**Nanotechnology: Boon for Disease Management in Different Crops**

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**Introduction**

Nanotechnology, conceived by Richard Feynman, represents the forefront of scientific exploration into materials that exhibit distinct attributes compared to their larger, macroscopic counterparts. Nano-materials possess the remarkable capability to operate on a molecular scale, manipulating atoms individually to assemble extensive structures with entirely novel molecular configurations. This intrinsic capacity underpins the diverse applications of nanotechnology, including its substantial impact on the realm of food production. The food industry has been greatly influenced by nanotechnology, contributing to the creation of fresh functional materials, innovative product design, and the establishment of methodologies and instruments for ensuring food safety and bio-security (Moraru*et al*., 2003). As outlined by the National Science Foundation (NSF) and the National Nanotechnology Initiative (NNI), nanotechnology entails the comprehension, governance, and manipulation of matter at both the atomic and molecular scales, as well as the supramolecular level encompassing molecular clusters ranging from 0.1 to 100 nanometers. The ultimate objective is the fabrication of materials, devices, and systems boasting entirely novel properties and functionalities attributable to their diminutive structural dimensions. This field embodies the manipulation and exploitation of materials, device systems, and matter properties at the nanoscale, as established by researchers such as Ajayan*et al*. (2003) and Astruc*et al*. (2010). Nanotechnology transcends disciplinary boundaries, drawing from physics, chemistry, biology, material science, and engineering, as evidenced by its interdisciplinary nature highlighted by Roco (2007). Centering on the distinct properties arising from nanometric proportions, nanotechnology holds immense potential for transformative impacts across domains including agriculture, food production, biomedicine, environmental engineering, water resource management, energy conversion, and many more. The integration of nanotechnology into agriculture and the food industry was initially addressed by the United States Department of Agriculture in 2003. Its applications encompass disease control, controlled release of pesticides, the development of diagnostic tools, and the design of functional food systems. This involves the creation of interactive, edible nanopackaging to deter pathogens, precision release of chemicals, comprehensive nanoscale surveillance, and the design of interactive agrochemicals functioning as herbicides and pesticides. Consequently, nanotechnology emerges as a promising avenue for combating plant diseases through precise delivery of functional molecules or serving as a diagnostic tool for disease detection, as elucidated by Tarafdar and Raliya (2012). The novel realm of nanotechnology, characterized by its distinct material properties compared to their macroscopic counterparts, holds substantial potential across diverse fields. Central to nanotechnology's essence is its ability to manipulate matter at the molecular scale, atom by atom, culminating in the assembly of extensive structures with unprecedented molecular arrangements. The overarching goal is to harness these distinctive properties by acquiring mastery over structures and devices at atomic, molecular, and supramolecular tiers, optimizing their efficient production and utilization. Nanotechnology has ushered in innovative solutions for challenges within plant science and food technology, particularly in post-harvest product preservation, while also presenting novel methodologies for raw material selection and processing, ultimately enhancing the quality of plant-based products. At the heart of nanotechnology lies the capability to condense tools and devices within the nanometer range, accumulating atoms and molecules into more substantial structures while maintaining their diminutive size.

**Properties of Nanoparticles**

Nanoparticles exhibit distinct properties that sharply contrast with their bulk counterparts, and these properties are harnessed for their applications in nanotechnology. These properties include:

• **Small Size (1-100nm):** Nanoparticles possess dimensions within the range of 1 to 100 nanometers.

**• High Surface-to-Volume Ratio:** Nanoparticles boast an expansive surface area relative to their volume.

• **Modifiable Chemical and Physical Properties:** The chemical and physical attributes of nanoparticles can be altered.

• **Size- and Shape-Dependent Changes:** Nanoparticles exhibit variations in chemical and physical properties based on their size and shape.

**• Structural Resilience despite Atomic Composition:** Nanoparticles maintain structural integrity despite being composed of individual atoms.

**• Enhanced or Delayed Particle Aggregation:** Aggregation tendencies of nanoparticles are influenced by surface modifications, leading to enhanced photoemission, heightened electrical and thermal conductivity, and improved surface catalytic activity (Roco, 2007).

These nanostructures can assume different dimensionalities, including zero-dimensional (nanoparticles), one-dimensional (nanowires), two-dimensional (thin films), or three-dimensional (arrays, hierarchical structures). A visual comparison of various nano and micro structures in biology is provided in Figure 1.

Source: Misra*et al.,* 2013

**Figure 1:** Scale showing the dimensions of different nanometric and micrometric objects in biological materials (A, T, G, C are nucleotides molecules).

**Techniques for Nanoparticle Preparation**

Nanomaterials are prevalent in nature, as biological organisms inherently operate on the nanoscale. Nanotechnologists aim to produce and employ both novel synthetic nanomaterials and certain naturally occurring nanomaterials on a larger scale and with greater uniformity in size. A range of techniques are employed to synthesize diverse nanomaterials. Nanostructures can be generated through two distinct methodologies commonly known as the top-down process and the bottom-up process.

The top-down approach typically involves the disintegration of larger material entities (either physically or chemically) into smaller units possessing desired shapes and dimensions. This is achieved through techniques such as mechanical milling and ion implantation, among others.

In contrast, the bottom-up approach employs self-assembly as a mechanism to construct nanostructures by precisely arranging individual atoms and molecules together. This approach is illustrated in Figure 2.



Source: Shakeel*et al.,* 2015

Fig.2 Bottom up (a) and top down (b) process.

Various Methods for Nanoparticle Preparation:

**a) Solvent Extraction/Evaporation:**

Nanoparticles of specific organic polymers can be synthesized through a process involving solvent dissolution, followed by sonication, evaporation, filtration, and freeze-drying. This method has been employed, for instance, in the creation of polymer nanoparticles using dichloromethane as a solvent (Zhang and Feng, 2006).

**b) Crystallization:**

The formation of nanoparticles can also occur via the crystallization process. In one example, hydroxyapatite-aspartic acid (or glutamic acid) crystals were generated by introducing varying concentrations of amino acids into solution (Boanini*et al*., 2006).

**c) Self-Assembly:**

Manipulating factors like pH, temperature, and solute concentrations can induce the self-assembly of molecules, resulting in the formation of fibrous nanostructures. This technique has been employed to create vesicles referred to as polymerosomes, which can encapsulate substances. Polymerosomes are self-assembled through the gradual evaporation of an organic solvent (Lorenceau*et al*., 2005).

