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Chemical and Biological Technologies in Agriculture

REVIEW

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Exploring the potential of nanomaterials (NMs) as diagnostic tools and disease resistance for crop pathogens

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Abstract

Food crops are attacked by microbial pathogens and insect pests, leading to significant yield reductions and economic losses. Conventional disease diagnosis and management approaches often fail to provide rapid and eco-friendly solutions. In the current situation, nanomaterials (NMs) serve a valuable role in both managing emerging pathogens and monitoring overall plant health. Nanotechnology has transformed the biotechnology industry including agriculture with specific applications such as nano-fungicides, nano-bactericides, and nano-pesticides. This review focuses on the use of various nanomaterials, including inorganic materials such as Ag, ZnO, CuO, and CeO, as well as carbon-based nanoparticles, nanotubes, nanowires, and nano-capsules. The application of NMs holds the potential to address various challenges in food security through novel applications like advanced nano-biosensors for rapid pathogen detection and targeted disease management strategies. This includes the potential to minimize reliance on chemical inputs and contribute to more sustainable agricultural practices. Nanomaterials (NMs) promise to deliver plant hormones and signaling molecules to plants, enhancing resistance inducers against major crop pathogens. NMs against newly arising pathogens through reactive oxygen generation, membrane damage, and biochemical interference are also reviewed. However, challenges regarding the stability, toxicity, and environmental impacts of NMs are discussed, along with recommendations on green synthesis and functionalization approaches. This article aims to investigate the role of nanomaterials (NMs) in managing emerging pathogens and monitoring overall crop health offering an insightful outlook for future generations. Further biosafety aspects and larger-scale validation of NM-based applications could enable their commercialization for improving global food security.

Keywords Nanomaterials, Disease resistance, Plant defense system, Plant pathogens, Disease identification, Eco-friendly

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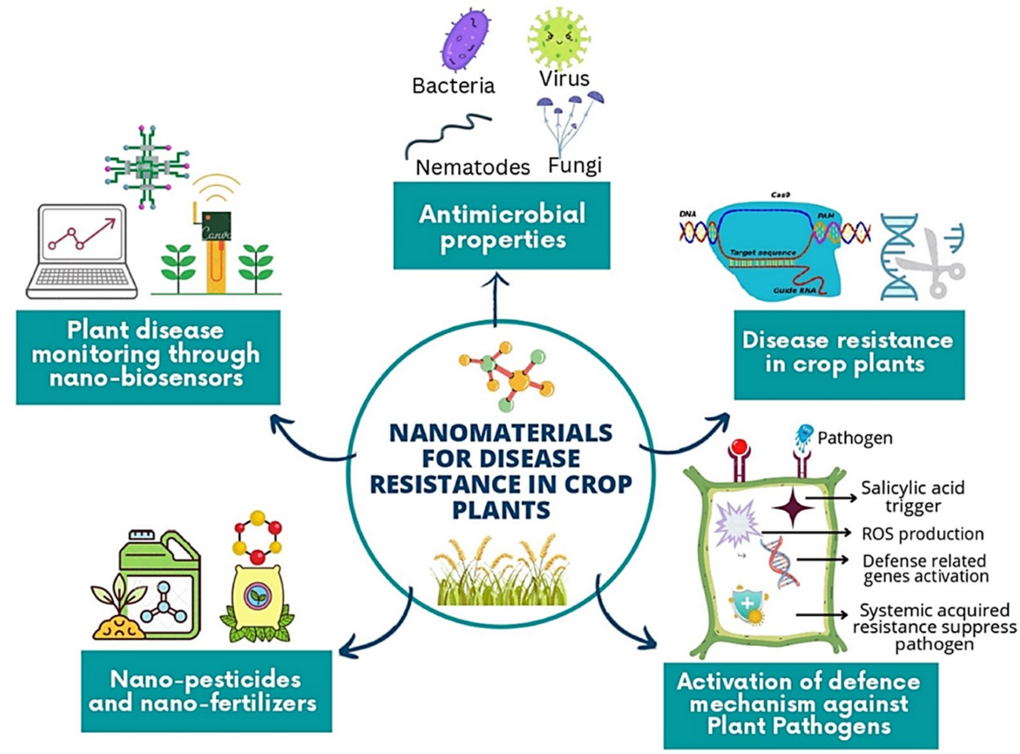
Graphical Abstract



Introduction

The global demand for food crops is approximately 2–3 billion tons, which is needed to feed the world's 7.9 billion people. However, this demand is expected to increase by 50% in the coming years, creating a significant challenge for agricultural production to keep up [1]. Food crops are facing severe threats from plant pathogens and the impacts of climate change [2]. Plant diseases are major barriers to agricultural development and food security, lowering crop yields, as well as the quality of the production in storage [1]. For instance, a historic epidemic of *Phytophthora infestans* that caused potato late blight disease in Ireland had substantial cultural and economic repercussions, leading to population movement and starvation in the 1840s [3]. Pathogens are responsible for significant agricultural output losses around 20%–40% [4], prompting ongoing research into managing fungal, viral, nematode, and bacterial diseases. Plant pathogens have evolved mechanisms to evade plant defense systems by secreting effector proteins that interact with specific plant target proteins, thereby suppressing plant immunity and facilitating disease development [5]. Plant diseases impart a substantial yield penalty on major global food crops. A recent study estimated average yield loss from diseases across potato (17.2%), soybean (21.4%), wheat (21.5%), maize (22.5%), and rice (30.0%) comprising over half of global calorie consumption crops. Worryingly, the maximum losses happened in food-insecure areas with high populations that also face emerging or re-emerging diseases aggravated by climate change. Targeted efforts to encourage sustainable agricultural productivity through minimizing yield gaps from pathogens will be critical to global food security [1]. In the past, agrochemicals have mitigated plant diseases, but their overuse has led to resistance, environmental harm, and unintended species accumulation. To address these challenges, alternative disease control strategies are imperative due to their profound implications for human health and the environment [6]. Novel control measures such as nanotechnology include nanomaterials, nanoparticles, nanocarbon materials, agrochemical-based nanomaterials [7], nano-biosensors, and small detection devices that could be used for the detection and management of plant pathogens. NMs can significantly contribute to revolutionizing agrotechnology because they are more efficient, defendable, and durable than currently used synthetic chemical products. For example, the development of nanosensors for early disease detection [8], nano-fertilizers, nanopesticides, nanoherbicides [9], growth promoters, and antimicrobials presents a hopeful path for sustainable crop protection and productivity improvement. Collectively, NMs present versatile opportunities to transform plant disease management and agriculture worldwide. These materials enable site-specific and controlled nutrient release, improving plant uptake efficacy while producing disease resistance and mitigating environmental impacts [10]. Various nanostructured materials, including nanoclays, carbon nanodots, nanotubes, nanofibers, carbon-based nanomaterials, and polymeric nanoparticles, serve as promising carriers for nutrients [11]. In recent years, the use of nanomaterials has been considered a suitable solution to control plant pests, including insects, fungi, and weeds [12]. Furthermore, NMs can act as stimulators for plant defense mechanisms helping to prevent disease [13]. For example, engineered nanomaterials (ENMs) were applied to a 5-mL suspension containing 50 or 200 mg L^{-1} concentrations of two metal-based nanoparticles (Fe_2O_3 or TiO_2) and two carbon-based nanomaterials (MWCNTs or C60). These ENMs were sprayed onto tobacco leaves every day for 21 days and fully developed young leaves were inoculated with turnip mosaic virus TuMV tagged with green fluorescent protein (GFP). The fluorescence images of TuMV abundance on the leaf surfaces indicated that NMs exhibited a substantial inhibitory effect on viral proliferation, as evidenced by reduced fluorescence intensity on the newly emerged leaves. Moreover, a noteworthy reduction ranging from 15 to 60% was observed in the relative quantity of TuMV coat proteins, providing additional insights into the mechanisms through which NMs exert their suppressive influence on viral infection [14]. In recent studies, nanoparticles, including zinc oxide (ZnO), titanium dioxide (TiO_2), gold (Au), silver (Ag), cerium oxide (CeO_2), silica oxide (SNP), and copper (Cu), have found extensive application in crop fields [15]. In prior studies, it was observed that metal oxide nanomaterials such as TiO_2 NPs and CuO NPs, as well as carbon nanomaterials like reduced graphene oxide (GO) and multi-wall carbon nanotubes (MWCNTs), exhibited distinct inhibition of the fungus *Podosphaera pannosa* when applied at high concentrations 200 mg/L in preventing infestation of rose leaves. However, when used at lower concentrations 50 mg/L, only CuO NPs demonstrated inhibitory effects on *P. pannosa*. Hence, a multidisciplinary technique is required to examine the impact of nanomaterials on plant diseases, including gene expression, transcriptomic analysis, proteomics, and secondary metabolites [16]. Furthermore, NMs have the capability to influence plant biochemical and molecular responses, such as activating antioxidant defense systems, accumulating osmolytes and hormones, and regulating gene expression. These mechanisms collectively enhance plant resilience against biotic stresses [17]. For example, nanoparticles like TiO2 induce the expression of P5CS1 gene, enhancing proline synthesis and stress resilience in plants [18–20]. Moreover, NPs (ZnO, Se, Si) boost antioxidant enzyme activity such as APX, CAT, and SOD, reducing drought-induced oxidative damage [21]. NMs have demonstrated potential antimicrobial effects through the modulation of several intracellular pathways. Their antimicrobial mode of action may involve suppression of ATP synthesis, disruption of cell membranes, alterations in membrane permeability, inhibition of enzyme production, interference with electron transport processes, disruption of cytochrome interactions, and enhancement of reactive oxygen species generation. Collectively, these diverse cellular impacts of NMs can effectively control microbial growth and viability through both membrane and metabolic perturbations [22]. The contribution of nanomaterials and nanotechnology in the agricultural field is still in its infancy. Therefore, a system-level approach is required to provide more precise information on the role of nanoparticles against plant pathogens. The use of several nanomaterials in crop protection for disease resistance and early detection via triggering defense responses to pathogens is reviewed in this article. Moreover, plant disease monitoring using nano-biosensors is an innovative and highly promising approach for the early detection and management of plant diseases. Nano-biosensors operate on various principles, including surface plasmon resonance, fluorescence, electrochemistry, and field-effect transistor technology. These sensors employ biorecognition elements such as antibodies, and DNA probes to selectively bind to pathogen-specific molecules ensuring high sensitivity and specificity. They can detect various plant pathogens from bacteria to viruses and fungi, offering advantages such as high sensitivity, specificity, real-time monitoring, and cost-effectiveness. For instance, case studies highlight their application in detecting plant pathogenic bacteria such as *Xanthomonas* and *Ralstonia* spp. [23] and fungal pathogens such as *Fusarium* and *Botrytis* spp. [24]. Despite their potential, challenges such as affordability and integration into agricultural practices remain to be addressed. Nevertheless, ongoing research improves biorecognition elements and sensor platforms making nano-biosensors a valuable tool for early and accurate plant disease monitoring. This technology empowers farmers and agriculturalists to proactively manage crop health, ultimately reducing yield losses. Furthermore, this review summarizes the effectiveness of different NMs used against various plant diseases and the safe applications of NMs in food crops. The review also elucidates the various biochemical and molecular mechanisms facilitated by nanoparticles (NPs) that promote pathogen resistance in plants. While existing research offers concise insight into NP characteristics, synthesis methods, and their contribution to pathogen detection and prevention, gaps remain in understanding their potential drawbacks in pathogenesis [25]. Therefore, this review aims to address existing knowledge gaps and highlight promising future research directions concerning the use of specific NMs in plant disease diagnosis and disease resistance. Areas of focus include exploration of biosafety considerations associated with NM applications and potential detrimental impacts on crop health. Understanding biosafety profiles and adverse effects is crucial to enabling safe development and deployment of NM technologies in agriculture. The review also discusses optimization of NM properties for effective disease detection and management while minimizing environmental risks. Overall, the review seeks to provide insights and perspectives to guide continued advancement of safe and sustainable NM solutions for improved plant health and productivity.

