

# NANOTECHNOLOGICAL APPLICATIONS IN HORTICULTURAL PLANT DISEASE MANAGEMENT

## Abstract

Plant diseases significantly challenge agricultural and horticultural productivity and food security worldwide. The emergence of resistant pathogen strains and environmental concerns associated with conventional disease management strategies have necessitated exploring novel approaches. Nanotechnology, with its unique properties and capabilities at the nanoscale, has emerged as a promising field for revolutionizing plant disease management. Nanotechnology offers innovative tools and materials for disease control in horticultural and agricultural systems. Nanomaterials, such as nanoparticles, nanocarriers, and nanosensors, exhibit distinct physicochemical properties that can be exploited for effective disease management. Nanofungicides and nano bactericides enhanced antimicrobial activity and targeted delivery, showing great potential in combating fungal and bacterial pathogens. Nanovaccines, utilizing nanocarriers for precise delivery of antigens, hold promise for stimulating plant immunity and developing long-term resistance against diseases. The use of nanotechnology also extends to disease diagnosis and monitoring. Nanosensors enable rapid and sensitive detection of pathogens, facilitating early disease identification and intervention. Nanoscale imaging techniques provide high-resolution visualization of pathogen-host interactions, aiding disease diagnosis and understanding resistance mechanisms. The targeted delivery system of the nanomaterials effectively reduces the need for excessive chemical application, contributing to sustainable and eco-friendly disease management practices. Despite the immense potential, nanotechnology in plant disease management faces challenges to standardization, safety assessment, and regulatory framework. Robust research efforts and interdisciplinary collaborations are

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essential for overcoming these challenges and ensuring responsible and effective implementation of nanotechnology-based solutions in agriculture. Nanotechnology presents exciting opportunities to revolutionize plant disease management, providing precise, sustainable, and practical approaches. By harnessing the unique properties of nanomaterials and exploiting their potential in diagnosis, treatment, and prevention, researchers and stakeholders can enhance disease control strategies and mitigate plant disease's economic and environmental impacts. Continued research and development in this field promise a more resilient and productive agricultural sector.

**Keywords:** Nanotechnology, Plant disease management, Nanoparticles, Nanofungicides, Nanobactericides, Nanovaccines and Nanosensors.

## I. INTRODUCTION

Horticulture, which includes fruits, vegetables, flowers, ornamental plants, and plantations, is an essential aspect of agriculture that serves the vital purpose of meeting the rising food demand and ensuring a well-rounded diet for the expanding global population. However, the increasing population and the impact of climate change present challenges that require inventive approaches to increase food production by 70 – 100 percent by 2050 [137] and minimize crop losses. This chapter investigates the possibilities offered by nanotechnology as an advanced technology in horticulture, aiming to address the imperative for sustainable crop management. Nanotechnology, derived from the Greek word for "dwarf," emphasizes materials between 1 and 100 nanometers in various scientific disciplines [70]. Nanotechnology is one of the six essential enabling technologies for sustainable development, according to the European Commission [104]. Nanotechnology involves manipulating or self-assembling individual atoms, molecules, or molecular clusters to create materials and devices with unique properties. Therefore, nanotechnology entails designing, producing, characterizing, and using devices, structures, and systems by precisely controlling their size and shape on the nanometer scale [71]. Initially utilized in electronic communications, textiles, the automobile industry, biomedical applications, biotechnology, and renewable energy [41, 104], nanotechnology has emerged as a promising tool in agriculture, including horticulture [43, 46].

The application of nanotechnology, its products, and devices are vitally important in the agriculture and horticulture sector for drug delivery, nano barcoding, developing the nanosensor for the simple deduction of pest and disease or abiotic stress, slow release of agrochemicals, enhancing seed germination, and the nano-vectors used for the efficient gene transfer [125]. Using nanomaterials offers new avenues for addressing critical crop production and management challenges. One of the primary objectives of nanotechnology in horticulture is to increase crop productivity while optimizing limited resources. Nanofertilizers and nanosensors enable precise delivery of nutrients and real-time plant health monitoring, improving plant nutrition, growth, and stress tolerance [60, 109]. Nanoparticles can enhance agrochemical efficiency, reducing usage and environmental impact.

Crop losses caused by biotic factors, such as diseases and pests, significantly impact horticultural productivity. Plant diseases pose significant challenges to agricultural productivity and food security worldwide. The emergence of resistant pathogen strains and environmental concerns associated with conventional disease management strategies have necessitated exploring novel approaches [90]. Nanotechnology offers novel solutions and revolutionizes disease management strategies in horticulture. Researchers are exploring innovative strategies to prevent, control, and mitigate plant diseases using nanomaterials and nanotechnology-based approaches. Nanomaterials, such as nanoparticles, nanocarriers, and nanosensors, exhibit distinct physicochemical properties that can be exploited for effective disease management [43]. Nanofungicides and nano bactericides enhanced antimicrobial activity and targeted delivery, showing great potential in combating fungal and bacterial pathogens. Nanovaccines, utilizing nanocarriers for precise delivery of antigens, hold promise for stimulating plant immunity and developing long-term resistance against diseases. Nanovaccines provide a means to stimulate plant immunity, bolstering disease resistance in horticultural crops.

Maintaining post-harvest quality and extending shelf life are critical considerations in horticulture. Nanomaterials can be utilized in packaging and coatings to enhance food safety, reduce spoilage, and minimize post-harvest losses. Nanoscale delivery systems ensure the controlled release of bioactive compounds, reducing deterioration and enhancing the sensory attributes of harvested produce. Nanotechnology contributes to precision farming, enabling site-specific management practices tailored to the needs of individual plants or areas within a field. The use of nanotechnology also extends to disease diagnosis and monitoring. Nanosensors enable rapid and sensitive detection of pathogens, facilitating early disease identification and intervention. Nanoscale imaging techniques provide high-resolution visualization of pathogen-host interactions, aiding disease diagnosis and understanding resistance mechanisms. Nanosensors facilitate real-time monitoring of environmental parameters, disease incidence, and nutrient status, allowing for targeted interventions. Precision farming practices enhance resource efficiency, minimize waste, and promote sustainability in horticultural crop production.

