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Nanotechnology and its role in plant pathology

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Abstract

Nanotechnology has application potential and prospects in plant pathology in many different aspects *viz.* direct application in foliage, seeds or soil for plant disease management along with accurate, reproducible, quantitative, reliable, specific, robust, and stable results. Extracellularly and intracellularly synthesis of NPs is carried out using microbes. Detection of plant pathogen plays an important role in successful management of many plant diseases, allowing the effective controlling of disease at various stages of disease development. By virtue of their small size, nanoparticles have exclusive chemical, photosensitive and electrical properties and offer improved surface-to-volume ratios. Nano particles finds application in regulation of pathogen concentration, its severity, rate of infection, and percentage of bacteria and fungi, viruses, actinomycetes and other pathogens. Nanoparticles causes induction of systemic resistance, reduction of cellular factors, inhibition of DNA replication, suppression of polymerase activity and lowering of infection rate. Emergence of nano particles has enormous concern in agriculture in relation to environment. Even though some of the nanoparticles have varied negative effect, which might lead to degradation of root membrane, remodeling agriculture and successful management of plant pathogens would not be possible without nanotechnology which directly reflects positively on the sustenance of food production and fulfil the increasing demands of food production in an efficient and cost-effective way. Moreover, nanotechnology plays important role in pathogen sensing and control and provides a remedy to the emerging agricultural challenges. Profound research studies, regarding the possible aspects of nanotechnology in plant pathology, is being carried out for exploring an economic, eco-friendly stabilized nanomaterials for effective management of plant diseases in the long run.

Keywords: Nanotechnology, plant disease management, biosynthesis

Introduction

The need of fulfilling the challenge of feeding the ever increasing population has instigated the need for a push in the global food production. However, a disturbance of the food production is instigated by the climate change (Elmer and White, 2018) [22]. The assured promise of food to everyone by the first green revolution has compelled the need for a second green revolution since agriculture is now experiencing a plateau (Singh, 2012) [94]. Hence agriculturist faced a daunting challenge. Globally, agriculture is facing a serious challenge due to biotic and abiotic stresses causing crop losses (Malandrakis *et al*., 2019) [59]. Efficient management of pests and diseases is carried out using the conventional agricultural techniques. However, changes in the soil water composition, along with the reduction in absorption rate of nutrients, organic matter content, soil texture and pH accompanied with the damage of leaf residue, target beneficial organism and pollinator species is often associated with the poor active ingredient delivery system drastically. Thus, the continuous reliance on synthetic agrochemicals for plant disease management is being questioned (Kumar *et al*., 2021a) [54]. The novel mode of action of nanoparticles such as slow and controlled release of active ingredient and multi-site mode of action along with alleviation of effectivenss in lower has significantly reduced the resistance of the pathogens along with the reduction in costs (Malandrakis *et al.*, 2019; Kumar *et al*., 2021a) [59, 54]. Thus nanoparticles such as copper, zinc titanium, magnesium, gold, alginate, silver have to play a potent role in the effective management of various bacteria, viruses an deukaryotic microorganisms in agriculture.

History of nanotechnology

The investigation on gold colloids by Michael Faraday in 1831 paved the way for the study on nanoparticles.

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After a gap of 125 years, Nobel Laureate Richard Feynman studied the potential of fabricating matter at nano levels. Norio Taniguchi, a Japanese researcher, coined the term ‘nanotechnology’ and engineered the materials at nano scale.

Tools of Nanotechnology

Nanosensors

Nanosensor, owing to their small and portable size, gives rapid response and real time processing. They also give with accurate, quantitative, reliable, reproducible, robust, specific and stable result. Nanotechnology has the potential to integrate into precision farming as it plays a crucial role in identifying infections in asymptomatic plants and subsequently delivering targeted treatments. Micromechanical cantilevers can be employed for the identification and detection of fungal spores. Proteins such as concanavalin A, fibronectin, and immunoglobulin are surface grafted onto both uncoated and gold-coated silicon cantilevers that have been micro-fabricated. The proteins have different binding abilities to the molecular structure present on the fungal cell surface. The dynamically operated cantilever arrays measures the change in resonance frequency due to spore immobilization and germination. The biosensors are capable of detecting fungi in the range of 103-106 cfu ml/l, and this method proves to be faster when compared to the conventional approach. (Nugaeva *et al*., 2005) [66].

Quantum dots (QDs)

Quantum dots are roughly spherical and few nm in diameter. The utilization of quantum dots has been widely employed in various applications such as cell labelling, cell tracking, *in vivo* imaging, and DNA detection (Sharon *et al*., 2010) [78].

Nanofabrication

Nanofabrication has been employed in the production of synthetic components of plants, including stomata and xylem vessels. The investigation of infection mechanisms and pathogen behavior within plant hosts involves the utilization of these materials. The production of micro nanoparticles can be categorized into two distinct methods: biological synthesis and biotemplated forming.

Biological synthesis of MNPs involve the use of magnetotactic bacteria which biomineralizes endogenous magnetite NPs. In the biotemplated forming method, microorganisms with diverse shapes and structures are directly employed as templates for the deposition of nanoparticles (NPs). This enables the fabrication of magnetic nanoparticles (MNPs) with standardized shapes and structures. Consequently, this approach harnesses the inherent diversity of microorganisms to create MNPs with varying shape and structure. (Gong *et al*., 2023) [30].

Smart Delivery

In the biological system, nanoparticles are used in smart or targeted drug delivery. P. Ehrlich proposed the utilization of nanoparticles for smart delivery, which he referred to as "magic bullets." These nanoparticles, composed of a magnetic Fe core, are directed towards a specific location within the body for targeted delivery.

The biocompatibility is provided by the carbon coating which acts as an adsorbing surface for various types of molecules. Nano particles can be used in transportation and penetration inside the whole plant. (Gonzalez-Melendi, 2008) [31]. Thus, the nanoaprticles can be used in delivery of substances to various plant pathogens.