Nanomaterial synthesis and characterization for crop plants

Nanotechnology produces nanoscale materials called nanoparticles or nanomaterials in various forms, such as ferromagnetic, ferroelectric, and superconducting materials. The characteristics of nanomaterials are dependent on their size, charge, and shape. Nanomaterials in crop plants have a size typically between 1 and 100 nm and can be synthesized using different methods depending on the material and application. They can be classified into inorganic, carbon, organic, and composite nanomaterials. Nanoparticles can be produced from metal or metal oxide using physical, chemical, and biological procedures, with the biological methods being environmentally safe. In the realm of nanomaterial synthesis, various methods including chemical reduction, sol–gel synthesis, and green synthesis, are employed for nanomaterial synthesis. Among them, green synthesis utilizing biological entities like plants and fungi to reduce metal ions is remarkable for its sustainability. Recent studies have successfully synthesized gold nanoparticles (Au-NPs) through this eco-friendly method [26]. Green synthesis of nanoparticles, which uses plant extracts and microorganisms, is frequently used to produce metal-based nanoparticles [27]. Numerous plants have been utilized for green synthesis of nanomaterials [28]. For example, a study evaluated 109 plant species from Middle Eastern traditional medicine, analyzing dried samples of different plant parts including bark, bulb, flower, fruit, gum, leaf, petiole, rhizome, root, seed, stamen, and above-ground parts. Out of 117 plant parts from 109 species across 54 families, 102 extracts showed the bio reduction of Au^{3+} to Au^{0}. This study unveiled 37 new plant species capable of AuNP synthesis [29]. According to the literature, leaf extracts from numerous plants including *Cocos nucifera* [30], *Psidium guajava*, *Garcinia mangostana* [31], *Ocimum sanctum*, *Bryophyllum* spp., *Cyperus* spp., *Hydrilla* spp., *Rosa rugosa*, and *Chenopodium album* [32], *Azadirachta indica* A. Juss [33], *Eucalyptus globules* [34, 35], etc., have been used to produce silver, gold, zinc, and copper, iron nanoparticles. Nanoparticles produced by plants are more stable and their synthesis rate is faster than that of microorganisms. Plant-based nanoparticles have been proven to be more beneficial due to the presence of biomolecule variability [36]. The plant biomolecules such as alkaloids, phenolics, terpenoids and proteins that leach out during nanoparticle synthesis play a critical role in modulating the NP size, shape, dispersity, and surface properties [37]. They form a protective biomolecular layer or "halo" around the NPs which likely facilitates their interactions with target pathogens [38]. Plant-based materials play a crucial role in green nanomaterial synthesis, using phytochemicals as reducing and capping agents, offering an eco-friendly alternative with enhanced biological effects and interactions with living systems. After the nanoparticles have been synthesized, they must be purified and characterized before being used to confirm their morpho-physical properties that may enhance their efficacy. Various techniques are used for this purpose, each tailored to address specific aspects of purification. Centrifugation, for instance, capitalizes on size and density inequalities to effectively separate nanomaterials from aggregates. Filtration with specific pore-sized membranes eliminates larger contaminants. On the other hand, dialysis aids facilitate the in the removal of small molecules and ions through selective diffusion across membranes. Precipitation methods, including solvent and co-precipitation separate nanomaterials from excess reagents. Additionally, surface modification strategies involving the use of ligands or coatings prevent aggregation and boost colloidal stability. The choice of purification method is influenced by nanomaterial properties, intended use, and desired purity levels that is guided by quality control measures [39]. Several approaches are involved in characterizing the size, shape, crystal structure, and atomic composition. The size and shape of nanomaterials are defined using innovative microscopy methods, including scanning electron microscopy (SEM), UV–visible spectroscopy [40], transmission electron microscopy (TEM), atomic force microscopy (AFM), X-ray fluorescence microscopy (XFM), and scanning transmission X-ray microscopy (STXM) [41]. The different structural characteristics of nanoparticles were determined using nuclear magnetic resonance (NMR). Another essential approach for nanoparticle characterization is X-ray diffraction (XRD). Likewise, the crystalline structures of nanoparticles in various nanomaterials can be revealed using XRD. Furthermore, the charge of NPs is crucial. For example, nanoparticles can carry either a positive (cationic) or negative (anionic) charge, as determined by the type of molecules on their surfaces. This surface charge can be deliberately adjusted during nanoparticle synthesis or functionalization processes [42]. Nanomaterials are rapidly acquiring interest from several scientific segments; therefore, there is a need for the development of novel, cost-effective, and easy approaches for the large-scale synthesis of nanomaterials.

Applications of nanomaterials in agriculture

Nanomaterials possess diverse physicochemical characteristics due to their small size, exhibiting higher reactivity, biochemical activity, and solubility owing to their elevated surface-to-volume ratio. Inorganic nanoparticles, particularly metal oxides like ZnO, TiO2, CuO, and CeO2, are predominantly employed in studies (79%), with metal nanoparticles, notably Ag, representing (25%). Carbon-based nanoparticles are less utilized, accounting for only 10% of studies [43]. These examples highlight the diverse real-world applications of nanomaterials in various aspects of agricultural management, including nutrient delivery, pest and disease control, diagnostics, and plant growth enhancement. Further, NMs have been explored for their potential in enhancing crop disease resistance by modulating plant biochemical and molecular responses, triggering the upregulation of defense-related genes. Several applications of NMs have been explored for controlled release of agrochemicals and nutrients, particularly micronutrients such as Fe, Mn, Zn, Cu, K, Ca, P, etc., enhancing plant biomass and growth [44, 45]. Mg nanoparticles (Mg-NFs) not only enhance seed germination and seedling growth attributes, but also serve as activators. Recent studies have highlighted the beneficial effects of various NPs, including carbon nanotubes (CNTs), silicon dioxide (SiO2), zinc oxide (ZnO), titanium dioxide (TiO2), and gold (Au) NPs, in promoting seed germination in wheat, pearl millet, tomato, soybean, barley, rice, and maize. For example, a study investigated the impacts of nano-biofertilizer on tomato crops affected by *Ralstonia solanacearum* caused bacterial wilt disease and its pest-resistant function against wilt disease [46].

Nanomaterials for enhancing crop resistance to pathogens

Approximately 14% of crops in the world are damaged by infectious diseases caused by plant pathogens, and yield losses could be as high as 20–40% globally [47]. Major cereal crops in the world, such as wheat, rice, barley, and maize, can be easily infected by fungal diseases. Fungal pathogens pose a significant threat to global crop production, accounting 70% of plant diseases. In addition, fungal diseases caused a significant loss of more than 3.41 million tons of wheat in China between 2000 and 2018 [48]. Nanomaterials are an integrated and sustainable approach because of their small size, nanoparticles (NPs) easily penetrate plant pathogens and can affect their disease-causing ability. More than 90% of applied pesticides are either lost in the environment or miss the target regions/microbes for effective disease control. This not only causes harmful impacts on the environment, but also increases the overall production cost for farmers. Therefore, one difficult area of agricultural research that still must be applied is the development of innovative crop protection formulations [10]. Nanomaterials have revolutionized agriculture by improving plant disease resistance. Some examples of how nanomaterials are being used to enhance plant resistance to pathogens have been reported [49]. A recent study investigates the efficacy of five distinct nanoparticles (NPs), namely Co_3O_4, CuO, Fe_3O_4, NiO, and ZnO, in combating Fusarium wilt and promoting common bean plant growth. In vivo experiments demonstrated that all NPs significantly improved resistance with respective disease control values of 92.84% (therapeutic) and 82.77% (protective). The plants were grown under greenhouse conditions. These results underscore the potential of nanomaterials in agriculture as nano-fungicides and nano-fertilizers with promising implications for sustainable agriculture and environmental preservation [50]. For instance, CuO nanoparticles have been used to improve disease resistance against Fusarium crown and root rot, Fusarium wilt, and Verticillium wilt in various plants. Copper and silver NP compounds are highly effective in eliminating several fungal pathogens, including *Aspergillus carbonarius*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus oryzae*, *Candida albicans* and *Cryptococcus neoformans* [51]. Similarly, the antifungal properties of Ag NPs and CuO have been reported to suppress the growth of powdery mildew in different crops. Similarly, the effect of nanoparticles has been studied on *Ustilaginoidea virens* caused by false smut infection of rice [52]. Recent attention has focused on eco-friendly nanoparticle production. This study successfully synthesized cerium oxide nanoparticles (CeO_2 NPs) using a green method involving quinoa leaf extract. The NPs were characterized as spherical clusters with sizes ranging from 7 to 10 nm. Testing of two wheat varieties revealed that higher CeO_2 NPs concentrations significantly reduced disease severity and incidence, particularly at 100 mg/L. These findings suggest promising antifungal potential for CeO_2 NPs against *Ustilago tritici*, offering a potential solution for global crop protection [53]. However, there is still a need for a critical review to discuss the most recent developments in this field and to clarify research areas on their ecological safety and difficult gaps. Overall, the application of nanomaterials against plant pathogens is a promising area of research with the potential to revolutionize the way we protect crops and increase yields. The different NMs could be used via foliar, seed treatment, soil application, and plant root applications against the plant pathogens discussed in Fig. 1.