In addition to their direct impact on disease control, nanomaterials offer environmentally friendly alternatives to conventional pesticides. Their targeted delivery systems minimize off-target effects and reduce the need for excessive chemical application, contributing to sustainable and eco-friendly disease management practices. Despite the immense potential, nanotechnology in plant disease management faces challenges to standardization, safety assessment, and regulatory framework. Robust research efforts and interdisciplinary collaborations are essential for overcoming these challenges and ensuring responsible and effective implementation of nanotechnology-based solutions in agriculture.

## II. NANOPARTICLES IN HORTICULTURAL DISEASE MANAGEMENT

Nano particles are diverse in shape and size at the nanoscale (10 – 100 nm). The size of the nano particles is the main restriction factor for the penetration of nanoparticles into the plant tissues. Plants only permit particles between 40 and 50 nm in size [136]. Engineered nanoparticles have the desired size and shape with specific properties to enhance the quality of pesticides and their delivery via adsorption, encapsulation, or conjugation [73]. Various nanoparticles commonly utilized in disease management include carbon, silicon, silver, copper, zinc, and non-metal or aluminum oxides [5]. These nanoparticles exhibit antifungal properties [60], antibacterial properties [39], and some also reveal antiviral activity [76]. By controlling plant pathogens such as *Xanthomonas*, *Aspergillus spp*, *Botrytis cinerea*, and *Fusarium*, carbon nanomaterials stimulate plant growth and development.

**1. Nano-Hybrids or Nano-Composites:** Developing nano-hybrids or composites can enhance the spectrum of action against plant pathogens [133]. These nano-hybrids have diverse components with different chemical origins, including biological-inorganic or natural/synthetic organic-inorganic materials [103]. The field of antimicrobial nano-hybrids has attracted much research to develop potent, effective, and multifunctional solutions. Specifically, alumino-silicate nanoplates have been incorporated into pesticide formulations due to their superior biological activity and environmental safety compared to engineered nanoparticles [50]. Therefore, nanoparticles and nano-composites can potentially combat fungal pathogens and disease outbreaks effectively. These nano-composite materials consist of multi-phase components, one of which is continuous, and the other has nanoscale dimensions. Nano-composites are created by incorporating nanoparticulate materials into long-chain or short-chain polymeric matrices. Adding

nanoparticles to polymers can considerably improve the polymer properties [50]. In addition, nano-composites serve as antimicrobial agents and sensors to extend the expiration life of food materials [139].

In a study conducted by researcher Wang et al. [144], the antifungal activity of a graphene oxide-iron oxide nano-composite was demonstrated against the downy mildew disease on grapes leaves. The nano-composite was applied as a pretreatment, followed by the inoculation of *Plasmopara viticola* sporangium. The nano-composite effectively inhibits spore germination by obstructing the water channels of sporangia through its surface adsorption mechanism, as demonstrated by the study's findings [115]. Silver-titanate nanotubes nano-composite stimulates Reactive Oxygen Species (ROS) cascades, causing harm and leading to the conidial mortality of *B. cinerea* isolated from tomatoes. Kaur et al. [68] discovered that combining silver and chitosan in a nano-composite substantially enhanced the inhibition of pathogens isolated from chickpea seeds. In addition, applying chitosan-silica nano-composites effectively suppressed grey mold disease in Italian and Benitaka grapes while maintaining the fruit's quality.

- 2. Nano Fungicides for the Protection of Horticultural Crops from Diseases:** Traditional agriculture and horticulture methods commonly involve using agrochemicals such as fertilizers, insecticides, fungicides, antibiotics, and nematicides to safeguard cultivated crops and promote sustainable growth. However, the prolonged application of these chemicals poses significant risks, such as developing pest resistance and indiscriminate toxicity, despite their role in ensuring food availability. Nanopesticides, which are pesticides containing active ingredients reformulated at the nanoscale to facilitate easy delivery and exhibit properties like biocompatibility, biodegradability, and environmental friendliness, have emerged as a potential solution [61]. Nanopesticides incorporate nanometer-sized surfactants, organic polymers, and inorganic nanoparticles [122]. Extensive research and studies have been conducted worldwide to explore the potential benefits of nano-pesticides. The studies on nano fungicides started in 1997, incorporating fungicides in solid wood against brown rot wood-decaying fungus *Gloeophyllum trabeum* [86]. Akther and Hemalatha [3] stated that metal oxide-based nanomaterials are more potent than conventional fungicides.
- 3. Mode of Action of Nano-Fungicides :** Studying the mode of action of nanoparticles/nano-composites against plant pathogens is necessary. Interestingly, nano-fungicides demonstrate superior antifungal activity even at low concentrations compared to bulk materials. According to Elizabeth A. Worrall et al. [147], two pathways are implicated in nanoparticle-mediated plant protection. The first method includes nanoparticles directly involved in plant protection, whereas the second involves nanoparticles functioning as transporters for existing pesticides.

In plants, nanomaterials are incorporated into cells through endocytosis, where the plasma membrane engulfs them to form vesicles. Alternatively, nanomaterials can create pores in the plasma membrane to directly access the cytosol. Another mechanism involves binding nanoparticles to carrier proteins, integral membrane proteins in the plasma membrane. These carrier proteins aid in the internalization of nanomaterials into the cell [32, 146, 113].

Plasmodesmata, specialized structures in plant cells, are crucial in transporting nanomaterials between cells and facilitating translocation through the phloem [152]. Furthermore, ion channels are the primary pathway for nanoparticle entry into the cell [113]. However, a key limitation of the ion channel pathway is the narrow size restriction of 1 nm, which necessitates modifications to enable proper nanoparticle entry [120].