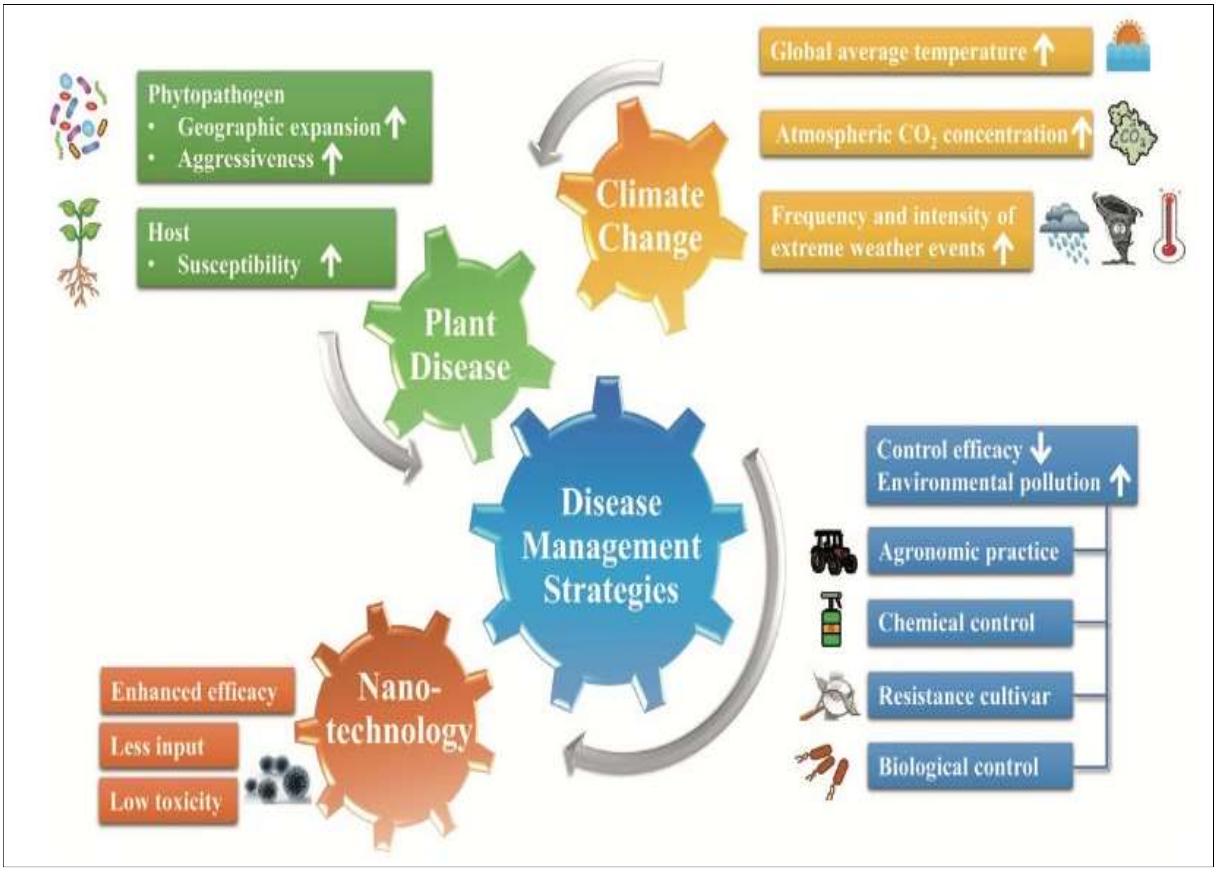

Fig 1: The schematic depiction of the consequences of climate change on plant diseases and the corresponding strategies for their management, along with the utilization of nanotechnology in the field of plant disease management, is presented. (Fu *et al*., 2020) [28].

Approaches involed in nanotechnology

The top-down methodology for producing nanoparticles involves the reduction of bulk materials into nanosized structures or particles through mechanical and physical methods such as grinding, milling, and crushing. This approach requires the manipulation of a small number of atoms or molecules to achieve the desired outcome. This technique is employed to create nano-composites and nano-grained bulk materials, such as metallic and ceramic nanomaterials, with a wide range of sizes (10-1000 nm). The synthesis methods utilized are an extension of those used for producing particles on a micron scale. The bottom-up approach involves the assembly of numerous molecules in parallel steps, based on their molecular recognition characteristics, resulting in the formation of more intricate structures from atoms or molecules.

This approach enables the production of nano materials with uniform control over their sizes, shapes, and size ranges. Typically, this method is utilized for the preparation of a majority of nano-scale materials (1-100 nm) and plays a crucial role in the production of nanostructures and nanomaterials.

Biological approach for synthesis of nanoparticles

Microorganisms are capable of synthesizing nanoparticles (NPs) through both extracellular and intracellular processes. In the extracellular synthesis method, the culture filtrate is obtained through centrifugation and then combined with an aqueous metallic solution. The resulting mixture is monitored for NP synthesis by observing any changes in the color of the solution.. In the event of intercellular synthesis, the biomass undergoes a thorough washing process after cultivating the microbes under optimal conditions. Subsequently, the biomass is incubated with the metal ion. The nanoparticles are then collected through a series of procedures including ultrasonification, centrifugation, and washing. Remarkably, both *Pseudomonas strutzeri* and *P. aeruginosa* exhibit survival capabilities even in highly concentrated metal ion environments. (Bridges *et al*., 1979; Haefeli *et al*., 1984) [8, 34]. *Thiobacillus ferroxidans*, *T. thioxidans*, and *Sulfolobus acidocaldarius* are capable of converting ferric to ferrous ion while utilizing elemental sulphur as their energy source. These bacteria exhibit the remarkable ability to thrive and acquire nutrients necessary for their survival and reproduction. Silver nanoparticles, on the other hand, are circular in shape and have a diameter range of 20- 200nm. *P. rhodesiae* has been reported to synthesize the AgNPs (Hossain *et al*., 2019) [37]. *B. cereus* SZT1 was employed to conduct the extracellular synthesis of AgNPs. The bacteria were obtained from soil contaminated with waste water. The resulting AgNPs exhibited a spherical morphology, with a diameter ranging from 18 to 39 nm. (Ahmed *et al*., 2020) [4]. The endophytic Pseudomonas poae's culture filtrate was utilized in the production of AgNPs, which had a size range of 19.8-44.9 nm (Ibrahim *et al*., 2020) [38]. Silver nanoparticles (AgNPs) were produced through the synthesis process using the culture supernatant of Stenotrophomonas sp. BHU-17. The bacterium was obtained from the soil of an agricultural farm. (Mishra., 2017) [63]. Stenotrophomonas, a newly discovered bacterium, has proven to be instrumental in the eco- friendly production of silver (Ag) and gold (Au) nanoparticles through a process known as green synthesis. Moreover, *Kocuria flava*, a marine strain of bacteria has been able to synthesize copper NPs with the size of 5-30nm (Kaur *et al*., 2014) [45]. Pseudomonas stutzeri Ag259 has demonstrated the capability to intracellularly synthesize a diverse range of nanoparticles, including silver (Ag), gold (Au), and iron (Fe).. (Srivastava and Constanti, 2012) [95]. CuONPs were synthesized using Streptomyces zaomyceticus Oc-5 and Streptomyces pseudogrosoulus Acc-11, both of which belong to the streptomyces bacteria genus. (Subramani *et al*., 2014) [83]. The biomass of three endophytic actinomycete strains, namely Streptomyces capillispiralis Ca-1, Streptomyces zaomyceticus Oc-5, and S. pseudogrisolus Acv-11, along with the filtrates derived from their biomass, were utilized as catalysts for the green synthesis of silver nanoparticles (AgNPs). The extracellular synthesis of AgNPs was achieved by employing the supernatant liquid obtained from *Streptomyces griseorube* (Ranjitha *et al*., 2017) [72]. The antimicrobial potentials of green synthesized nanoparticles were found to be superior to those of conventionally synthesized nanoparticles. This is attributed to the presence of certain biomolecules that act as capping and stabilizing agents during the synthesis process of the nanoparticles (Gahlawat and Choudhary, 2019) [29]. Fungi such as ascomycete and imperfect fungi produces more than 6400 bioactive compound. Fungi exhibit a significant level of resistance to heavy metals and have the ability to internalize and accumulate these metals. As a result, fungi can be employed in the reduction and stabilization processes during the synthesis of nanoparticles. Nevertheless, the successful synthesis of metallic nanoparticles is contingent upon the specific conditions of the fungal culture. Extracellular AuNPs were synthesized by *Trichothesium* species which reduces the Au ions. Similarly *Rhizopus stolonifer* synthesized the AgNPs in the size of 2.86, 25.89 and 48.43 nm. The production of AgNPs through biosynthesis has been observed in *Fusarium oxysporum* as well (Husseiny *et al*., 2015) [96]. *Penicillium fellutanum* has been used to synthesized AgNPs (Kathiravan *et al*., 2014) [43]. Yeast undergoes biosorption, biotransformation, bioaccumulation of metal ions by living microorganisms. The immobilization of metal ions encompasses several significant mechanisms (Siddique., 2015) [79]. It has been reported that AgNPs biosynthesis through transformation has been achieved using *Saccharomyces cerevisiae*. Additionally, *Yarrowia lipolytica*, a marine yeast, has also been utilized for the biosynthesis of AgNPs. Intercellular synthesis of AuNPs has been accomplished using *Pichia jadini*, where the growth and cellular activities of the organism were regulated to control the shape and size of the nanoparticles. Furthermore, extracellular synthesis of copper NPs has been reported using *S. cerevisiae*.