Copper nanoparticles (Cu NPs)

Copper-based nanoparticles have garnered significant attention for their potential in controlling a wide range of plant pathogens, including bacteria and fungi. Notably, several studies have demonstrated that copper nanoparticles can serve as a more effective and achieving comparable efficacy at lower concentrations. In addition, copper nanoparticles can be applied in a variety of forms, including as a foliar spray, seed coating, or soil amendment, making them versatile for different cropping systems and plant growth stages [54]. Researchers have been particularly fascinated by Cu NPs because of their distinctive biological, chemical, physical, and antibacterial properties. In addition, iron- and copper-based nanoparticles react with peroxides in the environment, generating free radicals that are highly toxic to microorganisms [55]. For instance, studies have demonstrated the significant antifungal activity of Cu NPs against common crop pathogens such as *F. oxysporum*, *F. culmorum*, and *F. equiseti*,

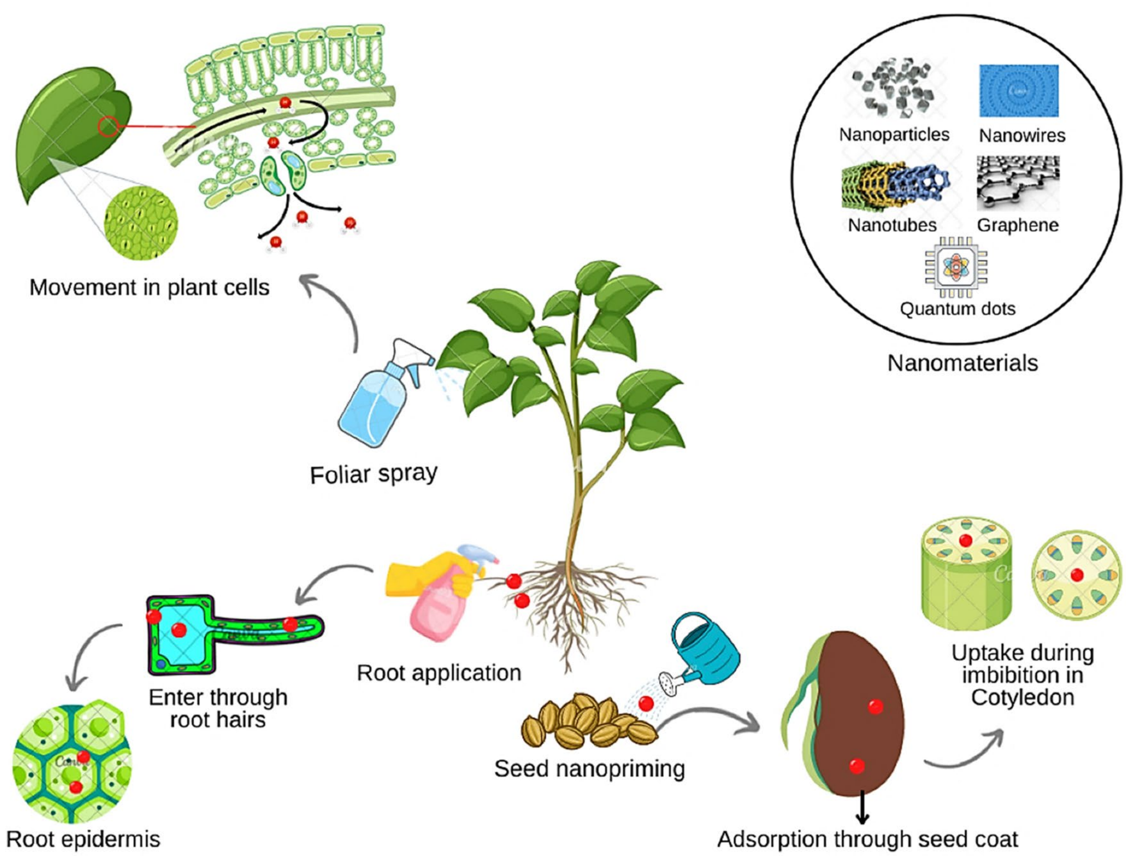


Fig. 1 Illustration describes the application of nanomaterials (NMs). The schematic represents the different ways in which nanomaterials can be applied to protect plants from pathogens. The most common method is through foliar spray, where nanoparticles penetrate plant tissue and provide long-lasting protection against fungal infections and insect pests. Another application is through seed treatments, where nanoparticles can be used to protect seeds from soil-borne pathogens before they are planted. These nanoparticles can form a physical barrier around the seed, preventing pathogens from penetrating and infecting the seed. By binding to the pathogen and preventing it from infecting the plant roots, these nanoparticles can help protect the plant from disease

[56]. Further, the study presents a promising approach for the development of environmentally friendly copper-based fungicides using neem leaf extract. It effectively controlled the pathogens of apple orchards, including *Alternaria mali*, *Diplodia seriata*, and *Botryosphaeria dothidea* [34, 35]. However, it is important to note that the use of copper nanoparticles for plant pathogen control is still in its early stages, and more research is needed to fully understand their effect on plants and the environment. Also, overuse of copper nanoparticles can lead to copper toxicity, which is harmful to plants.

Silver nanoparticles (Ag NPs)

The most prevalent inorganic NPs used for antimicrobial properties are Ag NPs. These were the first nanoparticles to be used in agriculture to combat plant pathogens [57]. Silver is known for its broad-spectrum antimicrobial properties and its ability to disrupt the cell membrane of pathogens. At the molecular level, research has shown that Ag NPs can enhance plant disease resistance by inducing the production of important defense compounds in plants. Studies report Ag NP treatment leads to increased biosynthesis of phytoalexins, phenolic compounds, terpenoids and polyphenols known to play critical roles in the plant defense response against pathogens [58]. Various soil-borne diseases have been controlled by using silver nanoparticles including *Phytophthora parasitica*, *Meloidogyne* spp., and *Fusarium* spp. [59]. During field tests**,** a reduction in the symptoms of powdery mildew in cucumber and pumpkin caused by the fungi *Golovinomyces cichoracearum* and *Sphaerotheca fusca* was reported after Ag NP sprays at 10–100 mg/L [60]. Additionally, at a concentration of 15 mg/mL, the biosynthesized Ag-NPs showed outstanding inhibitory effectiveness against *Curvularia lunata*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotinia sclerotiorum*, *Alternaria alternata*, and *Botrytis cinerea* [61]. Furthermore, biosynthesized nanoparticles from various sources have shown considerable antibacterial activity against pathogenic bacteria in vitro and in vivo. Similarly, plant pathogenic fungi such as *Bipolaris sorokiniana* and *Magnaporthe grisea* have been reported to be suppressed by several types of silver ions and nanoparticles [62]. However, silver nanoparticles (AgNPs) were synthesized using aqueous leaf extract obtained from *Aloysia citrodora* and evaluated for their antifungal activity against soil-borne and airborne pathogens, including *Pythium aphanidermatum, Paecillomyces formosus, Macrophomina phaseolina,* and *Botrytis cinerea*. The study demonstrated significant inhibition of mycelial growth. These findings suggest that environmentally friendly biosynthesized AgNPs possess more potential to combat phytopathogens in the agricultural sector [7, 63].

Zinc oxide nanoparticles (ZnO NPs)

ZnO-NPs as a sustainable alternative for controlling harmful plant pathogens and safeguarding global food security. In the literature, they have been found to be effective against fungal pathogens such as *Fusarium oxysporum* and *Botrytis cinerea*, bacterial pathogens such as *Pseudomonas syringae*, and viral pathogens such as tobacco mosaic virus (TMV) [64]. At the molecular level, ZnO NPs can interact with various cellular components in plant cells to enhance disease resistance. These interactions include interactions with plant cell membranes, proteins, DNA, and other cellular components that can inhibit the growth and reproduction of disease-causing microorganisms. Mechanically, ZnO NPs can also induce the production of reactive oxygen species (ROS) within plant cells, which can inhibit the growth of pathogens. Numerous scientists have studied zinc oxide nanoparticles and discovered that they effectively reduce fungal growth in crops. For example, ZnO NPs produced distortion in fungal hyphae and inhibited the production of conidiophores and conidia, according to scanning electron microscopy (SEM) photographs and Raman spectra [65]. Similarly, the effectiveness of zinc compounds in preventing wheat deoxynivalenol production and Fusarium head blight was investigated. The impact of pre-sowing seed application with metal nanoparticles (Zn, Ag, Fe, Mn, and Cu) on the development of resistance in wheat seedlings infected with *Pseudocercosporella herpotrichoides* was demonstrated by [66]. In a recent study, ZnO NPs were employed to protect tomato plants against Fusarium wilt. These ZnO NPs exhibited significant potential as inducers of plant physiological immunity against Fusarium wilt, reducing disease incidence by 28.57% and providing high protection by 67.99% against *F. oxysporum*. Furthermore, they enhanced various growth parameters and biochemical compounds indicating their effectiveness in controlling and fortifying plants against *fusarial* infection [67]. Hence, the exact molecular and mechanistic aspects of zinc oxide nanoparticles in plant disease resistance are still not fully understood, and more research is needed to fully understand their mechanisms of action.