Nanoparticles can affect fungal pathogens and plants in numerous ways, including direct internalization into the cell wall, receptor-mediated adsorption followed by internalization, and internalization via ion transport proteins [63]. Once internalized, nanomaterials inhibit  $\beta$ -glucan synthase, thereby interfering with N-acetylglucosamine synthesis in the fungal cell wall. Magnesium oxide nanoparticles induced the accumulation of superoxide molecules, increased the pH of the bacterial cells, triggered membrane damage and intracellular component leakage, and ultimately caused the death of the bacterial cells [56], which led to the cytoplasmic organelle dissolution and the cell wall separation from the cytoplasmic contents. Sometimes, plasmolysis may occur. Iavicoli et al. [49] discovered that silver nanoparticles harmed bacterial cells by creating holes and crevices in the bacterial membrane and fragmenting the cells.

The bacterial cell wall comprises a peptidoglycan layer and an outer membrane of negatively charged lipopolysaccharide molecules [130, 4]. The presence of negatively charged nanoparticles increases the likelihood that metals will bind to these structures, resulting in increased toxicity due to the concentration of inorganic nanoparticles [130]. Nanoparticles can enter plant cells when concentrated at high levels on their surface, depolarizing the cell membrane and increasing permeability. This enables nanoparticles to be taken up by the cell, resulting in their continued dissolution and intracellular damage. Most plant viruses have a rod-shaped structure with a capsid made of protein discs that form a tube-like structure called the coat protein. Most of the negative charge is located at one end of the virus and is governed by the viral genome [8]. By producing reactive oxygen species (ROS), nanoparticles may inhibit the replication of plant viruses by permeating plants and impeding virus replication. The interaction of inorganic nanoparticles with the surface of the plant virus prevents the virus from entering plant cells. They also bind to the virus coat protein and, due to their charge, exhibit a high affinity for the virus genome, thereby preventing replication. In addition, nanoparticles induce plant defense mechanisms against plant viruses, including the antioxidant system, resistance genes, and plant hormones [141].

In a culture medium, nanoparticles exhibit antimicrobial activity by releasing ions. The dissolution of ions depends primarily on the size and concentration of the nanoparticles and inhibits spore germination [100, 14, 59]. When utilized as carriers, nanoparticles offer increased shelf life, greater pesticide solubility, lower toxicity, and increased target-specific applications. Nanoparticles can be applied in various ways, including foliar spray, soil irrigation, seed treatment, and root application.

- 4. Delivery of Nanoparticles for Crop Protection:** Nanoparticles are transported inside the plant by apoplastic and symplastic movement. Apoplastic movement involves the extracellular spaces, the cell wall of adjacent cells, and xylem vessels. On the other hand, symplastic movement involves plasmodesmata and sieve plates for movement [118, 114]. Once they reach the plant's central cylinder, Nanoparticles may move toward the upper part of the plant through xylem vessels utilizing the transpiration mechanism [134]. When

nanomaterials are delivered through the irrigation system, they may overcome the barrier created by the band of suberin, an impermeable substance present in the endodermal cells (Casparian strips) of plant roots for the apoplastic movement [108]. The foliar application of nanoparticles crosses the cuticular barrier and follows the lipophilic or hydrophilic pathways for its movement [119]. Eichert et al. [27] reported that the nanomaterials chose the stomatal pathway than the cuticular pathway because the stomatal pathway sizes 10 nm and the cuticular pathway sized only 2 nm. The table below (Table 1) provides a list of different types of nano-formulations for nanoparticles:

**Table 1: Types of Nano-Formulation**

| <b>Formulation</b> | <b>Description</b>                                                                                                                                                                  | <b>References</b> |
|--------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|
| Nano Encapsulation | The fungicide active ingredient compound/molecule is embedded in a matrix or surrounded by a coating.                                                                               | [116]             |
| Nanoemulsion       | Emulsions are oil and water molecules with a particle size of fewer than 100 nm.                                                                                                    | [6]               |
| Nanorods           | Solid cylindrical nanoparticles ranging from 1 to 10 nm maintain uniformity in size for a few to hundreds of nanometers.                                                            | [87]              |
| Quantum dots       | Small semiconductor particles in the nanometer size range exhibit unique spectral properties compared to traditional dyes, commonly used in bioimaging and biosensing applications. | [9]               |
| Carbon nanotubes   | Carbon molecules consist of rolled-up sheets of a single layer of atoms. Single-walled or multi-walled carbon nanotubes facilitate the targeted delivery of agrochemicals.          | [112]             |

### III. NANO-FUNGICIDES AS PROTECTANTS OF HORTICULTURAL CROPS

Protectants are substances or agents that safeguard against detrimental elements like pests, diseases, environmental stressors, or harm. In agriculture, protectants encompass pesticides, fungicides, or other remedies employed to avert or manage the harm inflicted by pests or diseases on crops or plants. Silica, chitosan, solid lipids, stimuli-sensitive carriers, and double-layered hydroxides are commonly used carriers for plant disease management.

- 1. Nano Silica:** Silicon, abundant in the vicinity of plant roots, is an essential element that plants utilize in the form of mono silicic acid ( $H_4SiO_4$ ). In addition, silicon plays a crucial role in disease mitigation in vegetables [11, 69]. Nano silica is well-known as an anti-stress agent for various cultivated plants [29], and it strengthens plants by enhancing their disease resistance and stimulating their physiological mechanisms [105].

Treating pea seedlings with potassium silicate leads to increased activity of chitinase and  $\beta$ -1,3-glucanase, which effectively combats *Mycosphaerella pinodes*, the blight-causing agent in peas [21]. The application of nano silicon for controlling post-harvest vegetable diseases is also demonstrated in studies by Barman et al. [12] and

James and Zikankuba [54]. The synthesis of nano-silica is a straightforward method that allows for precise shaping, sizing, and structuring. These nanoparticles are typically spherical with pore-like openings. Porous hollow silica nanoparticles and mesoporous silica nanoparticles facilitate the encapsulation of pesticide-active ingredients within their inner core, protecting against UV light degradation and enabling the controlled release of the molecules [94].

