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Nano-Technology in Plant Disease Management

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Introduction

Pests and diseases cause significant reductions in crop production, with estimated global losses of 20%–40% per year (Flood J., 2010). The use of micronized chemical pesticides in controlling plant diseases is an age-old practice. In its comparison, nano-formulation of these chemicals is a recent introduction to the field of plant protection. The efficiency of a chemical can be improved by generating nanoparticles (NPs) and hence nanotechnology attains much attention. Nanoparticle engineering is one of the latest technological innovations that demonstrate unique targeted characteristics with elevated strength. As agriculture faces numerous and unprecedented challenges, such as reduced crop yield due to biotic and abiotic stresses, including nutrient deficiency and environmental pollution, the emergence of nanotechnology has offered promising applications for precision agriculture. Further, nanotechnology provides excellent solutions for an increasing number of environmental challenges. For example, the development of nanosensors has extensive prospects for the observation of environmental stress and enhancing the combating potentials of plants against diseases (Afsharinejad, A.et al.,2016, Kwak, S.Y.et al., 2017).

Benefits of Nanotechnology in crop protection

The use of nanoparticles to protect plants can occur via two different mechanisms such as (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 (Hayles, J. et al., 2017). Another possible nanocarrier benefit includes an increase in the efficacy of the activity and stability of the nano-pesticides under environmental pressures (UV and rain), significantly reducing the number of applications, thereby decreasing toxicity and reducing their costs. (Figure 1.)

Biological approaches for the synthesis of metallic nanoparticles

Nanoparticles (NPs) are organic, inorganic, or hybrid materials with at least one of their dimensions ranging from 1 to 100 nm (at the nanoscale). NPs that exist in the natural world can be produced from the processes of photochemical reactions, volcanic eruptions, forest fires, simple erosion, plants, and animals, or even by microorganisms (Love, J.C. et al., 2005; Dahoumane, S. et. al., 2017). The production of plant and microorganism-derived NPs, has emerged as an efficient biological source of green NPs that draw extra attention of scientists in recent times due to their eco-friendly nature and simplicity of production process. (Panpatte, D.G.et. al., 2016; Dahoumane, S. et. al., 2017; Kitching, M. et al., 2015; Iravani, S. 2011; Park, T.J. et al.,2016). For the exploitation of green nanotechnology, a number of plant species and microorganisms including bacteria, algae, and fungi are being currently used for NP synthesis. For example, *Medicago sativa* and *Sesbania* plant species are used to formulate gold nanoparticles. Likewise, inorganic nanomaterials, made of silver, nickel, cobalt, zinc, and copper, can be synthesized inside live plants, such as *Brassica juncea*, *Medicago sativa*, and *Helianthus annus* (Panpatte, D.G.et. al., 2016; Ghormade, V. et al., 2011; Kitching, Met al., 2015; Iravani, S. 2011). Microorganisms, such as diatoms, *Pseudomonas stuzeri*, *Desulfovibrio desulfuricans* NCIMB 8307 *Clostridium thermoaceticum* and *Klebsiella aerogens* are used to synthesize silicon, gold, zinc sulphide and cadmium sulphide nanoparticles, respectively.

This schematic shows different nanomaterials as either protectants or carriers for actives such as insecticides, fungicides, herbicides, or RNA-interference molecules, targeting a wide range of pests and pathogens. It also highlights the potential benefits of nanomaterial applications, such as improved shelf-life, target site-specific uptake, and increased solubility, while decreasing soil leaching and toxicity.