Chitosan nanoparticles

Chitosan nanoparticles show promise as biopesticides for crop protection due to their broad-spectrum antifungal activity, biocompatibility with plant materials, and low environmental toxicity. Their reported efficacy against an array of phytopathogenic fungi combined with benign safety profile make chitosan nanoparticles an attractive option for sustainable disease control in agricultural systems [68]. Due to its sustainable and safe properties, chitosan has become the material of choice to produce nanoparticles in agricultural fields. Previous studies suggest their high molecular weight, cationic charge, and surface hydrophobicity enable interaction with negatively charged fungal cell membranes. This interaction is pondered to disrupt membrane integrity through mechanisms such as increased permeability. For example, in a study by Zheng et al. [69], chitosan effectively enhanced resistance to *Phytophthora infestans* in potted potatoes. In vitro studies have shown that chitosan treatment can significantly reduce leaf lesion sizes. Chitosan nanoparticles exhibited substantially smaller lesion sizes compared to untreated control leaves when assessed 5–7 days post-infection. Chitosan at 0.5 g/L provided 46.0% protection, which was slightly higher than 0.25 g/L (35.5%). Furthermore, transcriptomics revealed chitosan-producing resistance, as confirmed by qRT-PCR analysis. A total of 11,410 differentially expressed genes (DEGs) were identified, with 6026 genes showing upregulation and 5384 genes showing downregulation. Chitosan appeared to upregulate these DEGs, indicating a positive response of potatoes to chitosan treatment. It also induced ROS- and SA-related gene expression, confirming disease resistance against *P. infestans*. Chitosan is essential to induce resistance in different crops. Studies have demonstrated that chitosan nanoparticles have antimicrobial activities and reduced disease severity against many plant pathogens, including *Fusarium graminearum*, *F. oxysporum*, *Phytophthora infestans*, *Xanthomonas campestris*, *Erwinia carotovora*, *Pseudomonas syringae*, and *Clavibacter michiganensis* [70]. For example, chitosan NPs have been found to be resistant against fungal pathogens including *Aspergillus niger*, *Alternaria alternata*, *Rhizopus oryzae*, *Phomopsis asparagi*, and *Rhizopus stolonifer* [71, 72]. In the literature, studies have shown that chitosan NPs can inhibit the growth of the plant pathogen *Clavibacter michiganensis* by disrupting its cell wall. Chitosan NPs can increase the production of defense-related enzymes such as peroxidases and catalases leading to enhanced resistance to pathogens [73]. Further, chitosan NPs have been found to induce the production of ROS and systemic acquired resistance (SAR) [74]. Similarly, Cu-chitosan nanomaterials have been tested for their ability to promote plant growth and enhance systemic resistance to the *Curvularia* leaf spot (CLS) disease of maize. Higher antioxidant (superoxide dismutase and peroxidase) and enzyme activities were evidence of a significant response in plants exposed to Cu-chitosan NPs [75]. However, it is important to keep in mind that the understanding of how chitosan nanoparticles work at the molecular and mechanistic level to enhance plant disease resistance is still ongoing, and further research is needed to fully understand their impact on plants and the environment. Several nanomaterials used for disease resistance in different crops are summarized in Table 1.

Nanomaterials as activators of immune responses in crops against pathogens

Plants possess natural immune systems that allow them to detect invading pathogens and launch tailored defense responses. Using pattern recognition receptors, plants can recognize signatures associated with bacteria and fungi. Upon pathogen perception, complex signaling networks are activated that initiate multifaceted immune responses aimed at protecting the plant and limiting infection spread [106]. They activate defense mechanisms including chemical responses and gene regulation which all work together to protect plants from diseases and infections. This process leads to the production of reactive oxygen species (ROS), antioxidants, and stimulation of important stress-related enzymes. Some nanomaterials can simulate the molecular patterns of specific pathogens without causing harm to the plant resulting in increased resistance to the disease. In the literature, chitosan, liposomes, and polysaccharide NPs have been reported to trigger plant immune responses that lead to the expression of defense-related genes and have been found to significantly reduce the severity of plant diseases caused by bacteria, fungi, and viruses [107, 108]. Plant viruses contribute significantly to agricultural losses, accounting for nearly 47% of crop damage. NMs show promise in combating several viruses like potato virus Y (PVY), cucumber mosaic virus (CMV), and bean yellow mosaic virus (BYMV). NPs serve as delivery vehicles for nucleic acids, sustaining plant immunity to develop resistance against viral infection via RNA interference (RNAi) [109]. Further, they act as standalone protectants or carriers for pesticides and RNA-interference compounds, while also exhibiting virucidal activity through mechanisms such as reactive oxygen species (ROS) production and interference with viral binding to manage plant diseases. Nanoparticles interfere with virus recognition by host cells through their interactions with virus surface proteins via glycoprotein receptors. For example, zinc oxide NPs (ZnONPs), iron oxide NPs ($Fe3O4$NPs), and Schiff-based nano-silver NPs have shown inhibitory effects against tobacco mosaic virus (TMV) infection [110]. Similarly, clay nanosheets loaded with plasmid DNA expressing artificial microRNAs (amiRNAs) have demonstrated efficacy against tomato yellow leaf curl virus [111]. Additionally, nanomaterials enhance the stability, translational efficiency, and cellular targeting of mRNA in plant genetic engineering, exemplified by BioClay-mediated protection against pepper mild mottle

Table 1 Application of nanomaterials for disease resistance in crops

Sr. no.	Nanomaterial	Pathogen	Activity	References
1	Ag NP	*Xanthomonas campestris and X. axonopodis*	Antimicrobial activity	[76]
2	Ag NPs	*Bipolaris sorokiniana*	Inhibited colony formation (in vitro)	[62]
3	Ag NP	*Botrytis cinerea (grey mold)*	Antifungal properties	[51]
4	Ag NPs	*Alternaria alternata, Macrophomina phaseolina,*	Strong antifungal activity	[77]
5	Ag NPs (solution)	*Sphaerotheca fusca*	Inhibited the fungus growth (in vitro and vivo)	[78]
6	Ag NPs	*F. culmorum*	Inhibited the fungus growth (in vitro)	[79]
7	Ag NPs	*Xanthomonas oryzae* pv. oryzae	Antibacterial activity	[80]
9	Ag NPs	*Fusarium oxysporum*	Fusarium wilt disease management	[81]
10	Ag NPs	*Bean yellow mosaic virus*	Mosaic disease	[82]
11	Ag NPs	*Phytophthora arenaria*	Crown and root rot disease inhibition	[83]
12	Ag NPs	*Pyricularia grisea*	Antifungal activity	[84]
14	Ag NPs	*Rhizoctonia*	Solani sheath blight	[85]
15	Ag NPs	*Fusarium graminearum, F. sporotrichioides, F. avenaceum*	Inhibited the development of fungus	[86]
18	Cu NPs	*F. culmorum, F. oxysporum*	Inhibited fungal growth	[87]
19	Cu NPs	*Alternate, P. destructiva*	Antifungal activity	[88]
20	Cu NPs	*Penicillium digitatum*	Green rot	[83]
21	CuO-NPs	*Colletotrichum gloeoesporioides*	Antifungal activity	[89]
22	ZnO NPs	*Burkholderia glumae*	Antibacterial activity	[90]
23	TiO_2 NPs	*Bipolaris sorghicola*	Target leaf spot	[91]
24	TiO_2 NPs	*Dickeya dadantii*	Antimicrobial agent	[92]
25	TiO_2 NPs	*Bipolaris sorokiniana*	Spot blotch disease	[93]
26	SiO_2 NPs	*Aspergillus flavus*	Reduced Ear rot disease	[91]
27	SiO_2 NPs	*Tobacco mosaic virus*	Mosaic disease	[94]
28	Chitosan NPs	*Xanthomonas, Erwinia strains*	Inhibited the growth of both pathogens	[95]
29	Chitosan-magnesium NPs	*A. oryzae and R. solani*	Antimicrobial agent	[96]
30	CS NPs	*Fusarium graminearum*	Antifungal agent	[97]
32	Titanium dioxide (TiO_2) NPs	*wheat rust (Ustilago tritici)*	Antifungal activity	[98]
33	Cu-chitosan	*Leaf and stem rust of wheat*	Inhibited to diseases	[99]
34	Chitosan	*Alternaria leaf spot disease*	Fungicidal properties	[44]
35	Chitosan	*C. gelosporidies, G. fujikori, P. capsici, S. sclerotiorum, F. oxysporum*	Antifungal activity	[95]
36	Chitosan NPs	*Pyricularia oryzae*	Antifungal activity	[100]
37	MgO NPs	*Rhizoctonia solani; Acidovorax oryzae*	Antimicrobial agent	[96]
38	MgO NPs	*Xanthomonas oryzae oryzae*	Antibacterial activities	[101]
39	Silicon dioxide NPs	*Xanthomonas oryzae pv. oryzae (Xoo)*	Antibacterial action	[102]
40	Al2O3 NPs	*Fusarium root rot*	Inhibited fungal growth	[103]
41	CWP-NPs	*F. oxysporum* f.sp. lycopersici	Antifungal activity	[104]
42	Se NPs	*Phytophthora infestans*	Antifungal activity, elicit resistance	[105]

virus (PMMoV) and CMV [112]. Furthermore, NMs target cell shapes, membrane integrity, essential biomolecules, enzymes, and pathogen-related proteins to exert inhibitory and anti-microbicidal effects. Scientists have postulated that NMs activate reactive oxygen species (ROS) and secondary signaling messengers that result in transcriptional regulation within plant secondary metabolism, but much work still needs to be done to clarify the mechanism [113]. Previously, scientists have used nanofibers, nano-capsules, and nanoparticles to successfully regulate gene expression. For example, the different changes in gene expression were studied by quantitative RT-PCR, and relative levels of expression of PR1, LoxA, Osm, and GluA were measured in roots and hypocotyls of plants at 12, 24, 72, and 120 h after treatment [114]. In the model plant *Arabidopsis thaliana*, ROS production condensed by Ag NPs lowered stress enzymes and induced autophagy. Multiple deformations on the spores of *A. brassicicola* were discovered using a scanning electron microscope [115]. Likewise, *Rhizoctonia solani*, *Macrophomina phaseolina*, and *Alternaria alternata* can all be inhibited by Cu-CS NPs [116].

