Management of phytopathogens by application of green nanobiotechnology: Emerging trends and challenges

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SUMMARY

Nanotechnology is highly interdisciplinary and important research area in modern science. The use of nanomaterials offer major advantages due to their unique size, shape and significantly improved physical, chemical, biological and antimicrobial properties. Physicochemical and antimicrobial properties of metal nanoparticles have received much attention of researchers. There are different methods i.e. chemical, physical and biological for synthesis of nanoparticles. Chemical and physical methods have some limitations, and therefore, biological methods are needed to develop environment-friendly synthesis of nanoparticles. Moreover, biological method for the production of nanoparticles is simpler than chemical method as biological agents secrete large amount of enzymes, which reduce metals and can be responsible for the synthesis and capping on nanoparticles.

Biological systems for nanoparticle synthesis include plants, fungi, bacteria, yeasts, and actinomycetes. Many plant species including Opuntia ficus-indica, Azardirachta indica, Lawsonia inermis, Triticum aestivum, Hydrilla verticillata, Citrus medica, Catharanthus roseus, Avena sativa*, etc., bacteria, such as* Bacillus subtilis, Sulfate-Reducing Bacteria, Pseudomonas stutzeri, Lactobacillus sp., Klebsiella aerogenes, Torulopsis *sp., and fungi, like* Fusarium *spp.* Aspergillus *spp.,* Verticillium *spp.,* Saccharomyces cerevisae *MKY3,* Phoma *spp. etc. have been exploited for the synthesis of different nanoparticles. Among all biological systems, fungi have been found to be more efficient system for synthesis of metal nanoparticles as they are easy to grow, produce more biomass and secret many enzymes. We proposed the term myconanotechnology (myco = fungi, nanotechnology = the creation and exploitation of materials in the size range of 1–100 nm). Myconanotechnology is the interface between mycology and nanotechnology, and is an exciting new applied interdisciplinary science that may have considerable potential, partly due to the wide range and diversity of fungi.*

Nanotechnology is the promising tool to improve agricultural productivity though delivery of genes and drug molecules to target sites at cellular levels, genetic improvement, and nano-array based gene-technologies for gene expressions in plants and also use of nanoparticles-based gene transfer for breeding of varieties resistant to different pathogens and pests. The nanoparticles like copper (Cu), silver (Ag), titanium (Ti) and chitosan have shown their potential as novel antimicrobials for the management of pathogenic microorganisms affecting agricultural crops. Different experiments confirmed that fungal hyphae and conidial germination of pathogenic fungi are significantly inhibited by copper nanoparticles. The nanotechnologies can be used for the disease detection and also for its management. The progress in development of nano-herbicides, nano-fungicides and nano-pesticides will open up new avenues in the field of management of plant pathogens. The use of different nanoparticles in agriculture will increase productivity of crop. It is the necessity of time to use nanotechnology in agriculture with extensive experimental trials. However, there are challenges particularly the toxicity, which is not a big issue as compared to fungicides and pesticides.

Keywords: Nanoparticles, pathogen detection, antimicrobials, nano-herbicides, nano-fungicides, nano-pesticides, toxicity

INTRODUCTION

Due to the extensive use of fungicides and pesticides there is rapid increase in ecotoxicity (Chen *et al*., 2015; Vu *et al.*, 2015) and development of resistance in plant pathogenic microbes (Dzhavakhiya *et al*., 2012; Alghuthaymi *et al*., 2015). The possible solutions include biological control of plant pathogens by using extracts of the plants or microbes. Although, biological control methods for the management of phytopathogens have been useful, several inherent challenges need to be addressed (Frampton *et al*., 2012). Therefore, there is a pressing need to search for alternatives for the management of phytopathogens. The emerging nanobiotechnology seems to be of paramount importance for the management of phytopathogens particularly in early detection of plant disease, as potential fungicides, in development of varieties resistant to fungal diseases, and also for smart delivery of fungicides to the plants. The fungi affecting agricultural production can be controlled by application of nanofungicides. The use of nanotechnology both in developing and developed countries will bring dramatic changes in agriculture. This would really be a journey from green revolution to green nanobiorevolution.

Nanotechnology (NT) is highly interdisciplinary technology with size range of 1-100 nm. NT involves physics, chemistry, biology, engineering, medicines, agriculture and all other sciences. It is also considered as enabling technology since the property and activity of nanoparticles changes with change in size and shape (Satalkar *et al*., 2015).