The production of Ag-NPs is achieved through the utilization of *Pseudomonas stutzeri* AG259, which is a bacterial strain that is resistant to silver. These cells amass significant quantities of Ag-NPs, reaching up to 200nm in size. The enzymatic process involving the exposure of aqueous Ag ions to *Fusarium oxysporum* in solution was utilized to synthesize Ag- NPs using fungi. This procedure resulted in the formation of a remarkably stable Ag hydrosol. The nanoparticles (NPs) have a size range of 5–15 nm and are kept stable in a solution by a fungus that secretes proteins. The process of synthesizing silver nanoparticles (Ag-NPs) involved using plant extracts to reduce an aqueous solution of $AgNO_3$, and the resulting NPs were then analyzed for their characteristics. (Rafique *et al*., 2016) [68].

Detection of plant diseases

The identification of plant pathogens is crucial for effectively managing plant diseases, as it enables the monitoring and control of diseases at different stages of their development. In the past, various biosensors have been employed for this purpose, primarily relying on serological assays, phages, DNA probes, and antibodies. However, the integration of nanotechnology into plant disease detection has the potential to revolutionize the field by facilitating the creation of advanced instruments that can rapidly and accurately detect plant infections at an early stage. Nanoparticles, due to their small size, offer enhanced surface-to- volume ratios and possess unique chemical, photosensitive, and electrical properties that are absent in their bulk counterparts (Mark *et al.*). Nanoparticles assists the biosensors in terms of selectivity, sensitivity and detection limits.

Metal nanoparticles possess distinct characteristics that set them apart from other materials. One notable feature is their exceptional melting points, which exceed those of many other substances. Additionally, these nanoparticles exhibit remarkable catalytic properties, enabling them to facilitate chemical reactions with great efficiency. Moreover, their exceptional toughness makes them highly durable and resistant to deformation. Lastly, metal nanoparticles display intriguing coloration, adding aesthetic appeal to various applications. (Singh *et al.*, 2017) [80]. Metal nanoparticles have a distinct advantage in pathogen detection due to their higher surface area to volume ratio. This characteristic enables them to detect pathogens with lower detection limits. Moreover, the detection of metal nanoparticles through electrochemical signals is a simpler and more cost-effective method compared to enzyme assays. As a result, metal nanoparticles are gradually replacing the enzyme labeling system in phytopathogen diagnostics. Among the commonly used metals for sample detection, gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), zinc sulfide (ZnS), lead sulfide (PbS), and cadmium sulfide (CdS) are widely employed.

The process of identifying single stranded DNA (ssDNA) specific to *Acidovorax avenae* sp. *citrulli* was accomplished through the utilization of colloidal AuNPs for labelling. A strip based DNA sensor was then constructed to detect the presence of the pathogen. In a colorimetric detection method of pathogen DNA molecules, AuNPs labelled DNA probes were employed to detect *Pseudomonas syringae* pathovars. A similar approach was used to detect the wilt causing bacteria, *Ralstonia solanacearum*. To detect fungal pathogens, an integrated universal primer mediated asymmetric PCR with AuNPs-based LFA was utilized for the detection of *P. infestans*. (Zhan *et al.*, 2018) [91] The development of a highly immunospecific sensor was achieved through the covalent immobilization of a polyclonal antibody on an Au- gold coated chip. This immobilization was facilitated by a mixed self-assembled monolayer of alkanethiols. The sensor was successfully utilized for the detection of *Pseudocercospora fijiensis*. In another study, an AuNPs-enhanced dynamic microcantilever (MCL) and isothermal recombinase polymerase amplification were employed for the detection of *Leptosphaeria maculans* in oilseed rapeseed. The use of AuNPs in virus detection offers several advantages, including ease of synthesis, surface modifications, stability, biocompatibility, and a high absorption coefficient, which enhances their applications in detection platforms. Additionally, a simple and sensitive label-free colorimetric detection method for Cucumber green mottle mosaic virus (CGMMV) was developed using unmodified AuNPs as a colometric probe. This method involved the binding of CGMMV target products from reverse transcription-polymerase chain reaction (RT-PCR) with species-specific probes, resulting in a change in color upon salt induction. Furthermore, AuNPs were conjugated to the primary antibody, and the limit of detection (LOD) for the detection of CGMMV was determined to be at a sap dilution of 10^3 virus particles. It is worth noting that there is typically an increase in resistance to NaCl-induced aggregation (Kulabhusan *et al.*, 2022) [52]. The functionalization of AuNPs and DNA hybridisation assay enable the detection of pathogen ranges up to 214 nm. A biosensor of the AuNPs type is utilized to detect bacterial canker, with the aid of a single plot hydrolysis (Fang *et al.*, 2014) [24]. The correlation between the concentration of the analyte and the intensity of color change can be observed by making certain modifications, these devices have the capability to identify volatile metabolites that are specific to pathogens.

