, which is brought on by nanoparticles (NPs) might harm DNA in microorganisms by resulting in oxidized DNA bases, single-strand breaks (SSBs), and double-strand breaks (DSBs). Additionally, NPs can affect DNA repair processes through their effects on gene expression, deregulate DNA repair protein functioning, and the deplete nucleotide pool required for DNA repair systems.

10. Nanomaterials and Regulation of Oxidative Stress

Since the natural pool of endogenous antioxidants, which may detoxify free radicals in a normal condition, is outweighed by the creation of ROS, oxidative stress is a phenomenon that occurs when a cell's redox equilibrium is upset [13]. Reactive oxygen species (ROS), which are highly reactive free ions or radicals created from molecular oxygen during redox reactions, are able to exist independently and have one or more unpaired electrons in their outer orbit or valency shell. A free radical's odd number of electrons makes it incredibly reactive, short-lived, and unstable. They can steal electrons from other molecules to become stable since they are extremely reactive. The attacked molecule becomes a free radical after losing an electron, which sets off a chain of events that ultimately damage the living cell. The most prevalent ROS in plant biological systems are singlet oxygen ($^1\text{O}_2$), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxy radical ($\text{OH}\cdot$). (Figure 5). These substances' high reactivity and toxicity make them capable of causing oxidative cell death [83, 84]. The electron transport chain (ETC) in mitochondria, the Mechlner reaction in chloroplasts, and photorespiration (C2 cycle) in peroxisomes are the three subcellular compartments that produce multiple reactive intermediaries promulgating ROS outburst [14, 85]. Environmental stress and increased ROS accumulation are directly connected. When an organism produces too many reactive oxygen species (ROS), it damages its physiological capabilities and ultimately leads to cell death. External stimuli such as heat stress, ionizing and non-ionizing radiation, heavy metals, xenobiotic chemicals, UV radiation, and different phytopathogens can upset cellular homeostasis, which in turn can trigger the generation of reactive oxygen species (ROS) in plant cells [12].

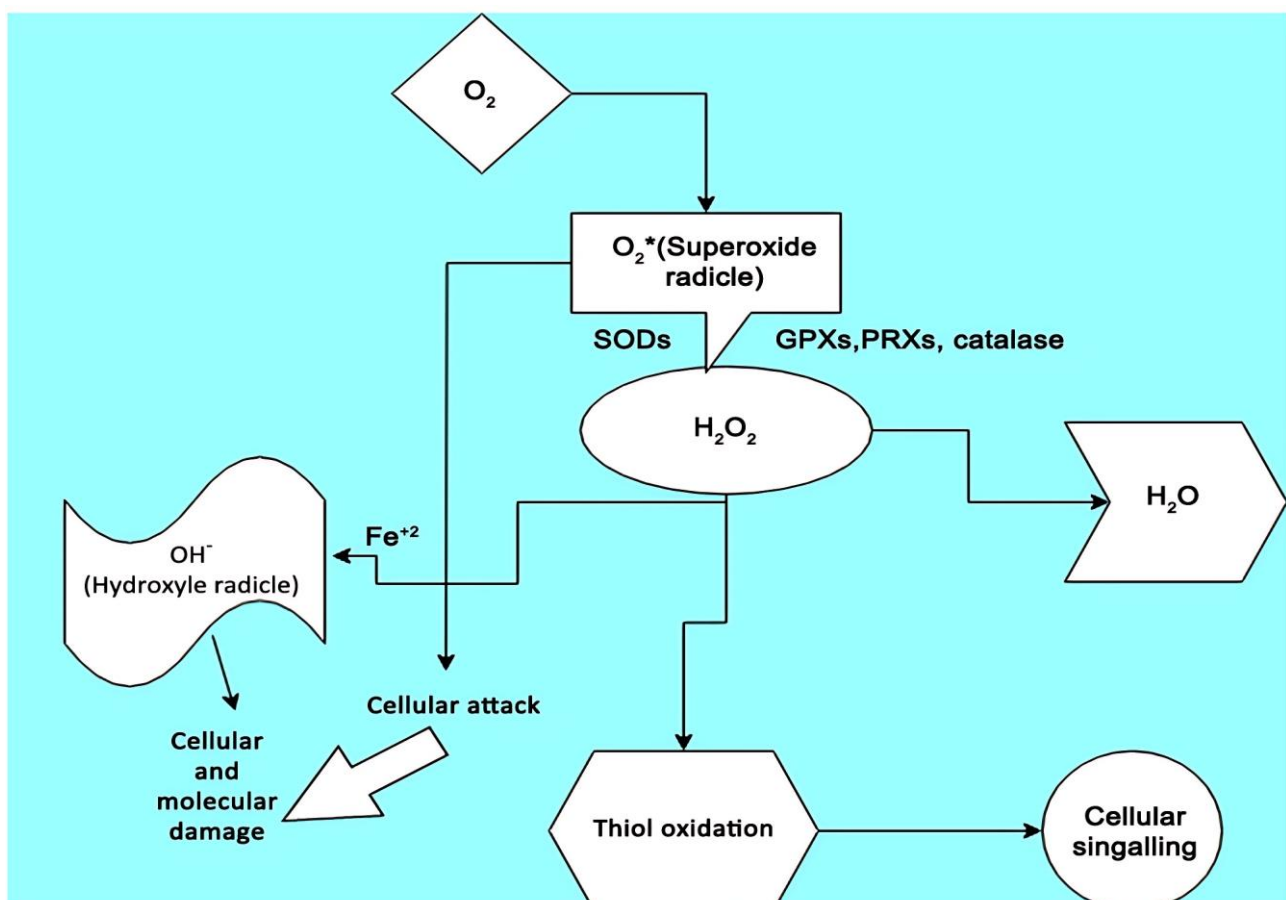


Figure 5. Schematic representation of ROS generation and their interconversions.