PR proteins

Upon pathogen detection, plants rapidly mobilize diverse defense mechanisms to combat infection. Among these, the timely induction of pathogenesis-related (PR) proteins plays a pivotal role in establishing early resistance against invading microbial pathogens. This is initiated within the cell by the identification of pathogen effectors through plant resistance proteins, frequently the nucleotide binding site (NBS)-leucine rich repeat (LRR) proteins. Furthermore, to manage plant diseases, systemic acquired resistance (SAR) also uses a natural signaling pathway comprising SA, reactive oxygen species (ROS), and nitric oxide (NO). Salicylic acid (SA) contributes to SAR by activating genes involved in pathogenesis (PR) [117]. Similarly, resistance genes are expressed in plants after pathogen infection when nanoparticles (NMs) are applied. For example, silicon nanoparticles (SNPs) demonstrate efficacy in activating tomato plant defenses via systemic acquired resistance pathways, as evidenced by the upregulation of crucial pathogenesis-related and antioxidant genes upon application. Ultrastructural analysis reveals SNP distribution in plant tissues directly correlates with inhibition of in planta pathogens. SNPs mitigate reactive oxygen species, membrane damage, and pathogen growth, underscoring their potential as a sustainable bioprotectant to enhance crop resistance against infection through diverse complementary mechanisms [118]. Similarly, bacterial growth on Arabidopsis leaves was assessed to quantify local systemic resistance to a virulent strain of *P. syringae* under control conditions. SiO_2 NP and Si (OH)4 application revealed a mechanistic insight into the processes involved in the induced triggering of SAR [119].

Reactive oxygen species (ROS)

ROS, including hydrogen peroxide (H_2O_2), superoxide (O_2^-), and hydroxyl radicals (OH·), are highly reactive molecules generated as part of the plant's defense mechanisms when opposed by pathogenic microorganisms such as fungi, bacteria, and viruses. These ROS have the capability to harm cell membranes, proteins, and DNA, resulting in the pathogen's death [120]. The integration of nanomaterials in agriculture has led to a new era of innovative strategies to combat plant diseases and enhance crop productivity. In this context, the role of ROS in plant pathogen interactions has attracted substantial attention. Few nanomaterials can imitate the molecular patterns of pathogens and trigger the production of ROS in plants. The production of ROS by these nanoparticles leads to an oxidative burst, which can cause damage to the pathogen's cell membrane and ultimately kill the pathogen [121]. Genetically, specific enzymes produce ROS under certain developmental or hormonal regulation to initiate or propagate signaling pathways [122]. For instance, the expression of defense-related genes, reactive oxygen species, and ATP-binding cassette (ABC) in wheat increased quickly after inoculation and pathogen attack. Similarly, ROS were activated during the application of NMs as the antimicrobial properties of graphene oxide nanoparticles were also observed, similar to pathogen membrane damage [123]. Conclusively, the production of ROS by nanomaterials can be an effective mechanism for fighting plant pathogens and can be an efficient way to reduce the use of chemical pesticides. However, the production of high levels of ROS can also be detrimental to the plant; therefore, it is important to find the optimal balance between activating the plant's defense mechanisms and not causing harm to the plant.

NMs for the induction of phytohormones and signaling molecules

Plants activate the immune system in response to pathogen attack, resulting in several physiological alterations in the plant body. The induction of immune responses is hypothesized to be regulated by phytohormones (jasmonic acid, methyl jasmonate, and salicylic acid) and signaling molecules (reactive oxygen species) [124]. Likewise, liposomes which are spherical formations consisting of a double layer of phospholipids have the capability to enclose and transport these molecules directly to plant cells. This action initiates the activation of genes associated with the plant defense system [68]. For instance, polymeric nanoparticles such as poly (lactic-co-glycolic acid) (PLGA) nanoparticles can be used to deliver phytohormones and signaling molecules to plants [125]. In addition, chitosan nanoparticles have been explored as a delivery vehicle for phytohormones and signaling molecules. These NPs can protect the delivered molecules from degradation and target them to specific cells in the plant leading to an enhanced defense mechanism as shown in Fig. 2.

Furthermore, the natural immune response is activated when pathogen-associated molecular patterns (PAMPs) are perceived by the host plants, which inhibits the development of plant infection. Plant hormones primarily control the events that affect how plants grow and develop. Abscisic acid (ABA), auxins, cytokinin (CK), gibberellins (GA), brassino steroids (BR), ethylene (ET), salicylic acid (SA), and peptide production have all been shown to vary in plants that are infected [127]. Similarly, the inhibition of the rice blast fungus *Pyricularia grisea*

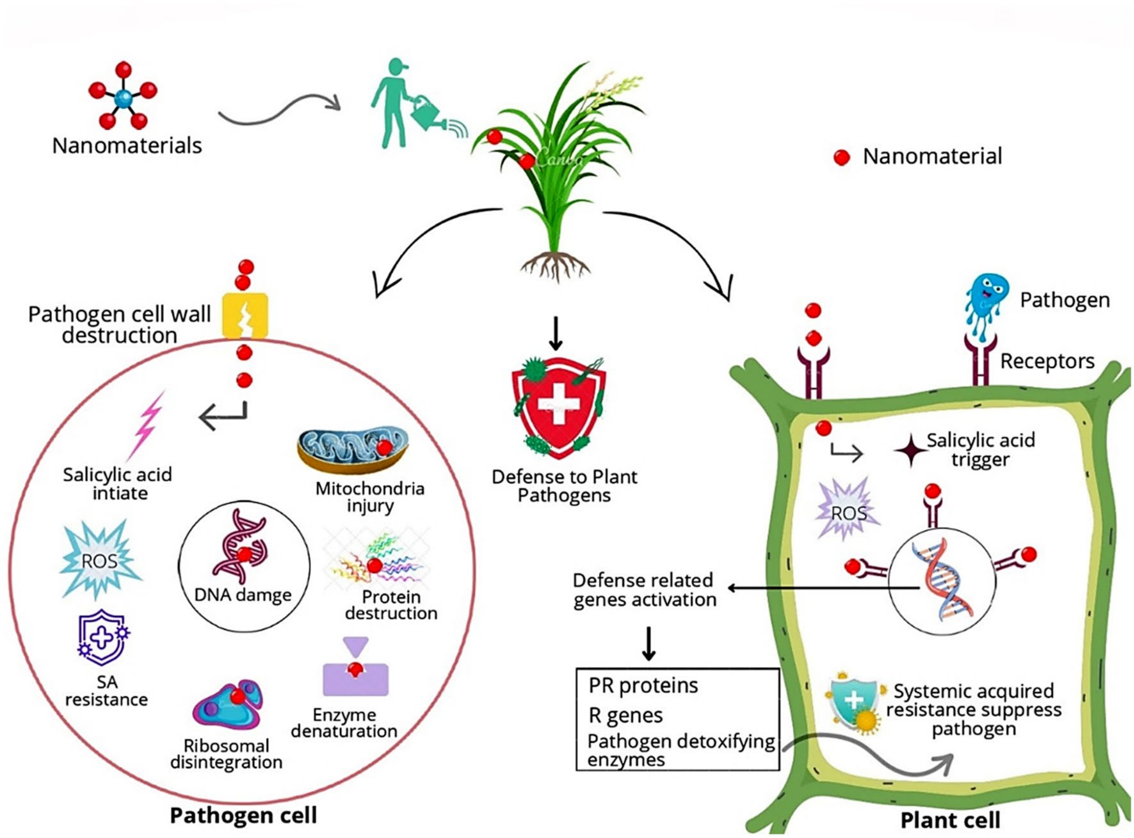


Fig. 2 Nanomaterials activate defense responses in crops against pathogens. The diagram provides a clear illustration of the role of nanomaterials in activating immune responses and suppressing plant pathogens. One example of this is the use of SiO_2 NPs, as demonstrated in a recent study [126]. Nanomaterials work through various mechanisms in both plant and pathogen cells, including the activation of immune responses and the destruction of pathogen cells. One important mechanism through which nanomaterials activate immune responses in plants is through the induction of systemic acquired resistance (SAR). Elicitor application to plants can also activate signals to distant tissues, and salicylic acid (SA) is a plant hormone that plays a significant role in the initiation of SAR by activating pathogenesis-related (PR) genes. Nanomaterials can also be effective in destroying pathogen cells by regulating signaling pathways and inducing the production of salicylic acid and reactive oxygen species (ROS). This can help suppress the growth and spread of plant pathogens and prevent damage to crops

by chitosan nanoparticles is one example and some polymeric nanoparticles can also induce resistance against diverse plant pathogens. Changes in gene expression such as those for peroxidase, phenylalanine ammonia lyase, catalase, superoxide, and polyphenol oxidase were linked to greater resistance against pearl millet downy mildew following treatment of seeds with chitosan nanoparticles [128]. Copper and silicon nanomaterials can increase the amount of both enzymatic and non-enzymatic plant immune chemicals in tomato, leading to increased disease tolerance to *Clavibacter michiganensis* and ultimately increasing tomato crop performance [129]. Similarly, other research examined the antibacterial activity of magnesium oxide nanoparticles (MgO NPs) and its implications on disease resistance in tomato plants against *Ralstonia solanacearum*. After treatment with MgO NPs, tomato plant roots and hypocotyls showed increased levels of salicylic acid-inducible PR1, jasmonate LoxA, and systemic resistance-related GluA [114].