**d) Layer-by-Layer Deposition:**

Layer-by-layer deposition involves the sequential application of layers of different materials. For instance, platforms supporting bilayer membranes suitable for protein analysis can be produced by depositing sodium silicate and poly ally amine hydrochloride onto a gold substrate, followed by calcination. Lipid bilayers can then integrate with the silicate layer, facilitating the detection of specific proteins (Phillips *et al.,* 2006).

**e) Microbial Synthesis:**

Living cells, including fungi and bacteria, can be harnessed to generate nanoparticles. For example, extracellular synthesis of silver nanoparticles has been achieved using the fungus Aspergillusfumigatus. Various other fungi and bacterial species have also been employed for producing gold and silver nanoparticles (Bhainsa and D'Souza, 2006; Bhattacharya and Gupta, 2005).

**Relevance of Nanotechnology to Plant Pathology**

The intersection of nanotechnology and plant pathology holds substantial significance. Nanotechnology offers innovative tools for disease control, such as the controlled delivery of functional molecules and enhanced disease detection. Additionally, it enables the development of nano-encapsulated agrochemicals, interactive nano wrappers to counter pathogens, and novel diagnostic tools. This convergence has the potential to revolutionize agriculture and address challenges related to plant diseases, while also impacting various other sectors through the unique properties and functionalities of nanomaterials.

**Detection and Diagnosis of Plant Pathogens**

The early identification of plant diseases has spurred the exploration of nanotechnology-based solutions to safeguard food and agriculture against bacterial, fungal, and viral agents. Researchers are envisioning the integration of autonomous nanosensors with GPS systems for real-time field monitoring, encompassing both soil conditions and crop health. By combining biotechnology and nanotechnology in sensor development, more sensitive equipment can be created, enabling faster responses to environmental changes and disease outbreaks. An imperative requirement exists for ultrahigh-sensitive diagnostic tools capable of rapidly detecting molecular defects at either the genomic or biochemical level. Bio-systems naturally possess functional nanoscale devices, including enzymes, proteins, and nucleic acids, which play a crucial role in detecting vital processes in plants. Disease diagnosis is challenging due to the extremely low concentrations of biochemicals and the limited amount of detectable viruses and various fungal or bacterial infections (Misra*et al*., 2013).

**Nanosized Metals as Diagnostic Probes**

While current diagnostic techniques are not yet perfected and are in their nascent stages for plant pathogen detection, researchers are striving to leverage the advantages of nanomaterials by addressing the limitations of existing diagnostic tools. Nanoparticles, when reduced to nanoscale dimensions (1-100 nm), exhibit unique properties that make them suitable for use as diagnostic probes (Sharon *et al*., 2010). Fluorescent silica nanoprobes show promise for rapid plant disease diagnosis. These nanoprobes, conjugated with the secondary antibody of goat anti-rabbit IgG, were employed to detect the bacterial plant pathogen *Xanthomonasaxonopodis*pv. *Vesicatoria*, which causes bacterial spot on solanaceous plants. Fluorescent silica nanoprobes incorporated with the organic dye tris-2,2'-bipyridyl dichlororuthenium (II) hexahydrate (Rubpy) emitted fluorescence and demonstrated photostability, making them suitable for diagnostics (Yao *et al.,* 2009).

**Nanoscale Biosensors/Nanosensors**

The development of small, portable nanosensors holds the potential for rapid, real-time, accurate, quantitative, reliable, and stable disease detection. Detecting infections in asymptomatic plants and subsequently delivering targeted treatments are vital components of precision farming. Nugaeva*et al*. (2005) demonstrated the use of micromechanical cantilever arrays for detecting fungal spores (*Aspergillusniger* and *Saccharomyces cerevisiae*). Functional proteins such as concanavalin A, fibronectin, or immunoglobulin G were grafted onto micro-fabricated silicon cantilevers, both uncoated and gold-coated. The cantilevers responded to the molecular structures on fungal cell surfaces, causing a shift in resonance frequency that was measured by dynamically operated arrays. This approach provided results in a matter of hours compared to several days using conventional methods. The shift in frequency was proportional to the mass of a single fungal spore, enabling quantitative estimation. The biosensors detected target fungi in the range of 103-106cfu ml-1 (Nugaeva*et al*., 2005).

**Quantum Dots (QDs)**

Quantum dots are fluorescent, crystalline semiconductor particles with diameters in the range of a few nanometers. These dots confine excitons in all three spatial dimensions. QDs have emerged as important tools for detecting specific biological markers with high accuracy, finding applications in cell labeling, tracking, in vivo imaging, and DNA detection (Sharon *et al*., 2010).

**Management of Plant Diseases Using Nanoparticles**

Nanotechnology is emerging as a potential solution for managing plant diseases, with nanoparticles of carbon, silver, silica, and alumino-silicates gaining attention. Carbon nanotubes (CNTs), for instance, have shown promise by not only penetrating the hard coats of germinating tomato seeds but also enhancing their growth. This growth promotion is attributed to increased water uptake facilitated by CNT penetration. CNTs could potentially serve as vehicles to deliver beneficial molecules during germination to protect seeds from diseases, as their growth-promoting nature poses no harm to plants. Several bacterial and fungal pathogens negatively impact vegetables, including *Erwiniacarotovora, Pseudomonas* spp., *Corynebacterium, Xanthomonascampestris, Alternaria, Aspergillus, Cladosporium, Colletotrichum, Fusarium*, and more. These pathogens lead to economic losses, and some even produce toxic metabolites affecting human health. Addressing the challenges posed by an estimated doubling of global food demand in the next 50 years is essential.

Nanotechnology provides potential solutions for crop protection, including the controlled release of encapsulated pesticides and fertilizers, as well as early disease detection through nanosensors. Utilizing nanomaterials in crop protection offers efficient approaches to manage plant pathogens. Several nanoparticles are employed in plant disease management:

**a) Biopolymer Nanoparticles:**

Developing nanoformulations for agrochemicals requires biodegradable, nontoxic, eco-friendly, and cost-effective materials. Biopolymers derived from natural sources fulfill these requirements and serve as an attractive alternative to petrochemical-based nanomaterials.