11. Production and Inter Switching of Reactive Oxygen Species in Microbial Cells

ROSs have an impact on living cells and tissues that including plants [12, 86] which are both positive and negative [86, 87]. ROS must be produced at a low to moderate level in the cellular compartments of aerobic organisms [13]. On contrary, elevated levels ROS out of abiotic and biotic stress can generate two distinct groups of reactions out of production of Reactive oxygen species (ROS: $\bullet\text{OH}^-$, $^1\text{O}_2$, O^-_2) and reactive nitrogen species, (RNS: $\bullet\text{NO}$, $\bullet\text{NO}_2$, ONOO^- , N_2O_3 , HNO_2 , NO^- , NO^+ , N_2O_4) (Figure 6) which can lead to: lipid peroxidation and protein carbonylation. Lipid peroxidation is a process that turns unsaturated lipids into saturated residues, such as hydroperoxide [70, 88]. When proteins are carbonylated, ROS can oxidize to the unsaturated nucleus of amino acids, including tyrosine, histidine, tryptophan, phenylalanine, etc. A downstream reaction produces a peroxide product in both cases, which can also progressively break down bimolecular fabrication of living cells. The surplus reactive oxygen species (ROS) generated within the cellular microcosm is scavenged by plants' efficient antioxidant defense mechanism under a variety of oxidative stress circumstances. The antioxidant safety net is composed of both enzymatic and non-enzymatic elements [89]. Flavonoids, tocopherol, carotenoid, polyamine, alkaloids, and phenolic compounds are examples of non-enzymatic components; glutathione reductase (GR), ascorbate peroxidase (APX), glutathione dismutase (SOD), and catalase (CAT) are examples of enzymatic components. Pizzino, et al. [87] and Rudenko, et al. [90] (Figure 6). This whole paragraph is rewritten as per instructions.

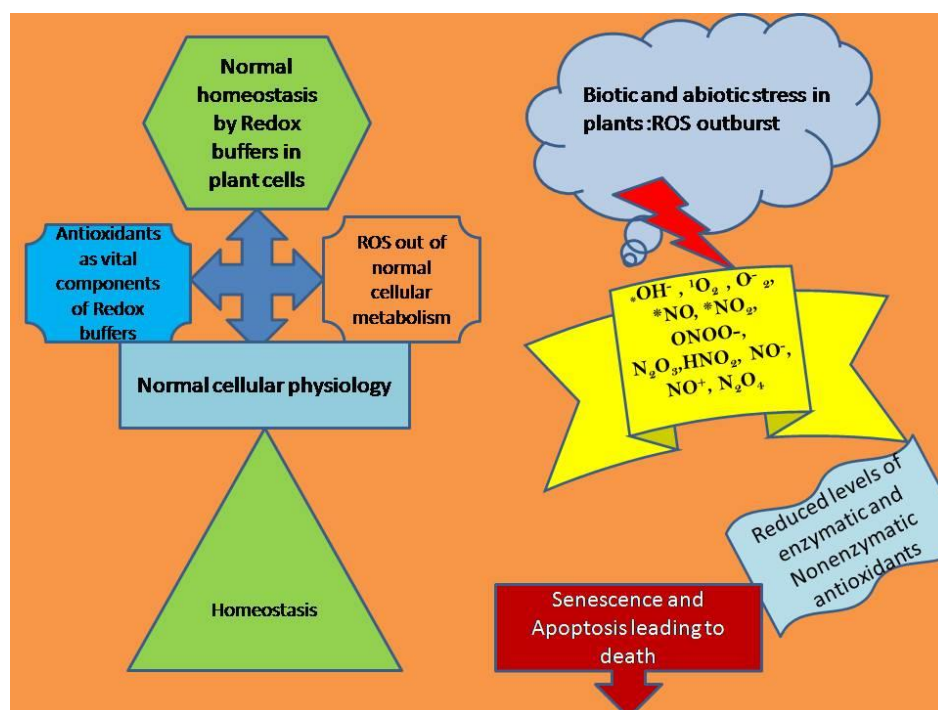


Figure 6. An imbalance between ROS and antioxidants brought on by abiotic stress causes an oxidative imbalance in live cells' cellular homeostasis.

The generation of reactive oxygen species (ROS), such as hydroxy radical ($\bullet\text{OH}$), is a key initiator of lipid peroxidation, which degrades lipoprotein and produces a cascade of end products including conjugated dienes and malondialdehyde (MDA) [70, 86, 88, 91].

12. Mechanisms of NP Uptake by Higher Plants

12.1. Passive Uptake of NPs

Understanding how nanoparticles (NPs) are absorbed by plants is critical for determining their interactions with plant cells and immunological responses. There have been several hypothesized methods for NP internalization, including passive uptake processes. Depending on their size, surface charge, and hydrophobicity, NPs can enter plants passively through diffusion and adsorption [92]. When NPs come into contact with a plant's surface, they can disperse through the stomata or cuticle and gather in different plant tissues. Their hydrophobicity facilitates passage through the lipophilic cuticle, while stomatal holes serve as entry locations. For example, gold nanoparticles coated with polyvinylpyrrolidone and citrate are passively absorbed by wheat leaves through cuticles [93]. However, additional research is required to understand how foliar NPs are translocated.

