



PLANT
PATHOLOGY

A REVIEW OF THE POTENTIAL OF SOME NANOPARTICLES IN CONTROLLING PLANT FUNGAL DISEASES

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ABSTRACT

The increasing threat of fungal diseases to global crop production necessitates innovative and sustainable control strategies. Fungicides used in conventional applications generate environmental pollution and create resistance challenges, making researchers pursue novel methods. This article investigates the utility of nanoparticles as plant disease protectors by examining their extensive protective range reduced vulnerability to resistance and their environmentally-friendly properties. The current evidence shows promising results although extensive research exists on their extended environmental impact alongside mechanisms of action and large-scale utilization limitations. The study evaluates new antifungal nanoparticle approaches combined with a review of sustainability challenges and future developments for agricultural uses in this field. The use of controlled release mechanisms for these agents enhances their effectiveness and reduces the overall quantity of chemicals required, thereby mitigating environmental impacts. Furthermore, nanotechnology has the potential revolutionized disease surveillance. By developing early detection techniques for rust and powdery mildew, It enable the detection of harmful spores in their initial stages, facilitating timely intervention and mitigating the development of severe illnesses. This proactive approach has the potential to substantially reduce the economic losses often associated with these diseases. Through the application of nanotechnology, the potential exists to confront the challenges posed by potato blight, tomato blight, root rot, rust, and powdery mildew. This approach holds promise for a future where plant diseases can be effectively managed with a high degree of precision, thereby reducing crop losses, decreasing reliance on chemical interventions and ultimately enhancing global food security.

KEYWORDS: Nanoparticles; fungal diseases; potato blight; tomato blight; root rot; rust; powdery mildew; Pakistan

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INTRODUCTION

Conventional fungal disease management struggles with resistance development, environmental contamination, high cost, and risk to human health and fungicides have a narrow spectrum of activity which increases the cost for farmers. These Challenges highlight the need for sustainable alternatives like nanoparticle-based antifungal strategies. Nanotechnology is quickly being utilized in different scientific and technological fields, positioning itself as an essential part of modern technology, frequently considered a “crucial technology of the 21st century.” (Spoiala *et al.*, 2020). Despite the abundance of information on nanoparticle toxicity to plant systems, very little study has focused on how exactly nanoparticles impact plant development and growth (Al-whaibi and Mohammad, 2015). The first definition of nanotechnology was given by Norio Taniguchi of Tokyo Science University in 1974. Essential components of nanotechnology, nanoparticles are solid colloidal

particles with dimensions between 10 nm to 1000 nm in at least one dimension (Shantamma *et al.*, 2021). At the 1959 American Physical Society annual meeting, physicist Richard Feynman famously proposed the idea of nanotechnology in his talk titled “There is plenty of room at the bottom” (Shojaei *et al.*, 2019). When a bulk material is reduced to nanoscale, its surface-to-volume ratio increases significantly, which can enhance its reactivity and result in the emergence of novel features that are absent in the macroscopic form (Khan and Rizvi, 2014). Nanoparticles can function as targeted carriers, encapsulating herbicides, nano-pesticide fertilizers, or genes, that are designed to selectively deliver their contents to certain cellular organelles in plants. Global production of nanoparticles in 2010 amounted to an estimated 260,000–309,000 metric tons, while the utilization of nanomaterials worldwide ranged from 225,060 to 585,000 metric tons between 2014 and 2019 (Cabral *et al.*, 2019). The synthesis of nanoparticles occurs through

biological, chemical, and physical methods but carries individual benefits and constraints. The use of plant extracts together with bacteria and fungi serves as reducing agents during biological synthesis which provides both sustainability and low toxicity levels. The chemical methods use precipitation and sol-gel processes (Zhang *et al.*, 2009) and hydrothermal techniques for precise control of particle size and composition yet demand dangerous chemical substances. Nanoparticles through mechanical or thermal energy emerge from physical synthesis via laser ablation and sputtering and ball milling methods which provide high purity at greater energy expenses. Biological synthesis has become prominent because it enables sustainable and large-scale production of nanoparticles while minimizing environmental impact (Ahmed *et al.*, 2022).

Possible important applications in the field of plant pathology include the use of nanomaterials to prevent plant diseases and enhance plant growth (Khan *et al.*, 2021). Nanotechnology has revolutionized agriculture by providing the opportunity to effectively control plant diseases. However, it is still in its first phases and necessitates additional investigation and analysis (Cabral *et al.*, 2019). Nanomaterials in agriculture can mitigate the overuse of hazardous pesticides employed for plant disease control (Shang *et al.*, 2019). The nano-sized universe is often quantified in nanometers, with 1 nanometer equivalent to 1-9 meters. Systems falling within this category typically have dimensions between more than 1 nm and less than 100 nm, which is bigger than molecular dimensions but smaller than macroscopic ones (Pal *et al.*, 2011). When it comes to plants, invasive pathogens like bacteria, fungus, and viruses are usually bad news because they can cause a host of diseases and lower crop yields. The global economy loses over \$220 billion due to plant diseases every year, says the Food and Agriculture Organization (FAO, 2020).

Even though NPs have a lot of benefits still they also present some environmental risks because they prove harmful to all living beings and they stay in the environment and lead to ecological contamination. Their tiny size enables them to enter biological systems where they might create oxidative stress conditions and harm DNA (Sajid *et al.*, 2015). Environmental sustainability advances occur through researcher development of eco-friendly NPs which use biopolymers and plant-based synthesis techniques. The methods strive to minimize dangers to biology through effective fungal disease control (Saraswat *et al.*, 2023). Nanotechnology has gained increasing recognition within the agro-food system, determined by recent positive reports over the past five years that

underscore the promising potential of nanomaterials in fortifying plants, bolstering crop resilience to environmental stressors, and enhancing plant defenses against pathogens (Silva *et al.*, 2022). In agriculture, nanotechnology acts as a biofertilizer factory, reducing the need for agrochemicals, strengthening plant resistance to diseases, optimizing nutrient utilization, and promoting robust plant growth. Several research has utilized nanotechnology to effectively address plant diseases in several crops, including wheat, corn, cucumber, and tomatoes. This has led to enhanced crop quality and increased output (El-Saadony *et al.*, 2021). Both industrialized and growing nations, including Germany, the USA, Brazil, China, India, France, and Korea, have demonstrated an increasing tendency to utilize nanomaterials for agricultural purposes. This is apparent from the increasing quantity of papers and patents in this particular domain (Bratovic, 2022). The intrinsic potential of nanoparticles makes them a promising candidate for direct application to plant seeds, leaves, or roots as a means of defending against insects, bacteria, fungi, viruses, and other pathogens. Certain nanoparticles have the potential to effectively manage plant diseases by inhibiting the growth of pathogens and enhancing the responses of plants to defense. Their exact mechanisms of action provide them with extremely promising tools for agricultural disease management. The strong antibacterial and antifungal properties of metal nanoparticles, such as those of titanium dioxide, copper, zinc oxide, and silver, have been extensively studied. Moreover, Kim *et al.* (2018) noted that these nanoparticles are well-known for their resistance to viruses. Silver nanoparticle utilization has increased significantly in recent years, primarily due to “green synthesis” produced by plants, bacteria, fungi, or yeast (Rafique *et al.*, 2017). Silver nanoparticles have been shown by Krishnaraj *et al.* (2012) to effectively control a variety of fungi, including *Alternaria alternate*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinerea*, and *Curvularia lunata*. By doing a well diffusion experiment, the inhibition was found. The sun-hemp rosette virus was completely inhibited when silver nanoparticles were applied to bean leaves (Jain and Kothari, 2014).