Parveen and Siddiqui [105] examined the effects of silicon dioxide ( $\text{SiO}_2$ ) nanoparticles on tomato plant growth characteristics and defense enzyme activities against bacterial and fungal pathogens. Under both in vitro and greenhouse conditions, the researchers used  $\text{SiO}_2$  nanoparticles as foliar sprays and seed preparatory treatments. Tomato plants sprayed with  $\text{SiO}_2$  nanoparticles at a concentration of 0.20 g/L showed the most significant increase in plant growth parameters, chlorophyll, carotenoid, proline levels, and the activity of defense enzymes. Additionally, this treatment showed the maximum reduction in disease indices. The leaves are suggested to absorb  $\text{SiO}_2$  nanoparticles through stomatal apertures and trichomes [140].

Due to the surface hydroxyl groups found in  $\text{SiO}_2$  nanoparticles, silica nanoparticles have been shown to have antifungal action by destroying the cell wall by creating hydrogen bonds with the lipopolysaccharides of the cell wall [17]. On the other hand, the potential antibacterial mechanism of  $\text{SiO}_2$  nanoparticles is associated with the presence of singlet oxygen and other reactive oxygen species (ROS) on the surface of  $\text{SiO}_2$  nanoparticles, leading to oxidative damage to bacterial membranes and subsequent DNA damage, ultimately causing bacterial death [131].

- 2. Nano Chitosan:** Chitosan is a hydrophobic substance that may be altered by graft reactions and ionic contacts thanks to its reactive amine and hydroxyl groups [84]. Chitosan is derived from chitin, an exoskeleton component of insects and crustaceans [18]. It is a biodegradable substance. Chitosan nanoparticles emerge as promising organic polymers used in agriculture and horticulture for the controlled release of agrochemicals due to their low solubility in aqueous media [67], allowing for prolonged contact on the plant's epidermis, facilitating the uptake of bioactive molecules by adhering intact [89] on the desired sites [1, 35, 98, 106, 126], improving stability and reducing the dosage of pesticides [67]. Because of its capacity to chelate both organic and inorganic molecules, chitosan is a good choice for enhancing substance stability, solubility, and biocidal activity. Chitosan was categorized as a "generally recognized as safe (GRAS)" chemical by the US Food and Drug Administration.

Chitosan nanoparticles exhibit potent antifungal activity against various plant pathogens, including fungi and bacteria. They accomplish this by binding to the negatively charged fungal cell wall, causing cell disruption and permeability alterations in the membrane. In addition, it also inhibits DNA replication, resulting in cell demise. Another possible mechanism is chitosan acting as a chelating agent and selectively binding to trace metal elements. This binding process inhibits microbial growth and reduces toxin production. Various intrinsic factors, such as the molecular weight and degree of deacetylation of the parent chitosan and the size and concentration of the nanoparticles, influence the effectiveness of this antimicrobial activity. Extrinsic factors, including pH, temperature, and reaction time, also play a role in determining antimicrobial properties [23, 52].



Sotelo-Boyas et al. [132] investigated the effects of chitosan nanoparticles and chitosan nanoparticles supplemented with lime and thyme essential oils in an in vitro study. The experimental results demonstrated that the chitosan nanoparticle supplemented with thyme essential oil effectively inhibited the growth of *P. carotovorum*, the pathogen responsible for soft-rot in carrots, by producing an inhibition zone measuring up to 2.4 cm in diameter. Additionally, various molecular weights of chitosan were tested against the bacteria causing head rot in broccoli, and it was observed that chitosan with nanoscale molecular weight effectively inhibited bacterial growth. The nanoscale chitosan nanoparticles disrupted the bacteria's cell wall more efficiently than microparticles [95].

Nanochitosan demonstrates varying levels of antimicrobial efficacy against bacteria and fungi, with the inhibition of mycelial growth observed in chili pepper seeds at a concentration of 0.6% w/v for *Rhizopus sp.*, *Colletotrichum capsici*, *C. gloeosporioides*, and *A. niger* [19]. The size and zeta potential of chitosan nanoparticles affects their inhibitory effect [52]. Zahid et al. [151] found that low molecular weight chitosan nanoparticles inhibit spore germination of *C. gloeosporioides*, the causative agent of anthracnose in fruits.

At the nanoscale, chitosan also acts as an effector molecule inducing systemic acquired resistance in plants against pathogens. Several studies have reported evidence of defense induction in tomato, cucumber, chili, strawberry fruits, and rose shrubs. Through a diffusion mechanism, nano chitosan is introduced to the agricultural ecosystem. This involves water penetration into the particulate system, which causes the matrix to enlarge and transforms the polymer into a rubbery or plasticized swollen matrix, thereby allowing the active constituents of pesticides or compounds to diffuse from the nano matrix.

- 3. Nano Silver:** Due to their unique properties and potential effectiveness as antimicrobial agents, silver nanoparticles (AgNPs) have garnered considerable interest. The mechanism by which AgNPs activate plant defense mechanisms remains unknown, necessitating further study. Recent research has shown that AgNPs and silver ions can induce cytotoxicity and genotoxicity in microbial cells [45]. Still, the green synthesis of AgNPs is gaining prominence [111]. AgNPs have enormous potential in the management of fungal and bacterial plant pathogens. The high surface area-to-volume ratio of AgNPs permits their direct interaction with microorganisms, which damages membranes by interacting with sulfhydryl groups of proteins. In addition, their interaction with bacterial DNA arrests the cell cycle in the G2/M phase, leading to apoptosis. Size, shape, and surface properties affect the antimicrobial activity of AgNPs [72]. Gupta et al. [42] found that silver nanoparticles inhibit fungal phytopathogens during outbreaks under field conditions.