12.2. Active Uptake of NPs

Plants may absorb nanoparticles (NPs) selectively through active uptake mechanisms, which allows for precise targeting and regulated responses. Endocytosis, in which NPs are engulfed by the cell membrane to form vesicles, allows plant cells to actively absorb NPs. Plants can control NP absorption and respond to environmental cues thanks to this widely used mechanism. Plant cells take up NPs more readily when they enter endocytosis pathways mediated by caveolin or clathrin. For example, tobacco protoplasts actively internalize gold nanoparticles via clathrin-dependent pathways [69]. For plant cells to absorb nanoparticles by endocytosis, their surface properties are essential. For instance, triethylene glycol-functionalized silica NPs were consumed by tobacco mesophyll protoplasts, while bare, unfunctionalized silica NPs were not able to penetrate plant cells [94]. Even with these results, there is still a paucity of direct evidence of endocytic NP uptake by plant cells, which calls for additional study to comprehend integrated mechanisms of NP uptake and translocation within the plant system.

12.3. Uptake and Translocation of NPs through Roots

Plants are mostly exposed to soil-applied nanoparticles (NPs) through their roots, where they interact with root hairs and epidermal cells. Plant roots are more likely to attract and retain positively charged nanoparticles (NPs) or those that chemically interact with the root cell walls. With the help of transpiration rates, NPs can move from inside the root to other plant portions through the xylem and phloem [95]. For example, it has been found that copper nanoparticles migrate with water in the xylem, making it easier for them to go from the roots to the aerial parts of maize. The variety of NP absorption mechanisms enhances the potential of these compounds as plant immune modulators by enabling targeted administration and controlled release of bioactive compounds. However, in order to choose application techniques and guarantee effectiveness in agriculture, characteristics such as the physicochemical properties of NPs, their intended use, targeted crops, and desired interaction with the plant system are essential [96]. Research into NP absorption pathways is progressing, and this could lead to the full realization of their potential in sustainable plant disease management. In addition to controlling oxidative stress and maintaining redox homeostasis, nanomaterials can also occasionally cause oxidative stress [97]. Owing to these two features, it is critical to comprehend the precise metabolic processes of NMs and their interactions with plants. Lipid peroxidation and electrolytic leakage were decreased as a result of antioxidants' production quenching the ROS level. Researchers such as Rizwan, et al. [98] discovered that foliar application of Si and TiO₂-NPs alleviated oxidative stress in rice plants under cadmium (Cd) stress by reducing lipid peroxidation and electrolytic leakage by enhancing the activities of antioxidant enzymes like SOD, CAT, and APX. Given that nanoscience is now being investigated as a discipline connected to redox biology and biochemistry, it is critical to comprehend the exact method by which nanoparticles (NPs) avoid ROS overproduction mitigating overall normal physiological processes.

13. How Nanomaterials (NMs) would emerge as 'Nano-Weapons' against Phytopathogens?

- The special optical characteristics of nanomaterials (NMs) enable the very accurate and practical detection of plant pathogens [16, 54, 99].
- NMs damage to the membrane transporter and nutrient absorption systems of pathogen, [100].
- Nanoparticles generate ROS inside the pathogenic cell and developed nanotoxicity which results damage of nucleic acids (DNA/RNA), uncontrolled cell signalling, cytotoxicity, genotoxicity and PCD [101].
- Toxic ions generated as a result of nanotoxicity impair membrane protein activity and cell permeability [102].
- Nanoparticles are designed to offer distinct and improved antibiotic activity against plant diseases, depending on their size and dose [46].
- NPs as bioactive chemical delivery agents: Many bioactive substances, including essential nutrients, plant hormones, RNA interference agents, and chemical protectants, may be transported via nanoparticles (NPs). This feature highlights how they could completely transform plant genetic engineering, growth regulation, and nutrition management [103, 104].
- Nanoparticles can improve nutrient delivery to plants by increasing solubility, stability and bioavailability. Nanofertilizers developed for this purpose preserve nutrients from degradation and could release them in a guarded manner, allowing for optimal welcome by the plant tissues. This technique has the potential to reduce nutrient deficits and increase agricultural productivity, hence addressing global food security issues. Zinc-based nanofertilizers, for example, stimulate seed germination and growth in a variety of crops, whereas copper oxide nanoparticles efficiently transfer copper to increase crop yields and control plant diseases such as wilt by *Fusarium* sp and bacterial Fruit Blotch Disease (FBD) [105]. Despite promising findings in greenhouse conditions, field experiments are required to evaluate their potential in real-world environments.
- Delivery of plant hormones: Plant hormones can be precisely delivered to specific areas of a plant using nanoparticles (NPs), which enables the plant to respond to disease or infection [106]. Mesoporous silica NPs, for example, had been created to supply abscisic acid (ABA) to *Arabidopsis*, whilst biogenic iron NPs had been utilized to therapeutically deliver salicylic acid (SA) into watermelon plants, for increasing resistance to wilt disease caused by *Fusarium* sp [107]. These nano-enabled delivery systems ensure that plants receive hormone signals at the optimal time, improving stress tolerance and utilization of the chief nutrients. Growth hormones, which stimulate root development, shoot growth, and flowering in commercially important plants, can also be distributed using chitosan-based nanocomposites [41]. Using NPs to deliver hormones offers the chance to control plant development and stress reactions, improving crop resilience and performance.
- Small interfering (Si-RNA) RNAs delivery through NPs: Plant cells can be directly exposed to small-interfering RNAs (siRNAs), which overwhelmingly mediate RNA interference (RNAi) responses and confer resistance against pests and diseases, through the use of nanoparticles (NPs), an adaptable auto-regulatory delivery system [108]. Researchers can regulate the expression of certain genes associated with disease susceptibility, insect resistance, or other agronomically important traits by using this capability, which enables targeted gene silencing. NPs increase the effectiveness of gene silencing by protecting siRNAs from degradation in the harsh extracellular environment and promoting cellular absorption [109]. Compared with naked dsRNA, pathogen-specific double-stranded (ds)RNA administered by layered double hydroxide nanosheets, for instance, remained stable for as long as 30 days and offered tobacco plants sustained protection against viral infections. This method has the potential to reduce the need for medicinal interventions by conferring resistance to diseases and pests. One useful method for precision farming and developing long-term crop protection strategies is the use of NPs as siRNA carriers [103].
- Chemical cyto-protectants delivery by NPs: The innovative application of nanoparticles (NPs) in crop delivery for chemical protectants represents a significant advancement in precision farming and disease control. It has been challenging to apply chemical protectants like fungicides and bactericides effectively and efficiently, despite the fact they are essential for shielding crops from phytopathogens. It is possible to