Elbeshehy *et al.* (2015) found that if silver nanoparticles were applied to bean yellow mosaic virus-infected faba bean plants 24 hours after infection, the results were noticeably better than if the nanoparticles were applied prior to infection or concurrently with inoculation. Significant effectiveness has been shown by silver nanoparticles in the treatment of bacterial and fungal-induced plant diseases. However, there are significant obstacles to the application of these chemicals, such as

those pertaining to toxicity, production, and soil effects (Mishra and Singh, 2015). Other metal nanoparticles that are frequently used are gold, copper, and titanium dioxide. Applications for nanoparticles in fertilizer, such as titanium dioxide and copper, are growing in popularity. However, research on their effectiveness in treating plant diseases is scarce (Worrall *et al.*, 2018). According to Worrall *et al.* (2018), chitosan nanoparticles strengthen plant tissues' ability to withstand viral infections caused by the mosaic virus in alfalfa, tobacco, peanuts, potatoes, and cucumbers. Chitosan nanoparticles are proven to have antibacterial properties; they have been shown to be effective against *Phyricularia grisea* in rice, *Fusarium crown* and root rot in tomato plants, and *Botrytis bunch rot* in grapes. But they are significantly less effective at fighting germs (Malerba and Cerana, 2016).

Major Plant Diseases and Their Mitigation by Nanoparticles

Potato blight and nanoparticles: *Solanum tuberosum*, a member of the Solanaceae family, holds significant worldwide importance as a widely consumed vegetable, carrying substantial economic and social significance. It is often ranked as the fourth most vital staple crop globally, trailing behind wheat, maize, and rice. According to research by Abdel-Razik *et al.* (2012), this crop is cultivated in various temperate and subtropical climates worldwide. The three leading potato-producing nations, namely China, India, and Russia, collectively account for around 45% of the world's potato production (Scott and Suarez, 2012). In India, potato cultivation primarily occurs on level terrains, with the West Bengal, Bihar, and states of Uttar Pradesh being the top contributors to potato production. These states individually contribute 26.94%, 14.56%, and 32.38%, to the overall production, respectively. These regions account for nearly 75% of the country's potato output (Pradel *et al.*, 2019). Unfortunately, the potato crop, scientifically known as *Solanum tuberosum*, is highly susceptible to a devastating disease called potato late blight. This disease is caused by a pathogen known as *Phytophthora infestans* (*P. infestans*), which falls into the group of organisms known as oomycetes (Valeeshouwers *et al.*, 2011). Nanoparticles utilize different methods to stop fungal infections. They penetrate fungal cell structures through their initial infiltration causing harm to both the cell wall and membrane and subsequent cellular contents leakage. The generation of reactive oxygen species (ROS) represents a second mechanism by which nanoparticles act as fungal inhibitors by causing harm to fungal DNA as well as proteins and lipids through oxidative stress. The interaction of nanoparticles with fungal enzymes and proteins modifies key metabolic

processes which results in growth-limitation of fungal pathogenesis. Nanoparticles deploy multiple simultaneous mechanisms to fight various fungal infections which lowers the chance of fungal resistance formation (Kutawa *et al.*, 2021).

The widespread occurrence of complete field devastation caused by late blight poses a significant global challenge, resulting in an annual economic impact of 3 to 5 billion dollars (Singh *et al.*, 2021). *Phytophthora infestans* sporangia exhibit two modes of germination: direct germination through a germ tube and indirect germination via the release of zoospores, as illustrated in figure 2. As per Tsedaley's 2014 research, secondary sporangia may also be produced through germ tubes, contributing to the prolonged viability of these spores. Sporangia germination can occur within a temperature range of 7 to 13 °C. Each sporangium has the potential to generate a variable number of mobile zoospores, typically ranging from 8 to 12. These microorganisms attach themselves to leaf surfaces and initiate infection within the host plant, as described by Kirk *et al.* (2013). According to research by Parsad *et al.* (2014), it is likely that particles like Ag (silver), ZnO (zinc oxide), Mg (magnesium), Si (silicon), and TiO₂ (titanium dioxide) exert a direct inhibitory effect on crop diseases due to their antibacterial properties, as detailed in Table 1. Copper and zinc are essential micronutrients and growth promoters for potato plants. In an experiment, biogenic Cu-Zn bimetallic nanoparticles were applied to the mycelium of *P. infestans* and observed under an electron microscope. These nanoparticles attached to the mycelium, causing it to shrink and develop an irregular surface. This led to the leaching of cytoplasmic material from the fungus cells and a decrease in viability (Manna *et al.*, 2017). The impact of Ag₂O/TiO₂ and Ag₂O on *P. infestans* (isolated from potato leaves) was investigated under a light microscope to understand their mechanism of action. Nanoparticle treatments resulted in the collapse of sporangia and the lysis of sporangiophores. According to (Elsharkawy *et al.*, 2022), Ag₂O/TiO₂ was more effective in reducing late blight severity in potatoes compared to Ag₂O alone, with no damaging effects on treated potato plants. Silver nanoparticles demonstrated a significant 75% suppression of *P. infestans* in laboratory settings, a pathogen responsible for late blight disease. Field trials confirmed that AgNPs treatment dramatically lowered the impact of late blight disease on potato plants, compared to untreated controls (Kim *et al.*, 2012).

Shantamma *et al.* (2021) reported a study where they applied colloidal silver particles varying in size from 100 to 1000 nm, to leaf samples of diseased potato plants. Out of all the concentrations tested, 500 ppm was the

most effective, stopping *P. infestans* mycelia growth by a significant 86.13%. Furthermore, the enzymatic functions of phenylalanine ammonia lyase, polyphenol oxidases, and peroxidase, were assessed in potato leaf samples that were infected with *P. infestans*. Zhan *et al.* (2018) developed a creative approach for pathogen detection that integrates a DNA amplification technique with a gold nanoparticle-based biosensor, as depicted in Figure 3. The aim of this approach was to attain a rapid, extremely sensitive, and visually prominent detection of *P. infestans*. The DNA was extracted from *P. infestans* mycelia, and in the UP-APCR cycling method, three primers were used: a universal primer at a normal concentration, and forward and reverse primers at a limited concentration. The results demonstrated that this biosensor possessed the ability to detect *P. infestans* at concentrations as low as 0.1 pg μL^{-1} within a time period of 1.5 hours.

The exact way magnesium oxide nanoparticles kill plant pathogens remains unclear, scientists have identified three main mechanisms for how metal nanoparticles might work: (I) directly attaching to the pathogen's outer shell (Chen *et al.*, 2020), (II) releasing harmful ions (Kasemets *et al.*, 2009), and (III) generating damaging molecules when exposed to light (Kumari *et al.*, 2017). Recent research by Wang *et al.* (2022) showed that MgO nanoparticles kill *P. infestans* filaments (hyphae), confirmed through electron microscopy. Their study suggests MgO nanoparticles work by causing several problems in the fungus: continuous oxidative stress, cell surface damage, disruption of transport processes, and hindered metabolic pathways. In addition, MgO nanoparticles modulate the plant hormone signal transduction pathway without inducing any detrimental effects on potatoes. In a study investigating the management of early blight in potatoes, silver nanoparticles were utilized at a concentration of 150 ppm along with Dipotassium sulfate (K_2SO_4 , 99) to evaluate the extent of the disease. Disease severity was evaluated using a 0-to-4 scale, allowing for a detailed observation of the varying degrees of leaf lesions. According to El-Batal *et al.* (2016), applying AgNPs directly to the leaves of potato plants at a concentration of 150 ppm was more successful in reducing the growth of early blight compared to a concentration of 100 ppm. Additionally, it was observed that the application of SeNPs (selenium nanoparticles) at a concentration of 0.5 $\mu\text{g}/\text{ml}$, AgNPs (silver nanoparticles) at a concentration of 150 $\mu\text{g}/\text{ml}$, and K_2SO_4 (potassium sulfate) at a concentration of 2% led to a disease severity of 12.63%. Furthermore, these treatments enhanced the physiological parameters and yield of the plants. Nanoparticles demonstrate superior fungicidal properties to conventional

fungicides because they show a broad spectrum of antifungal actions with complex multiple mechanisms and lower susceptibility to fungal resistance. The eco-friendly approach provides sustainable biodegradable plant disease control because nanomaterials replace conventional fungicides yet stand as promising tools for management (Jangjou *et al.*, 2022).