Defense-related enzymes

Molecular biologists stated that proteins perform all the tasks necessary for crop development, maturity, and immunity. Specific enzymes and peptides have antimicrobial properties. The researchers investigated defense mechanisms at the biochemical, cellular, and transcriptomic levels. Transcriptional analysis found mycogenic SeNPs upregulated important genes related to phenylalanine lyase, lipoxygenase, β-1,3-glucanase and superoxide dismutase, correlated with increased enzymatic activities important for biochemical defenses. Treated plants also accumulated significantly higher levels of important cellular defense molecules like callose, lignin and hydrogen peroxide compared to controls. These findings provided mechanistic insights showing mycogenic SeNPs activate robust biochemical and molecular defenses in tomatoes to combat late blight infection. Enzymatic and non-enzymatic anti-oxidative defense mechanisms are both activated in plants and combine to eliminate free radicals [130]. For instance, research has shown that silver NPs can induce the production of enzymes such as peroxidases and catalases in plants which can help to produce resistance against pathogens by breaking down harmful molecules and reducing oxidative stress. Similarly, NMs enhanced plant growth and boost the levels of self-protective enzymes including super oxide dismutase, CAT (catalase), and phenylalanine ammonia lyase (PAL), respectively [131]. The application of chitosan-based nanomaterials to maize improves resistance against plant pathogens by modifying ROS-scavenging enzymes such as CAT, peroxidase POD, and SOD [132]. Phenylalanine ammonia lyase is an important enzyme that produces antimicrobial molecules (like phytoalexins and pathogen-related proteins) and facilitates colonization around the infection point in plants [133]. The levels of lignin, callose, and hydrogen peroxide that act as the cells defense in the primed plants recorded a significant increase above the control plants. Similarly, Se NPs can be employed as a nano-bio stimulant antifungal to tomato plants by triggering immune responses against tomato late blight [105]. It has been reported by [134] that tomato-treated plants using chitosan nanoparticles increased the expression of SOD and CAT and protected the plants against bacterial wilt disease. Similarly, Sathiyabama and Indhumathi [135] conducted the latest study to determine how chitosan thiamine nanoparticles (TC NPs) affected the activation of innate immunity in chickpeas against stress brought on by the wilt pathogen *Fusarium oxysporum* f. sp. *ciceri* (FOC) under greenhouse conditions. In plants treated with TC NPs, there was more than 90% wilt resistance. In TC NPs-treated plants, histochemical staining revealed significant lignin development in the vascular bundles of chickpea stem cells. Several nanomaterials that induce immunity against plant pathogens are given in Table 2.

Nanomaterials as diagnostic tools for crop plant diseases

Identification of plant pathogens and disease incidence is important for managing plant disease and infestation. It allows for the observation and management of disease infections at different phases of the disease development cycle in open field and greenhouse studies. Plant pathogens have been widely detected using a variety of approaches including biomarkers, immunoassays, serological tests, and DNA-based techniques [145]. Hence, remote sensing tools are valuable for disease detection in plants. The core concept of remote sensing involves the use of non-contact, regularly monitoring instruments such as infrared red, chlorophyll fluorescence detection, and 3D scanning to collect information regarding activities occurring in both natural and human-engineered environments. For example, in a laboratory setting, Bawden noticed in 1933 the dramatic differences seen between necrotic leaf patches created by potato and tobacco viruses in an infrared image. The necrotic cells in potatoes contained chemical breakdown products, whereas the necrotic cells in tobacco were empty and differed in color from healthy leaf cells. These discoveries laid the foundation for the use of various spectral bands to identify variations in plant health [146]. Nanomaterials such as nanoparticles and nanocomposites have potential applications as diagnostic tools for plant disease

Table 2 Application of nanomaterials that induce resistance to crop plant pathogens

Sr. no.	Nanomaterial	Pathogen	Activity	References
1	Chitosan oligosaccharide nanoparticles (Cos-La)	*Magnaporthe grisea*	Defense response against rice blast	[136]
2	Fe_3O_4 NPs	*TMV*	Resistance against TMV through upregulation of SA genes	[110]
3	ZnO NPs	*TMV*	Deactivation of TMV particles and regulation of plant immunity	[137]
4	NiO NPs	*Cucumber mosaic virus CMV*	Reduced virus titer	[138]
5	Ag NPs	*PVY, ToMV*	Induce SAR, suppress their infection on tomato plants	[139]
6	Ag NPs	*Cucumis sativus* (crop)	Activated antioxidant defense systems	[140]
7	CuO NPs	*Lens culinaris* (Crop)	Defense enzyme activator	[141]
8	ZnO NPs	*Tomato mosaic virus* (ToMV)	Induce defense system activity	[142]
9	Elemental Sulfur NPs	*Fusarium oxysporum* f. sp. *lycopersici*	Salicylic acid-dependent systemic acquired resistance	[118]
10	Carbon-based NPs	*Fusarium wilt in tomato*	Antioxidant defense system	[143]
11	CuFe NPs	*Meloidogyne incognita and Meloidogyne*	Nematicide activity	[144]

identification. For instance, gold nanoparticles have also been explored as a potential tool for detecting specific plant pathogens [147]. Gold nanoparticles have also been employed for the detection of *Begomovirus* in chili and tomato plants, with the capability to detect 500 ag/µL of begomoviral DNA [148]. Furthermore, researchers have used this approach to detect specific plant pathogens such as *Phytophthora infestans*, a fungal pathogen that causes potato and tomato late blight, and *Xanthomonas campestris pv. vesicatoria*, a bacterium that causes bacterial spot disease in pepper and tomato. Moreover, nano-biosensors are very helpful such as molecular assays and smartphone apps for plant pathogen detection and disease monitoring in comparison with traditional methods [149]. Similarly, remote sensing techniques are used because plant infections and pest attacks normally alter how light interacts with leaves and branches. The use of nanosensors in plant quarantine and seed certification may prove to be an efficient and precise method for the identification of pathogen infections in plants. Therefore, the high sensitivity and specificity of NM-based biosensors, which are essential for the early diagnosis of plant pathogens, are just a few of their many advantages over conventional biosensors [150]. However, nano-biosensors are designed to interact with biological entities such as proteins, nucleic acids, cells, or even whole organisms enabling the detection of various biochemical processes, biomarkers, or pathogens [151]. These sensors revolutionize agriculture by swiftly detecting plant pathogens through nanomaterials and bioreceptors, enhancing accuracy. Nano-biosensors exhibit remarkable sensitivity detecting even trace amounts of pathogens, thereby aiding in early disease diagnosis. They are more useful for smart agriculture because of their low detection limit and high sensitivity [152]. The agricultural land could be monitored in real time using a nano-biosensor with a Global Positioning System (GPS). This technique allows for the early detection of the pathogen and information on crop growth. For example, a study reported that surface plasmon resonance can be used by nano-gold-based immunosensors to identify the pathogen *Tilletia indica* that causes Karnal bunt in wheat [153]. Similarly, gold NPs are used in nano-biosensors because of their transiting nature between optical and electrochemical methods for pathogen identification [154]. Furthermore, a metalloporphyrin-based e-nose offers a novel approach for accurately identifying and tracking Fusarium-infected wheat grains. Metalloporphyrins, as complex molecules with metal ions exhibit specific interactions with volatile organic compounds (VOCs) released by plants during stressful conditions including those generated as a response to pathogenic infections [154]. However, in plant tissue culture different NPs including ZnO NPs, TiO_2 NPs, and Ag NPs, are primarily utilized to limit microbial activity.

Electronic nose (e-nose) system

The relationship between electronic noses and nanomaterials lies in the development and improvement of sensors used in electronic nose systems. Nanomaterials can be employed in various ways to enhance the performance of e-nose sensors. A sensor-based intelligent device called an “e-nose system" is created to recognize and classify complex scents using various non-selective sensors. The electronic nose (e-nose) system is inspired by the human olfactory system, which has diverse applications spanning food quality assessment, environmental monitoring, and safety enhancement. In the context of plant pathology, e-noses offer a rapid and non-destructive approach for the early detection of plant pathogens. By using an array of gas sensors, these systems mimic human olfaction and detect volatile organic compounds (VOCs) emitted by plants in response to pathogen infections. This technology has the potential to revolutionize plant disease management by enabling proactive measures against the spread of diseases and crop losses [155]. For example, an e-nose system was developed to detect and differentiate between fungal pathogens affecting wheat crops based on the distinct VOC profiles emitted by each pathogen. The results demonstrated the system's ability to accurately classify infected wheat samples and identify the specific pathogens responsible for the infections. Another study focused on the use of an e-nose for the early detection of bacterial canker disease in tomato plants. By analyzing the VOCs released by infected plants, the e-nose system successfully discriminated between healthy and infected tomato samples, demonstrating its potential as an efficient diagnostic tool [156]. Similarly, E-noses are a reliable method for identifying fungal infections on rice grains before visible symptoms appear. Jiarpinijnun et al. [157] used to detect Aspergillus fungus presence on Jasmine brown rice grains. Furthermore, it has shown promise in real-world agricultural settings. An e-nose system was deployed to monitor and identify the presence of the fungus *Botrytis cinerea* in grapevine crops [158]. The system's accuracy in detecting the pathogen was demonstrated through its ability to detect infected plants even before visible symptoms were apparent, facilitating timely interventions to control the disease and minimize crop losses. Through its ability to accurately detect VOCs emitted by infected plants, e-nose offers a non-invasive, early detection approach that can aid in reducing the spread of plant pathogens and enhancing agricultural productivity.