Nanotechnology and biotechnology are two very important subjects, which are highly interdisciplinary. The fusion of these two subjects has given rise to Nanobiotechnology. The emerging science nanobiotechnology has revolutionized the world and it is believed that the present century will be the century of smart technologies represented by nanobiotechnology. There are various reports which provide evidence of *in vitro* efficacy of different kind of nanoparticles (Guo *et al*., 2015). But of course, the need of extensive experimental trials are necessary in order to utilize the fullest potential of nanobiotechnology. Different kind of nanoparticles in general and biodegradable nanoparticles in particular can be used for plant disease management (Chowdappa and Gowda, 2013).

Nanoparticles have higher surface area to the volume ratio. Therefore, they have more chances of interaction with the pathogenic microbes and killing them. As a matter of fact, they have better potential to kill the microbes as compared to bulk materials. The nanoparticles shows unique physical, chemical and biological properties due to their nanosize, and therefore can be utilized in gene transfer (Rai *et al*., 2012), for management of insect-pests in and pathogens in agriculture (Rai and Ingle, 2012; Mishra and Singh, 2015).

The bacteria and fungi are developing resistance to microbicides or fungicides and thus causing major problem for tackling the pathogens like *Phytophthora*. Considering this fact, it is a pressing need to develop alternative antimicrobial agents. Since ancient times, silver has been known for its antibacterial activity, and now silver nanoparticles are being used as antimicrobials (Rai *et al*., 2009). Encouragingly, AgNPs have been rightly called as nanoweapon against plant pathogens (Mishra and Singh, 2015). The American Biotech Labs have spent millions of dollars on testing the safety and efficacy of nanosilver technology products. Their studies have concluded that nanosilver products are not toxic to cells, animals or humans at low concentration.

Usually, the nanoparticles are synthesized by physical, chemical and biological methods. The first two methods are energy intensive and may require toxic chemicals whereas, biogenic technique is eco-friendly, clean, non-toxic and economically viable. However, the main draw-back of biogenic method is that it is difficult to achieve monodispersity, and control over the size and shape (Nayak *et al*., 2011). The formation of nanoparticles by using fungi (Ingle *et al*., 2009; Bawaskar *et al*., 2010; Kumar *et al*., 2012; Dar *et al*., 2013; Potara *et al*., 2015), plants (Narayan *et al*., 2008; Gade *et al*., 2010; Bonde *et al*., 2012; Rai and Yadav, 2013; Mallikarjuna*et al*., 2015) bacteria (Kumar *et al*., 2008; Shahverdi *et al*., 2009; Tiwari *et al*., 2014), actinomycetes (Golinska *et al*., 2014), algae (El-Kassas and El-Sheekh, 2014) have been reported by many researchers.

The main focus of the present talk is to discuss the role of green nanobiotechnology for the detection of diseases and also for the management of different phytopathogens in general and fungal pathogens in particular. In addition, the toxicity issue has also been addressed so that the nanofungicides may be formulated for the strategic management of plant diseases caused by different pathogens.

NANOTECHNOLOGY IN PATHOGEN DETECTION

There is increasing demand for the food production owing to the fast growing human population and therefore, food security has become an international issue. It is estimated that by 2050 an additional 70% food production is needed to fulfil the demand of growing population (Godfray *et al.,* 2015). Unfortunately, the food loss caused by bacteria, fungi and viruses ranges from 20 to 40% (Savary *et al*., 2012).

There are various recent methods for detection of diseases caused by microbial pathogens which are mostly laboratory-based (Fang and Ramasamy, 2015). These include polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), immunofluorescence (IF), fluorescence *in-situ* hybridization (FISH), flow cytometry (FCM) and gas chromatography-mass spectrometry (GC-MS). However, there is need of nanobiosensors for rapid detection of the pathogens (Rai *et al*., 2012). Many biosensors have been developed for different applications particularly in medical and environmental field. Such sensors can be developed for plant disease identification and their efficiency can be enhanced by the use of nanomaterials. Different nanomaterials, such as metal and metal oxide nanoparticles, quantum dots, carbon nanotubes, graphene, etc. can be used in nanobiosensors (*Table 1*). The nanoparticles are most widely used materials, for example, nanoparticles are used with antibodies for detection of *Xanthomonas axonopodis* (Yao *et al*., 2010).