Bacterial spot, a significant disease of tomatoes caused by *X. perforans*, frequently demonstrates resistance to copper-based bactericides commonly used for disease control. Kim et al. [78] report that silver nanoparticles are efficacious against the methicillin-resistant bacterium *Staphylococcus aureus*. Ocoy et al. [102] developed DNA-directed AgNPs grown on graphene oxide (Ag@dsDNA@GO), which exhibited outstanding antibacterial properties at a low concentration of 16 ppm. These AgNPs demonstrated enhanced stability, antibacterial activity, and adsorption characteristics [74]. The *Agrobacterium tumefaciens* and *A. rhizogenes* growth was effectively inhibited by the green synthesis of AgNPs using *Descurainia Sophia* [28, 66, 77]. Kim et al. [77] reported

that AgNPs at a concentration of 100 ppm in PDA media inhibited the mycelial growth of *B. cinerea*, *Cladosporium cucumerinum*, *Corynespora cassiicola*, *Cylindrocarpon destructans*, *F. oxysporum f.sp. cucumerinum*, *F. oxysporum*, *Fusarium sp.*, *Glomerella cingulata*.

Kale et al. [62] found that colloidal nano silver with a 1.5 nm diameter has antifungal activity against *Sphaerotheca pannosa* var. *rosae*, the causal agent of powdery mildew disease in roses. Using a chemical reaction involving silver ions, physical methods, reducing agents, and stabilizers, double-capsulated nanosilver can effectively eliminate unwanted microorganisms from planting soils and hydroponic systems, promoting plant growth.

It has been demonstrated that silver nanoparticles, stabilized with ammonia and having an average size of 52 nm at concentrations between 270 and 540 g/mL, inhibit the phytopathogen *Phomopsis spp.* in soybean seeds by compromising its membrane integrity [92]. Aguilar-Méndez et al. [2] report that spherical silver nanoparticles with diameters spanning from 5 to 24 nm, stabilized with gelatin as a capping agent, inhibit spore germination in *C. gloesporioides*. 24 hours following inoculation, spherical Ag nanoparticles (12 nm, 0.1 g/L) were applied to potato plants to combat Potato Virus Y (PVY). El-shazly et al. [31] discovered that this treatment timing is crucial for the antiviral activity of Ag nanoparticles, which interact with sulfhydryl groups in viral nucleic acids via the release of Ag<sup>+</sup> ions. In contrast, when Ag nanoparticles were applied with salicylic acid (at 0.1 g/L) three or seven days before PVY or Tomato Mosaic Virus (ToMV) inoculation, virus concentration, and infection rate were reduced. This indicates that resistance was already induced by salicylic acid and virus entry into the plant vascular system was prevented [31]. In addition, foliar irrigation of Ag nanoparticles at a concentration of 50 ppm seven days before PVY inoculation of tomato plants reduced infection [101].

AgNPs provide effective antimicrobial activity against various plant pathogens, making them a potential crop protection solution. Their mode of action, impact on plant defense mechanisms, and comparative efficacy with ionic silver salts require additional investigation. Addressing the potential environmental hazards associated with AgNPs will be essential to utilize their benefits for plant disease management when conventional methods prove ineffective.

- 4. Nano Zinc Oxide:** Antibacterial and antifungal properties of zinc oxide (ZnO) nanoparticles against pathogenic microorganisms have been demonstrated. Compared to chemically synthesized ZnO nanoparticles and other common antimicrobials, biologically synthesized ZnO nanoparticles inhibited bacterial growth more effectively [117]. Several mechanisms contribute to the antimicrobial activity of ZnO nanoparticles, including the production of reactive oxygen species (ROS), the release of Zn<sup>2+</sup> ions that disrupt cellular functions, and the generation of electron-hole pairs upon exposure to light [45]. Nanoparticles' antimicrobial efficacy is highly dependent on their size and concentration. Antibacterial performance is enhanced when particles are smaller, and concentrations are higher.

Additionally, ZnO nanoparticles have demonstrated antifungal activity against various fungal species. The mode of action differs depending on the fungal species,

causing deformation in fungal hyphae or inhibiting the growth of conidiophores and conidia. In addition to their effectiveness against bacteria and fungi, ZnO nanoparticles have been studied for their potential as a fungicide in agriculture and horticulture. They have dose-dependent activity against various fungal strains and could be utilized as a broad-spectrum fungicide.

Zinc oxide nanoparticles with concentrations greater than 3 mM/ml inhibit the post-harvest pathogens *B. cinerea* and *P. expansum* by inhibiting the cellular function in Botrytis and the conidiophore development in *P. expansum* [44]. ZnO and Ag nanoparticles inhibited *Sclerotinia homoeocarpa*, the pathogen that causes dollar spots on cool-season turfgrass. Exposure of the isolates to nanoparticles and ions significantly increased the expression of the stress response genes glutathione S-transferase (Shgst1) and superoxide dismutase 2 (ShSOD2) [82].

- 5. Nano Magnesium oxide:** According to research by Imada et al. [51], magnesium oxide (MgO) nanoparticles have been discovered to activate the salicylic, jasmonate, and ethylene pathways, leading to the induction of defensive mechanisms against *R. solanacearum* in tomatoes. The *R. solanacearum* can cause bacterial wilt in various plants, including potatoes, tomatoes, eggplants, groundnuts, olives, bananas, and ginger. When it detects its host, the bacteria enter the host plant and colonize the xylem vessels, obstructing the plants vascular system by producing excess polysaccharides and cell wall-degrading enzymes. The minimum concentration required to inhibit the growth of the bacteria (minimum inhibitory concentration) and the concentration required to kill the bacteria (minimum bactericidal concentration) with MgO nanoparticles is 200 and 250 µg/ml, respectively. MgO nanoparticles exhibit antibacterial activity against *R. solanacearum* by damaging the bacterial cell membranes, reducing their motility and ability to form biofilms, and causing DNA damage by accumulating reactive oxygen species (ROS) [16].
- 6. Nano Copper:** Copper is an essential micronutrient for plants as it plays a vital role in their growth and ability to resist diseases caused by pathogens. Specifically, copper produces crucial defense enzymes like plastocyanin, peroxidase, and copper multiple oxidases. When pathogens attack plants, these enzymes are responsible for mounting a response to combat the infection [33].