**b) Chitosan:**

Chitosan nanoparticles find multiple applications due to their biodegradability and non-toxic nature. Under acidic conditions, chitosan's free amino groups become positively charged, contributing to its effectiveness. Chitosan combats fungi through several mechanisms:

i) Interaction with fungal membrane components, altering membrane permeability and leading to cell death.

ii) Chelation with metal ions, disrupting fungal growth by depriving them of essential nutrients.

iii) Penetration of fungal cell walls, binding to DNA, and inhibiting protein and enzyme synthesis.

Chitosan and chitosan nanoparticles have demonstrated effectiveness against plant pathogens like *Fusariumsolani*, with particle size and zeta potential influencing their inhibitory effects. The use of low molecular weight chitosan nanoparticles with varying sizes exhibited antibacterial activity, highlighting the significance of zeta potential in binding to microbial membranes. The antimicrobial activities of chitosan, its derivatives, bound metal ions, and nanoparticles have been widely studied (Sanpui*et al*., 2008; Jagadish*et al*., 2012; Kaur *et al.,* 2012) (Fig. 3).

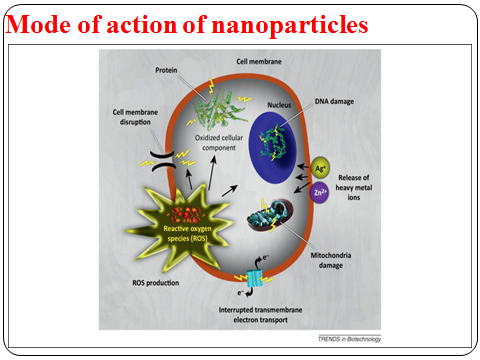


Fig. 3 Mode of action of nanoparticles.

**c) Metallic Nanoparticles:**

Metallic nanoparticles exhibit distinctive chemical and physical attributes, owing to their small size, significant surface-to-volume ratio, structural stability, and strong binding affinity to specific targets (Kumar *et al*., 2010). These nanoparticles offer a promising avenue as novel antimicrobial agents, providing an alternative to synthetic fungicides to impede or suppress the growth of various pathogens. The efficacy of metallic nanoparticles stems from their ability to exert multiple modes of inhibition.

**a) Carbon Nanoparticles:**

Recent scientific studies have highlighted the potential benefits of using carbon nanotubes (CNTs) to enhance plant growth and protect against diseases. In a notable experiment, tomato seeds were planted in soil containing CNTs, which not only penetrated the tough coat of germinating seeds but also exhibited growth-enhancing effects. This growth enhancement is attributed to the increased water uptake resulting from CNT penetration. The ability of CNTs to promote growth without toxicity makes them an attractive option for delivering protective molecules to seeds during germination. It's worth noting that high concentrations of carbon nanomaterials (CNMs) might obstruct water channels and potentially hinder water uptake and spore development, leading to plasmolysis.

It's also speculated that CNMs could influence the gating of existing water channels (aquaporins) in spores, potentially affecting biological pathways before spore development. While certain water channel genes, including the crucial LeAqp2 gene in tomato plants, were impacted by multi-walled carbon nanotubes (MWCNTs), spore-specific water channel aquaporins (Aqy1) were produced during later stages of sporulation. This suggests that CNMs might not regulate aquaporin expression. Therefore, CNMs' influence on spore water uptake, mainly blocking it due to surface-adsorbed CNMs, could be a significant factor contributing to plasmolysis and inhibiting spore germination.

In experiments, when subject to the highest dose of SWCNTs (500 µg/mL), spore germination of the fungus was inhibited by over 90.8%. Similarly, MWCNTs, GO (graphene oxide), and rGO (reduced graphene oxide) also exhibited inhibitory effects on spore germination, although to varying degrees. C60 and AC (activated carbon), on the other hand, showed no significant difference from the control conditions in terms of their effects on the spore germination of the fungus.

**e) Nano Alumino-Silicate:**

Leading chemical companies are exploring nanoscale formulations of efficient pesticides, including the use of Alumino-Silicate nanotubes with active ingredients. These nanotubes offer an advantage: when sprayed on plant surfaces, they are readily picked up by insect hairs. Insects that groom themselves inadvertently consume these pesticide-loaded nanotubes. This method offers biologically more active and environmentally safer alternatives to traditional pesticides. Mesoporous Silica Nanoparticles have been investigated for their ability to deliver DNA and chemicals into plants, introducing a powerful tool for targeted delivery into plant cells. Spherical nanoparticles with independent porous channels have been developed, featuring a honeycomb-like structure capable of holding chemicals or molecules (Wang *et al*., 2002). These nanoparticles possess a unique "capping" strategy that keeps the contents sealed until activated to release their cargo inside plant cells, providing precise control over timing. These nanoparticles have been employed successfully to introduce DNA and chemicals into various plant species, including arabidopsis, tobacco, and corn. Another advantage is their capability to deliver multiple biogenic species simultaneously.

**f) Silver Nanoparticles:**

Plant diseases continue to hinder agricultural production worldwide, leading to significant financial losses spent on disease control measures. While pesticides are commonly used, the environmental risks and residues associated with their application have prompted the search for alternative methods. Silver nanoparticles have gained attention as antimicrobial agents due to their potential to offer a more eco-friendly solution(Jo *et al*., 2009; Kim *et al*., 2012). Silver has been used for its antimicrobial properties since ancient times and is favored for its broad-spectrum activity and multiple modes of action against microorganisms(Wei *et al*., 2009). It demonstrates higher toxicity to microorganisms and lower toxicity to mammalian cells. The utilization of silver nanoparticles as antimicrobial agents is driven by their cost-effective production and versatile inhibitory effects on microorganisms(Clement and Jarrett, 1994). Among various nanoparticles, silver nanoparticles stand out due to their strong inhibitory and antimicrobial activities (Fig. 4). Their high surface area and a significant fraction of surface atoms contribute to their enhanced antimicrobial effects compared to bulk silver. Researchers have studied the antifungal potential of colloidal nano silver solution against rose powdery mildew caused by *Sphaerothecapannosa* var. *rosae*. This widespread disease affects both greenhouse and outdoor-grown roses, causing leaf distortion, curling, defoliation, and reduced flowering. A colloidal solution of double-capsulized nanosilver was prepared and effectively applied to an area affected by rose powdery mildew, resulting in over 95% reduction in disease severity within two days of application.