Tomato Blight and Nanoparticles: The tomato plant, a member of the Solanaceae family, is part of the Magnoliophyta division, Magnoliopsida class, Asteridae subclass, Solanales order, and Solanaceae suborder (Knapp *et al.*, 2004). The global tomato production as reported by FAOSTAT (2007), stands at a staggering 161.7 million metric tons, with an estimated cost exceeding \$59 billion (Nayik *et al.*, 2020). However, this impressive figure is marred by the significant threats to crop productivity, with an estimated 25% crop loss attributed to insect infestations and diseases (Nicoletto *et al.*, 2019). Among the diseases that affect the Solanaceae family, including *Solanum lycopersicum*, late blight and early blight are common and result in substantial crop losses (Zakharova *et al.*, 2017). Early blight, caused by several *Alternaria* species, is a notable infection that affects tomato plants. The *Alternaria* genus, a significant and diverse group of pathogenic fungi, is responsible for various severe illnesses. Kemmitt (2002) suggests that fluorescent light can facilitate the induction of sporulation in culture. Lesions ranging from dark brown to black in color and characterized by concentric rings on aged leaves are common signs of early blight in tomato plants. In addition, it is common to see stem lesions and fruit rot (Egidi *et al.*, 2019). This fungal genus is well-known for being one of the most aggressive soil-borne pathogens on the planet (Delgado-Baquerizo *et al.*, 2020). There is no indication of a sexual stage in the life cycle of *A. solani*; hence, asexual reproduction is the predominant form of reproduction for this fungus. The infectious agents might manifest as mycelia or conidia and can spread from one place to another (Foolad *et al.*, 2008). The development of germ tubes allows conidia to germinate. As seen in Figure 4, these germ tubes penetrate host tissue and start the infection process. It takes around two or three days after infection for lesions to start to appear. Production of spores then starts around three to five days after the lesion becomes visible. The fast dispersal of conidia by wind and rain splash at this stage allows the disease cycle to continue in either unaffected areas of the same plant or in other plants altogether (Adhikari *et al.*, 2017).

Research on the interaction between nanoparticles and plants in different environments has provided valuable insights into how plants adapt and respond. A study by Tripathi *et al.* (2015) indicated the synergistic

impacts of silicon nanoparticles and plants in mitigating UV-B stress and heavy metal stress. Yet, the potential impact of applying AgNPs to enhance plant resistance against biotic stress has not been investigated (Tripathi *et al.*, 2017). AgNPs exhibit various mechanisms that can hinder pathogen resistance, as reported by Ocsoy *et al.* (2013). In addition to its electrical and thermal conductivity, mechanical strength, optical characteristics, antimicrobial and antiviral capabilities, and more, AgNPs have a number of other desirable qualities (Ansari *et al.*, 2018). Consequently, they are considered promising nanomaterials. Nanotechnology allows effective fungal pathogen treatment across different types because of its distinct physical qualities which outperform traditional fungicides in versatility. Nanotechnology-based antifungals use two different action strategies that diminish the possibility of fungus creating resistance making them superior compared to traditional antifungal compounds. Green synthesis methods of eco-friendly nanoparticles present a sustainable option for plant disease management since these methods decrease environmental toxicities while promoting biological decomposition (Ghareeb *et al.*, 2022). This study examines the capacity of AgNPs generated using environmentally friendly techniques to stimulate the development and productivity of tomato plants, while also improving their ability to fight early blight disease. AgNPs were produced by utilizing a leaf extract derived from neem plants. Tomato plants that received treatment with AgNPs exhibited notable enhancements in multiple growth metrics. These enhancements included a 30% elevation in plant height, an increase in leaf count, a 45% boost in fresh weight, and a 40% increase in dry weight as compared to plants in the control group. In addition, plants treated with AgNP showed a major decrease in disease severity index by 73% and disease incidence by 69% compared to plants in the control group. The utilization of AgNPs generated by environmentally friendly techniques seems to be a feasible approach to enhance the growth and yield of tomato plants, while also offering defense against early blight disease (Ansari *et al.*, 2023).

Mesoporous materials, such as silica (MSN), have many applications, especially in the fields of disease diagnosis and treatment (Xie *et al.*, 2016). Utilizing MSN led to a substantial decrease in the severity of early blight disease in tomato plants, as well as noteworthy enhancements in multiple growth attributes of the tomato crop. Using MSN nanoparticles in lower quantities is anticipated to have environmentally sustainable effects and result in decreased agriculture operational expenses. The direct production of metal sulfide nanoparticles (MSN) presents a promising

method for creating different metal oxides that possess strong antifungal characteristics. Derbalah *et al.* (2018) propose that these metal oxides could be used as secure substitutes for traditional fungicides in the treatment of early blight in tomatoes. MSN demonstrates fungicidal activity through multiple ways. Several factors contribute to cell lysis in fungi, including hydrogen bond formation between surface hydroxyl groups and lipopolysaccharides in the cell wall, cell wall breakdown due to their small size, accumulation of MSN in the membrane, destruction of the electron transport chain by insoluble compounds produced in the fungal membrane, and oxidation of the cell membrane caused by electromagnetic attraction between MSN and the cell. MSN enhances the resilience and physiological characteristics of tomato plants, hence bolstering their ability to protect themselves against disease (Kanto *et al.*, 2004).

The studies conducted by Lahuf *et al.* in 2019 and Sabir *et al.* in 2014 provided evidence that zinc oxide nanoparticles (ZnO NPs) were remarkably successful in combating the pathogen responsible for early blight. The inhibition percentages observed in the experiments exceeded 50%, as indicated in the accompanying table. Zinc oxide (ZnO) nanoparticles have been recognized as promising substances for managing *Alternaria solani*, the microorganism responsible for early blight in tomato plants. This discovery is consistent with prior studies that have shown the effectiveness of ZnO nanoparticles in combating different plant diseases, such as those caused by *Penicillium expansum*, *Fusarium graminearum*, *Botrytis cinerea*, *Alternaria alternate*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Rhizopus stolonifer* (He *et al.*, 2011). ZnO nanoparticles possess not only high efficacy against dangerous bacteria but also exhibit low toxicity towards treated plants and have the capability to enhance soil fertility. Therefore, they can serve as environmentally favorable substitutes, thereby minimizing the ecological consequences of pesticide usage (Lahuf *et al.*, 2020). Hydrothermal synthesis with hydrophilic surfactants is preferred for the effective production of Cu-based NPs with potential applications as agrochemicals, due to their bio-applicability and solubility properties (Xiong *et al.*, 2011; Shah and Al-Ghamdi 2011). Figure 5 demonstrates that hydrothermal synthesis provides a direct approach to produce diverse Cu-based nanoparticles. Copper/copper oxide nanoparticles of small size (varying from 11 to 21 nm) were synthesized utilizing surfactants with low molecular weight (PEG 1000, Tween 20) and at temperatures of 90, 120, and 160 degrees Celsius. There is a limited amount of information available in the literature about small-sized copper-based nanoparticles (NPs) (Shahmiri *et*

al., 2013).

