



Nano-Bioformulation: A Spanking New Weapon for Plant Disease Management

Pranab Dutta, Alinaj Yasin*, Arti Kumari, Madhusmita Mahanta and Anwesha Sharma

School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University (Imphal), Umiam, Meghalaya (793 103), India



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Corresponding Author

Alinaj Yasin

✉: alinajyasin2020@gmail.com

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Abstract

Pests and diseases cause 20-40% of crops to be wiped out annually. Currently existing plant disease management strategies solely employ harmful pesticides, which are harmful for the environment and for people. When it comes to decreasing toxicity, extending the shelf life and making poorly water-soluble pesticides more soluble, nanotechnology is a blessing that may have a favorable effect on the environment. The fundamental unit of nanotechnology, nanoparticles, can be used in phytopathology to manage plant diseases in a variety of ways. They can be used as RNA-interference molecules, pesticide nanocarriers, or protectants. Furthermore, beyond their role as carriers for genetic material, probes and agrichemicals, nanoparticles hold potential as tailored biosensors, serving as diagnostic instruments. These days, biological organisms are a novel source for nanoparticle manufacturing. The nano-bioformulations are made up of the biological systems used to synthesize nanoparticles. Because of their exceptional efficiency and affordability, the adoption of plant extracts or microbial enzymes for the biosynthesis of nano-formulations is rapidly gaining momentum within the realm of nano-bioformulations. Owing to their tiny size (1-100 nm), an environmental risk assessment is necessary, especially when it comes to ingestion as food or feed. Agricultural applications have seen the commercialization of exiguous nanoparticle-based solutions, despite the numerous potential benefits linked to their use. Therefore, this demands nanotechnology be applied in farmer's fields to fill the voids in scientific research.

Keywords: Bioformulations, Nano-bioformulations, Nanoparticles, Nanotechnology, Phytopathology

Introduction

Fresh projections indicate that the world will need to double its food production by 2050 due to the growing global population. This alarming forecast takes on even greater urgency as climate change is expected to disrupt food production in various agriculturally sensitive regions. This disruption could occur through prolonged droughts and higher average daily temperatures, exacerbating the challenge of meeting future food demand (Elmer and White, 2018). Furthermore, pests and pathogens are responsible for substantial reductions in crop yields, resulting in estimated annual global losses ranging from 20% to 40% (Worrall *et al.*, 2018). The recent pest management

approaches predominantly rely on the use of pesticides, notably herbicides, insecticides and fungicides. While these chemicals offer benefits such as widespread accessibility, quick action and dependable results, they also inflict harmful consequences on non-target organisms. These consequences may encompass the revival of pest populations and over time, the emergence of resistance to these chemical agents (Worrall *et al.*, 2018). Besides, some estimations reports that 5-6% are utilised by pest after application and as remainder of 90-95% are lost either during or after their application. As a result, there is a pressing need to encourage the development of cost-effective, highly effective alternatives to traditional pesticides that are also environmentally friendly (Figure 1). Consequently, the challenges confronting plant

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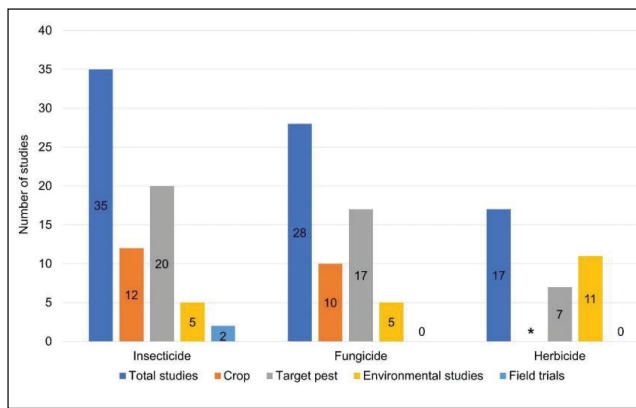


