



Nanotechnology in Plant Disease Management

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Plant pests and pathogens cause significant reductions in crop production, with estimated global losses of 20%–40% per year. Current pest management relies heavily on the application of pesticides, such as insecticides, fungicides, and herbicides. In spite of many advantages, like high availability, fast action, and reliability, pesticides have harmful side effects towards non-target organisms, the resurgence of the pest population, and the development of resistance. Furthermore, it is estimated that 90% of applied pesticides are lost during or after application. As a result, there is an increased motivation to develop cost-efficient, high-performing pesticides that are less harmful to the environment. Nanotechnology has led to the development of new concepts and agricultural products with immense potential to manage the aforementioned problems. Nanotechnology has substantially advanced in medicine and pharmacology, but has received comparatively less interest for agricultural applications. The use of nanotechnology in agriculture is currently being explored in plant hormone delivery, seed germination, water management, transfer of target genes, nanobarcoding, nanosensors, and controlled release of agrichemicals. Material scientists have engineered nanoparticles with desired characteristics, like shape, pore size, and surface properties, so that they can then be used as protectants or for precise and targeted delivery via adsorption, encapsulation, and/or conjugation of an active, such as a pesticide. As agricultural nanotechnology develops, the potential to provide a new generation of pesticides and other actives for plant disease management will greatly increase. The use of nanoparticles to protect plants can occur via two different mechanisms: (a) nanoparticles themselves providing crop protection, or (b) nanoparticles as carriers for existing pesticides or other actives, such as double-stranded RNA (dsRNA), and can be applied by spray application or drenching/soaking onto seeds, foliar tissue, or roots. Nanoparticles, as carriers, can provide several benefits, like (i) enhanced shelf-life, (ii) improved solubility of poorly water-soluble pesticides, (iii) reduced toxicity, and (iv) boosting site-specific uptake into the target pest. Another possible nanocarrier benefit includes an increase in the efficacy of the activity and stability of the nanopesticides under environmental pressures (UV and rain), significantly reducing the number of applications, thereby decreasing toxicity and reducing their costs.

Types of Nanoparticles for Plant Disease Management

Nanoparticles are materials that range between 10 to 100 nanometers (nm), and can be designed with unique chemical, physical, and biological properties, to distinctively differ from those of their molecular and bulk counterparts. Nanoparticles alone have the potential to be directly applied to plant seeds, foliage, or roots for protection against pest and pathogens, such as insects, bacteria, fungi, and viruses. Metal nanoparticles such as silver, copper, zinc oxide, and titanium dioxide have been intensively researched for their antibacterial and antifungal properties, and are known for their antiviral properties. This section gives a brief

overview and an update on current literature reviews pertaining to the individual nanoparticles that already exist. Recently, silver nanoparticles have increased in popularity, due to “green synthesis” production in plants, bacteria, fungi, or yeast. Silver nanoparticles have shown antifungal inhibition of *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinerea*, and *Curvularia lunata* by well diffusion assay. When silver nanoparticles were sprayed onto bean leaves, complete suppression of sun- hemp rosette virus was observed. Elbeshehy et al. further showed that faba bean plants challenged with bean yellow mosaic virus, and sprayed with silver nanoparticles, produced remarkably better results when the nanoparticles were applied 24 h post-infection, compared to spray application before infection, or simultaneously at the time of inoculation. Silver nanoparticles have shown immense potential for plant disease management against fungal and bacterial pathogens, but there are significant hurdles associated to them, such as production, toxicity, and soil interaction. Other commonly used metal nanoparticles include copper, titanium dioxide, and gold. Nanoparticles, such as copper and titanium dioxide, are more commonly being utilized for fertilizer with little research into plant disease management.

The research covering titanium dioxide, silver, and copper nanoparticles for antimicrobial properties, and aluminum nanoparticles as an insecticidal dust, has been highlighted. Furthermore, titanium dioxide nanoparticles in fertilizers have produced protection from bacteria and inactivation of viruses. Introducing poly-dispersed gold nanoparticles via a mechanical abrasive was seen to melt and dissolve the Barley yellow mosaic virus particles conferring resistance to the plant. Chitosan is another popular nanoparticle that has favorable biological properties, such as biodegradability, biocompatibility, non- allergenicity, and antimicrobial activity, with low toxicity to animals and humans. Chitosan nanoparticles induce viral resistance in plant tissues by protecting them against infections caused by the mosaic virus of alfalfa, snuff, peanut, potato, and cucumber. Chitosan nanoparticles have shown antimicrobial properties, such as controlling *Fusarium crown*, root rot in tomato, *Botrytis bunch rot* in grapes, and *Phyricularia grisea* in rice, but are less effective against bacteria. Malerba and Cerana summarized potential mechanisms that lead to the antimicrobial effects of chitosan, such as agglutination, disruption of the cell membrane, inhibition of H⁺ -ATPase activity, inhibition of toxin production and microbial growth, inhibition of the synthesis of messenger RNA and proteins, and blockage of nutrient flow. Antiviral effects have been observed in beans against Bean mild mosaic virus and, in tobacco, against Tobacco mosaic virus and Tobacco necrosis virus.