The dispersed and aggregated forms of AgNPs exhibit a noticeable transition in color, shifting from yellow to brown. To identify the presence of pathogenic bacteria *Xanthomonas axonopodis*, nanoprobes consisting of silica and conjugated with Ig antibody are employed.Additionally, the detection of *Phytoplasma aurantifolia* is facilitated by nanoparticles synthesized using indium, cadmium (Cd), and silicon (Si) (Singh *et al.*, 2020) [81].

Copper nanoparticles and their potential role in detection of plant diseases has been explored. Novel and portable biosensors utilizing copper as a foundation have been created using serological assays, phage, DNA probes, and antibodies (Chhipa, 2019; Kumar *et al.*, 2021b; Sharma *et al.*, 2021) [17, 54, 76]. The activity of *Sclerotinia sclerotiorum* has been detected using nano-biosensors based on copper (Cu). The nano biosensors has been successfully implemented for quantification of salicyclic acid for the estimation of the pathogen infection in case of mungbean leaf spot and bacterial blight disease.

ZnO NPs have gained global interest in management and detection of plant diseases since the synthesis of particles in manometer region is easy. ZnO NPs have plays extensive role in detection of plant diseases due to the enhanced specific surface area since the diminished particle size leads to increased particle size. Moreover, ZnO NPs possesses photocatalysis effect on plant pathogenic fungi. The reported study highlights the antifungal and antibacterial properties of ZnONPs against two specific pathogens, namely *Pseudomonas aeruginosa* and *Botrytis cinerea* (Bayat *et al.*, 2019) [6]

Table 1: Examples of nanomaterials as antimicrobial agents for disease suppression and control (Fu *et al*., 2020) [28]

Sl. no	Nanomaterials	Target pathogen	Application dose	Host and disease	Effect	Toxicity towards non target organisms
1.	AgNPs	*Botrytis cineria, Penicillium expansum,, Aspergillus niger, Alternaria sp., Rhizopus Sp.*	3 mg/L-1	Multiple disease in many crops	AgNPs can inhibit fugal growth in PDA Agar	No phytotoxicity
2.	AgNPs	*Fusarium oxysporum* f.sp. *ciceri*	10 micro gram	Chickpea, wilt disease	AgNPs show very high antifungal activity against FCC *in vitro.*	No phytotoxicity and no negative impact.
3.	CuO, Cu2O, NPs	*Phytophthora infestans*	27.78-43.87 g/Litre	Tomato late blight	Leaf lesion were significantly suppressed	No phytotoxicity
4.	Zno/Nano-copper nanoparticles	*Xanthomonas citri f.* sp. *citri*	0.22 Kg/Ha metallic copper	Citrus canker disease	In field trials, the ZnO-Cu compound exhibited potent antimicrobial properties against the phytopathogen responsible for citrus canker disease, surpassing the effectiveness of commercial copper oxide pesticides	No phytotoxicity
5.	Chitean NPs	0.0005% (w/v) *In vitro* 0.1% (w/v) for folair spar/ application *Fusarium oxysporum* f. sp. lycopersici	0.0005% (w/v) *In vitro* 0.1% (w/v) for foliar spar/ application	Tomato, Fusarium wilt	The prepared chitosan NPS exhibited excellent antifungal properties in *in vitro* conditions. The foliar applications of chitosan PS to tomato plant showed delay in wilt disease symptoms expression and results in the 81% protection of tomato plants from wilt disease.	

Chitosan, a natural polymer, has been found to be an effective bio elicitor that can induce systemic resistance in plants. However, its use as an antifungal agent is limited due to its low solubility in aqueous medium. To overcome this limitation, researchers have examined the use of chitosan in the form of nanoparticles for its phyto immunogenic activity. The induced enzyme of two different transcript activities were analysed, and it was found that plants exposed to chitosan particles showed higher expression of defense-related enzymes and genes. Additionally, there was an overexpression of cinnamte-4-hydrolase (C4H), flavonoid 3-hydrolase (F3H), and anthocyanidin reductase (ANR) genes in chitosan and CNP treated leaves, indicating the role of chitosan NPs in the secondary defense induction in plants. Chitosan-treated plants directly modulate the immune response in plants and play a key role in the simulation of defense response in the chitosan-treated plants. (Chandra *et al*., 2015) [15]. In chitosan-treated plants, a notable discharge of defense enzymes including PO, β-1,3-glucanase, and PAL was observed, alongside the stimulated activity of antioxidant enzymes SOD and CAT. Consequently, a simultaneous manifestation of genes associated with defense mechanisms, antioxidant enzyme coding genes, and flavonoid biosynthesis genes was observed (Chandra *et al*., 2015) [15].

QDs, also known as quantum dots, have been utilized in disease diagnosis through the implementation of the fluorescence resonance energy transfer (FRET) mechanism. This mechanism involves the transfer of energy between two light-sensitive molecules, enabling effective detection and diagnosis of various diseases (Grahl and Märkl, 1996) [32]. The technique was subsequently utilized for the recognition and characterization of *Aspergillus amstelodami*. (Kattke *et al*., 2011) [44]. The utilization of carbon nanotubes (CNTs) has been implemented in the detection of plant diseases, enabling the detection of plant metabolites. This is due to the fact that any phytopathological diseases can be linked to alterations in the metabolism of aromatic compounds. Nanodiagnostic imaging tools have the capability to visualize plant tissues and cells, thereby facilitating the diagnosis of phytopathogens.

Carbon-coated magnetic nanoparticles have been employed to observe the trajectory, accumulation, and movement of nanoparticles within the cellular structure of plant cells (González-Melendi *et al*., 2008) [31]. A highly sensitive nanosensor utilizing quantum dots (QDs) has been created for the purpose of detecting phytoplasma (*Candidatus Phytoplasma aurantifolia*) in lime trees that have been infected.

Management of plant diseases

Nanotechnology offers various potential avenues for the management of plant diseases. The predominant approach involves directly applying nanoparticles to foliage, seeds, or soil. This method is widely employed in plant disease management. Notably, the impact on non-target organisms, particularly mineral fixing solubilizing bacteria, holds immense importance when compared to chemical pesticides.

