

Nanoparticles as Alternative Pesticides: Concept, Manufacturing and Activities

Ahmed I. S. Ahmed¹ and Youn Su Lee^{2*}

¹Plant Pathology Unit, Plant Protection Department, Desert Research Center, Cairo 11753, Egypt

²Division of Bioresource Sciences, Kangwon National University, Chuncheon 24341, Korea

ABSTRACT : Nanotechnology, which has become an important area of science, has caused an enormous developmental revolution in many fields. In the last two decades researchers have focused on overcoming the obstacles encountered during the preparation of nanoparticles. This article highlights the nanotechnology, along with a brief description of the manufacturing, concepts and activities of nanoparticles as alternative pesticides against plant pathogens, some methods for evaluation of nanoparticles against phytopathogens *in vitro* and *in vivo*, and explains the importance of some common nanoparticle types used in agricultural applications and plant pathology.

KEYWORDS : Nanoparticles, Pesticides, Plant pathogens

Introduction

Nanotechnology has been defined as the manipulation of matter with at least one diameter sized from 1 to 100 nanometers and manufacture of devices on the scale of atoms or small groups of atoms. This definition resulted from a particular technological aim to a broad category of all research types and techniques that accord with the special characteristics of matter that occur as a threshold of given size. Nanotechnology clarifies the importance of quantum mechanical effects at an atomic and molecular scale [1, 2].

Manufactured nanostructures are the basis of modern nanotechnology where research activities are growing rapidly and attract essential funding either from public or private sectors [3].

The source of nanoparticles can be both natural and

anthropogenic (manmade). Natural nanostructures have existed in nature for millions of years and are generated by natural processes due to weathering, volcanic eruption, erosion, biological effects, and hydrolysis. Origins which have resulted in an increase in formation of anthropogenic nanomaterials [4] through the various actions that participated in the increase of nanostructures in the environment include coal fired combustion, transportation, welding operations followed by some recent processes where engineered nanoparticles are prepared and produced slowly [5].

The nanomaterials are physicochemically different and superior to the atomic and bulk materials of the same element [6]. For example, copper, which is impermeable at macroscale becomes completely opaque and transparent to visible light at the nano level [7, 8]. Metal nanostructures have also attracted interest due to their effective properties and applications in different fields such as plant pathology [9].

Many efforts have been focused on the impact of using nanoparticles in agriculture production, particularly in control of plant pathogens.

The main objective of this review is to clearly, quantitatively, and comprehensively describe the manufacturing, concept, and activities of nanoparticles as alternative pesticides against phytopathogens.

The concept of manufacturing and use of nanoparticles

Nanotechnology is dependent on the manipulation of

Kor. J. Mycol. 2015 December, 43(4): 207-215
<http://dx.doi.org/10.4489/KJM.2015.43.4.207>
pISSN 0253-651X • eISSN 2383-5249
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*Corresponding author

E-mail: younslee@kangwon.ac.kr

Received December 7, 2015

Revised December 8, 2015

Accepted December 8, 2015

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functional systems at the molecular scale. This includes the concepts of advanced works in the application of nanotech fields. In the natural environment, the particles concentration has increased gradually since tremendous amounts of products are being marketed and commercialized worldwide then released directly or indirectly again into the environment [10, 11]. As basics, nanotechnology refers to the techniques and tools used in construction of items from the bottom up using high performance products [12, 13].

Nanoparticles can be manufactured in two ways acting as bottom-up and top-down techniques (Fig. 1). Top-down technique involves breaking down the bulk material into nano structures while the bottom-up refers to creation of a material atom by atom and molecule by molecule, so that the nanoparticles have been manufactured using various chemical or physical methods depending on the size. Significant efforts have been focused on accurate control of the particles' size distribution; chemical pathways carried out in solution have also been very successful in obtaining nanoparticles with narrow size distributions [14-16]. Metal nanoparticles could be prepared by surfactant molecules in micro reactors formed like micelles or in polymer solutions as well as other reagents for coordinating that effect on the surface of nanoparticles and preventing its growth [17]. There are two main reasons for the qualitative differences in material properties of nano structures. First, a defining advantage at the nanoscale is the very great surface to volume rate of these nanostructures. This indicates that no atom is very far from a surface or interface and the action of atoms at these higher energy sites has a significant influence on the properties of the material. Second, mechanical and quantum active come into play at very small dimensions thus to new physics and chemistry. Therefore, the size and size distribution as well as the number and type of manufactured nanoparticles are most important in the properties and effects of nanostructures. Surface

properties with very high area can enhance surface phenomena to volume ratio, so that the atoms are on or near the surface and more reactive, and the quantum effects due the small dimensions of electrons leading to delocalized on the surface of the nanoparticles (NPs) as discontinuity behavior [18-22]. As a result of the tremendous progress in nanotechnology and its techniques for preparation of nanostructures, nanotechnology has become important in all areas of application, with promise to benefit society and considerably improve technology in environmental and industrial sectors (Fig. 2).