Root rot and nanoparticles: Root rot pathogens cause severe plant diseases on a global scale, impacting a vast range of crops (Gonzalez *et al.*, 2011). The primary issue with root rot is that its symptoms manifest below the soil surface, rendering them challenging to identify in the early stages. Various diseases have the ability to cause root rot, with certain ones being exclusive to certain hosts, while others can impact a broad spectrum of plants (Nzungize *et al.*, 2011). Out of these disease-causing agents, fungi are the most common offenders, with the ability to stay inactive and survive in waste and contaminated soil for many years (Berg *et al.*, 2014). Root rot is characterized by several symptoms, such as the browning and sooth of root tips, the development of root scrape in different sizes and colors (ranging from reddish ,brown and black), root decay, trining, yellowing and wilting of leaves, stunted growth of plants, decreased crop yield, and ultimately, loss of crops (Liberato *et al.*, 2011).

Citrus fruits have the greatest global production among all types of fruits, with an expected production of 95 million metric tonnes in 2019-20. This category includes varieties kinds such as grapefruit, lemon, oranges, and tangerines (Costa *et al.*, 2020). Nevertheless, as a result of inadequate pre and post-harvest management measures, developing countries face fungal damage rates ranging from 20 to 25%. Citrus Black Rot, a disease caused by fungus *Alternaria citri*, has a significant impact on citrus plants. The presence of black hyphae on plant surfaces caused by this phenomenon leads to a decrease in citrus yield. This disease in Pakistan results in annual economic loss rangies from 30% to 35% (Anwar *et al.*, 2020). The Chinese winter jujube, scientifically known as *Ziziphus jujube* Mill. cv. Dongzao, is a highly esteemed fresh food in China due to its exceptional nutritional content (Shi *et al.*, 2018). *Alternaria alternata* is a pathogenic organism that can have a remarkable negative impact on the quantity and quality of freshly harvested jujube fruits. Black Spot Rot is a disease that can affect jujube fruit at both the harvest and postharvest stages. *A. alternata* is a pathogen that can withstand cold temperatures and is able to germinate and develop even in settings as low as -2°C. The pathogen invades plant hosts and induces substantial harm to the exterior of jujube fruit, leading to the formation of dark brown lesions (Yuan *et al.*, 2019).

Black root rot is a disease caused by various fungal pathogens, including *Alternaria alternata*, which primarily affects young leaves through airborne conidia spores. The infection process begins with the presence of condiophores on young plant shoots, leading to leaf and stem infection. A specific toxin, called ACT,

produced by the tangerine phenotype *A.alternata*, is transported through the vascular system, causing necrotic lesions in detached calamandin, as shown in Figure known 6. The final stages involve the colonization and development of black and brown spots on young plant leaves (Chung *et al.*, 2012). In the case of black rot disease, which affects nearly all citrus species, dormant infections present on the fruit button can lead to black rot as the fruit ripens, infecting the columella and causing the characteristic black rot symptoms.

Fruits are susceptible to degradation caused by several pathogenic fungi due to their perishable nature (Chohan *et al.*, 2015). A previous study has shown the potential benefits of ZnO nanoparticles (NPs) in strengthening soil fertility, boosting plant productivity, and increasing the accessibility of zinc by Rossi *et al.* (2019). Khalil *et al.* (2018) discovered that including phytochemical preparation during the production of ZnO nanoparticles results in notable antibacterial characteristics. The XRD analysis revealed that the ZnO and CuO nanoparticles had diameters of 18 to 16.8 nm, respectively. The findings shows proof that ZnO nanoparticles, with a diameter less than 50 nm, may be chemically integrated into sweet oranges and demonstrate antifungal characteristics against *Alternaria alternata* when administered at a concentration of 6 mM Sardella *et al.* (2017) .

A study discovered that chemically synthesized copper nanoparticles, ranging in size from 45 to 48 nm, had antifungal activities against the *Fusarium solani* fungus in orange fruit Khamis *et al.* (2017). At physiological pH, the cell walls of the fungus contain carboxyl and phosphate groups, which give the fungal surface a negative charge. The study examined the antifungal properties of copper oxide, zinc oxide, and a combination of metal oxide nanoparticles . The nanoparticles that contained both CuO and ZnO exhibited the most potent antifungal activity at a concentration of 100 mg/ml, surpassing the individual antifungal effects of ZnO and CuO NPs. In addition, CuO and ZnO nanoparticles were produced using lemon peel extract as both the reducing agent and capping agent. This study investigates the effects of two different doses (100 and 200 mg/ L) of titanium dioxide nanoparticles composed when used as seed priming and foliar application by Anatase and Rutile). The study focuses on their ability to manage a disease complex in beetroot caused by *P. betavascularum*, *R. solani*, and *M. incognita*. The study investigated many aspects of plant growth, including chlorophyll fluorescence characteristics, chlorophyll content, defense enzyme activities, nematode multiplication, root galling, and soft-rot and root-rot indices. The researchers utilized

inductively coupled plasma mass spectrometry (ICP-MS) scanning to quantify the titanium levels in both infected and uninfected beetroot plants. There have been recent instances of bacterial stem and root rot disease in sweet potato, which is caused by *D. dadantii* (formerly known as *Erwinia chrysanthemi*). These occurrences have been recorded in important sweet potato farming areas in China (Shen *et al.*, 2018). This requires the assumption of efficient techniques to control the pathogen and halt the transmission of the disease. Silver nanoparticles are produced using the cell-free culture supernatants of plant growth. These nanoparticles possess the capacity to hinder the proliferation of bacteria and efficiently regulate their antibacterial efficacy against the disease *D. dadantii*. AgNPs produced using the cell-free culture supernatant of the bacterial *Pseudomonas rhodesiae*. The AgNPs shows a clear surface plasmon resonance peak at a wavelength of 420-430 nm. They are observed as Nano crystallites with dimensions ranging from 20-100 nm, as verified by transmission electron microscopy. The culture supernatant may contain functional groups that have the ability to decrease silver ions and contribute to the stability of AgNPs. There was a direct relationship between the increasing concentrations of AgNP and the effectiveness of its anti bacterial properties. AgNPs produced by environmentally friendly techniques can efficiently suppress the soft rot disease by impeding the pathogen's capacity to infect sweet potato seed tuber.

In recent years, silver nanoparticles have been used to control post-harvest infections, such as kiwifruit rot disease (He *et al.*, 2019). Previous research has shown that AgNPs can directly hinder the growth of mycelium and the germination of spores in *A.alternata*. In addition, they have the ability to impact the composition of the cell wall and the synthesis of sugar, protein, and N-acetylglucosamine (Al-Sheikh and Yehia, 2016). AgNPs primarily exert their antifungal effect by inhibiting the growth of mycelium and the germination of conidia. The deterioration of the cell walls was assessed using three replicates, and the experiment was replicated twice. The optimal concentration of AgNPs for inhibiting the growth of four strains was found to be 75 ppm. In order to prepare for each pathogen, a 100 µL spore suspension with a concentration of 1.0×10^6 spores per mL was introduced into tubes containing 1 mL of potato dextrose broth (PDB). The PDB is prepared by combining a solution of 200 grams of potato extract and 20 grams of dextrose per liter. The tubes were also infused with a concentration of 75 parts per million of silver nanoparticles (AgNPs), and the spores were subsequently adjusted to a final concentration of 1.0×10^5 spores per milliliter. The

negative control comprised the PDB solution without the presence of AgNPs. The effectiveness of AgNPs in killing fungi was assessed by measuring the size of fungal colonies (Wollenberg *et al.*, 2016). The inhibiting effects of silver nanoparticles with a size of 5 nm and a concentration of 5 mg /mL were seen on the growth of mycelium in four distinct infections. The average breadth of the lesion was 6.23 mm when AgNPs were sprayed before inserting PM-13, according to Li *et al.* (2022). Furthermore, the use of AgNPs not only reduce the average size of infected spots on the surface, but also prevents the propagation of infection to underlying layers of affected regions. Silver nanoparticles (AgNPs) successfully mitigated the symptoms of kiwifruit rot without leaving any detectable Ag+ residue on the peel and flesh.