Moreover, the use of specifically designed nano-biosensors can be developed to contribute to sustainable agriculture through the reduction of plant pathogens. A biosensor is designed to detect pathogens and signals when control measures are required. However, it does not possess the capability to directly control or mitigate the pathogens themselves. In addition, hyperspectral sensors streamline daily tasks by enabling them to be performed on smartphones, enhancing efficiency. Unlike conventional methods that require extensive field monitoring, farmers can now assess crops more efficiently using hyperspectral sensors. Farmers can effectively collect information such as the spectral signature of crops and interact with agricultural organizations to run their land from their homes [159]. A smartphone application called Dr. Lada was created in Malaysia by scientists at the University Kebangsaan Malaysia [160]. This program was employed to identify pathogens and pests in pepper. By enabling early detection of plant pathogens, these sensors can help prevent the spread of disease and reduce the economic losses associated with crop damage. Furthermore, pre-symptomatic and disease-specific identification and the impact on the environment remain significant issues in the electronic monitoring of plant pathology. By responding to questions, users could determine whether a pest or disease infection was present which reduced the need for farmers to rely on agricultural officers and allowed them to identify diseases on their own. Furthermore, it should be emphasized that managing farmers' demands must be the primary objective of electronic plant pathology. However, more research is needed to fully understand the potential of nanomaterials as diagnostic tools for plant disease identification and to ensure their safe and effective use in agriculture (Fig. 3).

Direct applications of nanomaterials against crop pathogens

The accurate method of direct application of nanomaterials against pathogens remains unclear. Usually, leaves, roots, and other vegetative portions of different plants absorb nanomaterials. NMs enter the plant via natural opening sites such as stomata, hydathodes, stigmas, and wounds [164]. Many assumptions on how nanomaterials work is consistent with their distinctive physicochemical characteristics, such as size, surface-to-volume ratio, and shape. NMs of smaller sizes have a greater surface area per unit volume which increases the possibility that they meet bacteria, viruses, and fungi which can cause cell death and damage. The exact mechanism of action of nanomaterials against plant pathogens can vary depending on the specific nanomaterial, pathogen type, and environmental conditions. However, many nanomaterials such as silver, zinc oxide, and titanium dioxide NPs can generate reactive oxygen species (ROS) when exposed to light or other forms of energy. These ROS can damage the cell membrane and DNA of pathogens leading to their death [165]. Similarly, chitosan nanoparticles can inhibit the activity of enzymes essential for the survival of pathogens. Nanoparticles can interfere with the metabolic pathways of pathogens, which makes it difficult for the pathogens to survive. Several studies have been reported and the in vitro antiviral activity of SiO_2 NPs and ZnO NPs against TMV was revealed in an investigation. It has been hypothesized that NPs could directly interact with viral capsid proteins leading to structural distortion that causes TMV aggregation and rapid viral particle inactivation [110]. In the case of viruses, nanoparticles (NPs) exhibit direct antiviral effects by interfering with viral genome replication and protein biosynthesis, particularly viral coat protein. They impede viral genome packaging and capsid protein degradation crucial for virus particle assembly. Additionally, NPs may disrupt plant cell electron transport systems, enhance cellular barriers, block viral entry, and inhibit viral DNA replication [109]. Moreover, little is known about the temporal changes in infection and efficient treatment plans. Each of these elements must be understood at a systematic and molecular level to fully investigate and recognize the potential of NM management techniques. It is also important to appropriately evaluate NPs including their placement, dosage, and timing to effectively counter pathogens. The use of nanoparticles in plant pathology is still under research and not widely used in commercial agriculture.

Biosafety perspective

The use of nanomaterials (NMs) against plant pathogens raises several concerns from a biosafety perspective. To fill the information gap about the hazardous consequences of NMs when they enter a different plant species, a deeper understanding of the biosafety issues is mandatory. For instance, particle size significantly impacts their physical and chemical characteristics as smaller nanoparticles (< 10 nm), higher concentrations, and specific shapes induce greater phytotoxic effects, contrasting with larger, lower-concentration, or more spherical nanoparticles [166, 167]. Variability in plant species sensitivity to nanoparticle exposure is observed; titanium dioxide NPs triggered higher reactive oxygen species production and DNA damage in wheat roots compared to soybean [168]. Conversely, zinc oxide NPs inhibited rice shoot and root growth more profoundly than cucumber, with cucumber displaying enhanced antioxidant activity against NP-induced oxidative stress [169]. However, the main use of nanomaterials is to reduce the need for agrochemicals fertilizers and fungicides while increasing production through effective management of plant pathogens and pests, though frequent use of fungicides can cause

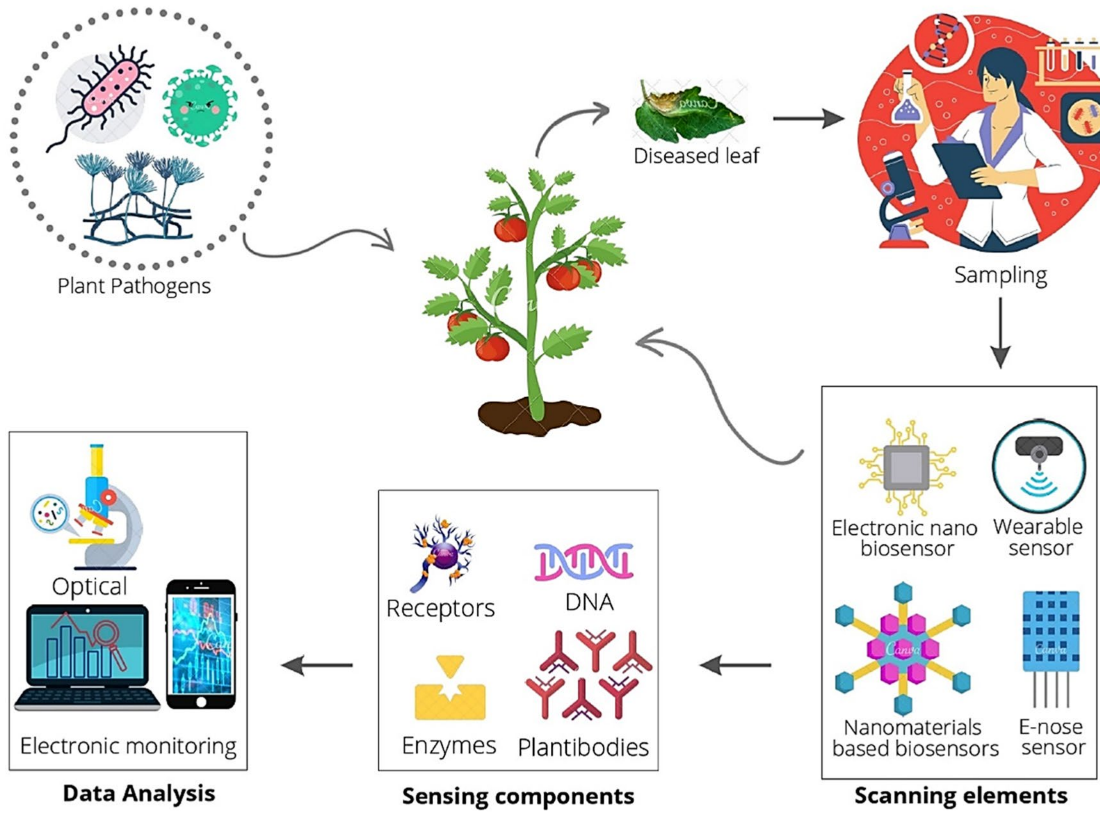


Fig. 3 Nanomaterials as diagnostic tools. This illustration describes the process of plant pathogen identification using electronic nano-biosensors and nanomaterial-based sensors. In the first stage, sensing components are used to detect pathogenic molecules in plant tissues. These sensing components are typically electronic devices that are functionalized with biological recognition elements, such as plantibodies*, that can specifically bind to pathogenic molecules. When the pathogenic molecule binds to the recognition element, it triggers a signal that can be detected by the electronic device. In the second stage, the scanning elements typically consist of nanomaterial-based scanning elements, such as carbon nanotubes or gold nanoparticles, which are functionalized with biological recognition elements. When pathogenic cells or spores meet the nanomaterials, they bind to the recognition element on the nanomaterials. This binding between the pathogenic cells and the nanomaterials triggers a change in the nanomaterials themselves. These changes can be their chemical properties, such as color or conductivity. Here is a highly sensitive device designed (nano-sensor) to detect even the smallest changes at the nanoscale. In the final stage, data analysis is used to interpret the signals generated by the sensing and scanning elements. This involves the use of algorithms and machine learning techniques to analyze the data and identify the specific pathogens present. (***** The concept of plant-produced antibodies, commonly known as "plantibodies", was first demonstrated by Hiatt and Duering in 1990 [161]. This term is used to describe antibodies produced and expressed in plants [162]. It can be used for various purposes, including pathogen detection, and immunizing the plant against pathogen infection [163])