provide these protectants precisely and regulated to particular plant tissues and pathogens by encasing or binding them in NPs [110]. For example, prochloraz had been transported via mesoporous silica nanoparticles, providing further defense against Rice Blast Disease (RBD) [111]. Similarly, silica nanoparticles loaded with azoxystrobin shown enhanced fungicidal activity against Tomato Late Blight Disease (TLBD) [112]. When compared to bulk counterparts, nanoscale fungicides were more effective against germs and prevented disastrous crop illnesses.

- Weeds and Arthropod Pests Management by NPs: several reports established that pests can move across cultivated and noncultivated plants, including weeds, posing issues for disease management. Insects play an important role in infection transmission, which complicates the situation. Traditional chemical control methods pose environmental problems, emphasizing the necessity for novel approaches such as nanotechnology. Nanoformulations show potential in improving pesticide efficiency while decreasing the environmental impact. For example, nanoencapsulation of atrazine boosted herbicidal efficacy tenfold, while light-triggered nanoformulations improved herbicidal effects while reducing toxicity to nontarget plants, such as *Glycine max* [113]. However, optimizing target delivery remains a difficulty, particularly given the similarities between weeds and crops. Carbon-based nanomaterials have been investigated for more effective and targeted pesticide delivery. Moreover, nanoparticles had shown useful roles in the management of insect pests. For example, encapsulated thiamethoxam has been effective in reducing Asian citrus psyllids [114]. Eco-friendly pest management solutions are offered by combining bioinsecticides with materials based on nanotechnology. Moreover, nanoparticles can be engineered to react to outside stimuli, which enhances their properties. For example, as temperature changed, mesoporous silica nanoparticles boosted insecticidal action against aphids. RNA interference technology paired with nanoclay effectively prevented virus transmission via insect vectors [115]. Overall, nanotechnology shows potential for transforming agricultural disease and pest management systems.

14. Environmental Risk Assessment of NPs

The rising use of man-made nanoparticles in the environment highlights the need for rigorous risk assessment and regulatory controls. Commercializing nanomaterials for agricultural applications involves a number of obstacles, including production scale, cost reduction and safety. To have a better understanding of the production, toxicity, and field application of nanomaterials in agriculture, more research is required. By producing nanopesticides, nanotechnology has the ability to completely transform agricultural pest management. These nanopesticides have various advantages, including enhanced solubility, reduced toxicity, targeted delivery and pH-dependent release. Nanopesticides are still in the early phases of development, and more study is needed to determine their effectiveness and potential toxicity to soil and the environment, despite their potential advantages. Although the use of nanoparticles (NPs) is expanding across many industries, there is a risk of environmental pollution from their release. They have the capacity to permanently contaminate the water, soil, and air [71]. Among the most popular sources are wastewater treatment facilities, electronics, paints, pigments, coatings, and cosmetics. Although nanoparticles can be used to clean up the environment, their extensive their extensive use might effectually jeopardize the ecosystems upon randomization. NP dispersion is aided by both nanosensors and nanofertilizers. In general, nanoparticles are challenging to control and contain due to their small size and reactivity. Moreover, NPs can be designed to release their payload in response to specific environmental conditions or triggers, which reduces the amount of fungicide needed overall and the amount of off-target effects. Chemical protectant delivery by nanoparticles (NPs) may change agriculture's approach to disease control; however there are safety issues and unintended consequences for other living things and the environment to take into account. All things considered, nanoparticles have shown promise as flexible means of delivering bioactive substances to plants, offering a novel approach to challenges in agriculture. The potential uses of NP-mediated smart delivery systems are expanding as nanotechnology research advances, providing new opportunities to increase agricultural output, nutritional value, and sustainability. However, concerns including NP biocompatibility, environmental impact assessment, and regulatory factors need to be carefully explored as this technology moves closer to being used in agriculture.