Prunus necrotic ringspot virus (PNRSV), which causes disease in *Prunus* species (peach, plum, apricot, sweet cherry and almonds) resulting into yield loss. Traditionally, the trees which suffer from disease have to remove from the orchard. Hence, the rapid and early identification of the pathogen is necessary in order to control this disease. In 2014, Zong *et al*. developed a new method for rapid detection of *Prunus necrotic ringspot virus* using magnetic-nanoparticle-based reverse Transcription loop-mediated isothermal amplification (RT-LAMP). The authors reported this technique to be highly specific and more sensitive to PNRSV than than reverse-transcription polymerase chain reaction (RT-PCR).

Table 1

List of different nanomaterials used in nanobiosensors

S.No.	Nanomaterials	Disease	Reference
1	AuNps-based optical immunosensor	Karnal bunt disease of wheat	Singh *et al*. (2010)
	Gold nanorods (AuNRs) functionalized by antibodies	Cymbidium mosaic virus (CymMV) or Odontoglossum ringspot virus (ORSV)	Singh *et al*. (2010)
2	Nanochips (made of microarray)	Bacteria and viruses	López *et al*. (2009)
3	Fluorescent silica nanoparticles (FSNPs)	*Xanthomonas axonopodis* pv. *vesicatoria*	Yao *et al*. (2009)
4	Quantum dots-based sensors	General disease detection, viruses	Algar and Krull (2008); Safarpour *et al*. (2012)
5	SnO_2 and TiO_2	General disease, detection of p-ethylguaiacol secreted by infected strawberry	Fang *et al*. (2014)
6	Polymers such as polypyrrole (PPy) nanoribbon modified chemiresistive sensors	Cucumber mosaic virus (CMV)	James (2013)
7	Magnetic-nanoparticl-based reverse transcription loop-mediated isothermal amplification (RT-LAMP)	Prunus necrotic ring-spot virus (PNRSV)	Zong *et al.* (2014)

NANOANTIMICROBIALS FOR TACKLING THE PROBLEM OF PLANT PATHOGENS

The use of metals as antimicrobials against pathogens is well known since ancient times. The metals such as copper, silver, palladium, ruthenium and their compounds have been used against human and plant diseases (Medici *et al.*, 2015). With the advent of nanobiotehnology, different nanomaterials including nanoparticles have been evaluated for their potential for the management of plant pathogens (*Table 2).* Cioffi and his collaborators in 2004 studied antifungal activity of nanocopper against plant pathogenic fungi. Gul *et al.* (2014) presented an informative review on role of nanotechnology in crop protection.

Lamsal and his colleagues in 2011 evaluated different concentrations of AgNPs against powdery mildew and found that 100 ppm silver nanoparticles (7-25 nm) demonstrated highest inhibition rate of the disease in cucumbers and pumpkins in field conditions. In 2011, they further studied different concentrations (10, 30, 50, and 100 ppm) of AgNPs on six species of *Colletotrichum* including *C. acutatum, C.dematium, C. gloeosporioides, C. higginsianum, C. nigrum* and *C. orbiculare* and reported the significant inhibition of growth of all the species of *Colletotrichum* tested in their experiment. They also reported that the treatment should be given to the plants before appearance of the symptoms on the host plants. Encouragingly, the highest percentage of inhibition was recorded with 50 ppm nanoparticles in field trials while 100 ppm AgNPs was needed *in vitro* inhibition.

Ocsoy and his colleagues (2013) on their study found leaf-spot disease caused by *Xanthomonas perforans* (Cu resistant) can be inhibited by DNA-directed silver (Ag) nanoparticles (NPs). The *in vitro* studies and nanoparticle-treated plants demonstrated that at 16 ppm the growth was inhibited, which provides evidence of remarkable antibacterial activity against *X. perforans*. The disease was significantly reduced, when 100 ppm Ag@dsDNA@GO was applied in green house experiment.