Future of nanotechnology in plant disease management

Nanotechnology offers major advances through faster and more-sensitive pathogen probes. Intuitively, nanoparticles can be used as rapid diagnostic tools in disease detection for bacterial, fungal, nematodal, and viral pathogens. The following provides a summary of how nanotechnology has enhanced the diagnostics of plant pathogens. Rispaal et al. found that super paramagnetic iron oxide nanoparticles differed from quantum dots after being applied to *F. oxysporum*. Although both nanoparticles attached to the hyphae, the super paramagnetic iron oxide nanoparticles attached only to the surface and were not absorbed where they could be visualized more readily. However, the quantum dots were taken up by the fungal hyphae and became toxic.

Gorny *et al.* developed a rapid and less expensive procedure for extracting DNA for quantification of *Meloidogyne hapla* from mineral soils. Adding the super paramagnetic iron oxide nanoparticles to the extraction lysate that combined a detergent lysis and polyvinylpolypyrrolidone, Gorny et al. maximized DNA yield while minimizing contaminants. Given the broad applications of super paramagnetic iron oxide nanoparticles in

detection of human bacteria and cancer cells, its utilization in plant diagnostics will grow; one new area would be in rapid detection of pathogens in irrigation water.

Biosensors are nanoanalytical devices that use a biological sensing element integrated into a physicochemical transducer to produce an electronic signal when in contact with the analyte of interest (pathogen). The biosensor can be loaded in sufficient quantity so that the electrical signal increases as a function of pathogen density. Thus, a biomolecular interaction is converted into a digital output. The construction of a biosensor is accomplished with metalloids or metal oxide nanoparticles and carbon nanomaterials (carbon nanotubes and graphene). However, more recent breakthroughs in nanotechnology have allowed biosensors to be prepared with different types of nanoparticles and nanostructures with fewer technical hurdles. A novel nanobiosensor used nano-Au functionalized with single-stranded oligonucleotides to detect as little as 15 ng of *R. solanacearum* genomic DNA in farm soil. During the past decade, several publications have demonstrated the capability of antibody-based biosensors for detection of plant pathogens. Nano-based biosensors have been developed for Cowpea mosaic virus, Tobacco mosaic virus, and Lettuce mosaic virus. The sensitivity of detection has been increased by two orders of magnitude over traditional ELISA methods. Biosensors were used to detect gram-negative bacteria that use N-acyl homoserine lactones in quorum sensing. The bacterial biosensors phenotypically respond when exposed to exogenous lactones. Singh and colleagues have engaged in a program to develop a nano-Au-based dipstick for rapid detection of Karnal bunt disease in wheat in the field.

Application of this dipstick could have far-reaching uses in enforcing quarantines. In addition to single biosensors directed at one pathogen, research has been ongoing to develop nanochip microarrays that contain multiple fluorescent oligo probes to detect small nucleotide changes in plant-pathogenic bacteria and viruses. Quantum dots are small semiconductor nanocrystals that have been used in biosensors to detect phytoplasma in lime trees. Using a fluorescence resonance energy transfer mechanism, a high sensitivity and specificity of 100% for approximately 5 Candidatus *Phytoplasma aurantifolia* per μL were achieved with consistent results. Quantum dot biosensors were useful in detecting rhizomania (Beet necrotic yellow vein virus) in *Polymyxa betae*. This exciting new approach of using nano-enabled biosensors can be coupled with robotics and GPS systems to create smart delivery systems that detect, map, and treat specific areas in a field prior to or during the onset of symptoms. This technology could reduce agrochemical inputs and increase yield and profits. Growers and scouts could perform diagnostics in situ once portable devices with biosensors are developed. An extremely valuable use for fast and sensitive biosensors is at ports of entry, where quarantined pathogens could be intercepted with greater efficiency. The value of rapid analysis in detecting food pathogens and mycotoxins is obvious.