Nanoparticle types and their classification

NPs can be classified into two main groups, on the basis of their origins, natural and artificial. The artificial group can be further subdivided into manufactured and accidental. NPs can also be divided according to their chemical structures into organic and inorganic [23].

Natural NPs are distributed throughout the atmosphere and found in many environments, and microorganisms could be prepared the natural nanoparticle in two ways, either directly to make the metabolic requirements available [24, 25] or indirectly from microbial activity results [26, 27]. NPs have been released due to human activities as a nano particulate matter for millennia as products of activities including agriculture, construction, mineral processing, and mining activities [28, 29]. The most important examples of natural and organic nanoparticles are humic, fulvic acids, viruses, fullerenes, organic acids, and nanoglobules, while examples of inorganic NPs include magnetite, Ag, Au, Fe-Oxides, allophane, and sea salt. Engineered NPs have been classified into C-containing and inorganic groups based on their core materials. C-containing NPs are classified as a combustion by-products and an engineered soot form. The inorganic NPs can be divided into by-products nanoparticles which composed of polymeric NP and combustion by-products and engi-

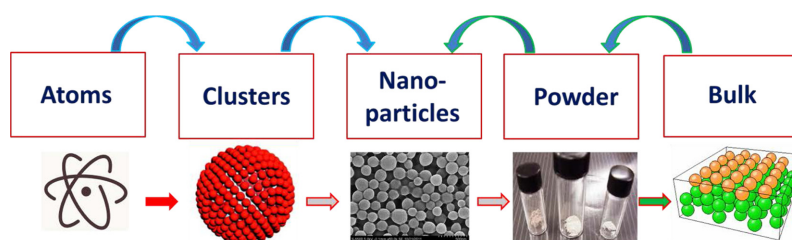


Fig. 1. Illustration of bottom-up and top-down techniques of nanoparticle preparation.

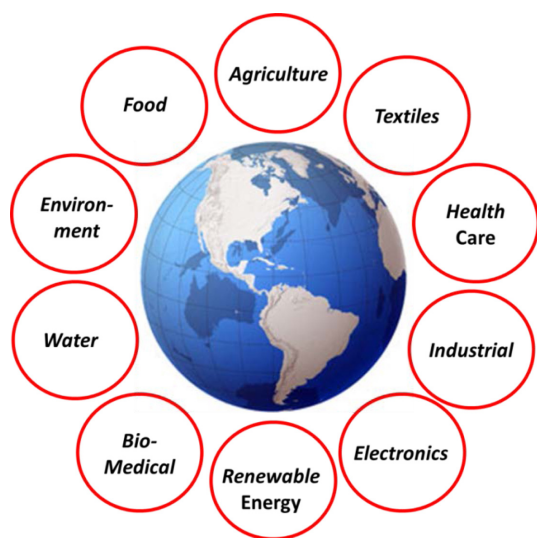


Fig. 2. Nanoparticle applications.

neered nanoparticles which composed of oxides, metals, salts and aluminosilicates.

The most important examples of engineered and C-containing NPs are nanoglobules, carbon black, and functionalized carbon nanotubes, and examples of engineered and inorganic NPs are polyethyleneglycol (PEG) NPs, platinum group metals, TiO_2 , SiO_2 , Ag, Fe, and metal-phosphates [23].