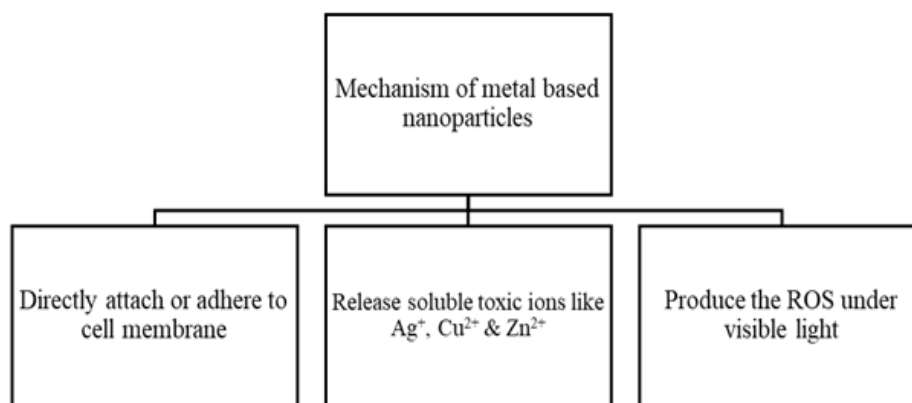
Rust disease and nanoparticles: Cereal crops are currently encountering significant challenges due to the increased proliferation of several diseases on a worldwide level. Rust, a fungal infection produced by the species *Puccinia graminis* f. sp. *Tritici*, is a major infection that impacts wheat harvests. This disease has the ability to affect various cereal crops, including but not limited to wheat, and can also appear as black rust. The spread of this fungal illness is aided by meteorological elements such as wind and humidity, in addition to other favorable environmental circumstances (Abdulridhaand Min, 2023). Rust is a widespread illness that is now impacting populations globally and may be classified into three primary kinds. Bhardwaj *et al.* (2016) identified three categories of rust: Brown/Leaf Rust, Yellow or Stripe Rust, and Black or Stem Rust. leaf rust is a major concern in multiple places, including as Asia (namely central, south, and southeast areas), the North African continent, Europe, and both the United States and Canada. Stem rust is a significant threat in the highlands of East Africa, but its importance is comparatively lower in the European Union, North America, Asian countries (both South and North), and other parts of Africa .Stripe rust is resulting in significant financial damages in various areas, such as the United States (particularly in the Pacific Northwest), East Asia (specifically southwest and northwest China), South Asia (including the countries of Pakistan, India, and Nepal), the Western Europe, Australia, New Zealand, and Eastern Africa. The life cycles of most rust fungus are intricate. An evident recurring pattern identified in the life cycle of rust fungus, renowned for its complexity, entails five unique spore stages that take place on two distinct plants that host it. The stages of life is distinguished by the generation of five distinct spore types, which are present on two hosts that lack genetic relation (see Figure 7). The life cycle of these fungus encompasses

both asexual and sexual phases, commencing with the asexual stage, succeeded by the uredinal, telial, and basial stages (Sood and Singh, 2020).

Green-synthesized silver nanoparticles (AgNPs) were applied to wheat plants, resulting in an enhancement of their antioxidant defense mechanism. This subsequently improved the plants' resistance to stripe rust disease. In a research done by Sabir and Arshad *et al.* (2022), many morphological parameters were investigated, including root area, leaf area, fresh and dry weight of plants, as well as physiological measurements such as Malondialdehyde Stress Index and chlorophyll content. Furthermore, they assessed non-enzymatic attributes such as soluble sugars, total protein, proline, phenolic, and flavonoid concentrations. Furthermore, the research investigated the operation of crucial antioxidant enzymes, including Superoxide Dismutase, Peroxidase, and Catalase.

The findings demonstrated that the utilization of 75mg/L quantities of AgNPs generated from green sources led to the stability of these parameters and provided wheat plants with resistance. Chitosan nanoparticles prolonged the duration of both the latent and incubation periods of stripe rust illness. Additionally, they decreased the infection type, size of pustules, and overall number of pustules in comparison to the control therapy. Hassan and Chang (2017) have shown that chitosan has the capacity to impede the growth, viability, sporulation, germination, and cellular alterations of infections. Moreover, it has been shown that it may elicit a variety of defensive responses in the plants it infects, either by promoting or inhibiting them. The antifungal effects of chitosan arise from its capacity to break the plasma membrane of fungal cells and hinder the functioning of fungal DNA and/or RNA. In addition, the application of chitosan nanoparticles has been discovered to boost seed germination, increase

water absorption and transportation, stimulate water channel proteins, and enhance nutrient absorption in plants (Elsharkawy *et al.*, 2022). Nevertheless, the alterations decreased as the concentration of chitosan nanoparticles escalated (Verma *et al.*, 2019). According to Elsharkawy *et al.* (2022), the utilization of chitosan nanoparticles along with salicylic acid led to a noteworthy elevation in the concentrations of superoxide and hydrogen peroxide. The quantitative assessments showed that the treatment with chitosan nanoparticles had greater amounts of superoxide and hydrogen peroxide in comparison to the control group. Under conditions that do not cause stress, both non-enzymatic and enzymatic antioxidants are capable of effectively eliminating reactive oxygen species (ROS). Recent research specifies that reactive oxygen species have the ability to behave as signaling molecules and enhance immune response in biological systems (Yang and Lian, 2020). In addition, it was shown that sulfur nanoparticles derived from pomegranate arils (PA-SNPs) had a notable rate of inhibiting spores, with approximately 74.70% inhibition against soybean rust disease. This inhibition rate was statistically similar to the rate reached with a concentration of 1000 ppm (74.08%). Furthermore, when the concentration reached 500 parts per million (ppm), the rate of spore inhibition was measured to be 61.09%. Moreover, the utilization of TiO₂ nanoparticles has been discovered to enhance the process of photosynthesis. Titanium dioxide (TiO₂) causes an increase or decrease in the production of proteins, which triggers defense-related reactions. For instance, in the pentose-phosphate pathways, which generate NADPH, it enhances the production of phosphor gluconate dehydrogenase, an enzyme involved in a number of these activities. NADPH is essential for enhancing the function of NADPH-oxidase, the core enzyme accountable for



**Fig. 1: Mechanism of action of metal-based nanoparticles
Major Plant Diseases And Their Mitigation By Nanoparticles**

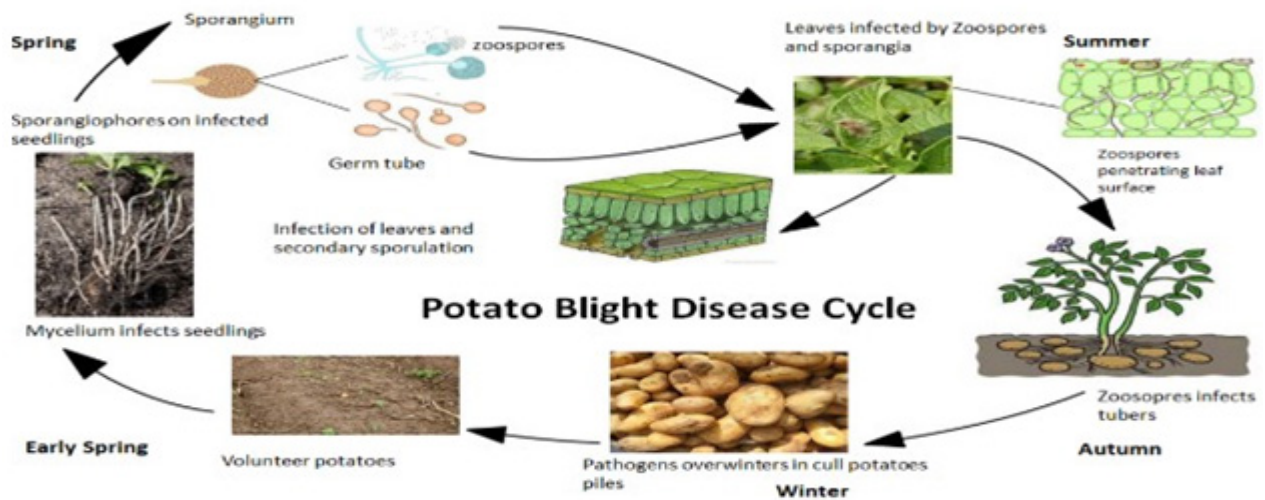


Fig. 2: Life cycle of Potato Blight.