Figure 1: An overview of approaches on studies made on the utilization of the insecticides, fungicides and herbicides (including biocides) loaded onto nanoparticles. *Experiments encompassing crop host were not included due to non-selective nature of some herbicides (Courtesy: Worrall et al., 2018)

pathologists and other agricultural experts are formidable. Nevertheless, nanotechnology emerges as an innovative and potent tool in our efforts to address the increasing challenges of disease management while simultaneously enhancing plant health (Dasgupta and Bachaspati, 2022; Figure 2). This has spurred the development of novel concepts and agricultural products with enormous potential to address and mitigate the aforementioned challenges (Worrall et al., 2018). While nanotechnology has made significant strides in the fields of medicine and pharmacology (Worrall et al., 2018), it has garnered relatively less attention in the context of agricultural applications. The application of nanotechnology in crop protection, genetic modification and diagnostics is still in its early stages and has only recently started to gain attention within the field of plant pathology (Elmer and White, 2018). In agriculture, this utilization encompasses various aspects of nanotechnology, including plant product delivery, water resource management, seed germination, nano-barcoding, gene transfer, nanosensors and controlled release of agricultural chemicals (Figure 3).

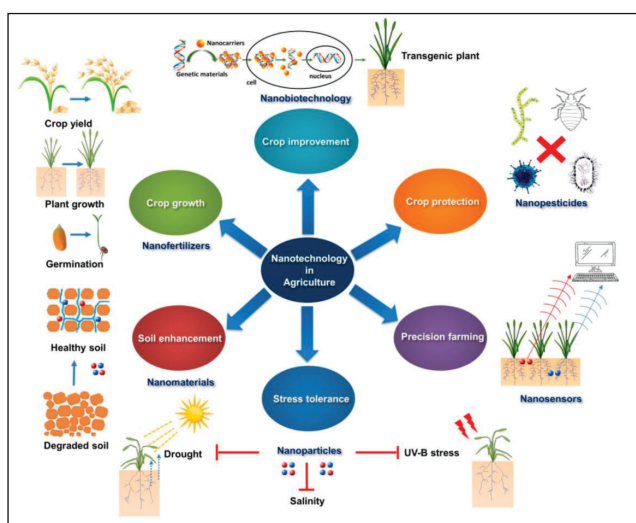


Figure 2: An overview of nanotechnology applications in agriculture (Courtesy: Shang et al., 2019)

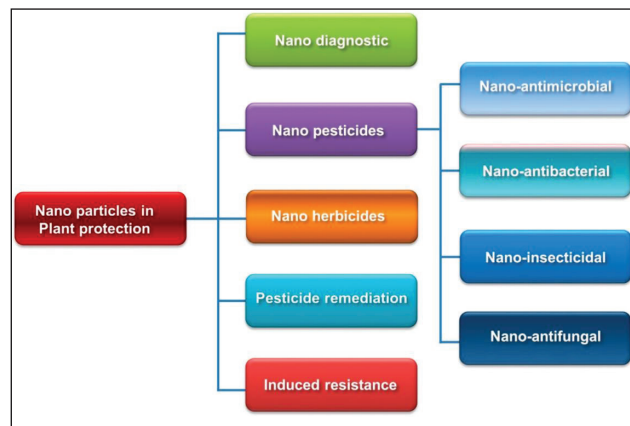


Figure 3: Overview of application of nanoparticles in plant protection (Courtesy: Worrall et al., 2018)

Evolution Timeline of Nanotechnology

One of the engrossing records cites the examples of utilising nanotechnology in the ancient world with the use of nanoparticles and structures by the personage in fourth century AD (Bayda et al., 2020), by the Roman (Table 1).