In another study, the biogenically synthesized nanoparticles by leaves and stem of *Piper nigrum* were tested against two bacteria, namely, *Citrobacter freundii* and *Erwinia cacticida* causing diseases on *Abelmoschus esculentus* and *citrullus lanatus*. The authors reported excellent antibacterial activity when silver nanoparticles impregnated antibiotic discs (Chloramphenicol) were used against the test bacteria (Paulkumar *et al.* 2014). Interestingly, Anusuya and Sathiyabama (2014) applied chitosan nanoparticles to induce antifungal hydrolases in turmeric plant (*Curcuma longa*). The author performed foliar spray of chitosan nanoparticles and found that chitinases and chitosanases were increased.

Table 2

List of different nanomaterials and nanocomposites used against phytopathogens

S.No.	**Type of Nanoparticles**	**Phytopathogens**	**References**
1	Copper nanoparticles	Antifungal	Cioffi *et al.* (2004)
2	Silica-silver nanoparticles	*Rhizoctonia solani, Pythium ultimum, Botrytis cinerea, Magnaporthe grisea* and *Colletotrichum gloeosporioides*	Park *et al.* (2006)
3	AgNPs	*Bipolaris sorokiniana* and *Magnaporthe grisea*	Jo *et al.* (2009)
4	AgNPs	Sclerotium-forming phytopathogenic fungi	Min *et al.* (2009)
5	AgNPs	Oak wilt pathogen *Raffaelea* sp.	Kim *et al.* (2009)
6	AgNPs	Stem-end bacteria on cut gerbera *Gerbera jamesonii*) cv. Ruikou	Liu *et al.* (2009)
7	AgNPs	*Fusarium culmorum*	Kasprowicz *et al.* (2010)
8	AgNPs	Powdery mildew on cucumber and pumpkin	Lamsal *et al.* (2011)
9	AgNPs	*Colletotrichum acutatum, C. dematium, C. gloeosporioides, C. higginsianum, C. nigrum, C. orbiculare*	Lamsal *et al.* (2011)
10	AgNPs	Cut *Acacia holosericea*	Liu *et al.* (2012)
11	AgNPs colloidal solution	*Alternaria alternata, A. brassicicola, A. solani, Botrytis cinerea, Cladosporium cucumerinum, Corynespora cassiicola, Cylindrocarpon destructans, Didymella bryoniae, Fusarium oxysporum* f.sp. *cucumerinum, Fusarium oxysporum* f.sp. *lycopersici, Fusarium oxysporum, Fusarium solani, Glomerella cingulata, Monosporascus cannoballus, Pythium aphanidermatum, P. spinosum, Stemphylium lycopersici*	Kim *et al.* (2012)
12	Nanosized Ag-silica-hybrid	*Pseudomonas syringae* pv. *tomato*	Chu *et al.* (2012)
13	DNA-directed silver (Ag) nanoparticles (NPs)	Bacterial spots caused by *Xanthomonas perforans* in tomatoes	Ocsoy *et al.* (2013)
14	Chitosan NPs	Rhizome-Rot Disease of Turmeric Caused by *Pythium aphanidermatum*	Anusuya and Sathiyabama (2013)
15	Silver – chitosan composite	Gray mold (*Botrytis cinerea*) in straberry	Moussa *et al.* (2013)
16	ß- D – glucan nanoparticles	Rhizome-Rot Disease of Turmeric Caused by *Pythium aphanidermatum*	Anusuya and Sathiyabama (2014)
17	Biogenic AgNPs	*Bipolaris sorokiniana* causing Spot Blotch Disease in Wheat	Mishra *et al.* (2014)
18	AgNPs	*Citrobacter freundii, Erwinia cacticida*	Paulkumar *et al.* (2014)
19	Silica NPs	*Fusarium oxysporum* and *Aspergillus niger*	Suriyaprabha *et al.* (2014)
20	Copper NPs	*Fusarium oxysporum*, *Curvularia lunata*, *Alternaria alternata*, and *Phoma destructiva*	Kanhed *et al.* (2014)
21	Nanotitania	*Alternaria brassicae*	Palmqvist *et al.* (2015)
22	AgNPs	*Phytophthora parasitica*, *P. infestans*, *P. palmivora*, *P. cinnamomi*, *P. tropicalis*, *P. capsici*, and *P. katsurae*	Ali *et al.* (2015)
23	Cu-Chitosan NPs	*Alternaria solani* and *Fusarium oxysporum* pathogenic fungi of tomato	Saharan *et al.* (2015)
24	Chitosan NPs	*F. oxysporum* f.sp. *lycopersici* in tomato	Sathiyabama and Charles (2015)