Engineering and manufacturing of metal oxides NPs

Different types of nanomaterial have been produced for different applications and commercial activities. Metal oxides are important inorganic types due to their physical and chemical properties and have demonstrated some size dependent desired characteristics which makes the nanostructures different from atomic scale and bulk materials of the metal [30, 31]. Nano structures of metals have an important role in many fields including catalysis, sensors, biomedical diagnostics, environmental remediation, and electronic materials [32-35]. Many metal nanoparticles also have antifungal and antibacterial effects against some plant pathogens [14, 36-38]. Nanoparticles have been fabricated using chemical synthesis routes under specific conditions. Therefore, some manufacturing methods for metal nanoparticles will be discussed as follows:

Nanoparticles from many systems have been prepared; important techniques for synthesis of nanoparticles include pyrolysis, attrition, and hydrothermal methods. In pyrolysis, the resulting solid is air classified for recovery of oxide particles from gases. Traditional pyrolysis results in

aggregates and agglomerates as single primary particles while the ultrasonic spray pyrolysis helps in avoiding formation of agglomeration [39]. In attrition, the nanostructures were grounded using a reducing mechanism like a ball mill or a planetary ball mill, and the resulting particles were air classified for recovery of nanoparticles. All thermal plasma temperatures were in the order of 10,000 K, so that the solid powder can be easily evaporated. Then thermal plasma nanostructures were formed due to cooling conditions while exiting the plasma region [31]. An example of application of thermal plasma in nanoparticle preparation is silica sand where it could be vaporized at atmospheric pressure. The resulting mixture could be rapidly cooled by quenching with oxygen thus ensuring the quality of the silica produced [33, 40].

Condensation of inert-gas is also frequently used in preparation of metal nanoparticles with low melting points by vaporizing the metal in a vacuum chamber under supercooling conditions and an inert gas stream. The super cooled metal vapor will be condensed into nanometer size particles and could be entrained in the inert gas stream then deposited and studied.

Nano structures could also be prepared by radiation chemistry techniques where radiolysis from gamma rays could create strongly active free radicals in solution. This technique is simple and economical because it uses a minimum number of chemicals. In this process, a scavenger chemical will preferentially interact with oxidizing radicals to prevent the re-oxidation of the metal and reducing radicals will drop metallic ions down to the zero-valence state; metal atoms also begin to coalesce into particles in zero-valence state, the particle surrounded by chemical surfactant and regulates its growth during the formation process; the interference with growing particle and nucleating to prevent agglomeration and to control size is important so that the surfactant molecules remain attached to the particle in plentiful concentrations therefore preventing formation of clusters [41, 42].

Most common NPs types in agricultural applications

The followings highlight on some of the most common types of NPs including iron oxide, titanium oxide, zinc oxide, sulfur, chitosan, copper, magnesium oxide and silver oxides.

Iron oxide

Different chemical techniques and methods have been used in manufacture of ultra-fine nanostructures of Fe_2O_3 ,

such as hydrothermal reaction techniques and chemical co-precipitation. The most common forms of iron oxide NPs are maghemite, β -Fe₂O₃ and magnetite, Fe₃O₄ which have a high potential for several applications. These types of iron oxides could be prepared by all known chemical techniques and methods and can be employed in many fields [43-45].

Titanium oxide

These nano structures of Ti can be synthesized by different techniques, including co-precipitation, sol-gel synthesis process, chemical vapor deposition, reverse micelle synthesis, microemulsion synthesis process, and hydrothermal reaction method [46-50]. Four crystal forms of Ti dioxide are natural structures called anatase brookite and TiO₂ [51], with desired properties including high refractive indicator, light absorption/spread and its chemical stabilization, and relatively low cost preparation of titanium dioxide.

Zinc oxide

Zinc oxides NPs have received the most attention in recent years due to their properties which are applicable in many fields including antibacterial agents and biomedical labels [52, 53]. Several manufacturing techniques are used in preparation of ZnO NPs, including thermal decomposition, laser ablation, chemical vapour deposition, sol-gel method, spray pyrolysis, molecular beam epitaxy, and hydrothermal synthesis [54, 55]. ZnO nanostructures can be synthesized on a large scale at low costs using simple chemical components as solutions in simple techniques such as chemical precipitation, sol-gel synthesis, and solvo-hydrothermal reaction [53]. Sol-gel synthesis and solvothermal/hydrothermal reaction have several influences over other processes, including use of basic equipment, economical cost, friendly environment, and lower risk. This technique has also been successfully employed in preparation of luminescent materials and ZnO in nanoscale, and the properties of particles produced from this technique can be controlled via a hydrothermal process by adjusting the temperature of reaction, concentration, and time of precursors [53].