Table 1: Evolution timeline of nanotechnology

Year	Events
2000 years ago	The Greeks and Romans utilized sulphide nanocrystals for the purpose of dyeing their hair.
1000 years ago	Gold nanoparticles of varying sizes were employed to create a spectrum of colors in stained glass windows.
1959	“There’s plenty of room at the bottom” by R. Feynman (Father of Nanotechnology).
1974	“Nanotechnology” term was used by Taniguchi, M. A. Ratner and A. Aviram (Molecular Electronics).
1981	Invention of STM by G. Benning and H. Rohrer, E. Drexler (Molecular Engineering).
1985	“Buckyball”- R. Smalley, R. Curl and H. Kroto discovered C ₆₀ .
1986	“Engines of Creation”- First book on nanotechnology by K. Eric Drexler.
1991	Sumio Ijima discovered multi-wall Carbon nanotubes.
1992	Sumio Ijima and Donald Betune discovered single walled C nanotube.
2000	President Bill Clinton announces US National Nanotechnology Initiatives.
2002	Cees Dekker (Carbon nanotubes functionalized with DNA).
2004	A. Geim and K. Novoselov discovered graphene (Discovery of fluorescent C dots).

What does Nanotechnology Means?

Nanotechnology is among the freshly functional budding technologies of the 21st century. Nanotechnology as defined by the National Nanotechnology Initiative (NNI) in the United States is “a science, engineering and technology conducted at the nanoscale (1-100 nm), where unique phenomena enable novel applications in a wide range of fields, from chemistry, physics and biology, to medicine, engineering and electronics”. This definition implies the existence of two distinct aspects of nanotechnology. The initial concern is the scale, as nanotechnology involves manipulating structures by controlling their dimensions on the nanometer scale. The other concern is with the novelty: nanotechnology deals with the merits of some properties perhaps because of its nanosize. Nanotechnology explores the ability of the nano science theory by converting it into desired applications through the observation, measurement, manipulation, assembly, control and manufacturing of materials at the nanometer scale (Worrall *et al.*, 2018). The potential benefits and opportunities offered by nanotechnology are limitless. These include enhancing agricultural productivity through the use of nanoporous zeolites for controlled and precise release of fertilizers and water (Kumari *et al.*, 2020). These also include the utilisation of nanocapsule as in case of smart delivery system besides detection of pest, pathogen and vector as nanosensors for pest and disease management.

Nanoparticles

The building blocks of nanotechnology are the nanoparticles. The preliminary step comprises the arrangement of atoms and thus manipulation of nanoparticles which influences their dimensions and orientation, ensuring effective interactions with the targeted tissues. The International Organization for Standardization (ISO), acknowledged as the world’s leading developer of standards, has defined nanoparticles as materials with external dimensions in the nanoscale or having internal or surface structures within the nanoscale range, where the length typically falls between approximately 1-100 nm, signifying the nanoscale (Kumari *et al.*, 2020; Pandey *et al.*, 2018). According to earlier literature the word “Nano” has its roots originally from the Greek word “nanos” meaning “dwarf”. It explains about the matter being extremely small, it translates to one-Billionth (10^{-9}) when quantified.

Two different approaches have explained two different possibilities of synthesis of nanostructures, captivating the interest of numerous scientists in this new emerging field. These manufacturing approaches comprise differences in degree of quality, speed, precision and cost. The top-down approach basically involves in breaking down bulk materials to obtain nano-sized particles (Bayda *et al.*, 2020). The utilization of recently developed and advanced techniques, such as precision engineering and lithography, has established the top-down approach as a successful one. Precision engineering plays a crucial role in the entire production process of the microelectronics industry and its high performance demands for the improvement in skills