Copper nanoparticles have been studied for their fungicidal properties. Nano-copper-based formulations have been developed and used in agricultural settings to control plant fungal diseases. Environmental Protection Agency of the US approved using Copper nanoparticles as antimicrobial agents. It is possible to create copper nanomaterials chemically, using copper nitrate and acetyl trimethyl ammonium bromide, or biologically, by reducing copper ions with ascorbic acid and sodium citrate [15]. These nanomaterials can create intermolecular cross-links by attaching to the targeted bacteria nucleic acids. Additionally, they can bind to the carboxyl or sulfhydryl groups of amino acids, disrupting the proteins that carry out biological functions [127].

The plant pathogens *Botrytis cinerea*, *Phoma destructiva*, *Curvularia lunata*, *Alternaria alternate*, and *F. oxysporum*, which are known to cause severe diseases in plants, have been demonstrated to have antifungal activity by chemically synthesized copper nanomaterials [88, 65]. Furthermore, Du et al. [24] demonstrated that copper-

alginate stabilized nanomaterials effectively inhibit the *Neoscytalidium dimidiatum* fungus, which causes brown spot disease in dragon fruit. Copper nanoparticle at the 0.2 ppm concentration was found to inhibit the bacterial blight (*X. axonopodis* pv *punicae*) of pomegranate by degrading the bacteria's cell wall and at the size of 8 nm. It controls *Penicillium chrysogenum*, *A. alternata*, *F. solani* and *A. flavus* [37].

Khatami et al. [75] discovered the potential of copper nanoparticles in controlling soil-borne pathogenic fungi through their research. Similarly, Kamel et al. [64] demonstrated the antifungal activity of copper nanoparticles at a concentration of 0.30 M against *F. solani*, the causative agent of cucumber root rot. The study revealed that copper nanoparticles inhibited the fungus effectively in both in vitro and greenhouse settings. This inhibition was ascribed to the induction of defense enzyme activities, such as catalase, peroxidase, and polyphenol oxidase, and the modulation of gene expression, especially PR-1 and LOX-1. In addition, the inhibitory activity of copper nanoparticles was associated with observed pathogen irregularities, alterations, twisting, plasmolysis, and spore contraction. These results emphasize the potential of copper nanoparticles as a promising strategy for fungus control and plant health promotion.

- 7. Nano Selenium:** Selenium (Se) is a semi-metallic element of the chalcogen group. Sodium citrate, sodium borohydride, and ascorbic acid are commonly used to synthesize selenium nanoparticles (SeNPs) via chemical methods [36, 79, 124]. SeNPs have demonstrated comprehensive antibacterial activity, effectively inhibiting Gram-negative and Gram-positive bacteria. In addition, SeNPs have antifungal properties by inhibiting spore germination [128]. Incorporating SeNPs into agricultural practices presents several potential approaches, such as soil supplementation with selenium, cultivation of plants in nutrient mediums enriched with SeNPs using hydroponic or aeroponic systems, pre-soaking seeds in SeNP solutions before planting, or applying SeNP solutions via foliar application [40].

Like nano silica, nano selenium has been recognized as an anti-stress agent for cultivated plants [29] and is considered a nano fungicide against phytopathogens [107]. Recent research by Taha et al. [135] demonstrated that nanoselenium exhibits antifungal activity against tomato leaf blight disease caused by *A. alternata* at a concentration of 100 ppm. This effect is achieved by inducing morphological changes in the hyphae and inhibiting mycelial growth. Moreover, applying nano selenium increased the activities of defense enzymes in tomato plants. In a separate study, Joshi et al. [58] found that Nano-selenium effectively inhibits late blight disease in tomatoes caused by *P. infestans*. This inhibitory effect is attributed to regulating differential gene expression related to cellular processes, biochemical pathways, and defense responses.

Lazcano-Ramírez et al. [81] conducted a study to evaluate the antifungal activity of nano selenium (SeNPs) obtained from *Amphipterygium glaucum* leaves (SeNPs-AGL) and *Calendula officinalis* flowers (SeNPs-COF) against plant pathogenic fungi (*F. oxysporum*, *C. cladosporioides*, and *C. gloeosporioides*) and bacteria (*Serratia marcescens*, *Enterobacter cloacae*, and *Alcaligenes faecalis*). According to the results, SeNPs-AGL, which has an average particle size of 8 nm, has antifungal activity at dosages of 0.25 mg/ml and higher. According to Huang et al. [48], smaller selenium nanoparticles (around 81 nm) demonstrated the highest inhibition against *S. aureus*. Additionally, Joshi et al. [59] reported that selenium nanoparticles at low concentrations

of 50 and 100  $\mu\text{g/mL}$  inhibited the growth of pathogens such as *C. capsici* and *A. solani* on chili and tomato leaves.

The antimicrobial activity of selenium nanoparticles can be attributed to their small size and high surface area/volume ratio. This allows them to interact closely with microbial cell membranes, facilitating intracellular diffusion and causing membrane damage or toxic effects on DNA. Another mechanism involves inhibiting cell proliferation through reactive oxygen species (ROS)-mediated processes [123, 55]. In the poisoned food technique, the pathogenic fungal culture treated with selenium nanoparticles produces a strong odor due to the release of volatile compounds like dimethyl selenide  $(\text{CH}_3)_2\text{Se}$ , which reduces the toxicity of selenium nanoparticles [129]. Hu et al. [47] suggest that pathogenic fungi, such as *Fusarium* sp., may produce mycotoxins like zearalenone, trichothecenes, deoxynivalenol, and fumonisins as secondary defense mechanisms to protect themselves from excessive ROS produced by selenium nanoparticles.