Note: AgNPs = Silver nanoparticles; NPs = Nanoparticles

These enzymes are responsible for defense of the host plants. The treated plants of *C. longa* were found to be resistant to *Pythium aphanidermatum,* the causal organism of rhizome-rot of turmeric. In 2014, they also applied ß-D-glucan nanoparticles, which reduced rot incidence by 23.3%. The authors found correlation between reduction on incidence of rhizome-rot and enhanced activity of defense enzymes such as peroxidases, polyphenol oxidases, protease inhibitors and ß-1,3-glucanases.

Mishra *et al.* (2014) synthesized AgNPs by using *Serratia* sp. and evaluated these AgNPs against spot blotch disease in wheat caused by *Bipolaris sorokiniana*. The AgNPs demonstrated remarkable antifungal activity when 2, 4 and 10 mg/ml concentrations were used. The conidial germination of *B. sorokiniana* was totally inhibited. In an interesting experiment, Suriyaprabha and colleagues (2014) treated maize with silica NPs (20-40 nm) to know the resistance against two important phytopathogens, *Fusarium oxysporum* and *Aspergillus niger.* The authors reported development of higher resistance in Silica nanoparticle treated maize plants than the bulk silica treated plants. In an interesting study, Palmqvist and colleagues (2015) used Titania NPs to understand the interaction between *Bacillus amyloliquefaciens*, a plant growth promoting bacterium and the host plant *Brassica napus* for providing protection against *Alternaria brassicae*. The authors observed increased number of bacteria on the roots of *B. napus* due to use of Titania NPs., which protects the test plant against infection caused by *A. brassicae.*

NANOTOXICITY: A MAJOR CHALLENGE

As discussed in the earlier sections nanoparticles have huge applicability for the management of plant diseases. But due to increased applications there is greater possibilities of getting accumulated in the environment and cause the harmful effects. By any kind of aqueous medium there are high chances of accumulation of nanoparticles in soil resulting into the soil ecosystem toxicity. Thus, if accumulated above certain limit they will bound to show the harmful effects. For instance, a study has shown that TiO_2 and ZnO nanoparticles have negatively affected the biomass of wheat growth and also inhibited the activities of soil enzymes such as protease, catalase and peroxidase activities, thereby affecting the soil quality and health (Du *et al.*, 2011). Yang and Watts (2005) were the first to report about the nanotoxicity to plants through the soil. According to their study Al_2O_3 conjugated with and without phenanthrene affected the root elongation of *Zea mays*, *Cucumis sativus, Glycine max*, *Brassica oleracea* and *Daucus carota*. Similar results were obtained by exposing MWNT, Al_2O_3, Al, Zn and ZnO on radish, rape, ryegrass, lettuce, corn, and cucumber. With affecting the root elongation, those nanoparticles also shown to affect the plant germination (Lin and Xing, 2007). TiO_2 were reported to reduce the water usage in *Z. mays* and changes the path of apoplast (Asli and Neumann, 2009). Palladium nanoparticles (PdNPs) were reported to get accumulated in leaves of barley (Battke *et al.*, 2008). Therefore, there is possibility of transfer of these nanoparticles in the animals. On the similar way, nano Fe_2O_3 were also found to get accumulated in tissue of pumkin (Zhu *et al.*, 2008).Through an interesting study Lin *et al.* (2009) reported the transmission of C_{70} into the progeny of nanoparticle exposed rice.

All of the reports suggest that as a consequence of presence of various nanoparticles in soil, there is possibility of threat to soil microorganisms and to plants by absorption of nanoparticles from soil. Moreover, such accumulation and/or toxic effects to plants may affect the complete food chain. The toxic effects to plants may further pass to animals such as fish, insects or mammals including humans who consume them (*Figure 1*). But still there is huge knowledge gap in the information on the mode of uptake of nanoparticles and consequences of their exposure to soil environment. There is also need to study the impact of surface functionalization, size, charge, agglomeration and stability of nanoparticles on the plants. Moreover, it is also essential to perform further studies on the transport of nanoparticles and the effects caused to food chain thereby affecting the humans and animals.