and techniques (Worrall *et al.*, 2018). The utilization of highly sophisticated nanostructures crafted from advanced materials like diamond or cubic boron nitride, along with sensors for precise size control, is complemented by the integration of numerical control and advanced servo-drive technologies (Bayda *et al.*, 2020). Lithography involves the creation of patterns on a surface by exposing it to light energy, ions or electrons, followed by the deposition of material onto that surface to create the needed structure (Bayda *et al.*, 2020). On the other hand, the bottom-up approach entails constructing nanostructures from the ground up, either atom by atom or molecule by molecule, employing physical and chemical techniques (Bayda *et al.*, 2020). These processes operate on a nanoscale range, usually falling within the range of 1-100 nanometers (Bayda *et al.*, 2020), achieved through controlled manipulation of the self-assembly of atoms and molecules. One commonly employed method is chemical synthesis, which entails the creation of raw materials. These materials can be harnessed either in their bulk and disordered form for direct use in products or as the fundamental building blocks for the creation of more sophisticated, ordered materials (Table 2). Self-assembly is a component of the bottom-up approach, where atoms or molecules autonomously arrange themselves into structured nanostructures due to chemical-physical interactions among them (Schubert *et al.*, 2011). The only technique in involves the gradual positioning freely of single atoms, molecules or cluster is positional assembly method.

The effectiveness and precision of these techniques have empowered scientists to engineer nanoparticles with specific characteristics, including morphology, pore size and surface properties (Mohamed *et al.*, 2021). Engineered nanoparticles can be employed as protectants or facilitate precise and targeted delivery through methods such as adsorption, encapsulation, and/or conjugation of active substances such as pesticides (Mohamed *et al.*, 2021). Recent advancements in agricultural nanotechnology hold the promise of ushering in a new era of pesticides and other active agents for enhanced plant protection (Worrall *et al.*, 2018). The future prediction states that their utilisation will greatly increase to benefit the farmer community within in the future ahead.

Bioformulation

Formulations that incorporate microbes are commonly referred to as “bioformulations.” In simpler terms, bioformulations are formulations made up of beneficial strains of microbes that are immobilized or captured on an inert carrier material. These formulations are designed to promote plant growth, combat plant pathogens and enhance soil fertility (Rani and Kumar, 2019). Bioformulations are more preferred and effective than synthetic chemicals credited to the benefits incurred by the formulations as there can be beneficial interactions by a one microbe with plant pathogens; besides these, bioformulations play a role in promoting plant growth and inhibiting diseases. Nevertheless, the harmful residual effects to the environment of the chemical are well known.

Table 2: Descriptions, applications and types of nanoparticles in plant pathology (Elmer and White, 2018)

Type	Definition	Potential use in plant pathology
Metalloids, metallic oxides, non-metals and their composites	Engineered metals at nanoscale in cubes, spheres, bars and sheets	Bactericides/ Fungicides; Nano-fertilizers; Delivery vehicle for antimicrobials and genetic material.
Carbon nanomaterials	Carbon allotropes with nanoscale design	Several applications.
Nanotubes with single or multiwalls	Single or more tubes made from folded graphene sheets	Antibacterial agents: carriers of genetic material and antibacterial agents.
Fullerenes (bucky balls)	60 carbon atoms arranged in a certain football configuration	Antimicrobial agents: carriers of genetic material and antimicrobials.
Graphene oxide sheet (reduced or oxide forms)	Graphene oxide sheet	Antibacterial agents: carriers of genetic material and antimicrobial agents.
Liposomes	A lipid covering a core of water	Delivery vehicle for genetic or antimicrobial products.
Dendrimers	Nanomaterial with tree-like appendages that radiate from a central core	Delivery vehicle for genetic or antimicrobial products.
Nano biosensor	A biological component combined with a nanoparticle for detection	As a research tool and for diagnostics.
Nano shell	Semiconductor-encased in a gold shell to form nanoparticles	Diagnostics, research tool.
Quantum dots	Crystalline, inorganic fluorescent semiconductor nanoparticles for biosensor applications	Diagnostics, research tool.

Nano-Bioformulation

The use of biological entities for the synthesis of nano-formulations is known as Nano-bioformulation. Microbes are widely utilized because of their natural capability to produce inorganic materials, either within their cells (intracellularly) or outside their cells (extracellularly) (Rani and Kumar, 2019). The nano-bioformulation should ideally exhibit all the desired properties, including the desired shape and size, minimal eco-toxicity and safe, straightforward mechanisms for transport, delivery and disposal (Das and Dutta, 2021). Moreover, the matters related to the bioformulations are often overcome with nano-bioformulations.