Copper nanoparticles have emerged as effective antimicrobial agents, exhibiting remarkable antimicrobial activities against a wide range of microorganisms, including those that are resistant to multiple drugs. Additionally, the abundance and affordability of copper make the synthesis of copper nanoparticles a cost-effective process. Copper nanoparticles have been utilized in the field of agriculture to effectively suppress the growth of fungi, including Phytophthora infestans, which is responsible for causing late blight disease in tomatoes. The conversion of copper metal into nanoparticles has significantly enhanced the fungicidal properties, thereby improving its efficacy against different pathogens such as *Phytophthora sp.* and *Corticium salmonicolor* (Kanhed *et al*., 2014) [42]. After 48 hours of incubation, the fabric coated with copper oxide nanoparticles (100–150 nm) exhibited a complete eradication of *E. coli, S. aureus* and *Aspergillus niger*, with a reduction rate of 100%. (Schrand *et al*., 2010) [74]. Copper nanoparticles exhibit efficacy against both Gram-positive and Gram-negative bacteria. These particles have the ability to eradicate bacterial cells by attaching to their membranes and generating reactive oxygen species (ROS). Consequently, the cell permeability increases, leading to the loss of control over the transportation of CuO through the cytoplasmic membranes of the bacterial cells.

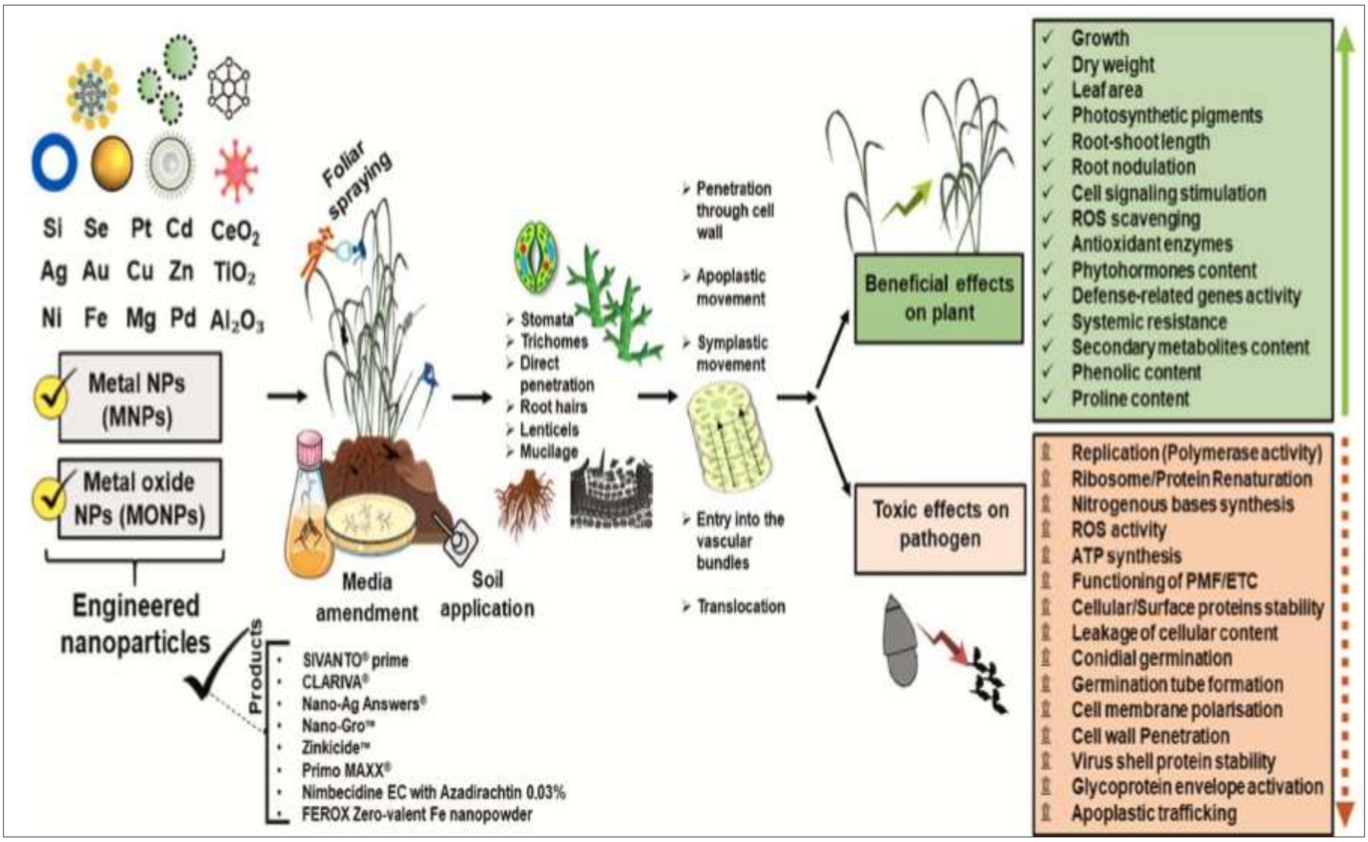

Fig 2: The utilization of engineered nanoparticles, specifically Metal NPs and Metal oxide NPs, in the management of plant diseases showcases both advantageous impacts on plants and inhibitory effects on pathogens.

lumina nanoparticles exhibited a slight inhibitory effect on growth, but only at extremely high concentrations, which could be attributed to the interaction of surface charges between the particles and cells. The antialgal effect of AgNPs was investigated on *Scenedesmus* sp. and *Chlorella* sp., and a noticeable decrease in chlorophyll content was observed in the cells treated with nanoparticles (Sadiq *et al*., 2011) [97]. The particles' ability to scavenge free radicals effectively hindered the disruption of cell walls, thereby exhibiting a significant antimicrobial effect (Sadiq *et al*., 2009) [98]. The colonization of two phytopathogenic fungi, namely *Magnaporthe grisea* and *B. sorokiana*, is suppressed by the AgNPs. Inhibiting disease development was found to be crucial through the direct exposure of Ag NPs to pathogenic spores and germination tube. The effectiveness of AgNPs, however, is dependent on fungi spore inoculation and is more effective when applied immediately after infection. Additionally, the production of sclerotia by the soil-borne fungi *Rhizoctonia solani* was significantly reduced due to the presence of silver AgNPs. Furthermore, AgNPs exhibit fungicidal activity against various molds and yeast (Bryaskova *et al*., 2011; Kim *et al*., 2012) [9-10, 50-51].