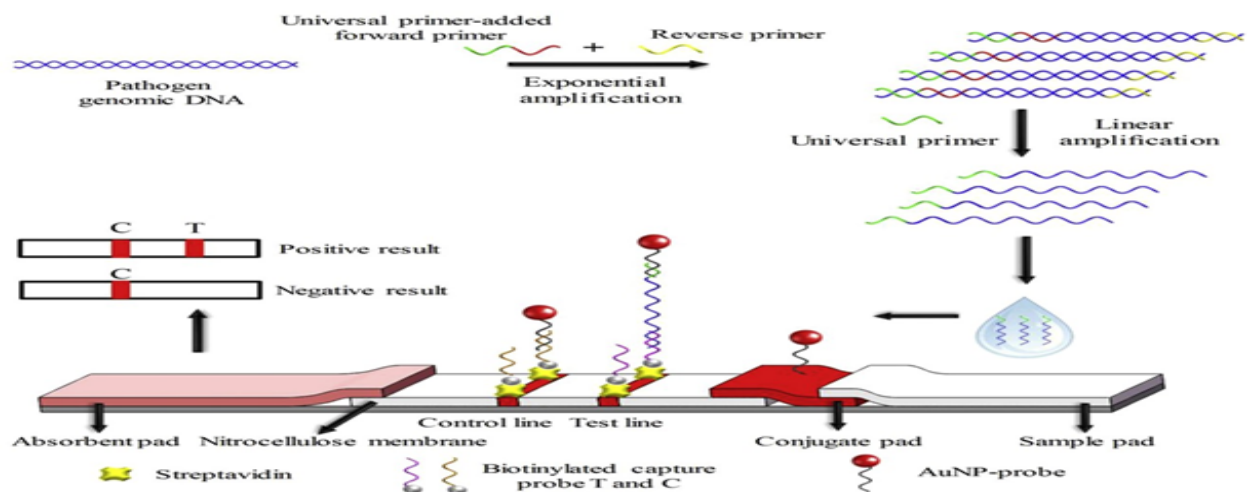
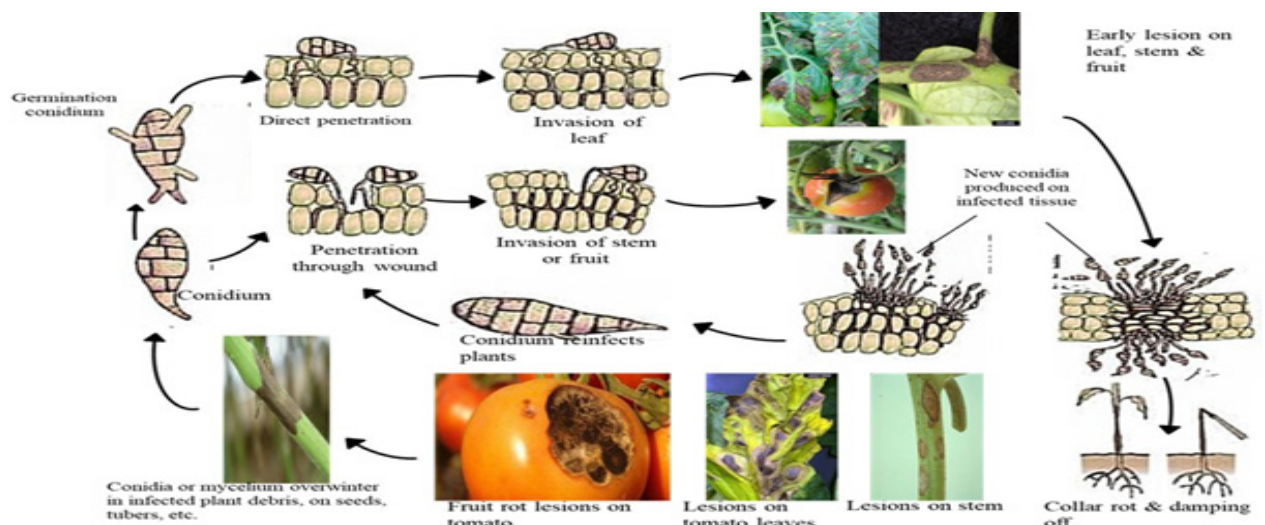
Fig. 3: Schematic illustration of gold-nanoparticle based lateral flow biosensor for early identification of *P. infestans* (Zhan *et al.*, 2018).

Fig. 4: Life cycle of tomato blight in host plant

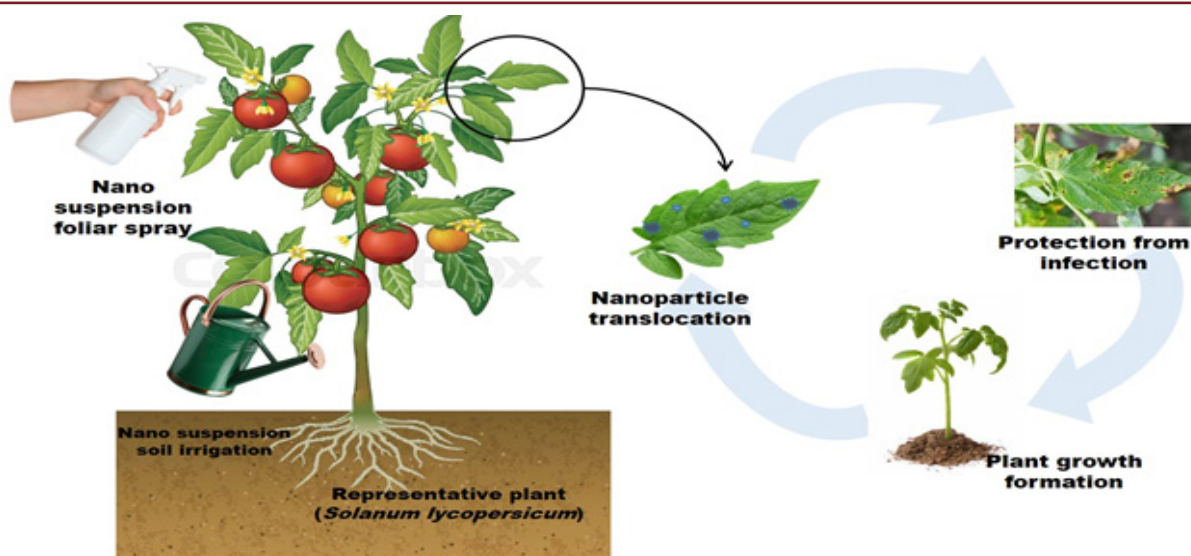


Fig. 5: Pathway of tomato blight and action of Nanoparticles.



Fig. 6: Life cycle of root rot in citrus fruit.