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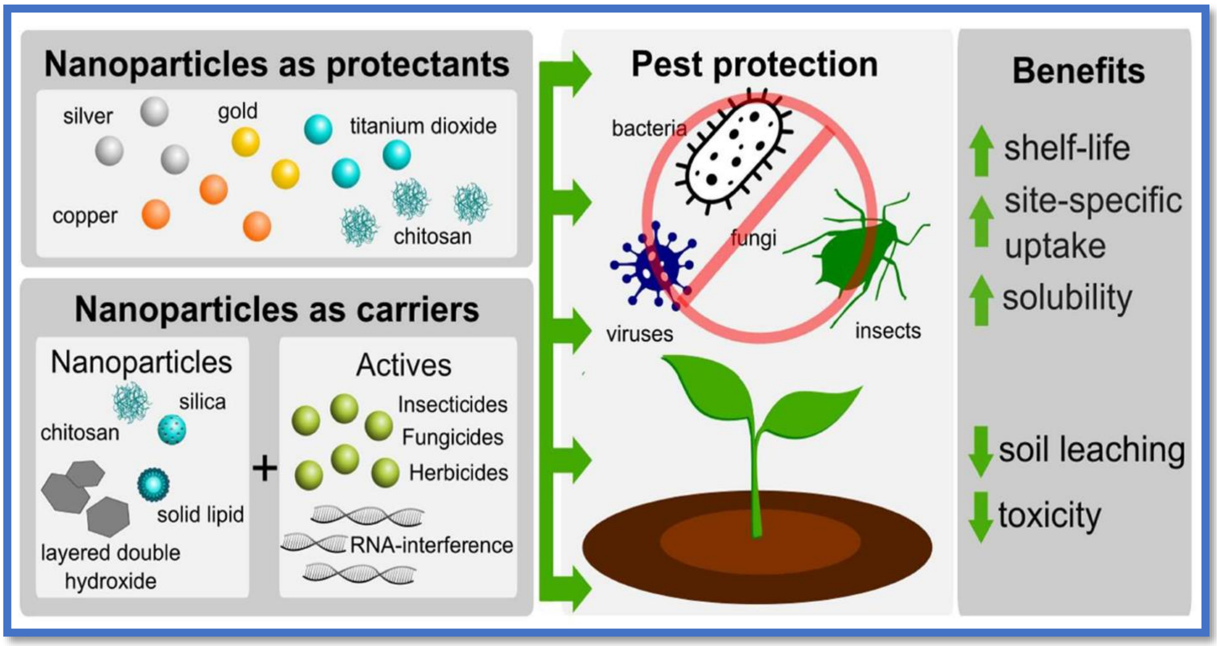

Figure 1. Nanomaterials as protectants or carriers to provide crop protection.

Of the various biological materials, fungi are taking the center stage in studies on the biological generation of metallic nanoparticles due to their tolerance and bioaccumulation ability (Sastry et al., 2003). According to Thakkar et al., 2010, the distinct advantage of using fungi in nanoparticle synthesis is the ease of their scale-up (e.g., using a thin solid substrate fermentation method). Fungi are efficient secretors of extracellular enzymes that facilitate obtaining large-scale production of enzymes. Further, the fungal mediated 'green approach' (green synthesis) of metallic nanoparticles includes economic viability and ease of handling biomass. The fungi, mainly*Verticillium* sp., *Aspergillus flavus*, *Aspergillus furnigatus*, *Phanerochaete chrysoparium*, and *Fusarium oxysporum* are considered to be the most efficient systems for the biosynthesis of metal and metal sulfide containing NPs (Panpatte, D.G.et. al., 2016; Kitching, M.et al., 2015). Of course, a significant drawback of using these bio entities in nanoparticle synthesis is that the genetic manipulation of eukaryotic organisms as a means of overexpressing specific enzymes is much more difficult than in prokaryotes. It was also emphasized that irrespective of the biological system used to its maximum potential, it is very essential to understand the biochemical and molecular mechanism of nanoparticle synthesis.

Types of Nanoparticles for Plant Disease Management

Nanoparticles as Protectants

Nanoparticles are designed with unique chemical, physical, and biological properties, to be distinctively different from those of their molecular and bulk counterparts (Yang, W. et al., 2008). Nanoparticles alone have the potential to be directly applied to plant seeds, foliage, or roots for protection against pests 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 (Kah, M. and Hofmann, T. 2014; Gogos, A. et al., 2012; Kim, D.Y. et al., 2018).

Silver nanoparticles (Ag NPs)