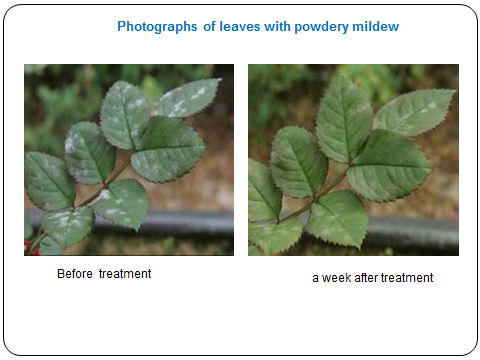
**Kim *et al*., 2008**

Fig. 4 Application of colloidal nano-silver solution against rose powdery mildew.

Nano silver colloid represents a finely dispersed and stabilized solution containing silver nanoparticles, which exhibit enhanced adhesion to bacterial and fungal surfaces, rendering them more effective as fungicides. This formulation proves effective in eradicating undesired microorganisms in both soil used for planting and hydroponic systems. It is also employed as a foliar spray to counteract mold, decay, and various plant diseases. Additionally, silver demonstrates its capacity as a catalyst for plant growth. The wide spectrum of applications for this nearly odorless, nearly tasteless, and colorless disinfectant and healing agent encompasses numerous indispensable functions.A composite known as Nano Silica-Silver emerges as a combination of silicon (Si) absorbed into plants to heighten their resilience to diseases and environmental stressors. The aqueous silicate solution applied to plants is recognized for its capacity to preemptively combat pathogenic microorganisms that give rise to conditions like powdery mildew or downy mildew in plants. This solution also supports enhanced physiological activity and growth in plants, fostering resistance to disease and stress. Nonetheless, while silica demonstrates these beneficial traits, it does not directly exhibit disinfection capabilities against established plant pathogens. The efficacy of silica is influenced by the specific physiological context, making it ineligible for registration as an agricultural chemical. In the realm of disinfection, silver's prowess is widely acknowledged. Its mechanism of action involves deactivating enzymes integral to the metabolic functions of unicellular microorganisms through a process known as oligodynamication. This attribute extends to inhibiting the proliferation of algae. When existing as ionic silver, its antimicrobial activity is remarkably potent. However, the reactivity of ionic silver renders it susceptible to oxidation or reduction, transforming it into a metallic form contingent upon its surrounding environment. This transition does not sustain continuous antimicrobial efficacy. In contrast, silver in the form of metal or oxide exhibits stability within the environment. However, its limited antimicrobial activity necessitates its usage in relatively higher quantities, which is not an optimal approach.A novel formulation consisting of nano-sized Silica Silver has been developed to address a range of plant diseases. This composition combines nano-silver with silica molecules and a water-soluble polymer, created through exposure to radioactive rays. In practical tests conducted in both field and greenhouse settings, the solution demonstrated antifungal properties and effectively controlled powdery mildew in pumpkins at a concentration of 0.3 ppm. Within three days of application, the infected leaves exhibited the disappearance of pathogens, leading to sustained plant health.Exploration of the effective concentration of nanosized silica-silver was undertaken to gauge its impact on the growth inhibition of various fungi. Notably, *Pythiumultimum, Magnaporthegrisea, Colletotrichumgloeosporioides, Botrytis cinerea*, and *Rhizoctoniasolani* demonstrated complete growth inhibition at a concentration of 10 ppm of the nanosized silica-silver. Similarly, *Bacillus subtilis, Azotobacterchrococum, Rhizobium tropici, Pseudomonas syringae*, and *Xanthomonascampestris*pv. *Vesicatoria* displayed full growth inhibition at a concentration of 100 ppm. Instances of chemical damage were reported when higher concentrations of nanosized silica-silver (3200 ppm) were applied to cucumber and pansy plants.In this study, the inhibitory effects of three different types of AgNPs (WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R) against various plant pathogenic fungi were examined in vitro. The results indicated that AgNPs possess the capability to inhibit these pathogens, albeit with variations dependent on the concentration and type of AgNPs employed. Most fungi exhibited pronounced inhibition at a concentration of 100 ppm of silver nanoparticles. Furthermore, the study revealed a higher rate of inhibition on potato dextrose agar (PDA) media in comparison to other media. Notably, WA-CV-WA13B displayed the most substantial inhibition effect among the AgNPs tested. In most instances, inhibition increased in direct proportion to the concentration of AgNPs applied. This heightened inhibitory impact could be attributed to the dense saturation and adherence of the solution to fungal hyphae, thereby deactivating plant pathogenic fungi.Existing reports on the inhibitory mechanisms of silver ions on microorganisms have demonstrated that the introduction of Ag+ leads to the impairment of DNA replication. This results in the inactivation of ribosomal subunit proteins and select cellular proteins and enzymes crucial for ATP production. An additional hypothesis suggests that Ag+ predominantly influences the function of membrane-bound enzymes, particularly those within the respiratory chain. To summarize, the potent antifungal effects of AgNPs on the tested fungi in vitro are likely due to the disruption of membrane integrity, underscoring the significant antifungal activity of AgNPs. A study conducted by Kim *et al*. (2008) evaluated the efficacy of colloidal nano silver solution in combating rose powdery mildew.Nano silver colloid exhibits enhanced adhesion to the surfaces of bacterial and fungal cells, making it a more effective fungicide due to its well-dispersed and stabilized silver nanoparticle solution. Notably, nano silver falls under the classification of a pesticide (Anwar *et al*., 2008, Baier, 2009). Given silver's exceptional antimicrobial properties, it has gained acceptance as a replacement for traditional agrochemicals. Furthermore, it serves as a plant-growth stimulator and contributes to the reduction of undesired microorganisms in both soil and hydroponic systems (Sharma *et al*., 2012). Although relatively few studies have addressed the use of silver for controlling diverse plant pathogens in a safer manner compared to synthetic fungicides (Park *et al*., 2006), nanoparticles, such as silver nanoparticles, offer efficient penetration into microbial cells. This enables the achievement of effective microbial control at lower concentrations. This is especially relevant for microorganisms that demonstrate reduced sensitivity to antibiotics due to limited antibiotic penetration into microbial cells (Samuel and Guggenbichler, 2004). Lamsal*et al*. (2011a) showcased the effective utility of silver nanoparticles as an alternative to commercial fungicides. Their investigation focused on the impact of silver nanoparticles on six *Colletotrichum* species associated with pepper anthracnose, revealing that the application of a 100 ppm concentration of silver nanoparticles inhibited fungal hyphal growth and conidial germination in vitro, in comparison to the control. In field conditions, silver nanoparticles exhibited notably high inhibition of fungi when applied to plants prior to disease outbreak. In a study by Aguilar-Méndez *et al*. (2011), the fungistatic activity of silver nanoparticles on *Colletotrichumgloeosporioides* was found to be dose-dependent. Jo *et al*. (2009) conducted tests involving various forms of silver ions and nanoparticles to evaluate their antifungal efficacy against two plant-pathogenic fungi, *Bipolarissorokiniana* and *Magnaporthegrisea*. Their findings in both in vitro and in planta evaluations demonstrated that silver ions and nanoparticles influenced spore colony formation and disease progression in fungi. Kim *et al*. (2012) reported the inhibitory effects of three distinct types of silver nanoparticles (WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R) against eighteen commercially significant plant pathogenic fungi on different media, including potato dextrose agar (PDA), malt extract agar, and corn meal agar. The inhibition of fungal pathogens by silver nanoparticles was dependent on concentration and nanoparticle type. Notably, the silver nanoparticle variant WA-CV-WA13B exhibited the highest inhibition effect.Investigations into the effects of silver nanoparticles on the growth of sclerotium-forming species, including *Rhizoctoniasolani*, *Sclerotiniasclerotiorum*, and *S. minor*, revealed a dose-dependent inhibition of hyphal growth. Microscopic observation of hyphae exposed to silver nanoparticles displayed severe damage, leading to the separation of hyphal wall layers and collapse of fungal hyphae (Bhat *et al*., 2009, Min *et al*., 2009). A recent study explored the in vitro and in vivo efficacy of silver nanoparticles against powdery mildew both before and after disease outbreak in plants under varying cultivation conditions. Results indicated maximum inhibition of fungal hyphae and conidial germination, with lower nanoparticle concentrations on cucumbers and pumpkins (Lamsal*et al*., 2011b). Both pre- and post-inoculation application of silver nanoparticles effectively reduced disease severity across all concentrations. Mechanistically, this antifungal activity is attributed to direct effects on fungal germination and infection processes. Focusing on *Magnaporthegrisea*, a foliar disease-causing organism, disease initiation involves the attachment of spores to plant surfaces and germ tube formation (Tucker and Talbot, 2001). Under favorable environmental conditions, spore germination and germ tube penetration occur within 24 hours. Antifungal efficacy of silver nanoparticles was observed at 24 hours post-inoculation, indicating the critical role of direct silver contact with spores or germ tubes in inhibiting disease development (Young *et al*., 2009). Notably, silver nanoparticles exhibited sustained antifungal efficiency even at 5 days post-inoculation, suggesting penetration of the plant cell wall and subsequent inhibition of disease progression. The use of silver nanoparticles proves effective in the management of rice blast disease and the prevention of detrimental infections, without causing apparent phytotoxic effects on rice.