#### IV. NANOTECHNOLOGY IN POST-HARVEST DISEASE MANAGEMENT

Most perishable horticultural products deteriorate during post-harvest handling, such as transport, storage and microbial contaminants. The microbes cause decay to the produces. Nanotechnology eliminates or reduces harmful pesticides and promotes alternate post-harvest disease management methods.

Applying clay-chitosan nano-composite at a 20 g/mL concentration is highly efficacious against *P. digitatum*. The nano-composite completely inhibits the growth of *P. digitatum* in vitro and reduces lesions in citrus oranges by 70% in vivo trials [150]. The grapes were covered with a chitosan-titanium oxide nano-composite film of 70 m thickness after harvest to prevent microbial deterioration. The composite film has a 22-day resistance to mildew growth [153]. The banana fruits coated with a nano-composite composed of soybean protein, cinnamaldehyde, and zinc oxide nanoparticle delay, maturation, reduce respiration rate and physiological weight loss, and preserve fruit firmness [83]. The metallic nano-composite of silver, nickel, copper, and magnesium nanoparticles prepared from ajwain and neem leaf extract regulates the anthracnose-causing pathogen *C. musae* on bananas [53]. Similarly, the thin film coating of nano-composite polylactic acid containing zinc oxide nanoparticles prevents fungal infections for 14 days on cut apple fruits stored at 4 °C. At 100 g mL<sup>-1</sup>, the chitosan-silver nanoparticles nano-composite completely inhibits the conidial germination of *C. gloeosporioides*, the pathogen that causes post-harvest anthracnose in mango [25].

In a study conducted by Liu et al. [85], it was observed that the use of nanosilver significantly impacted the vase life of cut flowers by suppressing the growth of bacterial pathogens. The vase life was extended by treating cut *Gerbera jamesonii* flowers with a solution containing 5 mg/ml of nanosilver for 24 hours, and bacterial infections were inhibited.

#### V. NANOSENSORS: DETECTING PATHOGENS AND CONTAMINANTS

Nanosensors are devices having a receptor transducer that can detect an organic molecule and display it physically [20]. Monitoring pests and diseases throughout the

growing season is an essential yield-enhancing strategy in horticultural production. For food safety and environmental protection, detecting pathogens and contaminants in food, water, and soil is crucial. However, conventional methods such as immunological and molecular approaches are time-consuming, costly, and require specialized laboratory skills. Rapid, robust, sensitive, and cost-efficient detection protocols or sensors are required to combat the spread of plant pathogens. Using nanotechnology, nanosensors provide a potent instrument for rapid and sensitive detection [26, 80]. These nanoscale sensors detect pathogens, pesticides, heavy metals, and other contaminants more precisely and quickly than conventional methods. Numerous nanosensors are founded on smartphone technology, making them more user-friendly and inexpensive. Multiple universities have contributed to developing nanosensors for food and environmental analysis [143]. Utilizing nanomaterials conjugated with nucleic acids, proteins, and other biomolecules in nanosensors is advantageous for the detection of pathogens rapidly.

One study [110] devised a quantum-dot fret-based nanosensor for diagnosing Candidatus *Phytoplasma aurantifolia*, which infects lime plants even at extremely low inoculum levels of 5 phytoplasma cells per microliter. Another scientist [149] created a fluorescent silica nanoprobe conjugated with biomarker/antibody molecules to detect *X. axonopodis* pv. *vesicatoria*, the agent responsible for spotting diseases in tomato and pepper. Ariffin et al. [7] created a nanowire biosensor using conventional photolithography and nanometer-scale structure formation techniques in their study. Schwenkbier et al. [121] developed a chip-based hybridization technique incorporating silver nanoparticles to detect *Phytophthora* spp. This biosensor was designed to detect the cauliflower mosaic and papaya ring spot viruses. The researchers could construct nanowire structures that facilitated the detection of these viruses by employing precise trimming techniques. This novel strategy can potentially improve virus detection methods and contribute to developing biosensing technology within the field of virology.

Graphene quantum dots, gold nanoparticles, and an antibody were coupled to create an immunosensor by Bhardwaj et al. [13] on an indium tin oxide (ITO) electrode-modified surface. This immunosensor exhibited high sensitivity for detecting Aflatoxin (AFB1) in food samples at extremely low concentrations. By incorporating nanoparticles within plant cells and chloroplasts, nanosensors are being investigated in precision agriculture to create bionic plants [38]. These bionic plants can detect or image objects in their environment, communicate with infrared devices, and some even have the potential to self-power as light sources.

Gold electrode nanosensors combined with copper nanomaterials have been employed to detect the phytopathogenic fungus *S. sclerotiorum* by monitoring the electrocatalytic oxidation of the phytohormone salicylic acid content in rape oilseeds [145]. The Silica nanoparticles combined with antibodies have been used to detect *X. axonopodis* pv. *vesicatoria*, the causative agent of bacterial spot disease in Solanaceae plants [149]. Rad et al. [110] developed quantum dots fluorescence resonance energy transfer-based immunosensors to detect *Phytoplasma aurantifolia*, which causes witches' broom disease in lime. The sensitivity of this detection method is approximately 5 *Phytoplasma aurantifolia* per  $\mu\text{L}$ . In another study, p-ethyl guaiacol was sensed using  $\text{TiO}_2$  and  $\text{SnO}_2$  nanoparticles and detected on screen-printed carbon electrodes as an electrochemical sensing system of volatiles in fruits and plants [34].