AgNPs have also been employed in the management of viruses. The utilization of AgNPs has been documented to effectively impede the activity of the sun hemp rosette virus. (SHRV) in *Cyamopsis tetragonoloba* and cause the complete suppression of the pathogen. The suppression of activities of yellow mosaic virus by AgNPs is also reported. Different concentrations of silver nanoparticles (AgNPs) have demonstrated antiviral properties and have shown effectiveness in treating Banana bunchy top virus (BBTV). AgNPs have also been successful in managing plant bacteria. They have been used against both gram-positive bacteria, such as *Staphylococcus aureus* and *Bacillus subtilis*, and gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Escherichia coli*. In another study, AgNPs with a spherical morphology and a size ranging from 20-100nm were observed to inhibit the growth of *Xanthomonas campestris* and *X. axonopodis* (Vanti *et al*., 2019) [86]. Brinjal plants were effectively protected against the root knot nematode, *Meloidogyne javanica*, by the application of Green AgNPs, which demonstrated strong nematocidal activities. Remarkably, the AgNPs exhibited their efficacy without causing any harm to the overall health of the plants. (Abdellatif *et al*., 2016) [2]. Thus, AgNPs foliar spray activates the systemic resistance in plants.

Various microorganisms, such as Gram-positive bacteria, Gram-negative bacteria, and certain pathogenic fungi, have been confirmed to be susceptible to the antimicrobial effects of AuNPs. These gold nanoparticles, synthesized through a green method and ranging in size from 45 to 75 nm, exhibit potent antifungal properties. They effectively combat wheat stem rust caused by *Puccinia graminis tritici*, as well as other fungal pathogens like *Aspergillus flavus, Aspergillus niger, and Candida albicans*. The standardized well diffusion technique is employed to assess their efficacy. Consequently, these AuNPs hold significant promise in the development of fungicides for the treatment of various plant diseases. (Jayaseelan *et al*., 2013) [40]. The unique properties of AuNPs, such as their ability to concentrate illumination, their strong positive attraction to the negatively charged plasma layer of organisms, and their ability to be conjugated with antimicrobial agents and antibodies, are responsible for this action. Additionally, AuNPs synthesized using green methods have shown promising antifungal activity against *A. niger* and *A. flavus* (Brakov, 2005) [7]. The eradication of viral diseases like yellow mosaic virus and yellow dwarf virus in barley is achieved through virus elimination, which effectively halts the spread of the pathogen. Meanwhile, the use of nanoparticles hinders the formation of peptidoglycan, leading to the breach of the cell wall and ultimately killing the pathogen. In addition, the inhibition of DNA uncoiling and transcription is observed with the use of AuNPs. On the other hand, green nanoparticles have been found to be effective in inhibiting the growth of fungus, specifically *Pseudomonas aeruginosa* (Rai, 2010).

ZnONPs have demonstrated remarkable efficacy as antimicrobial agents, exhibiting effectiveness against a wide range of microorganisms including bacteria, fungi, toxicogenic fungi, as well as thermophilic and barophilic spores. The antimicrobial properties of ZnONPs have proven to be highly successful, effectively combating various microorganisms such as bacteria, fungi, toxicogenic fungi, as well as thermophilic and barophilic spores. ZnONPs exhibit exceptional antimicrobial activity, displaying efficacy against bacteria, fungi, toxicogenic fungi, as well as thermophilic and barophilic spores. (Sondi and Salopek-Sondi *et al*., 2004) [82]. Zinc oxide (ZnO) has the potential to act as an effective antimicrobial agent for combating plant diseases. The disruption of the hyphal cell structure can be attributed to the excessive accumulation of nucleic acids and sugars, as ZnO nanoparticles (NPs) have the ability to impact cell physiology and stimulate the increased production of nucleic acids. Moreover, the heightened synthesis of nucleic acids can be viewed as a stress response by fungal hyphae, while the elevated production of starches may serve as a protective mechanism against the effects of ZnO NPs on the cells (Perez Espitia *et al*., 2012) [23].

The antimicrobial properties of metal nanoparticles have been investigated and found to exhibit antibacterial effects. (Kim *et al*., 2007; Shahverdi *et al*., 2007) [49, 75] and antifungal agents. Despite the significant financial interest, there is a scarcity of research reports on the efficacy of nano- based materials in combating plant viruses (Elbeshehy *et al*., 2015) [21].

Chitosan nanoparticles have demonstrated the ability to impede the spread of viruses and viroid's within plants and to boost the host's hypersensitive reaction to infection (Faoro *et al*., 2001; Chirkov, 2002) [25, 18]. The effectiveness of suppressing viral infection is directly proportional to the molecular weight of chitosan (Kulikov *et al*., 2006) [100]. Several reports have corroborated this problem across a range of viral pathogens, including potato virus X, tobacco mosaic and necrosis viruses, alfalfa mosaic virus, peanut stunt virus, and cucumber mosaic virus (Chirkov, 2002) [18]. Conversely, various reports have highlighted the broad spectrum of bacterial species that chitosan exhibits antibacterial activity against. (El Hadrami *et al*., 2010) [20]. The application of chitosan in Valencia orange has been observed to result in an increase in the activities of chitinase and β-1,3-glucanases (Canale Rappussi *et al*., 2009) [13]. Chitosan treatment has the potential to enhance fruit resistance by regulating ROS levels, inhibiting enzymes, and modulating the ascorbate-glutathione cycle. Additionally, it can boost the capacity of pathogenesis-related (PR) proteins through various mechanisms, such as activating cell surface or membrane receptors and influencing plant DNA compliance, thereby impacting gene translation (Hadwiger, 1999) [33]. The utilization of chitosan has been reported to enhance the activity of phenylalanine ammonia lyase (PAL) in fruit tissue that has undergone treatment.

Action of nanoparticles against plant pathogen-the mechanism

Nano particles are employed in the control of pathogen concentration, severity, infection rate, and the presence of various pathogens such as bacteria, fungi, viruses, actinomycetes, nematodes, and others in plant disease management (Liang *et al*., 2018) [56]. Nanoparticles causes induction of systemic resistance, reduction of cellular factors, inhibition of DNA replication, suppression of polymerase activity and lowering of infection rate. The mode of action of silver nanoparticles against pathogenic invasion involves several mechanisms. These include altering membrane structure and causing leakage of cellular content, ATP, and adhesion of cell membrane. Additionally, mitochondrial dysfunction, destabilization, and denaturation of protein and rhizomes inside the cell can occur. Oxidation of lipids, proteins, and mediation of cellular and ROS toxicity is also observed. Finally, silver nanoparticles can alter phosphorylation profile and simulate via modulation of cell signalling. (Sharma *et al*., 2019) [76].