Recently, silver nanoparticles have increased in popularity, due to "green synthesis" production plants, bacteria, fungi, or yeast (Rafique, M. et al., 2017). Silver has got broad-spectrum antimicrobial properties due to microbial colonization associated with biomaterial-related infections (Gristina, 1987). Many fungi (Velmurugan et al., 2009) including phytopathogens *Fusarium culmorum* (Kasprowicz et al., 2010), oak wilt pathogen *Rafflaelea* sp. (Kim et al., 2009), sclerotium forming fungi *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *S. minor* (Min et al., 2009), *Bipolarissorokiniana* and *Magnaporthe grisea* (Jo et al., 2009) are sensitive to AgNPs. Govindaraju et al., (2010) recorded the antimicrobial activity of *Solanum torvum* mediated AgNPs against the silkworm (*Bombyx mori*) pathogens viz. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus niger* and *A. flavus*. This antimicrobial activity of Ag is due to the formation of insoluble compounds by inactivation of sulfhydryl groups in the fungal cell wall and disruption of membrane-bound enzymes and lipids which cause cell lysis (Dorau et al., 2004). Silver nanoparticles have shown antifungal inhibition of *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Macrophominaphaseolina*, *Rhizoctonia solani*, *Botrytis cinerea* and *Curvularia lunata* by well diffusion assay (Krishnaraj, C. et al., 2012). When silver nanoparticles were sprayed onto bean leaves, complete suppression of the sunhemp rosette virus was observed (Jain, D. and Kothari, S.2014). Elbeshehy et al., 2015, 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 hr post-infection, compared to spray application before infection, or simultaneously at the time of inoculation. Antibacterial activity against both gram-positive bacteria *Staphylococcus* sp. and *Bacillus* sp. and the gram-negative bacterium *E. coli* is also known to have in AgNPs (Jaidev and Narasimha,2010; Marcato et al., 2010). Silver is also known to attack a broad range of biological processes in microorganisms including the alteration of cell membrane structure and functions (McDonnell et al., 1999; Pal et al., 2007 Sondi et al., 2004) inhibits the expression of proteins associated with ATP production (Yamanaka, et al., 2005).

It is obvious that Ag NPs have several antimicrobial functions to control various phytopathogens (Jo et al., 2009; Min et al., 2009; Kim et al., 2009). But some disadvantages of using AgNPs such as adverse effects on the host plants have also been reported. Studies on the seed germination and root growth of zucchini plants in hydroponic solution amended with AgNPs showed no negative effects whereas a decrease in plant biomass and transpiration was observed in prolonging their growth in presence of AgNPs (Stampoulis et al., 2009). The cytotoxic and genotoxic impacts of AgNPs were studied using root tips of onion and observed that AgNPs impaired the stages of cell division and caused cell disintegration (Kumari et al., 2009). However, it needs more cytotoxic and genotoxic evaluations by considering the properties of nanoparticles, their uptake, translocation, and distribution in different plant tissues.

Copper nanoparticles (CuNPs)

Nano-copper was found highly effective in controlling bacterial diseases namely, bacterial blight of rice (*Xanthomonas oryzae* pv. *oryzae*) and leaf spot of mung (*X. Campestris* pv. *phaseoli*). Nano-Cu application was found to be more effective against *Phytophthora infestans* compared to currently available non-nano Cu formulations in tomatoes (Giannousi, K. et al., 2013). But precautions must be adopted while using the CuNPs. Because, Environment Protection Agency (EPA) itself has recently recognized that, “Nano copper is more acutely toxic than micro copper.”

Studies on the effects of CuNPs on the growth of zucchini plants showed a reduced length of emerging roots (Stampoulis et al., 2009). However, the germination of lettuce seeds in the presence of CuNPs showed an increase in the shoot to root ratio compared to control plants (Shah and Belozerova, 2009). Different flora and fauna respond differently to nanomaterials and hence, it is necessary to evaluate the safe and effective concentration of each group of nanoparticles before their application that reduce the risks of ecotoxicity to a great extent.

ZnO, SiO_2, TiO_2, and MO nanoparticles

ZnO nanoparticles have recently been shown to provide effective growth control of *Fusarium graminearum*, *Penicillium expansum*, *Alternaria alternata*, *F. oxysporum*, *Rhizopus stolonifer*, *Mucor plumbeus,* and *A. flavus* as well as pathogenic bacteria *Pseudomonas aeruginosa* (Dwivedi, S. et al., 2016; Servin, A. et al., 2015; Vanathi, P. et al., 2016). Besides, Si and TiO2 have been found to promising to suppress crop diseases directly, through antimicrobial activity. Titanium dioxide nanoparticles in fertilizers have produced protection from bacteria and the inactivation of viruses (Sadeghi et al., 2017). MO nanoparticles inhibit the development of fungal conidia and conidiophores which cause the ultimate death of fungal hyphae.