**g) Silica Nanoparticles**

Silicon (Si) has been recognized for its capacity to enhance disease resistance and stress tolerance in plants (Brecht *et al*., 2004). It also plays a role in stimulating the physiological processes and overall growth of plants (Carver *et al*., 1998). Torney*et al*. (2007) explored the utilization of honeycomb mesoporous silica nanoparticles (MSN) with 3nm pores as a delivery system for DNA and chemicals into plant cells and leaves. Their approach involved loading the gene of interest and its chemical activator into the MSN structure, capping the ends with gold nanoparticles, and studying the controlled-release dynamics of the chemicals and the subsequent gene expression induction in plants. This study demonstrated the potential of silica nanoparticles in facilitating precise delivery of proteins, nucleotides, and chemicals within plant biotechnology contexts. Silicon (Si) absorption by plants has been linked to bolstered disease resistance and stress resilience, attributed to its promotion of physiological activity and growth. Aqueous solutions containing silicate have exhibited remarkable preventive effects against pathogenic microorganisms that trigger conditions such as powdery mildew and downy mildew in plants. Additionally, these solutions have shown promise in enhancing overall plant physiological activity and growth, thereby fostering disease and stress resistance.

**h) Copper Nanoparticles**

Copper-based fungicides are known to generate highly reactive hydroxyl radicals, which possess the ability to impair lipids, proteins, DNA, and various other biomolecules. This mechanism underpins their crucial role in preventing and treating a diverse array of plant diseases (Borkow and Gabbay, 2005). The integration of copper with chitosan nanogels has exhibited a potent synergistic outcome between chitosan and copper, effectively inhibiting the growth of the phytopathogenic fungus *Fusariumgraminearum*. The biocompatibility of these nanohydrogels positions them as a promising copper-based bio-pesticide generation, offering potential for their utilization as an effective delivery platform for copper-centered fungicides aimed at safeguarding plants (Brunel *et al*., 2013). Incorporating copper nanoparticles into low melting point soda-lime glass powder has demonstrated noteworthy antimicrobial effectiveness against a spectrum of microorganisms including gram-positive and gram-negative bacteria, yeast, and fungi. The enhanced antimicrobial action is attributed to the inhibitory synergistic impact of Ca2+ ions released from the glass (Esteban-Tejeda *et al*., 2009).

**i) Zinc Nanoparticles**

The mode of action of nano-ZnO derived from zinc nitrate against the notable fungal pathogen Aspergillusfumigatus has been elucidated, revealing a process involving hydroxyl and superoxide radicals. This interaction results in cell wall distortion and subsequent demise of the fungus due to the transfer of high energy (PrasunPatra and Goswami, 2012). Zinc oxide nanoparticles (ZnO NPs) have displayed potential as effective fungicides in both agricultural and food safety contexts. A recent investigation by He*et al*. (2011) showcased notable inhibition of two post-harvest pathogenic fungi, Botrytis cinerea and *Penicilliumexpansum*, through the utilization of ZnO NPs measuring approximately 70 nm in size and at lower concentrations. The mechanism of action was confirmed through scanning electron microscopy (SEM) and Raman spectroscopy. ZnO nanoparticles induce morphological changes in fungal hyphae, hinder conidiophore and conidial development, ultimately culminating in the demise of the fungal hyphae.