Many studies have reported on the effects of ZnO NPs against plant pathogenic fungi e.g., *Botrytes cinerea*, *Penicillium expansum* [56]; *Alternaria alternata*, *Fusarium oxysporum*; *Rhizopus stolonifer*; *Mucor plumbeus* [57]; *Fusarium oxysporum*; *Penicillium expansum* [58].

Also the scope of ZnO nanoparticles has been interest

for biologist due to their distinguished antimicrobial activity which has opened new trends to biological applications, particularly in its nanoscale form has a toxicity against wide range of microorganisms such as bacteria, fungi, algae and plants [59, 60]

Sulfur nanoparticles

Sulfur nanoparticles were prepared by different methods, including chemical precipitation, electrochemical method, composing of oil, micro emulsion technique, surfactant, co-surfactant, aqueous phases with the specific compositions and ultrasonic treatment of sulfur-cystine solution. This type of NPs has many practical applications in agriculture, particularly as antibacterial, fertilizers where sulfur can be used as fungicide against many plant diseases such as apple scab disease in cold conditions. Sulfur nanoparticles are also used in grape, strawberry, vegetables, and many other crops, and it is considered a high efficiency pesticide used in agriculture where it has a good effect against a wide range of plant diseases [61].

Chitosan nanoparticles

Chitin and chitosan are naturally occurring compounds which can be obtained through alkaline deacetylation of chitin, consisting of a β -(1,4)-linked-D-glucosamine residue with the amine groups randomly acetylated [62]. Chitosan has many properties and applications in different areas. Chitosan is safe and has no toxicity and can interact with poly anions to form complexes and gels [63]. Chitin and chitosan are known to have eliciting activities leading to enhanced defense in host plants as a response to microbial attack by the accumulation of pathogen related (PR) proteins, proteinase inhibitors, and phytoalexins, and thus have a distinguished role in control of fungal plant diseases.

Copper nanoparticles

Copper is one of the most widely used materials worldwide. Copper nanoparticles have an important role in control of several bacterial and fungal phytopathogens. Different techniques have been used for preparation of copper nanoparticles with controlled shape and size, including metal vapour deposition, radiolytic reduction, electrochemical reduction, mechanical attrition, thermal decomposition, and chemical reduction. Among these methods the solution method is the simplest and most versatile for nanoparticle preparation [64, 65], therefore copper nanoparticles were synthesized by chemical reduction

of Cu^{2+} in the presence of cetyl trimethyl ammonium bromide and isopropyl alcohol. Many publications have reported on the antifungal effect of copper nanoparticles against plant pathogenic fungi like *Alternaria alternata*; *Phoma destructiva*, *Fusarium oxysporum*, *Curvularia lunata* [66].

Magnesium oxide nanoparticles

Magnesium plays a crucial role in employment of important biological polyphosphates such as ATP, RNA, and DNA [67]. It is an alkaline earth metal with its ionic form Mg^{2+} important to all living organism. Magnesium oxide is an important type of nanoparticles and has many applications. Nanoparticles of MgO have unique properties, and are used extensively in catalysis, toxic waste remediation, and refractory materials industries [68-70]. These are also used as agents to induce systemic resistance against various plant pathogens [71]. Wani and Shah [57] mentioned the antifungal effect of magnesium oxide nanoparticles on some pathogens like *Alternaria alternate*, *Fusarium oxysporum*, *Rhizopus stolonifer*, and *Mucor plumbeus*, and reported the highest effect using the 30 and 50 nm nanoparticle size.

Silver nanoparticles

Among the different types of metal, silver nanoparticles

have been reported most in the literature due to their effects and rapidly growing applications in different production and environmental areas [72]. Various shapes and sizes can be designed through a variety of different synthesis approaches using various capping agents depending on aims and applications. In addition, Ag NPs are safe type of nanoparticle due to its residue could be changed to natural status in low pH or acidic environmental conditions [73]. Also Ag NPs are safe because there is no interaction between silver and living organisms during the preparation of nanoparticles [74].