15. Bacteria and Fungi Play Crucial Roles in Promoting Plant Growth through Various Mechanisms in Natural Environment

- Nutrient Cycling: Organic debris in the soil is broken down by fungi and bacteria, releasing nutrients that plants can absorb. This cycle of nutrients increases soil fertility and provides essential nutrients for plant development.
- Nitrogen Fixation: Certain bacteria build symbiotic relationships with leguminous plants, such *Rhizobium* species, and transform atmospheric nitrogen into a form that plants can utilize. Nitrogen is added to the soil through this process, which is necessary for plant growth.
- Mycorrhizal Associations: Mycorrhizal fungi create mutualistic relationships with plant roots, extending their reach and increasing nutrient intake, particularly phosphate and micronutrients. This symbiotic interaction can greatly enhance plant growth and health.
- Disease Suppression: By building systemic resistance in plants, manufacturing antibiotics, or engaging in resource competition, beneficial bacteria and fungi in the soil can inhibit the growth of dangerous diseases. This aids in preventing disease in plants.
- Stress Tolerance: Certain bacteria and fungi create chemicals that help plants withstand environmental challenges like drought, salt and heavy metal contamination. These bacteria improve plant health and resilience by increasing their stress tolerance.
- Biofertilizers and Biostimulants: In agriculture, several bacteria and fungus serve as biofertilizers and biostimulants. They can boost soil fertility, nitrogen uptake, root growth and plant vigor, resulting in higher crop yields.

- Overall, bacteria and fungi have various and crucial roles in encouraging plant development and health, thus they are beneficial.

16. Bacterial Nano-Toxicity by ENPs

Bacteria, which are essential for environmental processes such as fermentation and decomposition, are extensively researched in eco-nano-toxicity because of their customization for ecological sustainability. *Bacillus*, *Pseudomonas*, *Escherichia* sp and majority of nitrifying bacteria are typically employed in this research. Some engineered nanoparticles (ENPs) with antibacterial capabilities are included into consumer products and eventually released into the environment. Interaction with bacteria has an impact on their growth and ecosystem function. ENPs such as Ag, CuO and carbon-based ones reduce bacterial growth and change enzyme activity, mostly by membrane degradation and oxidative stress generation [116]. Studies have shown that ENPs have a wide range of favourable vis-à-vis toxicological effects upon several beneficial microbiota [117].

17. Protozoal Nano-Toxicity out of ENPs Accumulation

Protozoa are single-celled eukaryotic organisms that feed bacteria, thrive in damp environments, and are crucial to the development and maintenance of microbial communities. They are actively researched in ecotoxicity assessments. *Tetrahymena thermophila* is an excellent model for assessing the toxicity of engineered nanoparticles (ENPs). ENPs mostly enter protozoa by endocytosis and phagocytosis, generating toxicity by upregulating metallothionein genes, modifying membrane fluidity, causing oxidative stress and lowering viability and motility [118]. Protozoa also serve as indicators of ENP trophic level transmission in food chains, since studies have shown that cadmium is biomagnified from ENP-loaded bacteria to protozoa and beyond. In vivo bio-imaging shows ENPs' trophic transmission across various layers of the food chain.

18. Nano-Toxicity of Fungi out of ENPs Accumulation

In a variety of environments, fungi are essential decomposers and are crucial to the nitrogen cycle. Understanding the harmful effects of engineered nanoparticles (ENPs) on fungi is critical to ecosystem health. ENP exposure can harm cell membranes, impede development, lower cell density, cause cytoplasm blebbing and impair breakdown activity by influencing lignocellulolytic enzyme synthesis [119]. High doses of ENPs utilized in toxicity studies may not accurately reflect environmental exposure levels. In natural settings, fungal communities have been constantly exposed to lower concentrations of ENPs over longer periods of time, emphasizing the need for more representative nano-toxicity studies.

19. Harmful Effects of Nanoparticles (NPs) on Earthworms

Although snails and earthworms are beneficial and crucial parts of healthy soil ecosystems, their populations and activities need to be carefully controlled to reduce any potential harm to plant development and agricultural output. Earthworms, which belong to the phylum "Annelida," are invertebrate animals that live in soil and contribute significantly to soil quality by increasing plant nutrients. The species of earthworms found in the field affect the soil's nutritional value. Based on the range of soil types and horizons they inhabit, earthworms are divided into three ecological groups: endogenic species, like *Aporrectodea caliginosa*, which live in organic horizons and dig horizontal burrows; epigeic species, like *Eisenia fetida*, which live on the surface and in litter; and anecic species, like *Lumbricus terrestris*, which live in deep, vertical burrows and consume large amounts of soil. Numerous organic acids and enzymes secreted by earthworms interact with soil to promote the growth of bacteria, fungi, actinomycetes, and soil microflora. These microorganisms help fix nitrogen, solubilize nutrients, and increase plant growth and production potential. Earthworms are therefore essential to a sustainable ecosystem and are commonly known as "farmers' friends." According to research, earthworms' physiological processes and general environmental activities are negatively impacted by nanoparticles (NPs). Numerous scientific studies have found detrimental impacts on the digestive and immune systems, species diversity, and the growth, reproduction, and development of earthworms. *Pheretima posthuma*, the most prevalent species of earthworm, is frequently employed as a standard test organism to identify environmental pollution levels [71, 120].

20. Biochemical Pathways Leading to Toxicity of Soil Microflora and Macrofauna: Oxidative Stress and Cellular Death Occurrence after ENPs Exposure

When faced with environmental challenges, reactive oxygen species (ROS), which are produced during regular metabolic processes, function as signaling molecules. In rhizospheric microflora and fauna, engineered nanoparticles (ENPs) can lead to an excess of reactive oxygen species (ROS), which can induce oxidative stress, cellular damage, DNA fragmentation, dysfunction of proteins and enzymes, and finally cell death. To reduce ROS effects, organisms have antioxidant defense mechanisms that include both non-enzymatic (*e.g.*, ascorbate, proline and phenolics) and enzymatic (*e.g.*, catalase, peroxidase and superoxide dismutase) counterparts. However, high ENP concentrations can overwhelm these defenses, resulting in oxidative damage to cellular organelles. The efficiency of antioxidant responses to ENP-induced oxidative stress varies depending on the rhizospheric species and exposure conditions. Non-enzymatic antioxidants including ascorbate, proline and phenolics play important roles in scavenging ROS and protecting animals from oxidative damage. To fully comprehend the mechanisms of nanotoxicity in beneficial microflora and fauna, as well as the reactions of antioxidant defense systems to acute and long-term exposure to ambient deposition of manufactured nanoparticles, more research is required.