Morphological Representation of Various Nano-Formulations

Different types of nano formulations are commonly grouped based on their morphological features such as:

- **Nanoemulsions:** The particle size is below 200 nm. The term “Nanoemulsion” usually refers to a thermodynamically stable, clear dispersion of two immiscible liquids, such as oil and water, with stabilization provided by a surface-interacting film of molecules. Nanoemulsions typically contain about 5-10% surfactant, compared to 20% in microemulsions. However, a challenge arises when using nanoemulsions for delivering water-insoluble substances. Nevertheless, they have been developed to efficiently deliver naturally occurring compounds with insecticidal properties

that are poorly water-soluble or insoluble (Kumari et al., 2020; Worrall et al., 2018).

- **Nanogels:** Nanogels are generally cross-linked hydrophilic polymers which have the potential to soak up high volumes of water.
- **Nanospheres:** A morphologically spherical aggregate in which the active compound is evenly distributed within the polymeric matrix (Kumari et al., 2020).
- **Nanocapsules:** These aggregates consist of the active compound concentrated near the central core, surrounded by the matrix polymer (Kumari et al., 2020).
- **Metallic Nanoparticles:** Usually, a metal core comprises an inorganic metal or metal oxide, enveloped by a shell made of organic or inorganic material, or metal oxide. The dimension varies from 3 nm or smaller and usually possesses strong fluorescent properties.

Synthesis of Nano-Bioformulation

Being one of the fastest developing multidisciplinary field, nanotechnology encompasses wide and different field of science as demanded by its wide range of applications and gaining importance. This has resulted in the new findings in the research for procedures of different nanomaterials having different and unique properties. The nanomaterials synthesis techniques are classified as “top down” approach or “bottom up” approach (Iqbal et al., 2012). The former approach involves mechanical processes like grinding

milling, chemical etching, thermal or chemical electro-explosion, kinetic sputtering and thermal laser ablation that employ destructive approach. In the top-down approach, the process begins with a larger molecule that is broken down into smaller units, which are then transformed into appropriate nanomaterials. On the other hand, in the bottom-up approach, nanoparticles are derived from relatively simpler particles and it is therefore considered a building-up approach (Ding *et al.*, 2015).

To foster the development of nano-bioformulations, eco-friendly methods using a variety of single- and multicellular biological entities, including bacteria, actinomycetes, yeast, fungi and plants must be used. These biological entities can all be efficiently used to synthesize nano-bioformulations due to their varying degrees of biochemical processing capability (Boruah and Dutta, 2020). It's crucial to remember, nevertheless, that due to differences in intrinsic metabolic processes and enzyme activity, not all biological entities can be utilized for synthesis. As a result, it necessitates a suitable selection, which is required to create a precisely specified nano-bioformulation.

General steps for synthesis of nano-bioformulation comprise the following:

1. Preparation of plant or microbial extract for nanoparticle synthesis.
2. Mixing of metallic solution in plant or microbial extract for bio-reduction.
3. Bio-reduction of metal to metallic nanoparticles by the extract.
4. Formed nanoparticles analysed by UV- Vis spectroscopy.
5. Characterization of nanoparticles by SEM, TEM, XRD, FTIR, EDX.
6. Application in various paths.