The bactericidal activity of AgNPs was assessed, and it was found that they induce the production of reactive oxygen species (ROS), cause DNA damage, and inhibit DNA replication in phytopathogens. Additionally, the antibacterial activity of AgNPs can be influenced by the presence of thiol groups in L-cysteine residues, which interact with proteins and subsequently inhibit enzyme activity (Hernandez-Diaz *et al*., 2020) [87].

The root-shoot length, proline, leaf area, chlorophyll content, and phenol levels were enhanced in Phaseolus vulgaris and Zea mays plants infected with BBTV when exposed to AgNPs ranging from 20-60nm (Heba *et al*., 2020) [35]. The presence of ToMV and potato virus Y (PVY) in tomato plants has been found to result in an increase in their phenolic content (Mahfouza *et al*., 2020) [57]. Previous studies have reported that SiONPs and ZnOPs exhibit antiviral properties by causing damage to the virus shell protein, inactivating glycoproteins, and inducing aggregation of the virus-cell wall. (Vargas-Hernandez, 2020) [87]. Direct injury is caused to different virus like particles leading to deterioration and puffed VLPs caused by AgNPs in yellow dwarf virus in barley. The combination of SiO2NPs and ZnONPs demonstrated a beneficial effect on TMV infection under both *in vivo* and *in vitro* conditions. Consequently, the antimicrobial properties of these nanoparticles involve various mechanisms such as hindering ATP synthesis, degrading cell membranes, disrupting protein motive force, altering permeability, inhibiting enzyme activities, suppressing cytochrome activity, promoting the production of reactive oxygen species, denaturing ribosomes, and impeding nitrogen base function. (Chorianopoulos *et al*., 2011; Mehrdad *et al*., 2017; Khezerlou *et al*., 2018; Farias *et al*., 2018; Zhang *et al*., 2019; Khan *et al*., 2019; Chen *et al*., 2019) [19, 61, 47, 26, 92, 46, 16].

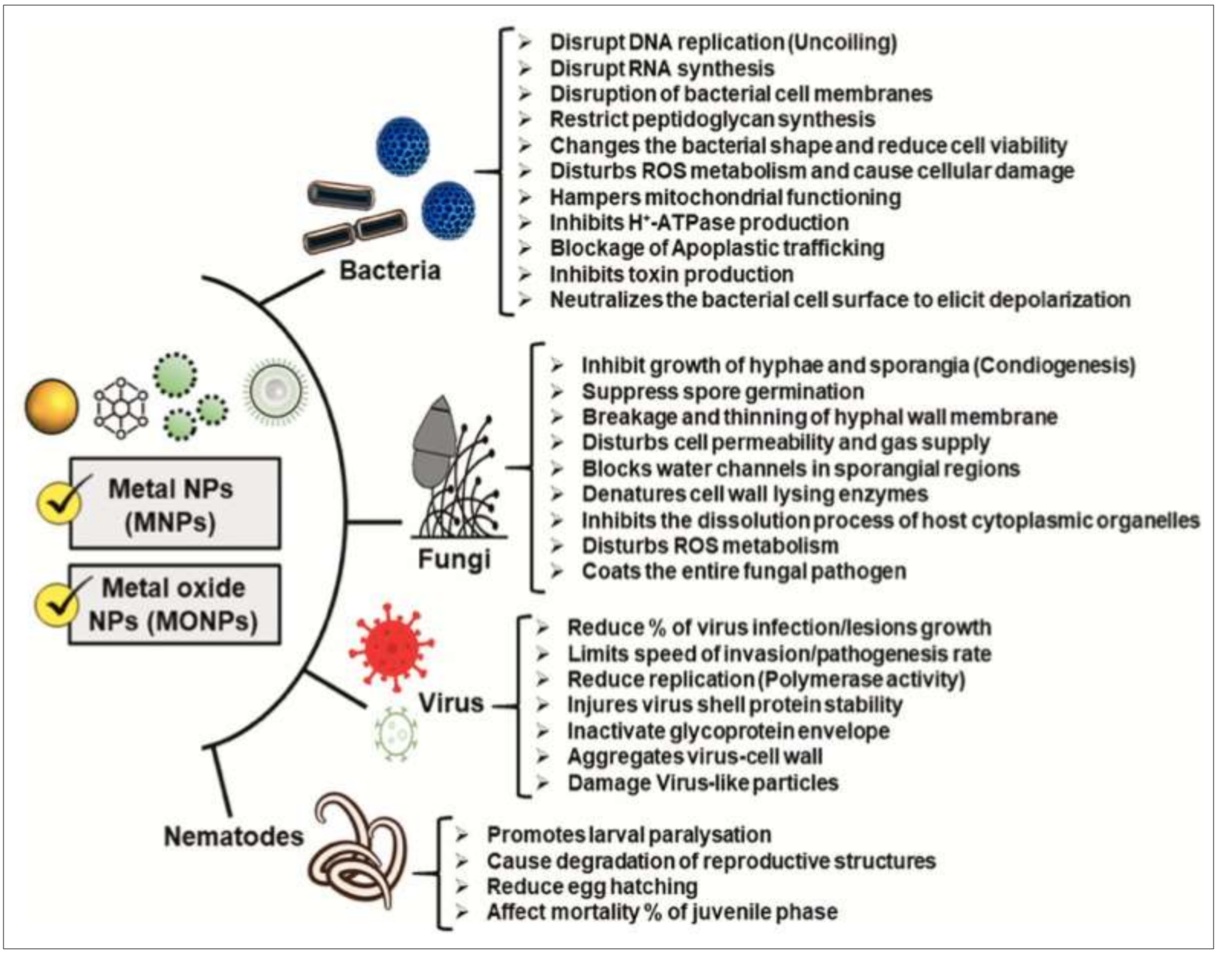

Fig 3: The utilization of engineered nanoparticles (Metal NPs and Metal oxide NPs) is demonstrated through a schematic illustration, which showcases their impact on four different types of pests, namely Bacteria, Fungi, Viruses, and Nematodes (Kumar *et al*., 2022).