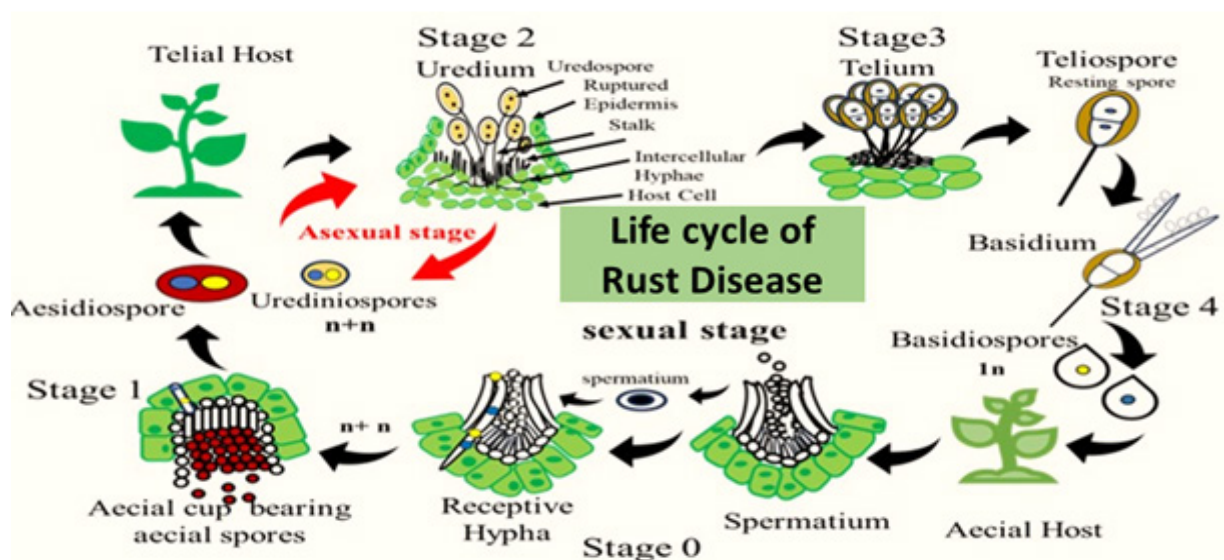


Fig. 7: Life cycle of Rust disease and interaction with host plants.

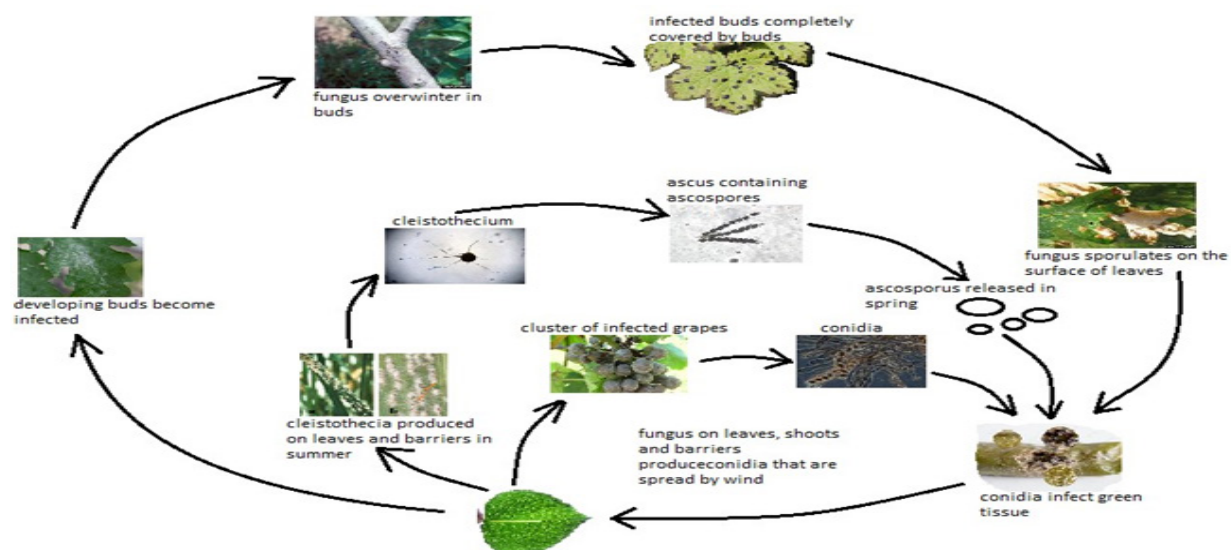


Fig. 8: Life cycle of powdery mildew and inoculation with host plants.

Table 1: Effect of NPs on plant diseases

S r . No.	Diseases	Host Plant	NPs	Method of Application	Effect	Reference
1	Late Blight	Potato	Ag ₂ O/TiO ₂	Foliar application	-100% radial growth inhibition of Phytophthora Infestans	Elsharkawy <i>et al.</i> (2022)
		Potato	Colloidal silver	Foliar application	-Reduced the severity at 500 ppm and activates the defence system in plants	Shantamma <i>et al.</i> , (2021)
		Potato	MgO	Foliar application	-Cell surface disruption -Suppression of both the ability to transport substances across the cell membrane and crucial metabolic pathways in <i>P. Infestans</i>	Wang <i>et al.</i> (2022)
		Potato	Au NPs	Biosensor method	-Reduce the risk of epidemic development.	Zhan <i>et al.</i> (2018)
		Potato	Cu and Zn Bimetallic	Foliar application	-Addition of growth promoting substances and control the Phytophthora	Manna <i>et al.</i> (2017)
2	Early Blight	Potato	Ag And Se	Petri dish (stock solution)	-Completely inhibited the growth of <i>A. Solani</i> -Antioxidant for enhancing plant immunity.	Ismail <i>et al.</i> (2016)
		Potato	Mycogenic ZnO	Foliar application	-Inhibition percentage (IP) is 47.05% -Increase the tuber weight as well as the number of tubers	Singh <i>et al.</i> (2022)
		Tomato	Ag NPs	Foliar Spray	-Increase in plant height (30%), number of leaves, fresh weight (45%) dry weight (40%) -Reduction in (DSI) (73%) and (DI) (69%)	Ansari <i>et al.</i> (2023)
		Tomato	MSN NPs	Foliar spray	-Reduce the severity of disease -Improve growth characters	Derbalah <i>et al.</i> (2018)
		Tomato	ZnO NPs	Foliar Spray	-Inhibition percentage exceeded 50% and decreased the percentage of disease severity to 17.04 and 20.13%.	Lahuf <i>et al.</i> (2019)
		Tomato	CuO and Cu ₂ O NPs	Foliar application	-Did not induce any irreversible damage to the plant	Giannousi <i>et al.</i> (2013)