If a virus infects soil it may have a number of regulatory impacts on plant development and health:

- **Nutrient Uptake:** Nanoparticles influence nutrient availability in the soil. If plants are infected with a virus, the nutrition cycle may be disrupted, resulting in deficits in key nutrients required for plant growth.
- **Toxicity:** Certain nanoparticles (Cd, Pb, Cr and Hg based) can be hazardous to plants. If a virus affects the composition or activity of nanoparticles in the soil, the toxicity levels may rise, affecting plant growth.

- **Microbial Interactions:** The microbial ecology in the soil, which is essential for the cycling of nutrients and plant health, can be impacted by nanoparticles. Unbalances in the soil ecosystem could result from a virus that affects nanoparticles and interferes with these microbial interactions.
- **Plant Immunity:** Nanoparticles have been investigated for their ability to boost plant immunity to diseases. If virus-infected nanoparticles disrupt this function, plants may become more susceptible to disease.
- **Soil Structure:** Nanoparticles can alter soil structure and water retention. A virus-induced change in nanoparticle activity may modify soil characteristics, influencing plant water availability and root growth.
- Overall, the impact would be determined by the individual virus, the nanoparticles used and their interactions with the soil microflora and plant population ecology Khan, et al. [50].

21. Sensing and Detection and Plant Pathogens in Agricultural Crops through Nanomaterials (NMs)

Plant resources are what sustain human civilization. There are three essential needs: shelter, food and fiber. These requirements are increasing every day in line with the global population growth. The yield of crops is greatly hampered by the shrinking agricultural lands brought about by the fast urbanization and industrialization of society. Another barrier that results in biotic stress in agricultural crops is plant diseases, which causes the plants to become partially impaired and eventually die. It presents a significant hazard to food production and security. Plant infections have wreaked havoc on some major disasters. For instance, *Phytophthora infestans* in Ireland produced the Irish Potato Famine in the 1840s, while *Helminthosporium oryzae* in rice caused the Great Bengal Famine in 1943. The majority of research on plant biological stress attempts to reduce appalling yield loss, either straight or circuitously [15]. The administration of previously discussed insecticides, fungicides, pesticides and fertilizers is the primary method used to manage biotic stress in agricultural crops. Engineered sensors (Figure 7) based on nanomaterials are used to track stress caused by pathogens as well as the environment. Researchers discovered that nanosensors worked well to identify fungal pathogens that cause plant illnesses that are soil-borne, air-borne, seed-borne and other. An electrochemical sensor that can identify the causative agent of bacterial specks of *Solanum lycopersicum* (*Pseudomonas syringae* DC3000) with a recognition limit scale-up of 214 pM [121]. In order to identify soil-borne pathogens such as *Ralstoniasolanacearum*, which causes bacterial wilt in potato disease, the researchers used Au-NPs [122]. Therefore, remote sensing technology based on nanosensors is a great way to identify, anticipate and control insects, pests and plant diseases in order to safeguard both commercial and agricultural crops and preserve the environment.

22. Nanotechnology Mediated Detection of Plant Pathogens at Early Stages of Infections

Researchers found that fungal pathogens that cause soil-borne, air-borne, seed-borne and other plant ailments may be effectively identified using nanosensors. With a recognition limit of 214 pM, an electrochemical sensor can determine the cause of bacterial specks of *Solanum lycopersicum* (*Pseudomonas syringae* DC3000) [121]. The researchers employed Au-NPs to identify soil-borne pathogens, such as *Ralstonia solanacearum*, which causes bacterial wilt disease in potato [122]. In order to protect both commercial and agricultural crops and maintain the environment, remote sensing technology based on nanosensors is an excellent approach to identify, anticipate and control insects, pests and plant diseases.