**j) Nano Composites**

While silver has primarily been investigated for its antibacterial properties, its potential as an antifungal agent is gaining attention. Pinto *et al*. (2013) explored the preparation and antifungal capabilities of composite films consisting of pullulan and silver nanoparticles (Ag NPs) using *Aspergillusniger* as a model system. Their findings revealed that these composite films exhibit robust inhibitory effects on fungal sporulation, a phenomenon confirmed through SEM visualization of disrupted spore cells. Silver, when in an ionic state, exhibits elevated antimicrobial activity (Thomas and McCubbin, 2003). In the realm of plant disease control, Park *et al*. (2006) introduced a novel nano-sized Silica-Silver composite with promising antifungal attributes. The composite demonstrated significant antifungal efficacy, causing pathogenic disappearance from infected leaves within three days of application and ensuring plant health thereafter. The researchers endeavored to ascertain the optimal concentration of these composites, effectively suppressing the growth of numerous pathogens. Nano composites exhibited complete growth inhibition of *Pythiumultimum, Magnaporthegrisea, Colletotrichumgloeosporioides, Botrytis cinerea*, and *Rhizoctoniasolani* at a concentration of 10 ppm. Similarly, *Bacillus subtilis, Azotobacterchrococum, Rhizobium tropici, Pseudomonas syringae*, and *Xanthomonascompestrispv. Vesicatoria* displayed 100% growth inhibition at a concentration of 100 ppm.Park *et al*. (2006) conducted tests using nanosized silica-silver (Si-Ag) particles against various fungal and bacterial pathogens. In vitro experiments indicated that silica-silver nanoparticles exhibited higher efficacy against fungi at a dosage of 10 ppm, causing complete inhibition of vegetative growth. Smaller silver nanoparticle sizes were found to be more effective against fungi. The majority of tested bacteria were fully inhibited with a mere 100 ppm of silica-silver nanoparticles. In field conditions, the application of nanosized silica-silver particles to control powdery mildew diseases in cucurbits led to 100% disease control after three weeks (Park *et al*., 2006). Notably, these nanoparticles displayed phytotoxicity only at exceptionally high doses of 3200 ppm when tested on cucumber and pansy plants. Further, nanosized silica-silver exhibited growth and development inhibition for both Gram-positive and Gram-negative bacteria.

**Nanoparticles in Post-Harvest Disease Management**

The escalating global population, diminishing natural resources, and the emergence of resilient pathogens have rendered the provision of ample and wholesome food a formidable challenge. This predicament is poised to intensify manifold in the near future. Consequently, the imperative to augment production efficiency and curtail post-harvest losses has arisen, necessitating the deployment of progressive technologies like biotechnology and nanotechnology in the realm of post-harvest products. Nanotechnology has been notably harnessed in the agricultural and horticultural sectors, contributing to prolonged shelf life, regulation of microorganism proliferation through nanofilms and coatings, attenuation of gas influence and detrimental UV rays, and the utilization of Nano biosensors to detect quality and spoilage indicators (Yadollahi*et al*., 2009).Nanotechnology's potential encompasses post-harvest operations such as drying, storage, and preservation of agricultural commodities. Chitosan, an enzymatically deacetylated derivative of chitin, emerges as an efficacious agent in mitigating post-harvest deterioration of fruits and vegetables (Liu *et al*., 2007). The application of chitosan at 1 g/L has demonstrated significant efficacy in curtailing the proliferation of diverse phytopathogenic fungi responsible for post-harvest spoilage (Hirano, 1997). Yu *et al*. (2012) delved into the impact of a 1% chitosan film augmented with 0.04% nano-silicon dioxide on the qualitative attributes of stored jujube over a 32-day period at ambient temperatures. Their investigation revealed that the coated samples exhibited diminished red indices, decay incidence, respiration rates, and weight loss. Shi *et al*. (2013) probed a novel chitosan/nanosilica hybrid film's influence on the preservation quality of longan fruits under ambient conditions. The application of this coating prolonged shelf life, curbed browning indices, mitigated weight loss, and restrained the accumulation of malondialdehyde and polyphenoloxidase activity in fresh longan fruit.

Another compelling avenue involves the utilization of nanosilver in post-harvest treatments. Liu *et al*. (2009) explored the impact of nanosilver on the shelf life of cut gerbera (*Gerbera jamesonii*) cv. Ruikou flowers. Their study revealed that a 24-hour pulse with a 5 mg/L nanosilver solution elongated vase life and curtailed bacterial growth in the vase solution over the initial two days as observed under microscopic analysis. Subsequent research extensively studied the post-harvest treatment potential of nanosilver. It emerged as a significant player in prolonging vase life by inhibiting bacterial growth in various cut flowers, including Rose, Gerbera, and Acacia holosericea (Lu *et al*., 2010; Li *et al*., 2012; Liu *et al*., 2012; Mohsen Kazemi., 2012; Nazemi Rafi and Ramezanian, 2013). In a recent study, the utility of CS-Ag Np composites as fruit coating material to mitigate mango anthracnose caused by *Colletotrichumgloeosporioides* was evaluated. The findings indicated that these nanocomposites yielded significant reductions in rotting fruit tissue (71.28% at 1% concentration). Further, the inclusion of 0.1% non-ionic surfactant tween 80 enhanced coating solution wettability and adhesion properties, leading to pronounced disease mitigation (84.55% at 1% concentration). Consequently, these nanocomposites could be harnessed as coating agents to avert quiescent infections of *C. gloeosporioides* in mango, thereby mitigating post-harvest losses (Chowdappa*et al*., unpublished data). The potential of nanomaterials bears profound implications for post-harvest disease management, with research findings underscoring their superior applicability and advantages over conventional packing materials in safeguarding the physicochemical and physiological quality of stored fruits, vegetables, and other horticultural produce.