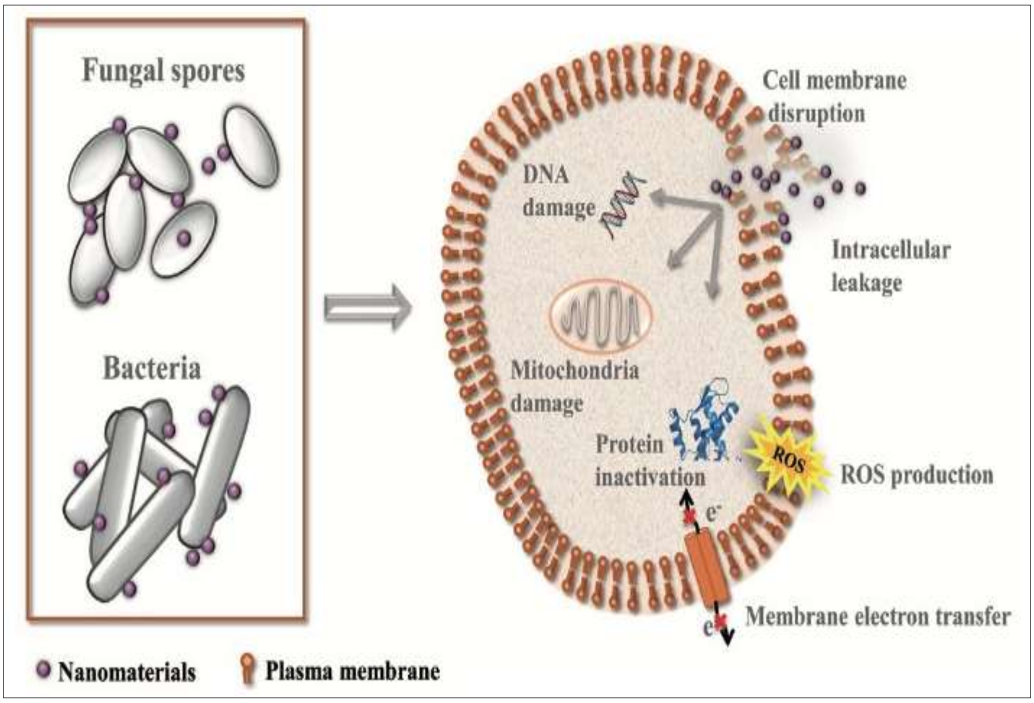

Fig 4: Proposed mechanism of toxicity against pathogenic bacteria and fungi. (Fu *et al*., 2020) [28]

In 2014, Zhao and colleagues presented findings on metabolomics data that highlighted the significant impact of $Cu(OH)_2$ nano pesticides in bolstering antioxidant defense and improving tolerance in maize and cucumber. Copper-based fungicides exert their inhibitory effects on microbes by targeting and damaging vital bio-molecules, including DNA, proteins, and lipids (Borkow and Gabbay 2005; Nimse and Pal 2015) [7, 64]. Copper toxicity is attributed to Fenton reactions, which involve the interconversion of free copper ions (Cu^{2+}) between Cu (I) and Cu (II). These reactions generate ROS that lead to the degradation of lipids, oxidation of proteins, and DNA damage. (Suresh *et al*., 2013) [101]. The adhesion of copper particles to bacterial cell membranes can lead to the destruction and death of the cells. This adhesion also triggers the production of reactive oxygen species (ROS), which further disrupts the cell's ability to control the transport of copper oxide (CuO) through its cytoplasmic membrane, ultimately causing the loss of control in managing CuO transportation. (Subramanian *et al*., 2014) [83].

Extensive research has been conducted on metal nanoparticles to explore their antimicrobial properties, which have demonstrated their effectiveness against bacteria. (Morones *et al*., 2005; Kim *et al*., 2007; Shahverdi *et al*., 2007) [99, 49, 75] and antifungal agents. However, despite the financial interest, there are very few reports on the effectiveness of nano-based materials against plant viruses (Elbeshehy *et al*., 2015) [21]. In principle, it is possible to analyse the antiviral activity of any metal. However, the focus is primarily on three types of metallic nanoparticles - silver, gold, and zinc nanoparticles - when testing the antiviral properties of nanomaterials.

Impact of Nanoparticles

Emergence of nano particles has enormous concern in agriculture in relation to environment. Different negative effects can be observed from nanoparticles like Zn and Cu. For example, ZnNPs can have an impact on plants when they are released into the soil and absorbed by the plant. In the case of *Vigna unguiculata*, the cationic ions of ZnNPs can move across the cell membrane through endocytosis, which ultimately leads to the degradation of the root membrane. Additionally, ZnO can negatively affect respiration, dehydrogenase activities, and the ammonification process. On the other hand, CuNPs with a size of 35nm can also affect the nitrification process. (Ali *et al*., 2018) [1]. In addition, the presence of nanoparticles can result in cellular toxicity as they can harm biomolecules such as proteins and nucleic acids, leading to chemical reactions. Furthermore, nanoparticles can cause oxidation of membrane components like lipids and the excessive production of reactive oxygen species. For instance, Zn nanoparticles have been found to exhibit genotoxicity, causing DNA damage in plants like onion, negatively impacting seed germination in mustard, and causing leaf damage in soybean at specific higher concentrations. (Ali *et al*., 2018) [1]. The metabolism, chloroplast ultra-structure, and stomatal conductivity are all suppressed by nanoparticles. Additionally, nanoparticles decrease the mitotic index and increase aberrations in cells. (Castiglione *et al*., 2011) [14]. Certain nanoparticles lead to increase production of free radicals, apoptosis and reactive oxygen species, which leads to mitochondrial dysfunctions. Thus, the production, implementation, storage, and residual activities of nanoparticles need to be closely monitored. In this context, there is a need for reliable investigation to measure the ecotoxicological effect of NPs and other factors (shape, size, chemical compositions, surface roughness, crystalline structure, angle of curvature, hydrophobicity, etc) accountable for NPs toxicity effect.

Future prospect and concluding remarks

Extensive research is being conducted on nanotechnology in plant pathology to create cost-effective, safe, and environmentally friendly stabilized nanomaterials. The use of nanotechnology in agriculture has the potential to significantly improve crop health and combat plant diseases, leading to increased production of healthy food and meeting demands efficiently and economically. Nanotechnology plays important role in pathogen sensing and control and provides a remedy to the emerging agricultural challenges. Major applications such as early detection of the pathogen, alleviation of pesticide solubility, bioavailability enhancement, nano pesticide with increase selectivity and lower resistance towards pesticides can revolutionize agriculture. Detection of pathogen at pre and post infection at lab and field condition is done by using nano sensors and devices at field and lab condition. With the monitoring with stringent laws, nano agrochemicals and their derived products can be exploited for the welfare of the farmers. For the successful implementation of NPs and the products derived from them, a single collaborative and multidisciplinary platform of scientific communities from different fields along with a comprehensive comprehension of the interaction between plants and pathogens within intricate nano systems is crucial. The majority of research on nanoparticle-based pesticidal control is still in its early stages, lacking sufficient reliability in real-world conditions and acceptance from the scientific community due to the absence of long-term trials.

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