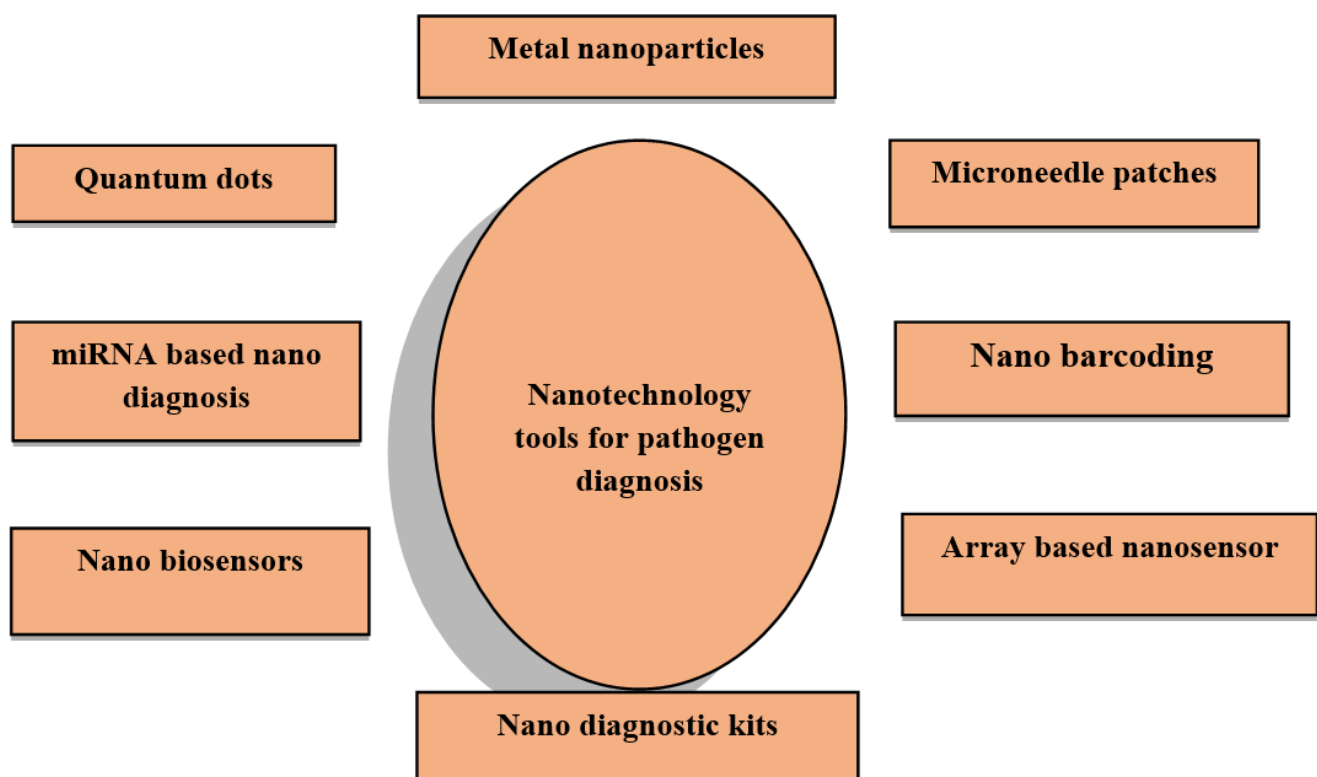


Figure 7. Flowchart shows the advent of different nanotechnology tools used for pathogen diagnosis.

23. Challenges and Limitations of Nanosensors

Nanotechnology holds great promise for revolutionizing pest management in agriculture through the development of nanopesticides. Numerous benefits are provided by these nanopesticides, including increased

solubility, increased effectiveness with decreased toxicity, targeted administration, and pH-dependent release. Nevertheless, study is extremely demanded to determine the efficacy and potential toxicity of nanopesticides to soil and the environment because they are still in the early phases of development, despite their apparent advantages. The precise definition of nanopesticides by regulatory bodies is still pending, and nothing is known about how they affect pesticide resistance. Challenges include the lack of regulatory standards, high manufacturing costs and public perception. Bridging the knowledge gap among farmers and stakeholders, as well as developing scalable solutions compatible with existing farming practices, is essential for successful implementation. To overcome these obstacles and fully utilize nanotechnology in agriculture, teamwork between companies and researchers is essential. For risk evaluation and regulatory approval, dependable data collection and long-term field experiments are required. Additionally, stakeholders should be informed about storage conditions and cytotoxicity concerns associated with certain nanoparticles and efforts should be made to utilize green and natural materials for nanoparticle synthesis to minimize environmental risks. Overall, interdisciplinary collaboration and comprehensive research are akin to advancing agricultural nanotechnology responsibly. Nanotechnology has potential for sustainable and environmentally friendly agriculture practices by introducing new methods of administering fertilizers, insecticides and other materials. However, current study indicates that nanoparticles could affect soil animals such as earthworms, increasing safety issues in food and agriculture. The environment's growing employment of artificial nanoparticles highlights the significance of stringent risk assessment and regulatory limitations. Several obstacles must be overcome in order to commercialize nanomaterials for use in agriculture, including those related to manufacturing scale, cost control, and safety. To fully comprehend the production, toxicity, and practical use of nanomaterials in agriculture, more study is required.

Nanomaterials (NMs) have special qualities that could make them useful in agriculture. These promising qualities include: I) increased bioavailability of the active ingredient; II) improved pesticide, fungicide, and insecticide solubility; III) releasing the active ingredient based on pH level; IV) transferring RNAi molecules for disease management; V) ROS detoxification and oxidative stress regulation in plants; and VI) epigenetic and epigenomic studies of animal stress regulation [123] etc.

Although nanomaterials (NMs) offer many potential uses and benefits in agriculture, particularly in terms of helping crops become more resilient to stress, they also have a number of harmful consequences on our ecosystem and living things [124]. Nanoparticles mediated pollution is danger to the natural flora and fauna including beneficial bacteria, fungi and soil-nematodes (Soil microflora) by causing long term nanotoxicity in their body, decreasing soil fertility, low level of nutrient solubilization and losing the activities of PGPR in soil [125].

Nano-agrochemicals continue to be denied authorization to be commercialized in the market due to their vulnerability towards unfavorable environmental attainments. When it comes to using fungicides, pesticides, herbicides, or other products based on nanoparticles (NPs), regulatory bodies or the pharmaceutical industry have failed to give the right ratio of nano substances and their maximum level of effective dose without endangering the environment. Furthermore, studies and researches are urgent for eco-friendly use of nanoparticles (NPs) in agriculture and its commercialization. Additionally, scientists ought to create fresh, workable solutions that our farmers can readily use without interfering with their current methods and practices [126]. In particular, more research using an in-vitro method is still needed to determine the appropriate dose level, establish regulatory requirements, and modify and use crops at the field level when using NMs to agriculture [127].