Chitosan

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 (Cota-Arriola, O. et al., 2013). 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 (Kochkina, Z. et al., 1994; Pospieszny, H. et al., 1991; Chirkov, S. 2002). Chitosan nanoparticles have shown antimicrobial properties, such as controlling *Fusarium* crown root rot in tomatoes, *Botrytis* bunch rot in grapes, and *Pyricularia grisea* in rice (Kashyap, P.L. et al., 2015), but are less effective against bacteria (Malerba, M. And Cerana, R. 2016). Malerba and Cerana 2016, 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.

Nanoparticles as Carriers for Fungicides

Similar to insecticides, nanoparticles were exploited to improve low-water-solubility issues, decrease volatilization, and improve stability while providing a slow sustained release. Pyraclostrobin, another low-water-soluble fungicide, was loaded onto chitosan–lactide copolymer nanoparticles at different concentrations (Xu, L. et al., 2014). Three- and five days post-application, they found that the nanofungicide was either similar to, or less efficient at preventing inhibition of *Colletotrichum gossypii* when compared to commercial pyraclostrobin. However, an increase in inhibition was observed at 7 days post-treatment, compared to active alone (Xu, L. et al., 2014). In another trial, kaempferol (another low-soluble fungicide) loaded onto lecithin/chitosan showed 67% inhibition efficacy after 60 days of storage on a *Fusarium oxysporum* infected Petri dish (Ilk, S. et al., 2017).

Essential oils with fungicidal properties evaporate too quickly for large-scale commercial use. Janatova et al. 2015 successfully encapsulated five individual essential oil components into mesoporous silica nanoparticles (MSN) to show higher antifungal activity 14 days post-Aspergillus *niger* infection, when compared to the bulk essential oil components.

Leaching, the movement of water and chemicals through the soil, is a major pesticide issue, but not many studies have been conducted on the soil. Wanyika H. 2013, loaded the fungicide metalaxyl onto MSNs and observed leaching in soil between free metalaxyl (76% release) and encapsulated metalaxyl (11.5%) within a period of 30 days. When tested in water, the encapsulated metalaxyl had an increased release rate of 47% compared to the 11.5% seen in the soil, highlighting the importance of testing within the farming environment. Campos et al., 2015, used two different types of nanoparticles, solid lipid or polymeric, and tested the cytotoxicity of carbendazim and/or tebuconazole loaded onto the nanoparticles. A decrease in toxicity with the nanoparticle-loaded pesticides was observed in preosteoblast and fibroblast mouse cell lines. In the soil leaching experiments, the addition of nanoparticles decreased the release rate in soil layer release experiments when compared to the commercial formulation.

Slow-release of the active molecules was achieved with nanosized calcium carbonate carrying validamycin (Qian, K. et al., 2011). Validamycin encapsulated in nanoparticles showed lower efficiency than validamycin alone, over a period of 1–7 days, in an in vitro assay against *Rhizoctonia solani*. However, two weeks later, the nanoparticle formulation showed slightly better results than the act alone. Kumar et al. 2017, found an increase in the fungal inhibition rate when carbendazim-loaded polymeric nanoparticles were tested against *Fusarium oxysporum* and *Aspergillus parasiticus*, compared to carbendazim alone. Phytotoxicity studies confirmed that the nano formulated carbendazim was safer for germination and root growth of *Cucumis sativa*, *Zea mays*, and *Lycopersicum esculentum* seeds.

Conclusion

Nanotechnology has got tremendous implications for the rapid detection of plant pathogens, biosensor related to the control of pests and diseases, soil management, etc. It is clear from the above discussion that nano pesticides represent an attractive advancement, owing to their potential advantages for the environment and human health. However, nanotechnology in the agricultural sector is not reaching the market. Most of the developed nanoparticle-based pesticides are at the very early stage of development, hence, the efficacy and toxicity of the nano pesticides on soil and the environment need to be studied in more detail. Another issue with the early stages of research on nanoparticles in plant protection is the current lack of long-term trials.

The potential of nanomaterials encourages a new green revolution with reduced farming risks. However, there are still huge gaps in our knowledge of the uptake capacity, permissible limit, and the ecotoxicity of different nanomaterials (He, X. et al., 2018; Li, M.et al., 2016). Therefore, further research is urgently needed to unravel the behavior and fate of altered agriculture inputs and their interaction with biomacromolecules present in living systems and environments.

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