## VI. NANOBIO TECHNOLOGY: MANIPULATING CROPS AT THE MOLECULAR LEVEL

The plant cell wall presents the most significant challenge for crop gene delivery. Traditional plant gene transfer technologies, including *Agrobacterium*-mediated gene transfer, electroporation, PEG-mediated gene transfer, particle gun bombardment, and others, are expensive, laborious, and disrupt cell development. Nanobiotechnology combines nanotechnology and biology to create innovative molecular-level crop monitoring and modification instruments. This emerging field provides a new foundation for comprehending plant biology and enhancing crop traits. Nanobiotechnology enables precise control over plant growth, tolerance to stress, and disease resistance. By manipulating nanoparticles and nanomaterials, scientists can enhance the expression of desirable traits in crops and develop novel ways to combat agricultural challenges. Nanotechnology offers valuable support for genome editing using nuclease-based techniques and is crucial in delivering nanoparticles for enhanced plant genetic engineering. These nanoparticles can potentially enter plant cells without needing external force, thereby improving the delivery of biomolecules. Furthermore, these particles possess adjustable physiochemical properties, allowing for the conjugation of various cargos and enabling their broad application in diverse contexts. Using nanoparticles to address the plant-pathogen interaction within agriculture offers fresh perspectives on safeguarding crops.

Mesoporous silica nanoparticles (MSN) have pores that are 3 nm in size, which makes it possible to transmit DNA and its chemical activator molecules into target plants simultaneously [138]. Extensive research has been conducted to increase the pore size of MSNs so that proteins can be delivered to specific plant locations. MSNs capped with gold (Au) nanoparticles convey plasmids with the GFP gene. Upon entry into plants, the uncapping process is initiated by the cleavage of bonds connecting Au nanoparticles to MSNs. This mechanism causes the gene to be released and initiates gene expression at the target site [91].

Gold nano particles at the size of 5-25 nm embedded nano carbon matrices employed for DNA delivery during transformation. It carries more genetic materials than microparticles [142]. The nano particles coated with the DNA will quickly enter the plant cell by size, increasing the transformation efficiency in both monocots and dicots. It also requires a low number of plasmids and gold particles than the commercial ones.

Since its discovery, the RNAi pathway has been a powerful tool for the genetic management of plant pests and diseases [93]. Plant virus-targeting RNAi-inducing chemicals are transported via a variety of nanoparticles. Layered double hydroxides (LDH), sometimes referred to as BioClay, are clay particles arranged into hexagonal sheets with active substances sandwiched in between the layers. LDH nanoparticles break down in an acidic environment when exposed to ambient water vapor and carbon dioxide. Positively charged delaminated lactate nanoparticles known as LDH help to transport genetic material across the plant cell wall by breaking down its barrier [148, 93, 10]. According to Mitter et al. [93], the dsRNA-loaded LDH nanoparticles efficiently shielded plants from the cucumber mosaic virus and the pepper mild mottle virus for 20 days. In plant cells with a high capacity for intracellular transfer, Demirer et al. [22] have shown the effectiveness of carbon nanotubes as a delivery platform for siRNA and polyethyleneimine-coated silver nanoparticles.

In the field of nanobiotechnology, recent advancements have focused on utilizing nanomaterials for the delivery of the CRISPR-Cas9 system. This delivery system includes single guide RNA (sgRNA) to improve delivery efficiency, reduce off-target effects, and enhance the specificity of the CRISPR/Cas system. By employing cationic arginine gold nanoparticles assembled with Cas9En (E-tag)-Ribonucleo proteins, nano assemblies can deliver sgRNA and achieve approximately 30% effective cytoplasmic/nuclear gene editing efficiency in cultured cell lines. This advancement holds excellent promise for future research in crop development. The nano assemblies are designed to fuse with cell membranes upon contact, releasing the Cas9En-RNP directly into the cytoplasm and nucleus. These nanoassemblies are approximately 475 nm in diameter [97].

## **VII. ENVIRONMENTAL IMPACTS AND SAFETY CONSIDERATIONS**

While nanotechnology holds great promise for agriculture, it is crucial to understand the potential risks and ensure the safe use of nanomaterials. Researchers are actively studying nanotechnology's human and environmental health effects on food, agricultural, and biological systems. By comprehensively understanding the potential risks associated with nanomaterials, policymakers can make informed decisions regarding their manufacturing and use. Ongoing research focuses on minimizing the environmental impact of nanotechnology and ensuring its long-term sustainability.

## **VIII. REAL-WORLD APPLICATIONS AND SUCCESS STORIES**

The application of nanotechnology in agriculture extends beyond horticulture crop production. Researchers have made significant advancements in various areas, such as product authentication, explosive compound detection, disease treatments, and oil spill classification. For instance, nanoparticles have been used to verify product authenticity, detect explosive compounds, fight antibiotic-resistant bacteria, and classify oil types in seawater. Nanomaterials have also been employed to enhance the efficiency of solar cells, contributing to the development of renewable energy solutions.

## **IX. EDUCATION AND TRAINING: EMPOWERING FUTURE INNOVATIONS**

Education and training play a crucial role in harnessing the potential of nanotechnology in agriculture and horticulture. Researchers and institutions actively develop and share educational materials, participate in workshops and conferences, and collaborate with global alliances focused on rapid diagnostics and biosensors. The agricultural community fosters future innovations and leadership in nanotechnology by training students and scientists worldwide. The recognition received by project members and their students further facilitates new projects and strengthens collaborations.

## **X. CONCLUSION: A PROMISING FUTURE FOR NANOTECHNOLOGY IN AGRICULTURE**

Nanotechnology offers immense potential to address the agricultural industry's challenges, particularly in horticulture crop production. Developing and applying nano fertilizers, nano pesticides, nanosensors, and nanobiotechnology can significantly enhance crop productivity, increase stress tolerance, and minimize environmental pollution. Collaborative efforts between researchers, industry partners, and government entities are



essential to driving agriculture adoption and commercialization of nanotechnologies and nano fungicides. By embracing the opportunities nanotechnology offers, we can pave the way for a sustainable and resilient future in agriculture.

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