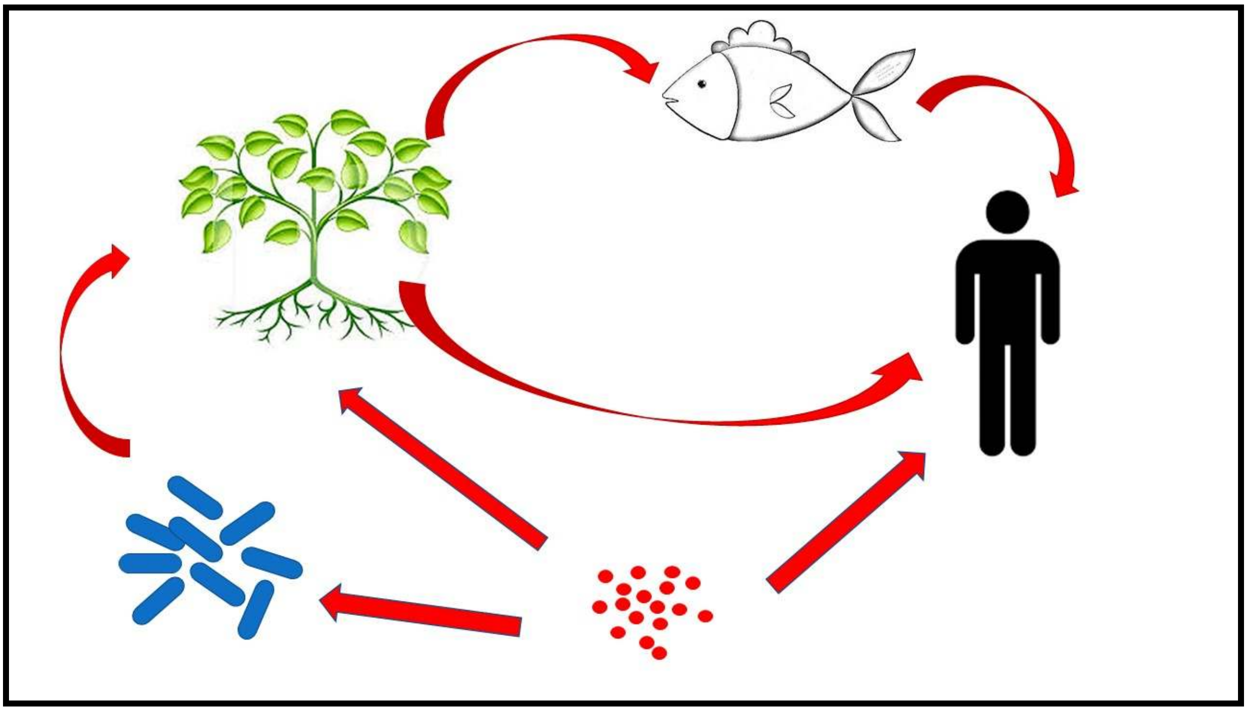

Figure 1: **Fate of nanoparticles in the soil environment. The nanoparticles may exert toxic effects to soil bacteria and plants, and if accumulated inside them, they can be ultimately transferred to animals and humans**

The straight red colored lines show the direct effect of nanoparticles. The curved red lines show the pathway of nanoparticles in food chain.

CONCLUSIONS

It can be concluded that extensive application of chemical fungicides and antimicrobials have generated huge pollution resulting into toxicity to all the living flora and fauna. Considering this fact, it is felt that there is an urgent need to search for alternatives to manage the phytopathogens for the sustainable plant production. The application of biocontrol agents for the management of different diseases is an interesting strategy but there are several inherent issues, which prompts us to search for other viable alternatives, and therefore, the use of eco-friendly green nanobiotechnology has become the main focus of the scientists all over the world. The synthesis of nanomaterials by biogenic methods mainly microbes and plants is green, eco-friendly, rapid and economically viable. These can be utilized for the development of nanobiosensors, which can be used for the detection of plant diseases. Moreover, different types of nanomaterials in general and nanoparticles in particular may solve the problem of microbes resistant to microbicides or fungicides. In addition, the pollution and toxicity of fungicides can be avoided. However, extensive experimental field trials are needed to understand the toxicity of the nanoparticles in food chain. Finally, the application of green nanobiotechnology may open up new avenues for research pertaining to the management of plant pathogens.

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