24. Conclusion and Future Perspectives for Future Smart Agriculture

Nanotechnology is a vast field of study that combines aspects of environmental biology, ecotoxicology, physics, chemistry, botany, biotechnology, and agricultural science. With atomic sizes ranging from 0.1 to 100 nm, nanomaterials (NMs) can have both positive and negative environmental effects. In order to apply NMs to their biological roots, researchers should concentrate on using them by highlighting and improving their ecologically favorable properties in the proper amounts. Nanomaterials are widely used in agriculture to boost high productivity and sustainability. NMs are mostly used as nanopesticides, nanofertilizers, nano-insecticides, nanofungicides, and nanoherbicides to protect agricultural crops from pests and boost their fertility. This paper discusses these applications. Our study also demonstrates how NMs help cells defend against redox homeostasis imbalances, which cause ROS to be produced in excess and activate the antioxidant defense system. Additionally, NMs would be improved as a tool for producing heat shock proteins [13] balancing physiological homeostasis, and enhancing epigenetic memory [123] in plants to fight off biotic and abiotic stressors. The type of NMs, the dosage required for each type of abiotic stress (drought, cold, salinity, etc.), and their effects on over ten different plant species were all documented. To enhance comprehension, scientific examples are provided on the use of NMs in sustainable agriculture, the production of ROS and their interconversion, and antioxidant defense mechanisms. Despite a number of innovative research initiatives, there are still several gaps in the field of nanoscience. How precisely nanomaterials (NMs) regulated and activated the antioxidant system against reactive oxygen species (ROS) is still unclear and contradictory, for example. Thus, before moving into the new and more sophisticated stage of NM-based agriculture, it is essential to have a precise understanding of how plants and NMs interact under different stressful conditions and how this affects long-term exposure. An organization that conducts research on nanotechnology should create suitable rules for the safe handling of nanoparticles (NMs), their variety of uses in biological systems, and their appropriate disposal to avoid harm to the environment or public health. Furthermore, NMs are used as sensitive and efficient pathogen diagnostic tools in the form of miRNA-based diagnosis, nano-biosensors, nano-barcoding, and quantum dots, among others. The writers of the review paper anticipate that it will meet the fundamental needs of upcoming students and researchers in nanoscience who want to concentrate on the function and use of NMs in agriculture. As nanotechnology research progresses, the potential applications of nanoparticle-mediated smart delivery systems are growing, opening up new avenues to enhance sustainability, nutritional value, and agricultural output. However, issues including NP biocompatibility, environmental impact, and regulatory considerations must be appropriately addressed as this strategy becomes more and more viable in agriculture. Through the introduction of innovative techniques for applying fertilizers, insecticides, and other items, nanotechnology has the potential to support ecologically friendly

and sustainable agricultural practices. However, recent studies indicate that if nanoparticles are not applied carefully, they may partially damage beneficial microorganisms in the rhizosphere, including earthworms. This may possibly augment raising safety concerns in food and agriculture. Regulatory bodies have yet to adequately define nanopesticides and there is a dearth of understanding about their consequences on pesticide resistance. Challenges include a lack of regulatory standards, expensive manufacturing costs and public opinion. Bridging the knowledge gap between farmers and stakeholders, as well as designing scalable solutions that are compatible with current farming techniques is critical for effective adoption of Nano-based strategies for future smart agriculture. To overcome these obstacles and realize the full potential of nanotechnology in agriculture, industry and researchers must work hand-in-hand. Risk assessment and regulatory approval depend on long-term field experiments with reliable data. In addition, stakeholders should be informed about the storage conditions and cytotoxicity problems connected with specific nanoparticles and efforts should be made to use green and natural materials for nanoparticle production to reduce environmental dangers. Overall, interdisciplinary collaboration and comprehensive research are crucial to developing agricultural nanotechnology ethically.

List of Abbreviation:

NMs: Nanomaterials; NPs: Nanoparticles; PGRs: Plant growth regulators; ROS: Reactive oxygen species; CuO: Copper oxide; ZnO: Zinc oxide; MgO: Magnesium oxide; MgOH: Magnesium hydroxide; Al₂O₃: Aluminum oxide; TiO₂: Titanium dioxide; Au: Gold; CuONPs: copper oxide-based nanomaterials, ZnONPs : zinc oxide-based nanopesticide, MgOHNPs: magnesium hydroxide-based nanopesticide, MgONPs : magnesium oxide-based nanopesticides, 1O₂: Singlet oxygen; O₂⁻: Superoxide; H₂O₂: Hydrogen peroxide; OH[·]: Hydroxy radical; BYMV: Bean Yellow Mosaic Virus., PCD: Programmed Cell Death., PGPR: plant growth-promoting rhizobacteria. ETC: Electron Transport Chain; SOD: Superoxide dismutase; CAT: Catalase; APX: Ascorbate peroxidase; GPX: Glutathione peroxidase; GR: Glutathione reductase; MDA: Malondialdehyde; Fe₂O₃: Ferric oxide; Ag: Silver; Si: Silicon; Ni: Nickel; CeO₂: Cerium oxide; Cd: Cadmium; ELISA: Enzyme linked immuno sorbent assay; PCR: Polymerasechain reaction; miRNA: micro RNA; SiRNA: Small interfering RNA, HSPs: Heat Shock Proteins.

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