**Nanostructures in Association - Colloidal Forms for Functional Ingredient Delivery:**

Nanostructures, specifically surfactant micelles, vesicles, bilayers, reverse micelles, and liquid crystals, have emerged as optimal nanomaterials for facilitating nano-dispersions and nano-capsulation to deliver functional ingredients. A colloid is a stable system wherein small particles are dispersed throughout a liquid medium. Association colloids have been employed extensively to transport polar, non-polar, and amphiphilic functional ingredients (21-24). The nanoparticle sizes in colloids typically range from 5 to 100 nm. However, a notable drawback of colloids is their tendency to spontaneously dissociate upon dilution.

**Nano-emulsions:**

Nano-emulsions, composed of two or more immiscible liquids, such as oil and water, are characterized by their limited propensity to mix. Within nano-emulsions, the diameters of the dispersed droplets are 500 nm or smaller. These nano-emulsions have the capacity to enclose functional ingredients within their droplets, thereby facilitating a reduction in chemical degradation.

**Nanoparticles as Intelligent Delivery Systems:**

Syngenta, a prominent agricultural company, has harnessed the potential of nanoemulsions in their growth regulator, Primo MAXX®. This innovative approach involves applying the product prior to stress triggers like heat, drought, disease, or traffic. By doing so, the physical resilience of turfgrass is enhanced, enabling it to endure various stressors throughout the entire growth season. Another product from Syngenta, Karate® ZEON, utilizes encapsulation to deliver a wide-ranging spectrum of pesticides. Upon contact with leaves, the encapsulated structure breaks open, releasing the active compounds. Notably, the encapsulated product "gutbuster" responds to alkaline environments, selectively releasing its contents upon encountering such conditions. The ultimate goal is to customize these products for controlled release in response to diverse cues, such as magnetic fields, heat, ultrasound, moisture, and more. Ongoing research also aims to enhance plant efficiency in utilizing water, pesticides, and fertilizers, thereby reducing pollution and promoting environmentally-friendly agricultural practices.

**Role of Plant Pathogens in Nanoparticle Biosynthesis:**

Nanoscience and nanotechnology research encompass the creation and utilization of nanoparticles comprising various elements and compounds. Among their diverse applications, nanoparticles are gaining attention as antimicrobial agents for managing plant diseases. The generation of nanoparticles can be achieved through various processes, either of physical or chemical nature. An especially safe approach for nanoparticle production is through biological systems, particularly microorganisms (Mansoori, 2005). Microorganisms offer numerous benefits in this context, including: i) tailored outcomes achievable through biotechnology, ii) ease of manipulation, particularly with fungi (Vigneshwaran*et al*., 2006), iii) cost-effectiveness, iv) potential for scalable production, v) high efficiency (Goodsell, 2004), vi) simplicity, and vii) alignment with green chemistry or eco-friendliness. Microorganisms have been recognized as "biofactories" for generating metallic nanoparticles.

**Fungi:**

The utilization of fungi for nanoparticle synthesis is a relatively recent trend. A transition from bacteria to fungi as natural "nanofactories" has been observed due to advantages like straightforward downstream processing, ease of handling (Mandal*et al*., 2006), and their capacity to secrete substantial quantities of enzymes. Nevertheless, fungi, being eukaryotic organisms, are less amenable to genetic manipulation compared to prokaryotes. Thus, genetically modifying fungi for enhanced nanoparticle synthesis might present challenges. Several fungi have been explored for their ability to synthesize metallic nanoparticles, with diverse outcomes in terms of shape, size, and other material properties (Table 3). Understanding the mechanisms of nanoparticle synthesis within microbial systems is crucial for exerting control over the desired characteristics of the resulting nanomaterials.

**Bacteria:**

Among microorganisms, prokaryotes have been at the forefront of attention for nanoparticle biosynthesis (Mandal*et al.,* 2006), as evidenced by examples presented in Table 1. Bacteria have been harnessed for the biosynthesis of various nanoparticles, including silver, gold, FeS, magnetite, and quantum dots like cadmium sulphide (CdS), zinc sulphide (ZnS), and lead sulphide (PbS).