Mode of Action

Nanoparticles exist naturally in the environment and are continually formed through processes such as mineralization, natural disasters and the geological recycling of materials (Boruah and Dutta, 2020). In recent times, nanoparticles and their applications have gained significant attention and has become an extensive subject of research in various fields. The application of nanomaterials across various industries undoubtedly increases the likelihood of releasing nanoparticles into the environment. Consequently, studies have been conducted to investigate the various ways in which nanoparticles interact with living organisms and their surroundings. Some extensively reported modes of action of nanoparticles include antimicrobial activity, cytotoxicity induced by reactive oxygen species (ROS), genotoxicity and promotion of plant growth, among others. It has been convincingly demonstrated that the actions of nanoparticles are influenced by factors such as their morphology, dosage and concentration. Nevertheless, a comprehensive mechanism explaining the actions of nanoparticles has not yet been definitively established. The utilization of

nanoparticles in plant protection can take place through two distinct mechanisms: (i) Nanoparticles themselves can directly provide crop protection and (ii) Nanoparticles can act as carriers for existing pesticides or other active agents, such as double-stranded RNA (dsRNA) (Worrall *et al.*, 2018). These nanoparticles can be applied through methods like spray application or drenching/ soaking onto seeds, foliar tissue, or roots (Worrall *et al.*, 2018).

Advantages of Nanotechnology

Specifically, nanotechnology can be the advance guard in the arena of crop protection under agricultural applications, with the potential to revolutionize existing technologies. The unprecedented advantages served by the utilisation of nanotechnology are as follows:

- Increases the solubility of pesticides that are not very soluble in water, overcoming the solubility barrier.
- Reduces pesticide toxicity and increases pesticide efficacy when loaded onto nanoparticles, all while reducing bioavailability (Mittal *et al.*, 2014; Worrall *et al.*, 2018).
- Provides a targeted release of actives and prolongs shelf life (Worrall *et al.*, 2018).
- Delivery of the active compounds to specific targets at specific pH levels (Duhan *et al.*, 2017) and at specific targets (Worrall *et al.*, 2018).
- RNAi molecules are transported frontline for crop protection (Hayles *et al.*, 2017).
- Nanoparticles integrated into formulations as rain-fastness and UV stabilizers, acting as transporters to attenuate the detriment of active compounds (Worrall *et al.*, 2018).
- By using nanoparticles as nanopesticides, pesticide resistance is overcome and selective toxicity is improved (Kah and Hofmann, 2014; Worrall *et al.*, 2018).

Disadvantages of Nanotechnology

Agricultural nanotechnology is a field having both advantages and disadvantages, much like any other coin. Some of the negative aspects or shortcomings are as follows:

- There is insufficient data to conclude with confidence whether certain nanotechnologies are hazardous or completely safe for human health (Agrawal and Rathore, 2014).
- However, there are always dangers involved when farmers are exposed to nanomaterials with unidentified life cycles on a long-term basis (Agrawal and Rathore, 2014; Kookana *et al.*, 2014).
- Interactions with the biotic or abiotic environment and their potential for increased bioaccumulation effects have not been taken into account thus far (Agrawal and Rathore, 2014; Torney *et al.*, 2007).
- Commercializing of the formulations involves high processing cost as the utilization at farmers level is still in its infancy.
- Research and development for prototype and industrial

production has challenges related to scalability (Agrawal and Rathore, 2014).

- The public's view of environmental health and safety hazards has not been assuaged by this technology (Agrawal and Rathore, 2014).

Conclusion

The impact of the efficacy and toxicity of nano-bioformulations on soil and the environment underscores the need for further in-depth exposure, studies and experiments to better understand these effects (Arora et al., 2010). However, nanobioformulations may be viewed as environmentally friendly and sustainable, offering a novel arsenal for combatting diseases in organic crop production practices (Prasad et al., 2017). Nanoparticles can also serve as quick diagnostic tools for detecting diseases influenced by bacterial, fungal, nematode and viral pathogens. When integrated with robotics and GPS systems, these technologies can create intelligent transport and delivery systems capable of detecting, mapping and addressing specific areas within a cultivation plot either before or during the initial phases of symptom development (Suffredini et al., 2014). The potential applications of this technology are vast and wide-ranging. Multidisciplinary and collaborative research will play a pivotal role in establishing a strong foundation for realizing the practical and attainable utilisation of nanotechnology in plant protection.

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