3	Root Rot	Citrus Fruit	ZnO and CuO NPs	Media plates application	-Completely inhibit the fungal growth.	Sardar <i>et al.</i> (2021)
		Apple	Ag NPs	Petri plates culture	-Reduce the diameter and fresh weight by 77.4% and 84.4%, respectively	Talie <i>et al.</i> (2020)
		Cherry	Fe ₂ O3 NPs	Foliar application	-A. <i>flavus</i> growth can be significantly reduced.	Zubair <i>et al.</i> (2022)
		Sweet Potato	TiO ₂ NPs	Foliar Application	-The restriction of soft rot disease	Hossain <i>et al.</i> (2019)
4	Strip Rust	Wheat	Ag NPs	Foliar Application	-Enhanced the disease resistance	Sabir <i>et al.</i> (2022)
					-Stabilized the morphological and physiological parameters, non-enzymatic attributes, major antioxidant enzymes	
		Wheat	TiO ₂	Foliar Application	-Disease index reduced by upto74%	Satti <i>et al.</i> (2022)
					-Disease incidences decreased by up to 65%.	
		Wheat	Se NPs	Foliar application	-Improve the morphological length and physiological parameters.	Shahbaz <i>et al.</i> (2022)
					-Raised levels of total proline, phenolic compounds, and flavonoids	
		Wheat	(CeO NPs	Foliar application	-Improved SOD and POD activity	Shahbaz <i>et al.</i> (2022)
					-Enhanced morphological, physiological, biochemical, and antioxidant parameters while alleviating illness severity.	
5	Rust	Coffee	CuO NPs	Foliar application	-Reduces coffee rust by more than 90%	Leal <i>et al.</i> (2023)
					-Inhibit the germination of H. Vastatrix spores	
		Soybean	Ch-ZnO NPs	Cavity slide method	-Inhibit 70.25 (56.92) % uredinio spores germination against <i>Phakopsorapachyrhizi</i>	Ashtaputre <i>et al.</i> (2023)
		Leaf	Ch-NPs	Foliar application	-Reduction in infection type, pustule size, and pustule count	Elsharkawy <i>et al.</i> (2022)
					-The maximum increase in enzyme activities	
		Soybean	Sn NPs	Cavity slide method.	-Maximum 74.70 (59.78)% inhibition of spore germination	Ashtaputre <i>et al.</i> (2023)
6	Powdery mildew	Wheat	Si andTi NPs	Photo catalyst technique.	-Reduced the severity by	Farhat <i>et al.</i> (2018)
					91.00%	
		Pepper	MgO and ZnO NPs	Foliar spray	-Inhibition and alterations in the fungal structures	Ismail <i>et al.</i> (2021)
					-Suppressed the germinating conidia	
		Cucumber	Ag NPs	Aerial spray method.	- Inhibits growth and duplication of those fungi and becteria that cause sore, ichiness, odour and infection.	Lamsa <i>et al.</i> (2011)

manufacturing reactive oxygen species (ROS) during pathogen invasion (Satti *et al.*, 2022).

Powdery mildew and nanoparticles: Powdery mildews are a notable category of plant pathogens that are recognized for their ability to form white powdery layer on the leaves, stems, fruits of flowering plants (angiosperms) and flowers (Braun 2012). They are a significant and widespread category of disease-causing fungi that may be found in nearly all regions

of the world. These fungi are responsible for causing different illnesses in economically significant plants (Braun, 2011). In Pakistan, the understanding of powdery mildews is restricted to a mere 10 genera and 42 species under the Erysiphaceae family. The powdery mildew (PM) fungus mostly target the aerial portions of higher plants for infection. These organisms are resulting powdery mildew illnesses in both wild and cultivated plants, resulting in potential

decreases in crop productivity of more than 30%. PM fungi are categorized as actual ascomycete (fungi) and are part of the Erysiphaceae. there are five tribes within this family (Golovinomycetinae, Blumerieae, Phyllactiniaee, Erysipheae, Cystothecae), along with subtribes and 10 genera (Braun *et al.*, 2002). Leading method of spreading for powdery mildew is through the release of conidia from surface ascospores or conidiophores from fruiting bodies (Braun and U, 2012). This Infection takes place when the conidium or ascospore starts to grow on a host that is vulnerable, resulting in the evolution of a primary germ tube leading to the creation of basic or lobed appressorium. The appressorium subsequently generates a penetration pin and haustorium, usually within the epidermal cell of the host (Figure 8). generally, a conidium can produce up to four hyphae that form colonies. Three of these hyphae derive from the horns of conidium, while one or more than one may emerge from the extreme appressoria (Cook and Braun, 2009).

Nanoparticles (NPs) offer a novel approach to developing fungicide alternatives because of their distinct chemical and physical properties, frequently differing crucially from their size counterparts (Köhler *et al.*, 2008). Bamboos face various pathogenic threats, with fungi being a major contributor to leaf diseases, stem diseases, rhizome diseases, and sheath diseases (Kulabhusan *et al.*, 2022). Research has specified that silicon increases plant opposition to heavy metal stress across different species. Tissue culture experiments were managed to evaluate the impact of silica nanoparticles on growth of bamboo species and growth under controlled conditions polluted with phytotoxic levels of lead (Emamverdian *et al.*, 2020). When lady finger's leaves are infected with *E. cichoracearum*, they exhibit characteristic symptoms of powdery mildew. different sulfur fungicides had a notable impact in reducing the germination of *E. cichoracearum* conidia when compared to the control group (study conducted by Gogoi *et al.* (2013). Sulfur nanoparticles shown much superior fungicidal activities in comparison to regular sulfur against the food contaminant *Aspergillus Niger* (Choudhury *et al.*, 2010). Subsequent investigations were conducted to examine the efficacy of recently developed nanosulfur battle against crop diseases such as lady finger powdery mildew that caused by *Erysiphe cichoracearum* (Gogoi *et al.*, 2013). Cucumber powdery mildew is a rotting disease which results in a decrease in crop output of 20-40% by disrupting important plant processes like photosynthesis and respiration. Plants subjected to high levels of silver nps showed a notable decrease in disease occurrence. The inhibitory effect became more pronounced as the concentration of silver nps become greater (Lamsa *et al.*, 2011). The study examined the

impact of silver nanoparticles and copper nanoparticles on growing indices, the severity of powdery mildew takeover leaves, and the occurrence of spontaneous ectomycorrhizal colonization in oak. The research that managed by Olchowik *et al.* (2017) focused on two frequently encountered metal nanoparticles, namely silver nanoparticles and copper nanoparticles. The nanoparticles were immediately obtainable from the market and comprised around 100 atoms dispersed in demineralized water at a 50 ppm concentration. The cotton bollworm is very damaging insect pest that inflicts harm on a range of crops, such as cotton, cabbage, and tomato. The overutilization of chemical insecticides results in the emergence of contamination and resistance of the environment (Shaker *et al.*, 2017). The study involved preparing titanium dioxide nanoparticles powder based on the active ingredient (ppm). Six different TiO₂ nanoparticle concentrations were created by diluting with distilled water. A control treatment used distilled water without NanoTitanium particles. The research evaluated the effect of LC50 on various biological activities, such as pupal and larval duration, % of pupation and adult hatching, and larval and pupal malformation, by applying titanium particles to leaves for 48 hours (Shaker *et al.*, 2017).

CONCLUSION

This review study examines the potential use of nanotechnology in addressing several plant diseases, specifically targeting potato blight, tomato blight, root rot, rust, and powdery mildew. Different categories of nanoparticles, such as metallic NPs and polymer-based nanoparticles, exhibit unique mechanisms of action that allow for tailored and targeted treatments against diverse fungal diseases. The research provided a comprehensive analysis of the inherent characteristics of nanoparticles that contribute to their high efficacy in combating fungal infections. The unique physicochemical features and small dimensions of nanoparticles enable the targeted administration of antifungal medications to specific areas of plant tissues affected by infection. Within the framework of a dynamic environment and a growing worldwide need for food production, nanotechnology emerges as a potentially fruitful approach to tackling diverse challenges linked to disease control. Nanotechnology has the potential to contribute to sustainable solutions in this field by reducing dependence on chemical inputs and enabling rapid pathogen detection.

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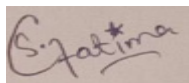
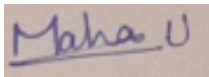
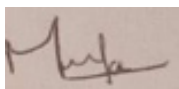
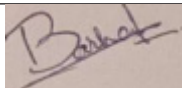
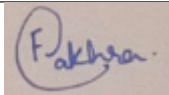
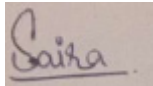
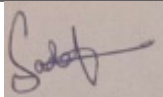
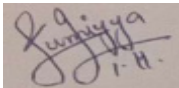
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3.	Maliha Usman	Identified relevant studies and publication	
4.	Barha Nasir	Categorised articles based on predefined manuscript section	
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