environmental contamination and pathogen resistance. Further, alterations in microbial diversity and activity can disrupt nutrient cycling, soil fertility, and plant–microbe interactions, compromising crop health. Soil microbes, essential for nutrient cycling and disease suppression, may be adversely affected by nanomaterial exposure, leaving crops more vulnerable to pathogens [18, 19]. Also, some engineered nanomaterials, like silver and zinc oxide NPs exhibit antimicrobial properties, causes microbial imbalances which can lead to increased susceptibility to diseases in crops by affecting plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi [170, 171]. The coexistence of natural and engineered nanomaterials in soil poses contamination risks, with highly produced ENMs like TiO_2, ZnO potentially surpassing toxic thresholds [172]. Consequently, nanomaterial presence alters the plant rhizosphere, affecting microbial communities, enzyme activity and plant health. Studies indicate toxicity in rice plants [14, 173] and microbial communities exposed to nanomaterials, underscoring the need for cautious agricultural nanomaterial use. Therefore, green-synthesized NMs are needed to produce eco-friendly alternatives, such as chitosan, a natural polymer that has been shown to be an effective substitute for defending hosts from fungal infections [174]. For instance, it has been recommended that chitosan may increase the expression of 1,3-glucanase and chitinase genes in addition to the activity of defense-related enzymes in tomato and *Arabidopsis* [175]. Furthermore, nano-based agricultural products play a crucial role in managing plant pathogens as discussed in Table 1 and 2. Their appropriate handling, long-term storage capability, ease of transport, non-toxic nature, and high effectiveness position them as an ideal choice for farmers compared to traditional chemicals [176]. Nano-based chemicals are rapidly gaining global interest and seeing significant growth in commercialization. Similarly, commercial nano-formulations are increasingly employed in agriculture. Nano-Ag Answers® (Urth Agriculture) utilizes silver nanoparticles as a potent biocide against predatory pests. Nano-GroTM (agro nanotechnology corporation) incorporates nanomaterials to stimulate plant immune system pathways and boost defense mechanisms. ZinkicideTM, employing zinc nanoparticles, demonstrates effectiveness in managing citrus scab, canker, and melanoses [91]. In addition to these developments, various regulatory laws and policies have been established in the field of nanotechnology. A noteworthy achievement in 1998 was the national science and technology council's initiative which resulted in the creation of the national nanotechnology initiative (NNI). The primary goal of NNI was to conduct research and development to address and build awareness about nano-based products within the community [91]. However, a scientific advisory panel under the federal insecticide, fungicide, and rodenticide act in the United States has raised concerns about the environmental risks posed by nano-silver oxides in pesticides. The panel suggests that there is inadequate potential to mitigate these effects. In Europe, comprehensive regulatory frameworks both vertical and horizontal have been established to screen and manage the risks associated with nanoparticles (NPs) to both the public and the ecosystem. Moreover, plan protection products (PPPs) are primarily regulated by rule (EC: 1107/2009), necessitating previous agreement before being introduced to the commercial market [177]. The authorization process involves two steps, with the European Food Safety Authority (EFSA) analyzing the operating parts used in PPPs, and European member states screening and approving the product nationwide. However, regulatory frameworks and policies in this regard are yet to be developed [178]. Additionally, comprehensive analytic tools perform an important role in the evaluation of regulatory laws for risk assessment. To manage pesticide resistance, there is a need for the rotation of pesticide groups to prevent the emergence of new strains. Also, a diverse array of nanopesticides is anticipated to be commercially available in the coming years. However, the development of nanoproducts faces challenges due to a limited understanding of their performance in field trials, high production costs, significant volume demands, and concerns regarding regulatory and public perceptions [179]. However, it is still necessary to solve their drawbacks in terms of price, preparation, particle dispersion, and ingredient delivery.

Nanomaterial challenges against crop plant pathogens

The risk assessment of nanomaterials in crop health is pivotal for ensuring safe agricultural practices. Currently, there are no standardized methods or regulations for assessing the toxicity and safety of nanomaterial-based agrochemicals. For instance, nanoparticle-induced phytotoxicity was evaluated using seeds of various crops, including *Allium cepa*, *Zea mays*, *Cucumis sativus*, and *Lycopersicum esculentum* [180]. Furthermore, regulatory frameworks must be established to oversee nanomaterial usage, ensuring adherence to safety protocols [181]. In vitro studies underscore the cytotoxic effects on seeds and seedlings such as mitotic index changes, chromosomal aberrations, and DNA damage, highlighting the imperative for comprehensive environmental risk assessments in crops [182]. It is evident from the literature that nanomaterials could be used for years for plant disease monitoring and resistance enhancement in crop plants to ensure food security. However, there are always objections regarding the adoption of new technology; hence, further in-depth research on their implications is urgently needed. Few hazardous effects of NMs may vary depending on the bulk substance, particle size, and dose employed to create them. Similarly, several studies found that exposure to single-walled carbon nanotubes, ZnO NPs, Ag NPs, and Fe nanomaterials led to reduced seed germination and downregulated gene expression in wheat, maize, barley, ryegrass, and soybeans. Likewise, increased silver ion concentrations can damage DNA and limit its ability to replicate. This results in the inactivation of ribosomal subunit proteins as well as other cellular proteins and enzymes that are required for ATP generation. Ag NPs have been proven in previous studies to have powerful antifungal effects on fungi by destroying membrane integrity [183]. Correspondingly, the use of NMs to develop plant disease resistance is a difficult task. Plant growth and development may be influenced by NMs, although their absorption, transport mobilization and targets in plant tissues are still poorly understood. At some doses, NPs may cause toxicities by altering the physiological and morpho-anatomical genetic components of plants [184]. The vacuole, apoplast, phloem tissues, and xylem of plant roots and shoots are among the sites where nanoparticles can be absorbed, dispersed, and accumulated. The use of nanomaterials (NMs) in crop plant pathogens is still an emerging field, and there are several limitations to their use. NMs can have potential toxicity to plants, and their long-term effects on the environment are not yet fully understood. However, the use of green synthesis techniques for nanoparticle production could be a cost-effective and ecologically favorable alternative.

Concluding remarks and future perspectives

This article pioneers the exploration of this exciting frontier, envisioning a future where nanomaterials exhibit diverse biocidal activities, including fungicidal, bactericidal, and virucidal properties, among others. This has the potential to revolutionize disease resistance for crop pathogens, both in vivo and in vitro, indicating a new era of crop protection. Nanomaterials have the potential to revolutionize agriculture and lead to breakthroughs in plant disease management. They can stimulate plant immunity and inhibit the growth of pathogens by producing antimicrobial compounds and secondary metabolites. However, the use of nanotechnology in food and agriculture is still in its promising stage, and certain faults and risks exist such as phytotoxic behavior which needs to be thoroughly understood and determined at different plant growth stages. Despite these challenges, the use of nanomaterials in plant disease management can provide several advantages over traditional methods and can be tailored to target specific pathogens. Nanomaterials' potential applications throughout the food chain from farming to packaging are getting attention due to their unique properties. These properties provide opportunities to enhance food quality, safety, and sustainability. In addition, crop diseases threaten plant growth by disrupting biochemical and molecular processes, yet NMs offer significant potential in enhancing plant performance and resistance to pathogens. NMs improve membrane stability, nutrient uptake, and protect photosynthetic apparatus from pathogen damage. They also enhance the accumulation of stress-protective compounds and upregulate stress-responsive genes, boosting plant defense mechanisms. Recent research extensively explores NMs' role in inducing tolerance to crop pathogens. However, investigations into the influence of NMs on proteomics and genetic factors remain limited at molecular level, highlighting the need for further investigation in future studies to better understand these aspects. Further research and regulation will be necessary to ensure the safe and effective use of nanomaterials in agriculture. In terms of future policies, nanotechnology shows promise in addressing various challenges related to plant characteristics, productivity, and resistance to pathogens. However, concerns regarding their long-term ecological impacts, including bioaccumulation in food chains and toxicity to environmental organisms, necessitate thorough environmental risk assessments. Integrating green synthesis methods for nanoparticles can mitigate their toxicity. Biogenically synthesized nanoparticles exhibit enhanced bioactivity against pathogenic microbes, surpassing their chemically synthesized counterparts [185]. Additionally, diverse nanomaterials, such as nano clays and nanotubes, offer unique properties like enhanced sensitivity and rapid response times, with polymer-based nanocarriers such as silica and chitosan serving as protective reservoirs for encapsulating pesticides [186]. In addition, nano-sensors and nano-devices are emerging as innovative tools for real-time pathogen monitoring in plants with potential applications spanning pre- and post-infection detection under both laboratory and field conditions. This surge in interest agrees with the rapid growth of the NMs industry, fueled by large-scale production and heightened demand for NMs-derived products like nanoscale carriers and biosensors. However, unlocking the full potential of nano-agrochemicals requires a balanced approach of responsible exploitation, stringent regulatory frameworks, and continuous monitoring.

Though, it is important to thoroughly examine how nanomaterials interact with crops and assess their ecological and toxicological impacts before considering their implementation, including the assessment of gene expression patterns. Combining nanomaterials with other disease management strategies can also lead to synergistic effects. Despite existing applications, the vast potential of NMs in plant protection continues to lie dormant. Emerging research on their antimicrobial capabilities highlights their immense promise for revolutionizing crop health through novel diagnostic and sustainable management solutions.

Abbreviations

Ag NPs	Silver nanoparticles
ZnO NPs	Zinc oxide nanoparticles
CuO NPs	Copper oxide nanoparticles
ZnO NPs	Zinc oxide nanoparticles
TiO_2 NPs	Titanium dioxide nanoparticles
SiO_2 NPs	Silicon dioxide nanoparticles
CS NPs	Chitosan nanoparticles
S NPs	Silicon nanoparticles
CuCh NPs	Cu-chitosan nanoparticle
MgO NPs	Magnesium oxide nanoparticles
Al_2O_3 NPs	Aluminum oxide nanoparticles
CWP NPs (chitosan)	Cell wall polymer-based nanoparticles
Se NPs	Mycogenic selenium nanoparticles
Fe_3O_4 NPs	Iron oxide nanoparticles
NiO NPs	Nickel oxide nanoparticles
CeO_2 NPs	Cerium oxide nanoparticles
CuFe NPs	Copper/iron nanoparticles
TC NPs	Chitosan thiamine nanoparticles
ROS	Reactive oxygen species
PR	Pathogen related proteins
SAR	Systematic acquired resistance
TMV	Tobacco mosaic virus
CMV	Cucumber mosaic virus

TSWV	Tomato spotted wilt virus
PVY	Potato virus Y
ToMV	Tomato mosaic virus

Author contributions
M.J. wrote the manuscript and worked on figures. M.A.A. and A.Z. reviewed the manuscript. F.A. and G.M.D. contributed to literature search. S.H. and M.I. carried out reference searching. S.M.: data curation and investigation. L.G. funded, supervised, edited, and approved the final manuscript.

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