**Table 1. Nanoparticles synthesized by fungi and bacteria.**

|  |  |  |  |
| --- | --- | --- | --- |
| **S.No.** | **Nanoparticle** | **Fungus** | **Reference** |
| 1 | Silver nanoparticles | *Verticillium*sp | Sastry*et al*. 2003 |
| 2 | Silver nanoparticles | *Phoma*sp*.* | Chen *et al*. 2003 |
| 3 | Silver nanoparticle | *Fusariumoxysporum* | Duran *et al*.. 2005 |
| 4 | Silver nanoparticles | *Phaenerochaetechrysosporium* | Vigneshwaran*et al*. 2006 |
| 5 | Silver nanoparticle | *Aspergillusflavus* | Vigneshwaran*et al*. 2007 |
| 6 | Nano crystalline silver | *Trichodermaasperellum* | Mukherjee *et al*. 2008 |
| 7 | Silver nanoparticles | *Fusariumsemitectum* | Basavaraja*et al*. 2006 |
| 8 | Silver nanoparticles(3-30 nm) | *Aspergillusniger* | Gade*et al*. 2008 |
| 9 | Silver nanoparticle | *Fusariumsolani* | Gade*et al*. 2009 |
| 10 | Silver nanoparticle | *Fusariumoxysporum* | Khosravi and Shojaosadati. 2009 |
| 11 | Silver nanoparticle (10-100 nm), extracellular | *Cladosporiumcladosporioides* | Balaji*et al*. 2009 |
| 12 | Silver nanoparticle (5-50 nm) | *Pleurotussajorcaju* | Nithya and Ragunathan, 2009 |
| 13 | Silver nanoparticles | *Alternariaalternata* | Gajbhiy*et al*., 2009 |
| 14 | Silver nanoparticle | *Penicilliumbrevicompactum* | Shaligram*et al*. 2009 |
| 15 | Silver nanoparticle | *Bipolarisnodulosa* | Saha*et al*. 2010 |
| 16 | Silver nanoparticle (5-40 nm) | *Trichodermaviride* | Fayaz*et al*. 2010 |
| 17 | Silver nanoparticle (10-25 nm) | *Aspergillusclavatus* | Verma*et al*. 2010 |
| 18 | Silver nanoparticles (3-30 nm) | *Aspergillusniger* | Jaidev and Narasimha. 2010 |
| 19 | Gold nanoparticles | *Colletotrichum*sp*.* | Shankar *et al*. 2003 |
| 20 | Gold nanoparticles | *Verticilium*sp | Mukherjee *et al*. 2001 |
| 21 | Gold and gold-silver alloy nanoparticles | *Fusariumsemitectum* | Sawle*et al*. 2008 |
| 22 | Bimetallic gold-silver alloy nanoparticle | *F. oxysporum* | Senapati*et al*. 2005 |
| 23 | Gold, silver (5-50 nm) and gold-silver alloy nanoparticle (8-14 nm) | *F. oxysporum* | Mandal*et al*. 2006 |
| 24 | Cadmiunsulphide | *Coriolusversicolor* | Sanghi and Verma. 2009 |
| 25 | Cadmium sulphidenanoparticles | *Fusarium*sp*.* | Ahmad *et al*. 2002: Reyes *et al*. 2009 |
| 26 | Zirconia nanoparticles | *Fusariumoxysporum* | Bansal*et al*. 2004 |
| 27 | Nanoparticulate magnetite | *Fusariumoxysporum and Verticillium*sp*.* | Bharde*et al*. 2006 |
| **Bacteria** | | | |
| 28 | Silver nanoparticle | *Clostridium versicolor* | Sanghi and Preetiverma, 2009 |
| 29 | Silver nanoparticle (5-60 nm) | *Bacillus subtilis* | Saifuddin*et al*., 2009 |
| 30 | Silver nanoparticle (50 nm) | *Brevibacteriumcasei* | Kalishwaralal*et al.* 2010 |
| 31 | Silver nanoparticle (1-100 nm) | *Escherichia coli* | Gurunathan*et al*., 2009 |
| 32 | Silver nanoparticle (1-100 nm) | *Staphylococcus aureus* | Nanda and Saravanan. 2009 |
| 33 | Silver, silver sulphide | *Pseudomonas stutzeri* | Slawson*et al*. 1992 |
| 34 | Silver, gold, and alloy of silver and gold | *Lactobacillus* | Nair and Pradeep. 2002 |
| 38 | Triangular gold nanoprisms | *Actinomycete* | Shankar *et al*., 2004 |
| 36 | Gold nanoparticle | *Rhodococcus*sp*.* | Ahmad *et al*. 2003 |
| 37 | Gold nanoparticle (5-25 nm) | *Bacilussubtlis*168 | Fortin and Beveridge, 2000 |

**Plant Viruses:**

Plant viruses, particularly spherical/icosahedral viruses, serve as remarkable examples of naturally occurring nanomaterials or nanoparticles. Among them, the smallest known plant virus is the satellite Tobacco necrosis virus, which measures a mere 18 nm in diameter (Hoglund, 1968). Intriguingly, plant viruses offer a unique avenue for advancing nanoscience and nanotechnology due to their inherent properties. These viruses consist of single or double-stranded RNA/DNA genomes, encapsulated within a protein coat. This protein coat, often resembling a container, serves both structural and functional roles, carrying the nucleic acid cargo from one host to another.

The remarkable abilities of plant viruses to infect host cells, deliver their nucleic acid genomes to specific cellular sites, replicate, package nucleic acids, and exit host cells in a well-organized manner have made them valuable candidates for nanotechnology applications. Plant viruses have been employed as templates for synthesizing diverse types ofnanomaterials (refer to Table 3). A comprehensive review detailing the utilization of plant viruses as biotemplates for nanomaterials and their broader applications has been undertaken by Young *et al*. (2008).

**Table 2. Nanoparticles synthesized by viruses.**

|  |  |  |  |
| --- | --- | --- | --- |
| Sl. No. | Plant virus | Application | Reference |
| 1 | *Cowpea mosaic vrus* (CMV). an engineered CMV | Iron-platinum nanoparticle (30 nm diameter) synthesis | Shah *et al*. 2009 |
| 2 | Cowpea chlorotic mottle virus (CCMV) | Gold nanoparticle synthesis | Slocik*et al*. 2005 |
| 3 | Cowpea chlorotic mottle virus (CCMV) | As reaction vessel for nanomaterial synthesis | Douglas and Youg. 1998 |
| 4 | Tobacco mosaic vrus (TMV) | Ag and Ni nanoparWe synthesis | Dujardin*et al*.. 2003 |
| 5 | Tobacco mosaic vrus (TMV) | Synthesis of nanowre of nickel and cobal | Young eraf.. 2008 |
| 6 | Tobacco mosaic virus (TMV) | Synthesis of twnetaicaloys of CoPl CoPt3 and FePta nanowires | Tsukamoto et at., 2007 |
| 7 | Brome mosac virus | Gold nanoparticle synthesis | Chen *et al*.. 2005; Dragnea*et al*. 2003: Sun *et al*.. 2007 |
| 8 | -do- | Iron oxide synthesis | Huang *et al*.. 2007 |
| 9 | Red cbver necrotic mosac virus | Au. CoFeA- and CdSenanopartides synthesis | Loo *et al*, 2007 |

**Conclusion**

In conclusion, nanotechnology presents a new frontier in agricultural research, offering innovative tools and nanodevices that have the potential to replace various cellular processes with enhanced efficiency. While the full extent of nanotechnology's impact on the agricultural and food industry is yet to be fully realized, significant strides are being made from theoretical understanding to practical application.The integration of smart sensors and intelligent delivery systems into agriculture holds promise for effectively combating crop viruses and pathogens. The development of nanostructured catalysts is expected to enhance the effectiveness of pesticides, enabling precise dosages to be applied as needed. Nanotechnology offers the potential for precise and controlled delivery of agrochemicals, thereby enhancing disease resistance and crop production.

The synergy between nanotechnology and biotechnology has significantly expanded the range of applications for nanomaterials in crop protection and cultivation. As the size of materials and devices continues to decrease, computing power will increase, resulting in stronger materials and more efficient utilization of fungicides for rapid disease control.Nanotechnology's ability to operate at the molecular and atomic scales will undoubtedly shape the future of agriculture. The transformative potential of nanotechnology in revolutionizing agricultural practices is vast, promising to usher in a new era of precision, efficiency, and sustainability in crop management and protection.

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