Silver NPs have been used against different fungal plant pathogens and their suppressive effects on growth and structures of fungi have been reported in *Alternaria alternata*, *Rhizoctonia solani*, *Botrytis cinerea*, *Curvularia lunata*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina* [35], *Cladosporium cladosporioides* [75]; *Alternaria alternate*; *Alternaria brassicicola*; *Alternaria solani*; *Botrytis cinerea*; *Cladosporium cucumerinum*; *Corynespora cassicola*; *Cylindrocarpum destructans*; *Didymella bryoniae*; *Fusarium oxysporum* f. sp. *cucumerinum*; *F. oxysporum* f. sp. *lycopersici*; *F. oxysporum*; *Fusarium solani*; *Fusarium* sp; *Glomerella cingulate*; *Monosporascus cannonballus*; *Pythium aphanidermatum*; *Pythium spinosum*; *Stemphylium lycopersici* [37]. *Golovinomyces cichoracearum*, *Sphaerotheca fusca* [38]; *Colletotrichum* spp. also; *Sclerotium cepivorum* [76];



Fig. 3. Illustration of some fungal pathogens that are suppressed by silver, with influential sizes of nanoparticles (NPs).

Sclerotinia sclerotiorum; *Rhizoctonia solani*, *Sclerotinia minor* [38, 77]. *Pythium altimum*, *Manginomyces grisei*, *Colletotrichum gloeosporioides*, *Botrytis cinerea*; *Fusarium culmorum* [78]; *Fusarium culmorum* [79]; and *Colletotrichum gloeosporioides* [80] (Fig. 3).

Kim et al. [81] studied the antimicrobial activity of silver nanoparticles against *Acidovorax citrulli* and they found that the growth of five strains of *A. citrulli* was checked by 99% with the combination of Ag/Glucose 1,000 ppm. Also, Paulkumar et al. [82] confirmed that the silver in nano scale has shown excellent antimicrobial activity against plant pathogens *Citrobacter freundii* and *Erwinia cacticida*, therefore, they concluded that the silver nanoparticles will have a beneficial application in crop improvement and protection in agricultural nanoscience.

Some methods for evaluation of nanoparticles against fungal phytopathogens

Basic steps for examination of nanoparticles as control agents against fungal plant pathogens [37]:

- 1) Prepare spore suspension in different concentrations of nanoparticles to evaluate their effect on the spore germination of fungi:
- 2) Prepare the fungal inoculates on potato dextrose agar (PDA) media (a common microbial media for culturing fungus) at 28°C in petri plates.
- 3) Prepare spore suspension of each isolate of fungi containing at least 20~30 spores per microscopic field from 10 day old fungal culture.
- 4) Place one drop, approximately 0.1 mL of spore suspension in a cavity glass slide containing a drop (approximately 0.1 mL) of different concentrations of nanoparticles.
- 5) Keep these slides in a moist chamber by placing two folds of filter paper on both sides of petri-plates.
- 6) Incubate these petri plates at $24 \pm 2^\circ\text{C}$ for 24 hr. Replicate each treatment four or five times to confirm the obtained results. Record the percent spore germination using the following formula:

$$\text{Percent spore germination} = \frac{\text{No. of spores germinated}}{\text{Total no. of spores examined}} \times 100$$

Assay for sclerotium forming phytopathogenic fungi

- 1) Refresh the sclerotia by incubating the fungal plates at room temperature without treatment.
- 2) Wash the sclerotia (2~4 weeks old) with sterilized

distilled water and rinse with 70% alcohol. The sclerotia must be 2~4 weeks old.

3) For testing the sclerotial germination growth, one sclerotium is placed in the center of petri plates containing malt extract agar (MEA) medium supplemented with either concentrations of the nanoparticles or an equal volume of water.

4) Incubate the plates at 24°C, and use for measurement of sclerotial germination growth.

5) Determine the sclerotial germination rate by measuring the diameter of mycelial colonies.

Bio-assay method for evaluation of nanoparticle effects on foliar diseases in greenhouse:

- 1) Artificial infection of host plant with pathogen.
- 2) Use nanoparticle at different concentrations.
- 3) Use the aerial spray method for application of nanoparticles around the shoot portion of the whole plants 3~4 weeks before the outbreak of the disease and after disease occurrence.
- 4) Distilled water was used as a control.
- 5) Calculate the disease index by counting the number of infected leaves out of 100~150 leaves among the treated plants.

Acknowledgements

This article was supported by Golden Seed Project (Project No. 213002043SBS10), Ministry of Agriculture, Food and Rural Affairs (MAFRA), Ministry of Ocean and Fisheries (MOF), Rural Development Administration (RDA), and Korea Forest Service (KFS). This article was also supported by the University-Industry Cooperation Foundation of Kangwon National University & Science and Technology Development Fund (STDF), Egypt.

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